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# Hypothetical Soil Thresholds for Biological Effects of Rare Earth Elements

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**Abstract**

*Objectives*: Anthropogenic exposures to rare earth elements are poorly known and there is limited information on their toxicity and ecotoxicity. At the same time, world production of rare earth elements has doubled every 15 years over the last half-century, and high environmental concentrations of gadolinium and lanthanum have already been found. The current review aims to give some estimates of overall exposures and an initial in-depth appraisal of thresholds for effects on agricultural soil. The results are envisaged to be used in initial assessments of agricultural soil where the natural concentrations have been anthropogenically enhanced.

*Methods*: An extensive review has been made of available scientific literature. Criteria have been established for the selection and analysis of eligible research. For instance, only effects on soils with vegetation have been included in the assessment of biological effects. A species sensitivity distribution based on 25% inhibition of organism functions has been used to establish thresholds for effects on soil organisms.

*Results*: Around the year 2000, mean anthropogenic contributions of lanthanides in European soil regions were at most a few per cent of the total soil content. Since then, they should have increased considerably. The proposed hypothetical threshold for agricultural soils is 1125 mg total rare earth element per kg of soil. This threshold is about 8 times the natural soil concentration.

*Conclusions*: If this result holds up to scrutiny, it implies that general anthropogenic pollution by rare earth elements will not be a threat to agricultural sustainability for the coming generation. A preliminary assessment suggests that this threshold would also protect humans from adverse effects due to secondary exposure.

**Keywords:** natural elements, rare earth elements, lanthanides, soil threshold, concentration ratios, species sensitivity distribution

## 1. Emerging Contaminants With Little Toxicity Information

### *1.1 Rare Earth Elements Production Has Doubled Every 15 Years But Environmental Consequences Poorly Known*

*What are rare earth elements?* The rare earth elements comprise 17 elements, all belonging to group 3 of the periodic system. They include Sc in period 4, Y in period 5, and 15 lanthanides in period 6.

*Doubling every 15 years.* Rare earth elements play a critical role in advanced technology, from smartphones to radar systems, from catalysts for the cracking of crude petroleum to electric cars. The world production of rare earth elements has doubled every 15 years over the last half-century (United States Geological Survey [USGS], 2021).

*High concentrations found locally.* Production is dominated by China where significant environmental damage has occurred in the areas surrounding mining and processing operations (United States Environment Protection Agency [USEPA], 2012). High environmental concentrations have also been found elsewhere for gadolinium, which has been extensively used as a contrast agent in magnetic resonance imaging. Even in Mediterranean bottom water far off the coast of Sicily, gadolinium has had anomalously high concentrations (Censi et al., 2010). High concentrations of lanthanum have been revealed near a production plant for fluid catalytic cracking catalysts on the Rhine river (Kulaksiz & Bau, 2011), and major contributions to atmospheric particles have been found near optoelectronic industries (Yu et al., 2020). Several-fold increases have been found for many light rare earth elements in the water in the San Francisco Bay area (Hatje, Bruland, & Russel Flegal, 2016).

*Poor knowledge of general anthropocentric contamination.* However, the degree of anthropocentric contamination of the soil with rare earth elements is poorly known. This is contrary to the knowledge for some other metals, where for instance maps have been made of European contamination with heavy metals (Toth, Hermann, Da Silva, & Montanarella, 2016).

*Poor knowledge of toxicity.* The rare earth elements are poorly known with respect to toxicity and ecotoxicity, but there are concerns about adverse health effects to the respiratory tract (Rim, 2016), and environmental exposure has been associated with impaired intelligence in children (Zhu et al., 1996). In general, major databases such as those of IFA (2021), and Canadian Centre for Occupational Health and Safety (2021) lack occupational threshold limit values for rare earth elements except yttrium.

*A hundred-fold range in earlier suggested thresholds for biological effects on soil organisms.* A critical value of 30 mg rare earth element per kg of yellow cinnamon soil was early proposed by Tang, Sun, Xia, Wen, and Zhang (2004). Based on much more information, a threshold of 50 mg/kg was found for effects on soil organisms by Li, Verweij, and van Gestel (2018). This was based on 5% of organisms affected but did not account for aging of the elements in soil. In contrast, Li, Jiang, Chen, Zou, and Zhang (2006) reported on a three-year study in a bean field, where there was no effect on the number of species and the number of animals at rare earth element concentrations up through 3000 mg per kg of soil.

*The vision: understanding consequences of anthropogenic contamination.* Knowledge of the degree of anthropogenic contamination and the associated thresholds for effects may be important to assess hazards to the ecosystem, and to human health via food consumption. An example relating to the latter was provided by the work for heavy metals by Toth et al. (2016). They found that while most European agricultural land can be considered adequately safe for food production, an estimated 6.24% or 137,000 $km^2$ needs local assessment and eventual remediation action. A similar assessment for rare earth elements is needed. This would require further elucidation of anthropogenic exposures, and of thresholds for effects on soil organisms and human health.

### *1.2 Objectives*

The main objective of the current work was to find thresholds for the biological effects of rare earth elements on soil organisms. These would meet needs for management of contaminated soils by providing general benchmarks for soil quality that will be protective of biota in soil systems, accounting for organisms and pathways. It should be seen as a first component, to be later followed by a similar assessment of effects on human health.

In the process, the goal is also to critically assess the existing data, find gaps in what is available, and identify research needs. Further, the degree of anthropocentric contamination of European soils will be explored.

Thresholds for environmental effects will be sought for all rare earth elements and put into the context of thresholds for other elements including nanomaterials. An initial hypothesis is that the effect of all rare earth elements, atom for atom, will be the same since they all belong to group 3 of the periodic system.

## 2. Methods

An extensive review has been made of available scientific literature to find data pertinent to anthropogenic exposures to rare earth elements, and to thresholds for their biological effects in soils. Three different subsets of data have been reviewed, concerning:

(1) Concentrations in soil (Section 2.3). Natural concentrations define thresholds to which biota are adapted, and anthropogenic concentrations are a basis for estimates of risks from the emissions of rare earth elements.

(2) Transfer from soil to biota (Section 2.4). Effect levels in soils might potentially be inferred from effect levels under laboratory feeding conditions using concentration ratios from soil to food.

(3) Effects on biota (Section 2.5). Effects on many species are reviewed and used to establish species sensitivity distributions from which thresholds for effects on soil organisms can be derived.

Criteria have been established for the selection and analysis of such data. Many factors need to be considered for the setting of thresholds (Section 2.5.1). In the following, the criteria are discussed, and available data reviewed. The final selection of data is reported in the ensuing Section 3. Results. For brevity in the main text, details of the reviewed data are given in three appendices corresponding to items 1, 2, and 3 above.

### *2.1 Selection of Elements and Anticipation of Aberrant Properties*

Of the lanthanides, Pm is radioactive. It is only seen extremely rarely in nature as a fission product with a dominant half-life for Pm-147 of 2.6 years; there are data on its pathways in nature but not on its effects. Information on the other 16 rare earth elements will be sought.

There are differences in atomic properties and relative soil concentrations between the elements, as detailed below. Because of these differences one might expect that Sc could be aberrant from the lanthanides with respect to environmentally relevant properties, Y lie somewhere in between, and properties of the lanthanides Pr, Tb, Dy, Ho Er, Tm, and Lu might be quite similar, with La, Ce, Nd, Sm, Eu, Gd, and Yb possibly showing different properties.

#### 2.1.1 Atomic Properties of Rare Earth Elements

The atomic properties are cornerstones influencing concentrations of elements in soil and food, as well as health and environmental effects. Among the rare earth elements, atomic properties tend to be similar. Some properties are aberrant, however:

- Sc: the lowest ionic and covalent radii, smallest atomic volume, lowest density, and second-lowest elastic modulus, and the highest specific heat capacity and electronegativity among rare earth elements.
- Y: intermediate between Sc and the lanthanides for many properties
- Ce: has a cubic crystal structure (along with only Eu and Yb), the dominant oxidation states include 4 in addition to the common 3, and Ce has the lowest elastic modulus and the lowest melting point of all rare earth elements. Besides the Eu and possibly Yb anomalies (see Aberrations for Ce, Eu, and Yb in Section A2.3.1), a Ce anomaly is well-known in geology. Unlike the Eu and Yb anomalies, there is no trend anomaly for European soil concentrations, but a major anomaly for Moroccan apatite (Ramos et al., 2016).
- Sm: the only rare earth element with a rhombohedral crystal structure
- Eu and Yb: the dominant oxidation states include 2 in addition to the normal 3, the crystal structure is cubic rather than hexagonal, the boiling and melting points are rather low, the covalent radius and atomic volumes are rather high consistent with rather low density, the third ionization energy is high, and they have exceptionally low elastic moduli. In geology, a europium anomaly is well-known, and one can be seen in dietary concentrations (see Section A2.3.1). In chondrite normalized European soils, there are deviations from a smooth atomic number dependence of soil concentrations for Eu and Yb (Ramos et al., 2016). There are also large deviations in a sedimentary apatite from Morocco.
- Gd: there is a large drop in third ionization energy from the preceding Eu. This is compensated by rather high first and second ionization energies. Gd also has a high specific heat capacity.

Further, there are clear trends with the atomic number for electronegativity, elastic modulus and density (increasing), as well as covalent and ionic radii and specific heat capacity (decreasing). Such trends might be associated with trends in environmental properties of heavy versus light lanthanides. The following review will show to what extent data on soil and biota concentrations and effects on living organisms will reflect the differences in atomic properties.

#### 2.1.2 Aberrations in Soil Concentration Over the Periodic System

The natural soil concentrations might also be important for biological effects. For instance, in the periodic system of elements, lanthanides are so related to the group 2 element calcium that they have been called “super-calcium” (Brown, Rathjen, Graham, & Tribe, 1990). Further, cerium plays a role similar to that of the other major group 2 element, magnesium (Guo, Nazim, Liang, & Yang, 2016). Interaction with phosphorus may also be important (Kovaříková, Tomášková, & Soudek, 2019). In the calcium case, the interaction is due to some trivalent lanthanides being similar in ionic radius to divalent calcium, which they can replace in enzymes and other functional proteins. The soil concentration for Ca in group 2 of period 4 (25 000 mg/kg for Europe) by far outweighs that of the sum of all rare earth elements (about 200 mg/kg), and of the sum of all other group 2 and 4 elements in periods 4-7 (4 400 mg/kg). Ca thus provides the dominant competition. The links to such competitions need further research.

### *2.2 Availability of Suitable Data*

Data on exposures such as concentrations in soil or water or daily intakes can be found for almost all elements of interest.

Data on environmental effects are scarce and above all available for the more abundant elements Y, La, and Ce. Several sets of data on effects on single organisms are available. For the generalization of data to be meaningful, it should ideally start from a set of element data that has been derived coherently, preferably under the scrutiny of many different scientists. For the current analysis, this ideal cannot be realized with respect to thresholds for effects on soil organisms, and the conclusions are hypothetical.

*2.3 Soil Concentrations of the Selected Elements*

Thresholds for effects of natural elements on soil organisms are closely related to natural soil concentrations for elements in groups 5-12 of period 4, and some systematic dependence also appears to exist for periods 5 and 6 (Bengtsson, 2019). It is of interest to explore if similar relations might hold also for the rare earth elements in period 3, groups 4 (Sc), 5 (Y), and 6 (lanthanides). Little information has been found for effects on biota exposed in soils. Effect levels in soils might however potentially be inferred from effect levels under laboratory feeding conditions using concentration ratios from soil to food. Soil concentrations are reviewed in Appendix 1 and transfer from soil to biota in Appendix 2. In summary, the soil concentration of rare earth elements may vary by about a factor of 1000 between different locations. Higher soil concentrations of lanthanides are linked with several times lower relative concentrations of heavy lanthanides than lower soil concentrations. The mean rare earth concentration in European soils increased several-fold with the mean soil fractions of sand and total organic carbon, while no strong dependence on pH was found. Mean concentrations among different countries may vary by about a factor of 5. For elucidating health and environmental risks, a world mean sum lanthanide soil concentration of 133 mg/kg was selected. This can be compared with an average sum concentration of 126 mg element per kg dry soil for Europe and a sum concentration of 177 mg element per kg dry soil for China.

Around the year 2000, the anthropogenic contributions to rare earth concentrations in Europe at most amounted to a few per cent of the natural ones (Sections A1.2 and A1.3).

*2.4 Transfer from Soil to Biota*

Soil to biota transfer data is available for many parts of the transport from soil to food. Conceivably, they might be related to thresholds for effects on biota. The discussion in Appendix 2 starts with an elaboration of some uncertainties and defines the *concentration ratio* for soil to biota transfer, which is the ratio of the element concentration in the biota (mg/kg dry mass) to that in the soil. Appendix 2 continues with the differences among the elements and then discusses the extent of transfer from soil to biota. Finally, the transfer of Sc and Y is examined. The results on transfer are presented in Section 3.1.

*2.5 Selection of Threshold Data*

Recent reviews on effects in soils of rare earth elements include those of Gonzalez et al. (2015), Ramos et al. (2016), Adeel et al. (2019), and Blinova, Muna, Heinlaan, Lukjanova, and Kahru (2020). Conflicting results on sensitivities abound. One reason for this is that rare earths generally tend to enhance organism growth in the soil at low concentrations. Only at higher concentrations will the negative effects on growth and development dominate. Another difficulty has pertained to the study of aquatic species. The formation of insoluble species in some highly complexing media likely leads to changes in the soluble concentration of lanthanides during some tests (Gonzalez et al., 2015). Similar difficulties might also adhere to many studies of soil organisms made in nutrient or other solutions, and are further discussed by Blinova et al. (2020). To add to the complexity, rare earth elements may also enhance the taste and nutritional value of crops such as oranges (Cheng, Ding, Li, Zhang, & Wang, 2015).

The purpose of the analysis below in this Methods is to explore the extent to which effect concentrations vary among rare earth elements and discuss methods to establish soil thresholds. The lanthanides are treated separately from the other rare earth elements Sc and Y. Only exposures in real soil have been selected, thus no exposures in hydroponic solutions or the like; it is known that natural soils are far more heterogeneous and complex than homogeneous materials, and retention of engineered nanomaterials is always higher in natural materials (Cornelis, Hund-Rinke, Kuhlbusch, van den Brink, & Nickel, 2014).

For microorganisms, only experiments where soils have borne crops have been used. The intention is to establish thresholds for environmental effects of continuous exposures of the kind encountered in the environment after year-long emissions, and short-term experimental exposures should be corrected for insufficient aging.

2.5.1 Principles for Setting Soil Thresholds

General soil thresholds are often used in investigating and remediating contaminated sites (Gaudet, Bright, Adare, & Potter, 2002), and may have a role together with detailed site assessments in different stages of management, such as preliminary and detailed site investigations, as well as in site management planning and implementation

(Western Australia Department of Environment and Conservation, 2010). Many factors need to be considered when soil thresholds for the protection of the environment are set. Gaudet et al. 2002 provide an illustrative discussion of the issues at stake in the Canadian case. In the following, the parameters involved are the type of land use, the pathways of exposure, the desired degree of protection, the special attention needed for microorganism processes, the availability of data, and the aging phenomenon. In a more detailed assessment at later stage of managemnt, additional factors need to be considered such as soil texture, organic matter content, and soil pH, as well as potential secondary poisoning of for instance birds and humans.

(1) Type of Land Use

Land can be used for different purposes, *e.g.*, agricultural, residential, and industrial. In the current discussion, the use of agricultural land has been chosen. Common policy is that the level of protection for commercial and industrial land use does not need to be as stringent as for agricultural or residential/parkland land uses.

As an example, the widely used Finnish set of soil thresholds (Ministry of the Environment, Finland, 2007) encompasses three levels of contamination:

- Threshold: triggers assessment of soil contamination and remediation needs.
- Lower guideline: defines the area as contaminated for general areas.
- Upper guideline: defines industrial, storage, or transport areas as contaminated.

On average, the lower guideline concentration is 4 times the threshold concentration, and the upper guideline is 10 times the threshold concentration.

(2) Pathways of Exposure

Exposures may occur through different pathways, *e.g.*, soil contact for invertebrates or food ingestion for animals. For the discussion below, soil contact tends to be the most limiting. In the Canadian case for agricultural soil (Gaudet et al., 2002), consideration is given to no-effects and effects on soil nutrient cycling processes, invertebrates, crops/plants, and livestock/wildlife.

(3) Degree of Protection Aimed For

For the Canadian case, the desired degree of protection is discussed in detail in a protocol of some 200 pages (Canadian Council of Ministers of the Environment, 2006). The overall objective is to achieve a level of ecological functioning that sustains the primary activities associated with the chosen land use. Adverse effects are identified that undermine a species' ability to survive and reproduce under normal living conditions. Selected endpoints normally include those considered critical to the maintenance of soil-associated plants and animals, such as mortality, reproduction, and growth (Gaudet et al., 2002). The procedure for deriving soil quality guidelines contains several options. One of these, pertaining to soil contact, involves finding the concentration below which 25% of the species concerned are protected. If necessary, a safety factor can be applied before this option is used as the threshold effects concentration. Other options can have a starting point in the lowest observed effect concentrations or lethal concentrations. It is important to note that the protection aimed for is not total protection against all kinds of effects on all organisms.

For the current discussion, the lowest observed effect concentrations *per se* are not useful as policy thresholds, besides their weaknesses on statistical grounds (Laskowski, 1995). The Benchmark Dose concept for assessment of human health risks (European Food Safety Agency [EFSA] Scientific Committee, 2016) has gained increased recognition, and is a more interesting model. It is commonly applied to a Benchmark Dose-Response of 25%. In the current evaluation, effects at 25% have been sought to provide the basis for the assessment of ecological effects. Validation studies show that the ranking involved in species sensitivity distributions reflects expected relative ecological impacts (Posthuma & De Zwart, 2014).

(4) Separation of Species and Functions for Microorganisms

When it comes to microorganisms, the species approach will not give useful results. Microorganisms play a major role among soil organisms. They are extremely versatile and the composition among thousands of species is constantly changing. Effects on microorganisms in themselves may therefore not be an indication of harmful effects on the functioning of the soil ecosystem. As an example, lanthanum additions stimulated the growth of actinomycetes, fungi, and cellulolytic bacteria at different rates at different concentrations (Tang, Sun, Xia, Wen, & Zhang, 2004). Only when the overall functions of the microorganisms in the soil ecosystem are affected, however, may the functioning of the soil ecosystem be threatened. Indicators of such overall function effects have been used in threshold assessments, and effects on species and functions have been treated separately. Examples of such indicators include a total number of bacteria, bacterial biomass, and dehydrogenase activity.

The term function sensitivity distribution has been used to describe the management of effects on soil process functioning (Suter II, Traas, & Posthuma, 2002).

(5) Availability of Data and Ranking

Data is often scarce and disparate. In the current application, a species sensitivity distribution is used. A species sensitivity distribution for a chemical is commonly based on the logarithm of a measure of an ecotoxicity endpoint, for instance, the concentration at which 50% of the organisms die. The species sensitivity distribution curve is fitted to the cumulative frequency distribution of these logarithms across organisms for which data is available. The minimum size of the data set that can be employed for establishing such a distribution and the minimum quality of eligible studies is disputed (Posthuma, van Gils, Zijp, van de Meent, & de Zwart, 2019), and further elaborated in Section 2.5.1(7). For lanthanides, marginally enough data is available for the aquatic toxicity of several La and Ce compounds to enable environmental risk assessment using species sensitivity distributions (Posthuma et al., 2019, Supplemental data), but no corresponding major data compilation has been found for terrestrial organisms. A toolbox has recently been made available for the calculation of species sensitivity distributions (Center for Computational Toxicology and Exposure, EPA's, 2021).

Note that microorganisms are treated separately from other species (Section 2.5.1(4)).

(6) Aging and Long Exposures

As mentioned in Section 2.5.1(3), the overall objective is to achieve a level of ecological functioning that sustains the primary activities associated with the chosen land use. The length of exposure involved is in most found studies a few weeks, in some cases months, and very rarely years. For interpretation of study results, the duration of exposure must be related to the objective of sustaining activities, in the current case for agricultural purposes. Two different occurrences are involved:

- Aging of compounds added to the soil, which are moved to soil matrices where they become less available for organisms. The aging phenomenon is well known (Section A.2.1). As a crude hypothetical correction for lack of aging, sensitivities have been reduced by a factor of 1-3.4 if the equilibration time has been less than 6 months.
- Exposure of organisms through their life cycle. If the mean residence time in organisms is short compared to the life span, element concentration in the organism will reach equilibrium before the life span is attained. The most relevant studies should be the ones where exposure occurs during periods that are approximating to or beyond the mean residence time for the element in critical organs of the organism.
    - For *microorganisms* with their short life cycles, equilibrium concentrations are generally attained.
    - For *short-lived plants*, the found studies often comprise the period from seed to plant maturity, and the later decay and withering is less significant from the perspective of sustaining soil activities. For *long-lived plants* such as trees, results from study periods of a few weeks may underestimate the adverse effect of a continuous exposure.
    - For *invertebrates*, the studies of interest mainly concern arthropods with life cycles of a few months to a few years. Results from study periods of a few weeks must be discussed in the light of the short fraction of the lifetime of arthropods that is involved. Little and conflicting information has been found on turnover times.

      According to a review of earthworm metal concentrations (Richardson, Görres, & Sizmur, 2020), earthworms exposed less than 2 weeks generally had lower concentration ratios to soil than those exposed their entire life. For exposures 3-20 weeks, however, concentration ratios could vary a factor of 100 up or down those of lifelong exposures. Factors like aging, toxicity, and essential element interaction were given as potentially contributing to the variations. The review comprised only one element, uranium, in periodic system group 3, with a concentration ratio near 1 for lifelong exposures.

      This scarce information does not give grounds for assuming continued enhancement of rare earth element concentrations in arthropods after 4 weeks of exposure.
    - For *mice*, *rats*, *pigs*, and *rabbits* there may be significant losses of ingested rare earth elements over the lifetime.

      Scarce evidence suggests that a large fraction is lost from 5 to 8 weeks after ingestion for rats (Cao et al., 2020). A general retention time of 45 days was given by Nakamura, Tsumura, Tonogai, Shibata, and Ito (1997). When the exposure time was increased by a factor of 6 from 25 to 150 days, the concentration of Yb in the liver increased by a factor of 3 and in the femur by a factor of ~~a~~ 9 (Feng et al., 2007). The mean residence time in rats and rat liver of injected Ce was about a month (Norris, Lisco, & Brues, 1956), and this also applied for the retention of Y between 16 and 64 days (Hamilton, 1944).

In humans, rare earth elements tend to concentrate in bones, about 100 times more than in the liver, kidneys, lungs and testes (El Ramady, 2010). The residence time of Gd in the human bone may be of the order of 8 years (Darrah et al., 2009), consistent with the accumulation in the rat femur.

In piglets daily fed a commercial additive containing rare earth elements, the only major accumulation of rare earth elements after 126 days occurred in bone and not in heart, liver, kidney, muscle, or skin + subcutaneous fat (EFSA, 2019). Similarly, for pigs, the accumulation rate of La and Ce in the muscle, liver, and kidneys was very low after feeding the rare earth element diet for 3 months (He, Ranz, & Rambeck, 2001).

Thus, while bones may serve as accumulators of rare-earth metals, the concentrations in other organs influencing growth, survival, and reproduction might be reaching semi-equilibrium within a few months, suggesting that exposures of such length may not grossly underestimate these effects. This should be considered a hypothesis until further research can give better information.

o For *ruminants* such as sheep, goats, and cows, the found study periods of a couple of months are far shorter than the typical life span of 10-20 years. The significance of such short exposures in the sustainability context must be discussed. Little relevant information has been found.

This limited information suggests that the study periods of a couple of months are too short to entail a full scale of effects from sustained exposures over ruminant lifetimes, and that effective concentrations may be overestimated.

(7) Number of Studies

Concerning the number of studies required, the principles enumerated by the Canadian soil guideline protocol (Canadian Council of Ministers of the Environment, 2006) have been consulted for guidance. According to this protocol:

- Data for plants and invertebrates should, where possible, be evaluated separately.
- At least ten data points from at least three studies are required.
- A minimum of two soil invertebrates and two crop/plant data points must be represented. Single studies reporting data for multiple species and/or multiple endpoints will be considered as separate data entries.
- Data points for the same species that are redundant should be combined into a single composite response concentration calculated as the geometric mean of the individual values.
- For data points with the same concentration, it is recommended that these be assigned separate, sequential ranks.

All requirements could be met in the current assessment.

2.5.2 Assumption of Little Variation in Effects Among Lanthanides and Rare Earth Metals

Different lanthanides are similar in many respects as discussed in Section 2.1.1. One might expect rather similar thresholds for effects with possibly significant deviations for Ce (Section 2.1.2). Most studies reviewed by Blinova et al. (2020) support the similarity hypothesis. There are, however, signs of atomic number dependencies. For aquatic toxicity, heavy lanthanides have been predicted to be more toxic than light ones. For instance, Blinova et al. (2020) quote the example of the marine bacterium *V. fischeri*, the sensitivity of which to Gd was 7-fold higher than that to La. Blaise, Gagné, Harwood, Quinn, and Hanana (2018) studied the freshwater invertebrate cnidarian *Hydra attenuata*. Lethality to 11 lanthanides varied within about a factor of 2 and morphological changes occurred at concentrations between one-fifth and 2.7 of the mean. A detailed discussion of variations in effects among rare earth elements is given in Appendix 3.

Nonetheless, many experimental results are contradictory. There may be flaws in the chemical speciation in ecotoxicological test media that might entail marked underestimation of lanthanide ecotoxicity (Gonzalez et al., 2015). Another difficulty is related to the failure of certain fibroblasts to respond to lanthanide induced growth stimulation which may reflect the physiological state of the cells in question rather than a unique response to the lanthanides (Jenkins et al., 2011). More studies are needed to elucidate the possible existence of common mechanisms or modes of action across the lanthanide series. Pending more convincing information, the similarity hypothesis is applied for the setting of thresholds (Section 3.5.2).

2.5.3 Examples of Lanthanide Effects on Organisms Exposed in Soils

Major reviews concerning adverse effects on soil organisms include those of Hu, Shen, and Zhao (2006), Redling (2006), El-Ramady (2010), Rim (2016), Kovaříková, Tomášková, and Soudek (2019), and Agathokleous, Kitao, and Calabrese (2019). Examples of adverse effects for major classes of organisms are given in Appendix 3.

It should be noted that many studies encompass the influence of rare earth exposures on microorganisms in soil without vegetation. Such effects may be quite different from those where the microorganisms have a vegetation environment. For instance

- The dehydrogenase activity was up to 15 times higher in soil bearing maize or oilseed rape compared with that for soil without vegetation (El-Ramady, 2008);
- Nano-$CeO_2$ did not affect soil bacterial communities in unplanted soils, but 100 mg/kg nano-$CeO_2$ altered soil bacterial communities in planted soils, indicating that plants interactively promote nano-$CeO_2$ effects in soil (Ge et al., 2014).

Studies on microorganisms without vegetation were deselected and for the current assessment, only studies on soil with vegetation were used for the evaluation of thresholds.

For rare earth elements, no thresholds have been found relating to mammals in a soil environment. However, many effect thresholds for intakes of rare earth elements by mammals are available. These might be translated to soil concentration thresholds if the concentration ratio soil-mammal-diet can be determined, as discussed in Section 3.4. There are, however, large uncertainties in the concentration ratios (Section 3.2).

Even less information has been found for birds.

2.5.4 Examples of Effects of Sc and Y on Soil Organisms

Little information may be found on the effects of scandium and yttrium. For instance, in compiling data for a major review, Gwenzi et al. (2018) could not obtain any literature on the toxicity of Y in humans, and information on biological effects was scarce in the eChemPortal (Organisation for Economic Co-operation and Development [OECD], 2021). Because of this, the selection criteria applied for lanthanides could not be upheld for Sc and Y.

Judging from atomic properties (Section 2.1.1), Y can be expected to be intermediate between Sc and the lanthanides for many properties. Concentration ratios might also give some clues (Table A2.1); the concentration ratios are not significantly different between Sc, Y, and lanthanides but the variation is large.

There is some information on the effective concentrations for Sc and Y relative to those for lanthanides (Section A3.3.2). This points in the direction of the same effect concentrations for Sc and Y as for lanthanides per mol, within a factor of 2. The limited information on the effects of Sc and Y suggests that soil concentrations of about 1000 mg/kg might not be harmful to plants or to rodents living in the soil environment.

## 3. Results

As mentioned in the introduction to Section 2. Methods, the data reviewed concern:

- Concentrations in soil,
- Transfer from soil to biota, and
- Effect concentrations for soil organisms. A special assessment has been made for mammals since there is no information of effect concentrations for mammals in a terrestrial habitat.

The information is summarized below while details are given in the respective appendices. The information is used to derive hypothetical thresholds for initial assessments of the status of agricultural soil where the natural concentrations have been anthropogenically enhanced.

### *3.1 Concentrations in Soil*

Under geological conditions, the relative concentrations of the different lanthanides in soil may be very changeable. The emphasis could be on light, intermediate, or heavy elements (Section A1.5). Similar variations occur in the aquatic environment (Section A2.3.3). Light lanthanides tend to be clearly enhanced at high total lanthanide concentrations (illustrated in Figure A1.1 of Section A.1.5).

Anthropogenic depositions were explored in appendix A.1.2. From that discussion, it appears that soil properties are the main factors behind relatively high topsoil concentrations, rather than unusually high depositions. It should be noted that the underlying soil measurements are about two decades old. In the years between 2000 and 2020, the world production of rare earth elements has increased 2.6 times (USGS, 2021), and this should have resulted in more significant anthropogenic contributions to topsoil concentrations.

*3.2 Transfer From Soil to Biota*

The transfer from soil to biota is discussed in detail in Appendix 2. There, the potential to relate soil-to-biota transfer data to the threshold for effects on biota was elaborated for lanthanides, Sc, and Y. The concentration ratios for lanthanides from soil to biota vary about 100 000-fold, with a range from 0.000 002 to 0.2 (Section A.2.4). Section A2.1 gives examples of extremely high local concentrations in a few biota samples, *e.g.*, for Dy and Er. An extreme case concerns a food sample with 20 000 times higher Er concentration than the 90th percentile.

The concentration ratios biota/soil are enhanced for light lanthanides in human hair and wheat seeds, maize seeds, and legumes (Section A2.3.3). In contrast, the concentration ratio tends to vary among lanthanides less than a factor of 2 or so from soil to many fruits, vegetables, and plants as well as many parts of the human body (Section A2.3.2).

The concentration ratios for Sc and Y fall within the range for the lanthanides, and are not significantly different from those, but the variation is large (Section A2.5).

*3.3 Effect Concentrations for Soil Organisms*

To sum up the assessment in Appendix 3, when it comes to aggregated complex effects such as growth or death, there are in many cases little differences in toxicity between lanthanides. Low standard deviations in the percentage range have been noted for some monocellular organisms and male mice. Low standard deviations in the tens of per cent range have been demonstrated for female mice, plants, and soil invertebrates. It is thus a reasonable hypothesis that for effects on soil organisms that affect soil productivity and ecotoxicity, all non-radioactive lanthanides can be assumed to be equally effective on a molar basis. This is consistent with the findings of Xu and Wang (2001) that the influence of individual rare earths in mixtures on two discussed microbial processes can be additive. It does not exclude the possibility that different lanthanides have different effectiveness under other circumstances, as exemplified for luminescence of monocellular organisms. A beautiful example of systematic variation comes from the influence of lanthanides on red blood cell deformability (Alexy et al., 2011): the change in shear stress due to La, Sm, Eu, Dy, and Er varied from 0.1 to 0.7 and was very clearly related to the lanthanide ionic radius which only varied between 1.03 nm and 0.89 nm.

There are known anomalies for Ce, Eu, and Yb in the trend of soil concentrations versus atomic number (Section 2.1.2). Since Eu and Yb hold less than a few per cent of the sum concentration in soil, any aberration may not be significant for the health or environmental effects compared to effects of the sum of lanthanides. In contrast, Ce accounts for 40-60% of the total lanthanide concentration in soils, and differences in the health and environmental effects of Ce in relation to other lanthanides could lead to important differences in the total lanthanide effects. Potential such differences should be reviewed when threshold concentrations are discussed.

*3.4 Effects on Mammals*

The thresholds for effects of rare earth intakes on mammals (Section A3.2.4) can be translated to thresholds for soil concentrations using the concentration ratios (Appendix 2).

According to the experiences of feed supplement for cows, sheep, and goats (Redling, 2006), addition of 400 mg rare earth element per kg feed does not seem to entail any adverse effects. The found concentration ratios for pastures have a range of 0.002-0.2 (Section A2.4). If the geometric mean 0.02 is assumed to represent common conditions, the no-observed adverse effect level would be at least 400/0.02 = 20 000 mg rare earth element per kg soil. Pigs and poultry would in nature be expected to have larger shares of feed with low concentration ratios such as grains and correspondingly higher soil thresholds.

Two studies with chronic exposures of rats and mice and one for rabbits are summarized in Appendix A3.2.4. None of them suggest any reduction in body weight or other adverse effects if they had been feeding in nature at a soil rare earth element concentration below 1000 mg per kg soil.

However, several studies suggest the lowest observed adverse effect level for neurotoxic effects in the range of only 1-10 mg rare earth element daily per kg body weight for chronic peroral administration to mice and rats. A level in the lower end of this range was found for neurological effects on the ensuing pups after shorter in utero exposure of rats (Xiao et al., 2020). Using the above-mentioned common concentration ratio for pastures of 0.02. the lowest concentration of 1 mg/kg body weight may correspond to a soil concentration of 1/0.02 = 50 mg La per kg soil. For a mixed diet, the threshold should be considerably higher.

### *3.5 Proposed Hypothetical Thresholds*

#### 3.5.1 Applicability

The proposed hypothetical soil thresholds are envisaged to be used in initial assessments of soil where the natural concentrations have been anthropogenically enhanced. They could be used together with detailed site assessments as described in Section 2.5.1. The use of agricultural land is the primary focus. Only effects on the environment have been considered, and the overall objective is to achieve a level of ecological functioning that sustains the primary activities associated with the chosen land use.

#### 3.5.2 Assumptions for the Derivation

It is assumed that the effect is determined by the molar sum of all rare earth elements since effect concentrations are quite similar across all rare earth elements on a molar basis (Section A.3.3.2). There may be deviations from this pattern for Ce which require special scrutiny (Section 3.3). The potential influence of competition from Ca may also need attention (Section 2.1.2).

The ecological functioning is assumed to be sustained at a concentration below which 25% of the species concerned are protected. Such a concentration is assumed to be approximated by the 25$^{th}$ percentile of species sensitivity distributions obtained from a combination of concentrations giving 25$^{th}$ percentile effects. Some support for the assumption is given by Posthuma and De Zwart (2014) who showed that the ranking involved in species sensitivity distributions reflects expected relative ecological impacts.

When it comes to microorganisms, the species approach will not give useful results (Section 2.5.1(4)). Only when the overall functions of the microorganisms in the soil ecosystem are affected, the functioning of the soil ecosystem may be threatened. Indicators of 25% inhibition of such overall function have been used in threshold assessments.

Aging (Section 2.5.1(6) and Appendix A.2.1) is a real effect that is rarely dealt with in ecotoxicological studies. It is here handled by a correction for aging. This correction is not applied to data encompassing more than 6 months of soil equilibration and organism growth. For shorter studies, the effective concentration is multiplied by a factor that is 3.4 at 0 months duration and the correction factor then decreases linearly with time through 6 months.

#### 3.5.3 Important Data Used

The most important information from the studies used for derivation of soil thresholds is summarized in Table 1. Generally, the studies are of too short duration to have accounted for the effects of aging of the supplied rare earth elements in soil. The exception is the 8 month study on invertebrates. There are 16 eligible entries for plants but only 2 for invertebrates and 4 for microorganisms.

The thresholds are expressed in mg rare earth element per kg soil. If they had been in terms of mol/kg, this would mainly have impacted the results for:

- 5 plants studied by Carpenter, Boutin, Allison, Parsons, and Ellis (2015) and Thomas, Carpenter, Boutin, and Allison (2014), where Y was also used. The mean $IC_{25}$ of those plants across the elements would have been impacted by less than 10% and the derived threshold by about 2%
- the earthworm study of Wu, Feng, and Qian (2012), where the use of molar concentrations would have had a similar minor impact.

Therefore, the accounting for molar weights was omitted. There were no data for Sc where the correction by mol would have been larger.

In the later discussion, the results are compared to those of nanomaterials (Section 4.1), and no-effect findings (Section 4.2).

#### 3.5.4 Proposed Hypothetical Thresholds

The data in Table 1 were fed into the United States Environmental Protection Agency toolbox for species sensitivity distributions (Center for Computational Toxicology and Exposure, EPA's, 2021). Data were fitted to a lognormal distribution using a maximum likelihood approach. The resultant 25$^{th}$ percentile of the distribution was 1125 mg rare earth element per kg soil (95% confidence interval 765-1674).

On a side note, the same data but uncorrected for aging gave the 25$^{th}$ percentile of 421 mg rare earth element per kg soil (95% confidence interval 279-676).

## 4. Discussion

Much research has been devoted to the effects of rare earth elements on organisms. For the development of thresholds, only a few studies have been selected, compatible to illustrate a level of ecological functioning that sustains the primary activities associated with the chosen land use. Some deselected studies could however give perspective on the proposed thresholds. These include investigations using nanomaterials, studies where no effects have been demonstrated, and deselected studies on invertebrates and mammals. In the following sections, results from such work are discussed as well as the validity of the used assumptions and future research needs.

### *4.1 Results From Nanomaterials*

When nanomaterials came into use a few decades ago, there were fears that their small size would entail especially hazardous effects on organisms. Much work has been devoted to addressing such concerns.

Table 1. Biological effects of rare earth exposures re-layout

| Organism | Elements studied | Effect | Exposure period | Corr. factor | Threshold mg/kg | Reference |
|---|---|---|---|---|---|---|
| ***Microorganisms*** | | | | | | |
| Microorganisms | La, Ce, Pr | Dehydrogenase activity | Plant growth 9 w | 2.56 | 691 | El Ramady, 2008 |
| Microorganisms | REE mixture | Microbial biomass | 16 w | 1.91 | 2174 | Zhou, 2003 |
| Microorganisms | La | Microbial carbon, nitrogen, $CO_2$ | 16 w | 1.91 | 2212 | Chu, 2001 |
| Microorganisms | La | Bacterial count | 75 d | 2.40 | 2880 | Jiang, 2008 |
| ***Invertebrates*** | | | | | | |
| Soil fauna | La (Ce, Pr, Nd, Sm) | Mean of species richness, diversity & evenness index | 8 months | 1.00 | 4510 | Huang, 2009 |
| Earthworms | Y | Death | 2 w | 3.20 | 1120 | Wu, 2012 |
| ***Terrestrial plants*** | | | | | | |
| *Asclepias syriaca* L. | Y, La, Ce, Pr, Nd, Sm, Tb, Dy, Er | Biomass reduction | About 8 w | 2.56 | 721 | Carpenter, 2015; Thomas, 2014 |
| *Brassica napus* | Mean of La, Nd, Ce | Suppressed leaf growth | Growth 70 d | 2.56 | 768 | Xiong, 1997 |
| *Brassica napus* | REE mixture | Plant growth* | Emergence + 2 w | 3.12 | 5086 | Zhang, 2001 |
| *Desmodium canadense* | Y, La, Ce, Pr, Nd, Sm, Tb, Dy, Er | Biomass reduction | About 8 w | 2.65 | 530 | Carpenter, 2015; Thomas, 2014 |
| *Glycine max* | Mean of La, Nd, Ce | Suppressed leaf growth | Growth 37 d | 2.91 | 4651 | Xiong, 1997 |
| *Glycine max* | REE mixture | Plant growth* | Emergence + 2 w | 3.12 | 2808 | Zhang, 2001 |
| *Oryza sativa* | Mixture | Biomass reduction | Assumed 4 months | 1.80 | 1080 | Wang, 2006 |
| *Oryza sativa* | Mean of La, Nd, Ce | Suppressed leaf growth | Growth 33 d | 2.96 | 8584 | Xiong, 1997 |
| *Oryza sativa* | REE mixture | Plant growth* | Emergence + 2 w | 3.12 | 3245 | Zhang, 2001 |
| *Oryza sativa* | La (red soil) | Mean for ground biomass and yield | 120 days | 1.8 | 272 | Zeng, 2006 |
| *Oryza sativa* | La (paddy soil) | Mean for ground biomass and yield | 120 days | 1.8 | 612 | Zeng, 2006 |
| *Panicum virgatum* L. | Y, La, Ce, Pr, Nd, Sm, Tb, Dy, Er | Biomass reduction | About 8 w | 2.65 | 1258 | Carpenter, 2015; Thomas, 2014 |
| *Raphanus sativus* L. | Y, La, Ce, Pr, Nd, Sm, Tb, Dy, Er | Biomass reduction | About 8 w | 2.65 | 2737 | Carpenter, 2015; Thomas, 2014 |
| *Solanum lycopersicum* | Y, La, Ce, Pr, Nd, Sm, Tb, Dy, Er | Biomass reduction | About 8 w | 2.65 | 2499 | Carpenter, 2015; Thomas, 2014 |
| *Triticum aestivum* | Mean of La, Nd, Ce | Suppressed leaf growth | Growth 70 d | 2.47 | 4119 | Xiong, 1997 |
| *Triticum aestivum* | Mixture | Biomass reduction | Assumed 4 months | 1.80 | 3060 | Wang, 2006 |

*Note.* The chemical form has been chloride except for the soil fauna for which oxides were used. In all cases the endpoint has been 25% inhibition of functions such as growth or enzyme activity. The concentration in soil with vegetation at which such inhibition occurs has been corrected for aging by the indicated multiplication factor. References are given as first author and year of publication. d = days, w = weeks. * mean of 3 soils.

In particular, nanosilver has been extensively studied and some general conclusions from that work may have a bearing on rare earth nanomaterials. Early on, an increasing number of studies had found that the release of ionic silver could not alone account for the toxic effects observed from nanosilver exposures. However, a special European assessment of nanosilver (European Chemicals Agency [ECHA], 2018) concluded that there was no reason to classify the nano forms of silver more stringently than the easily soluble silver nitrate. Among other things, the conclusion was based on results for three different soil types showing that silver nitrate was equally or more toxic to soil microorganisms as compared to silver nanoparticles.

Different rare earth oxide nanoparticles can have strongly differing effects on living organisms. For instance, Ma et al. (2010) studied effects on plants of nanomaterials with Ce, La, Gd, and Yb and found a diversity of effect concentrations for root elongation and growth processes. Studies on cucumber (Ma et al., 2015) showed that $La_2O_3$ acted in the ionic form while $CeO_2$ displayed the behaviour of particles or particle–ion mixtures. Many studies of rare earth nanomaterial effects have concerned nanoforms of $CeO_2$ and these dominate the detailed discussion in the appendix (Section A3.4).

The results for nano-lanthanides are not inconsistent with the proposed hypothetical threshold for non-nano forms of rare earth elements of 1125 mg rare earth element per kg soil as corrected for aging. There is a discrepancy, however, for neurotoxic effects for chronic peroral administration to mice and rats which were observed in the range 1-10 mg rare earth element daily per kg body weight for non-nano forms of lanthanides (Section 3.1.5). A study for nanoform $CeO_2$ did not show any effects on rats (including neurotoxicity) after daily administration of 1000 mg/kg (Lee et al., 2020).

### *4.2 Results From No-Effect Studies on Microorganisms, Invertebrates, and Plants*

Five results for arthropods are not inconsistent with the proposed threshold of 1125 mg rare earth element per kg soil:

- The three-year field plot experiments of Tang, Sun, Xia, Wen, and Zhang (2004, see Section A3.2.1) failed to show any significant reduction in the total number of bacteria, actinomycetes, or fungi below 700 mg/kg of mixed rare earth chlorides. In this case, no correction for aging is needed.
- Li, Jiang, Chen, Zou, and Zhang (2006) reported on a three-year study in a bean field where rare earth chloride was applied and sufficient soil aging should have been attained. Many invertebrate species were recorded. There was no effect on the number of species and the number of animals at rare earth element concentrations up through 3000 mg per kg soil.
- Huang (2009) found only increases in the number of invertebrates after the addition of 1000 mg/kg soil of La or Nd in a horticultural vineyard.
- Li, Hong, Yin, and Liu (2010) found a correlation between reduced numbers of some soil fauna (*Carabidae*, *Dermaptera*), but not others (*Formicidae*, *Coleoptera*, *Orthoptera*) at sum rare earth elements concentrations up to 27 000 mg per kg soil in a rare earth mining district.
- Effects on the fruit fly *Drosophila* were noted above 6 mg rare earth element per kg food (Huang, Li, Wang, & Hu, 2010). The concentration ratio from soil to orange juice and fruit, in general is about 0.000 5 (Section A2.4). If such food would be representative for *Drosophila*, a no-effect concentration would be 12000 mg element per kg soil.
- Three terrestrial plants (oat, oilseed rape (*Brassica napus*, dicotyledon), and soybean) were exposed for 21 days to a concentration of a commercial rare earth feed additive of 1,000 mg per kg soil dry weight (EFSA Panel, 2019). No adverse effects were seen on seedling emergence, the survival of emerged seedlings, and the shoot fresh weight. The corresponding lanthanide concentration is 269 mg per kg soil. Applying the template correction for aging of 3.1, the no-effect level would be 834 mg/kg. This may not be inconsistent with the proposed threshold of 1125 mg/kg.

### *4.3 Thresholds Suggested in Other Studies*

Li, Verweij & van Gestel (2018) put their results for La exposure of 5 invertebrate species in the context of a species sensitivity distribution which also encompassed 7 plants and one bacterium. No correction was made for the aging of the elements in soil, and further the bacterium effect concerned bioluminescence. The $25^{th}$ percentile of the distribution was 155 mg La per kg soil. In relation to the proposed hypothetical threshold of 1125 mg/kg soil in Section 3.5.4, the $25^{th}$ percentile of Li, Verweij, and van Gestel (2018) differed in several respects, for instance:

- No correction for aging was made *vs.* a correction that would enhance the result of Li et al. (2018) by a factor of about 3.
- The basis for the species sensitivity distribution was $IC_{10}$ *vs.* $IC_{25}$; application of the latter would enhance the result of Li et al. (2018) by a factor of less than 2 for the invertebrates and about 2 for the plants of Thomas et al. (2014).

- For bacteria, one special effect (photoluminescence) was studied in one species while the current assessment has used the combined effects (total number, total nitrification, etc.) on all species that were judged to influence the functioning of the ecosystem.

Such differences might explain the numerical differences between the two estimates.

For the five invertebrates of Li et al. (2018), the 10% effect was in the range of 350-1120 mg La per kg soil. The 25% effect concentration would be nearly the same due to the steep dose-response curves. After correction for aging this would fall well into the range given in Table 1. The exception was the weight gain of the woodlouse *Porcellio scaber*, for which the 10% effect concentration was only 69 mg/kg. After aging correction by a factor of 3, the effective concentration of 207 mg/kg is lower than any entry in Table 1. It should be noted that the test conditions were quite artificial and far from those in natural vegetated soil.

Effects on mammals were summarized in Section 3.1.5. The suggested threshold for effects on mammals should be about 20 000 mg rare earth element per kg soil based on the use of rare earth elements for feed supplement, 128 000 mg/kg based on chronic exposures of rats and mice, and 12 000 mg/kg for exposure of rabbits. Much lower thresholds would be expected for neurotoxic effects in mice and rats, but these are not likely to affect the functioning of ecosystems. It should be noted that the concentration ratios used for the conversion from feed to soil concentration ratios are uncertain by about a factor of 10 up or down.

For the protection of humans, as a first approximation, the concentration ratio for cereals of about 0.0002 (Wang, 2020; Jiang, J. Yang, Zhang, & J. D. Yang, 2012) could be used; lower concentration ratios can be derived from a total diet study in Canada (Health Canada, 2007; La in 2001, 2002, 2005, 2006, 2007; Y and La in 1993, 1999), and a study of human feces in Europe (Ulusoy & Whitley, 2000). An acceptable daily intake of about 3 600 microgram rare earth elements per day was proposed by Zhu et al. (1996). That level was based on effects on the intelligence quotient, in line with the result from mammals that suggest that mammals are more sensitive to neurological effects than to others. The intake limit proposed by Zhu et al. (1996) would correspond to a soil concentration of 45 000 mg/kg, assuming a dry food intake of 0.4 kg/day. The limit should be lower for populations with a high intake of vegetables for which the mean concentration ratio might be about 7 times higher than for cereals. There should still be ample margin to the proposed soil threshold of 1125 mg/kg, in line with the assessment of Jiang et al. (2012) that the 90$^{th}$ percentile of intake would correspond to 3.5% of the acceptable intake.

For birds, only one eligible study on broiler chicken was found. While the result may not be at variance with the proposed threshold, more studies are necessary to corroborate this.

*4.4 Potentially Different Threshold for Cerium*

The cerium anomalies might conceivably also lead to important differences in the total lanthanide effects on health and the environment (Section 3.3). The paucity of data precludes any conclusions in this respect, but there is no support for comparatively higher toxicity of cerium:

- Indeed, the addition of up to 300 mg cerium oxide per kg feed of lying hen diets had positive effects on egg production, feed conversion ratio, and egg shelf life (Bölükbaşı et al., 2016), and the same addition to feed of rabbits did not affect their feed conversion factor (Adu, Akinmuyisitan, & Gbore, 2013).
- Application of 80 mg Ce per kg soil did not influence soil enzyme activities of maize and oilseed rape 66 days after sowing (El-Ramady, 2008).
- Improvements of plant growth or quality are known, for instance, Ce-induced growth enhancement of tomato plants by counteracting *Fusarium* wilt infection (Adisa et al., 2018).

*4.5 Thresholds in Relation to Natural Soil Concentrations*

Organisms are known to adapt to natural concentrations of elements in the environment. For instance, a significant correlation has been found between antibiotic resistance genes and soil metal concentrations (Knapp et al., 2011; Knapp et al., 2017). It has been recommended that future design of toxicology experiments should attempt to incorporate the dosage rate or the dietary influx rate to facilitate inter-comparison of the results of different studies (Wang, 2013). It is thus a natural hypothesis to assume that thresholds for health and environmental effects would be closely related to the natural exposures of the elements.

It has also been shown that thresholds for effects on soil organisms for many metals have been set at about 1-5 times the soil concentration for elements in period 4 of the periodic system (Bengtsson, 2019). For 3 elements in period 5, the thresholds were at about 10-20 times the natural concentration. For period 6, a threshold was only found for Hg, at about 50 times the natural concentration. Similar data were reported for much of period 4 and 5 elements concerning thresholds for human intake (upper limits of intake) divided by mean dietary intakes. Here more information was available for period 6 elements, confirming that their limits were more than 50 times the mean natural intake.

The proposed hypothetical threshold (Section 3.5.4) of 1125 mg rare earth element per kg soil corresponds to 8.46 times the mean global soil concentration of 133 mg/kg soil (Section 2.3). This would apply to the dominant rare earth elements in period 6, the lanthanides. For Sc in period 4 and Y in period 5, the threshold of 1125 mg/kg corresponds to 112 and 56 times the natural concentrations (taken from Table A1.1, Section A1.6). This is in line with the high ratios of upper limits of intake to dietary intakes at groups 4-6 of Bengtsson (2019).

*4.6 Validity of the Used Assumptions*

Effects of rare earths on plants depend highly on a great variety of factors (Redling, 2006), such as soil properties (pH, organic matter, cation exchange capacity, clay contents), rare earth contents in soil, contents of interfering elements such as Ca and P, application methods and their rates and timing as well as climatic and plant conditions (species, growth stage).

For data-rich elements, a threshold calculator has been developed taking partly into account corrections for pH, organic content, clay content, cation exchange capacity, background zinc concentration, aging, background concentration, secondary poisoning, and bioavailability (Oorts, 2020). Data enabling such a comprehensive evaluation are only available for a handful of elements (Cd, Co, Cu, Mo, Ni, Pb, Zn) belonging to group 9 of the periodic system or higher, excepting Mo in group 6. Inferences with respect to rare earth elements in group 3 will thus be farfetched.

Any suggestion for a single threshold for rare earth element effects must necessarily be a gross simplification without much possibility of a proportionality check. To achieve such a simplification, several assumptions were enumerated in Section 3.5.2. Some of the weaknesses of such assumptions are the following.

*Life-time exposure is approximated.* The representativity of less than life-long exposures was discussed in Section 2.5.1(6). While bones may serve as accumulators of rare-earth metals, the concentrations in other organs influencing growth, survival, and reproduction might be reaching semi-equilibrium within a few months, suggesting that exposures of such length may not grossly underestimate these effects. This should be considered a hypothesis until further research can give better information.

*The effect is determined by the molar sum of all rare earth elements.* Sections A3.2 and A3.3 give many examples for which the molar effect ratio is within a factor of 1.5 among lanthanides and Sc/Y. There are some examples of larger differences. For instance, the concentration in soil that caused 25% reduction in plant biomass ($IC_{25}$) for Y relative to the lanthanides had a range of 1.1-2.9.

*The ecological functioning is assumed to be sustained at a concentration below which 25% of the species concerned are protected.* The 25% mark is used for instance in Canada (Gaudet, Bright, Adare, & Potter, 2002) and Sweden (Swedish Environmental Protection Agency, 2016). The 5$^{th}$ percentile using the current data is 509 mg per kg soil, as compared to 1125 for the 25$^{th}$ percentile.

*Correction for aging of metals in soil is in the range 1-3.4.* This can be compared with the factor in the range 1.2-4 for the laboratory to the field (aging or aging + leaching) used in European REACH dossiers (Registration, Evaluation, Authorisation, and Restriction of Chemicals) (Oorts, 2020), which is of the same order of magnitude.

*4.7 Research Needs*

*Similarities in effects among lanthanides.* More studies are needed to elucidate the possible existence of common mechanisms or modes of action across the lanthanide series.

*Role of competing elements.* The links between thresholds for rare earth elements and concentrations of other elements, mainly Ca but also Mg and P warrant further studies.

*Hazards from longer exposure times.* The significance of exposure times approaching the life length and influencing growth, survival, and reproduction of long-lived plants, invertebrates, and mammals is poorly understood and deserves further study.

*Secondary poisoning.* Secondary poisoning of avian and mammalian wildlife needs further elucidation.

*Microorganisms together with vegetation.* Microorganisms behave quite differently in soil with vegetation (Section 2.5.3) compared with unplanted soil, and vegetation is the normal state for agricultural soils. Only a few studies were found with vegetation, so there is a need for further studies on microorganisms in vegetated soils, particularly at high concentrations of rare earth elements.

*Year-long studies of invertebrates.* Only one long-term study was found with effects on invertebrates, in addition to one short-term study, one where no effect was found, and one for unplanted soil**.** Research is needed on effects in planted soils over years on invertebrates, at rare earth element concentrations high enough to produce effects on the soil fauna.

*Plants in equilibrated soils.* The aging correction for the current eligible plant studies enhances the effect concentration about three-fold. Studies are needed with longer duration, and in soil where rare earth elements have been supplied in such time that they have become equilibrated in soil matrices.

## 5. Conclusions

Around the year 2000, mean anthropogenic contributions of lanthanides in European soil were at most a few per cent of the total soil content. Since then, they are likely to have strongly increased. Sewage sludge concentrations may be more than hundred-fold the unavoidable dietary contributions, with important inputs from the unintentional mobilization of lanthanides via fertilizers and detergents.

Rare earth concentrations in soils may vary more than a thousand-fold depending on location. The ten heaviest lanthanides may account for more than 30% of the total at low total concentrations, but less than 5% at the highest totals. Scandium and yttrium have relatively low concentrations.

Concentration ratios from soil to plants and animals may vary in the range of 0.000 002 to 0.2. Extreme variations may occur, such as a food sample with 20 000 times higher erbium concentration than the 90th percentile.

The proposed hypothetical soil thresholds are envisaged to be used in initial assessments of agricultural soil where the natural concentrations have been anthropogenically enhanced. Only effects on the environment have been considered, although a preliminary assessment for human neurological effect has been made. The overall objective is to achieve a level of ecological functioning that sustains agricultural activities. This functioning is assumed to be sustained at a concentration below which 25% of the species concerned are protected. The proposed hypothetical threshold for agricultural soils is 1125 mg total rare earth element per kg of soil. This threshold is about 8 times the natural soil concentration. The proposed threshold is based on a species sensitivity distribution of only 22 eligible studies but is not inconsistent with many no-effect studies and studies on nanoforms of rare earth elements. Some deviations justify continued scrutiny, such as the low thresholds for effects on *Porcellio scaber*. If the threshold proves tenable, it implies that general anthropogenic pollution by rare earth elements will not be a threat to agricultural sustainability for the coming generation.

The proposed hypothetical threshold builds on several assumptions:

- less than life-long exposures are useful,
- the effect is determined by the molar sum of all rare earth elements,
- aging of the elements in soils can be corrected in a simplistic way.

More research is needed to provide more eligible studies, particularly for invertebrates and mammals, and assess the validity of the assumptions.

## Appendix 1

**Soil Concentrations of the Selected Elements**

The soil concentrations of different lanthanides depend on the geologic parent mineral and processes in the soil development, where many soil migration pathways have been isolated (M. T. Aide & C. Aide, 2012). Analytical methods may influence the apparent concentrations. Downward migration processes may generally but not always lead to reduced concentrations in the topsoil relative to those of the subsoil. The topsoil may also contain significant contributions from human activities. All these circumstances may lead to varying total soil concentrations of lanthanides and changing patterns of soil concentrations among the different lanthanides. These topics are elaborated below, and the discussion is concluded with a look at the soil concentrations of the non-lanthanide rare earth elements scandium and yttrium.

*A1.1 Influence of Analytical Methods and Statistical Parameters*

Sometimes the alleged relative concentrations depend on the analytical method used. For instance, *aqua regia* digestion may almost completely recover light lanthanides in Alfic Haplorthod soils but only to a small extent recover heavy lanthanides (M. T. Aide & C. Aide, 2012).

Another possible source of error may occur from the ashing of organic samples. The temperature in the sample may be inhomogeneous and the recovery of elements from ashing may be unpredictable. Factors involved include losses due to evaporation, insoluble residues, and material attachment to the used crucible (Mader, Száková, & Miholová, 1998; Harju et al., 2004).

One has also to watch out for the chosen statistical parameter. Concentration distributions may be quite wide and arithmetic, as well as geometric means may be used for their characterization. The difference might be substantial, as shown by several examples under Section A2.1.

*A1.2 Anthropogenic Contributions Derived From Emissions*

The anthropogenic contribution derived from emissions, mainly due to coal combustion, is discussed below and is in the following Section A1.3 compared with estimates from the topsoil/subsoil concentration ratio.

A1.2.1 Mean European Anthropogenic Soil Excess up to 3% Estimated From Emissions, Large Areas up to 30%

The mean lanthanide anthropogenic component for European topsoils has been estimated to be near 1.1% of the natural concentration except La 1.3%, Ce 1.8% and Eu 1.6% (Bengtsson, 2018). The dominant contribution comes from coal combustion. The calculation may not be grossly in error since the calculated excess was within about a factor of 3 from that estimated from topsoil/subsoil concentration ratios for other elements than lanthanides (A1.3.2). Some assumptions behind the lanthanide figures may imply underestimations:

- The assumed period for emissions was 1900-1999; 10-30% may additionally have accrued from emissions in the 19$^{th}$ century.
- Coal use statistics may be uncertain, particularly for countries in Eastern Europe, and other sources of statistics suggest a higher use by up to a factor of 2.
- Bengtsson (2018) pointed out that the assumed emissions are very approximate for Germany, where a large share of the coal use comes from lignite. Several trace elements may have much higher concentrations in lignite than the ones used in the calculations. Özbayoglu (2011) reported about 5 times higher concentrations of Ce and Nd than those assumed by Bengtsson (2018). Laudal, Benson, Palo, and Addleman (2018) report total lanthanide concentrations in the range 40-400 mg/kg, to be compared with the concentration 56 mg/kg used by Bengtsson 2018. Lower concentrations have however also been reported, (Životić et al., 2019; Adamidou et al., 2007).
- The assumed mean emission factor from coal of 80% may have been exceeded for some plants without emission limitation.

With these circumstances taken together, there is a possibility that lanthanide excess concentrations may be up to twice the estimate by Bengtsson 2018, or about 3% as a mean for European soils. Large areas may be exposed to 10 times the mean level (Bengtsson, 2019), that is, up to 30%. This is still not inconsistent with the estimate derived from topsoil/subsoil concentrations.

A1.2.2 High Concentrations of Lanthanides in Sewage Sludge

Indications for significant contamination with lanthanides also come from their concentrations in sewage sludge. The ratio of the mean national sludge concentration of some elements and the unavoidable dietary component of the sludge due to human intakes was 60-100 for 10 lanthanides (Ce, La, Er, Sm, Nd, Ho, Gd, Dy, Pr, Tm) for the least contaminated set of sludge which emanated from Sweden 2016 (Bengtsson 2018). Typically, sludge from the major cities of Chongqing and Xiamen in south-east China had about 5 times (range 3-13) higher lanthanide concentrations than the mentioned Swedish ones (Suanon et al., 2017). A major source of such enrichment was likely to be phosphate coming from detergents and fertilizers, but erosion and weathering processes of rocks were thought to be the main responsible for the lanthanide contents according to Folgueras, Alonso, Folgueras, and Lage (2018). The phosphate contribution is a reminder that unintentional mobilization of lanthanides can be of the same order of magnitude as the intended global production (Emsbo, McLaughlin, Breit, du Bray, & Koenig, 2015).

*A1.3 Topsoil Versus Subsoil Reveals Minute Anthropogenic Contribution*

The concentration of many metals in superficial soil layers decreases with time due to downwards transport (Bengtsson, 2015). Important parameters controlling the transport rate are pH, the concentration of sand versus clay and silt, and concentration of organic matter, for instance in the form of total organic carbon, TOC (Bengtsson, 2015; Sadeghi & Andersson, 2015). These parameters were measured for both topsoil and subsoil at about 800 European sites (Salminen et al., 2005). The concentration ratio topsoil/subsoil for lanthanides shows some correlation with the corresponding pH, sand fraction, and TOC ratios. The interpretation of those correlations needs to account for several circumstances:

- The topsoil may have had recent additions from the deposition of lanthanides.
- Recently deposited lanthanides may be more loosely bound and transported down through the topsoil at greater rates than the original lanthanides deposited in residual soil from geological processes
- Lanthanide transport through the subsoil may be controlled by complex processes. In general, the rate may be higher than the topsoil rate, but special conditions such as intervening clay layers may lead to slower transport of metals. In uncontaminated control soils, the metal concentration ratio topsoil/subsoil may in some cases exceed 1 (Bengtsson, 2015).

From the discussion in Appendix 1, Sections A1.2 and A1.3, it appears that soil properties are the main factors behind relatively high topsoil concentrations, rather than unusually high depositions.

A1.3.1 Equal Transport Conditions for Topsoil and Subsoil May Be Used to Infer Recent Depositions

The lanthanide concentration ratios topsoil/subsoil of Salminen et al. (2005) were analyzed for topsoil/subsoil ratios near one with respect to sand fraction, pH, and total organic carbon (TOC), on the assumption that the same soil parameters in topsoil and subsoil would imply equal conditions for downward transport. The dependencies on sand fraction, pH, and TOC were assessed in 3 tiers. To avoid undue influence of extreme outliers among the hundreds of samples, the 5 highest (range 3.9-18) and 5 lowest (range 0.08-0.31) lanthanide topsoil/subsoil ratios were deselected from the assessment.

- The dependence on pH was assessed first since the topsoil/subsoil pH ratio had a narrow range of 0.60-1.45. With the pH ratio averaged over 0.1 units intervals the lanthanide topsoil/subsoil ratio was,

  Lanthanide ratio = $-1.979\,0 \times pH^2 + 4.110\,6 \times pH - 1.1389$, with a coefficient of determination $R^2 = 0.78$.

  In the pH ratio interval of 0.8-1.2, the mean lanthanide ratio had no significant variation with a range of 0.90-1.05 and a mean of 0.96.

- For the selected pH ratio range 0.8-1.2, the mean ratio of lanthanide concentrations in topsoil and subsoil averaged over sand fraction topsoil/subsoil ratio intervals of 0.2 depended on the sand fraction ratio according to,

  Lanthanide ratio = $0.957 \times (\text{Sand fraction ratio})^{-0.446}$, with a coefficient of determination $R^2 = 0.95$.

  For a sand fraction ratio of 1, the lanthanide topsoil/subsoil concentration ratio was 0.957.

- The lanthanide topsoil/subsoil concentration ratio was corrected to correspond to a sand fraction ratio of 1. The corrected ratio averaged over a factor of 2 intervals depended on the TOC topsoil/subsoil concentration ratio as,

  Lanthanide ratio = $0.956\,3 \times (\text{TOC ratio})^{-0.042}$, with a coefficient of determination $R^2 = 0.92$.

  For a TOC ratio of 1, the lanthanide topsoil/subsoil concentration ratio was 0.956.

After the third tier, the lanthanide topsoil/subsoil concentration ratio corresponding to equal levels of pH, sand fraction, and TOC in the topsoil and subsoil was estimated to be 0.96.

The corresponding ratio for a Chinese agricultural test site was 0.92-0.96 (Liu, Wang, & Zhang, 1997). A relatively low ratio would be expected since the agricultural topsoil would have a rather high TOC concentration compared to the subsoil.

A1.3.2 Non-Lanthanides Topsoil/Subsoil Ratio May Give Clues to Expected Ratios of Lanthanides

Mean anthropogenic components of natural elements in European topsoils have been estimated by Bengtsson (2018). Generally, the dominant contributions come from coal combustion. The calculation may not be grossly in error; the calculated excess was within about a factor of 3 from that estimated from topsoil/subsoil concentration ratios for elements with large anthropogenic components. Non-lanthanide elements were identified from the. data set of Salminen et al. (2005) with soil concentration data determined with inductively coupled plasma analysis and with relatively low detection limits. Of these, 5 elements in the periodic system groups 3-5 (Sc, V, Hf, Ta, and U) had a predicted anthropogenic fraction in the topsoil of less than 2.3% with a mean of 1.7%. For these elements, soil samples with topsoil/subsoil ratios in the range 0.9-1.1 for pH and sand fraction, and 0.8-1.3 for TOC were selected, resulting in 17 soil samples with similar topsoil and subsoil parameters. The mean topsoil/subsoil concentration ratio was 0.946 (95% confidence interval 0.925-0.969) while that for the lanthanides was 0.930 (0.915-0.945) with an estimated mean anthropogenic fraction of 1.2%.

A1.3.3 Residence Time Relatively Long for Anthropogenic Contributions

Over the decades preceding the measurements of Salminen et al. (2005), the world production of rare earths had increased exponentially with a doubling time of about 15 years (USGS, 2021). In the same period, emitted fractions were reduced at a similar rate (Bengtsson, 2018). Anthropogenic contributions to soil concentrations related to consumption might therefore be decades old. The larger contributions from fossil fuel combustion would have peaked around 1975 (Bengtsson, 2018) and typically be a few decades old at the time of the measurements of Salminen et al. (2005). Most of these contributions would have stayed in the 25 cm topsoil if penetration followed the conditions for other cationic metals (Bengtsson, 2015). Larger losses might be associated with a combination of low pH, high sand fraction, and high TOC concentration (Section A1.3.1).

*A1.4 Total Concentrations of Lanthanides*

Results on the mean soil concentrations of Europe (Salminen et al., 2005) have been important sources for understanding lanthanide soil concentrations. As discussed in the preceding section, the anthropogenic component has been estimated to be at the percentage level (Bengtsson, 2018) and thus insignificant to assess health and environmental effects.

The mean national sum lanthanide concentrations divided by the mean concentration of the upper continental crust (Salminen et al., 2005) have a variation from about 0.4 for Ireland to 1.6 for Croatia, with Denmark as an outlier at 0.2.

China with rather high soil concentrations (Hu et al., 2006) had an average of about 0.8 of that of Croatia. In China, lateritic red earth soils stand out with about three times higher rare earth concentrations than the typical ones (Liang et al., 2005).

Analysis of the European topsoil data showed that the mean total lanthanide concentration increased strongly with the sand fraction, from 34 mg/kg at a sand fraction around 0.05 to about 150 mg/kg at sand fractions above 0.6. There was also a TOC dependence, from 53 mg/kg at TOC $< 0.5\%$ to about 130 mg/kg with large variations at TOC $> 5\%$. No strong dependence on pH was found.

For elucidating health and environmental risks, a world soil average sum lanthanide concentration of 133 mg/kg was used (Table A1.1). This can be compared with an average sum concentration of 126 mg element per kg dry soil for Europe (Salminen et al., 2005) and a sum concentration of 177 mg element per kg dry soil for China (Liang et al., 2005).

*A1.5 Relative Concentrations Among Lanthanides*

Generally, the lighter lanthanides La, Ce, Pr, and Nd have higher soil concentrations than the heavier ones. Depending on the geologic origin, however, the concentration distribution among lanthanides can be very variable. This has been illustrated for the mineral francolite which almost alone bears the rare earth elements of phosphate-rich rocks (Emsbo, McLaughlin, Breit, du Bray, & Koenig, 2015). The shale-normalized concentrations can vary from being very nearly independent of lanthanide element (Pleistocene-recent, upper Ordovician Richmondian) to having a very pronounced peak at Sm-Eu-Gd (upper Mississippian Serpukhovian) to having a large dip for Ce (middle Mississippian Visean). For the francolite, the highest total lanthanide concentrations are associated with the Sm-Eu-Gd peaking distribution implying comparatively low contributions from light lanthanides, while the lowest total concentrations exhibit relatively flat shale normalized concentrations.

For other minerals, other distributions occur. Xenotime minerals may have extremely high fractions of heavy lanthanides, while bastnäsite may be extreme the other way towards light lanthanides (Dostal, 2017). The mineral distributions are reflected in soil concentrations.

Heavier lanthanides tend to be less prevalent in soils when the total concentration of lanthanides is high. For carbonatites and alkaline igneous rocks, this has been demonstrated for La versus Yb (Dostal, 2017). The circumstance can be illustrated by the relation of some heavy lanthanides (Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) to the sum of all lanthanides (Figure A1.1). With the large variation among different minerals, it is not surprising that the ratios of heavy to total lanthanide soil concentrations are highly variable. In areas with high sum concentrations of lanthanides, such as in areas where rare earth elements are being mined, comparatively small contributions can be expected from heavy lanthanides.

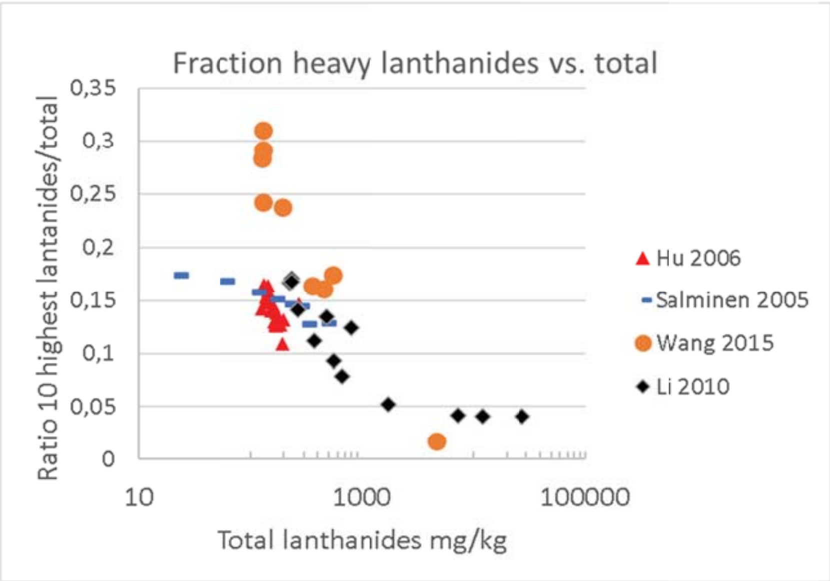


Figure A1.1. Ratio of topsoil concentrations of heavy and total lanthanides versus the total lanthanide concentration, for soils in Europe and China

*Note.* For Europe, data were obtained using inductively coupled plasma mass spectrometry (Salminen et al., 2005), for China using various techniques (Hu et al., 2006). The European data pertained to many soils and were assessed for 50 mg/kg intervals of total lanthanide concentrations. Data of Hu et al. (2006) also concerned many different soils. The results of Wang and Liang (2015) concerned soils in many directions within several kilometers from the Baodou mine site. The results of Li, Hong, Yin, and Liu (2010) concerned the same site but about 10 km in the downwind direction and with a different composition where the heavy lanthanides included Y but not the heaviest elements Ho, Er, Tm, Yb, and Lu; the latter comprised 29% of the 10 heaviest elements in European soils (Salminen et al., 2005).

Besides the just mentioned shifts with total lanthanide concentrations, the distribution of the soil concentrations among rare earth elements tends to be similar for the 25 European countries reviewed by Ramos et al. (2016).

*A1.6 Soil Concentrations of Sc and Y*

Soil concentrations of Sc and Y have been reviewed by Ross, Wood, Copplestone, Warriner, and Crook (2007) and Salminen et al. (2005). Their data is compared in Table A1.1 with the sum concentrations of lanthanides and with the concentrations in the Upper Continental Crust UCC.

Table A1.1. Sc and Y concentrations in topsoil and upper continental crust UCC compared with the sum concentration of the lanthanides

| Region | Mean concentration Sc (mg/kg) | Mean concentration Y (mg/kg) | Mean concentration sum lanthanides (mg/kg) |
|---|---|---|---|
| Soil Europe[a] | 9.1 | 22.7 | 126 |
| Soil China | 15 (5-28)[b] | 22 (11-39)[b] | 177[c] |
| Soil World | 10 (7-15)[b] | 20 (14-25)[b] | 133 (112-154)[d] |
| Upper continental crust[e] | 14 | 21 | 148 |

*Note*. The ranges in parentheses are only indicative. The rounded mean values indicated for World are used in the current assessment. [a] Salminen et al. (2005); [b] Ross et al. (2007); [c] Liang et al. (2005); [d] Ross et al. (2007; their quoted data from Bowen, 1979; Laul et al., 1979; Kabata-Pendias, 2000; Govindaraju, 1994); [e] Rudnick and Gao (2003).

There is some trend for Europe to exhibit low concentrations and China to exhibit high ones. Topsoil concentrations tend to be lower than upper continental crust concentrations, as expected from the migration patterns in topsoil (M. T. Aide & C. Aide, 2012). Sc concentrations tend to be about one-tenth of the sum lanthanide concentrations, Y concentrations about one-fifth.

**Appendix 2**

**Transfer From Soil to Biota**

Soil to biota transfer data is available for many parts of the transport from soil to food. Conceivably, they might be related to thresholds for effects on biota. The discussion below starts with an elaboration of some uncertainties and defines the terms used. It continues with the differences among the elements and then discusses the extent of transfer from soil to biota. Finally, the transfer of Sc and Y is examined.

*A2.1 Uncertainties in the Soil to Biota Transfer and Biota Effect Thresholds*

*Aging*. The aging phenomenon is well known. If sufficient time of several months has not been allowed for equilibration of the rare earth elements in soil, aging processes have not been completed, involving fixation of the elements in matrices from which they are less available to biologic matter. The median lowering of sensitivity for a large number of studies (Smolders et al., 2009) was 3.4, and differences up to one-hundredfold were noted. Many factors affect the aging and account for a large variability, as illustrated by a major study for molybdenum (van Gestel et al., 2012). Correction factors are available for data-rich metals (Oorts, 2020), but the only general correction given is for the element lead with a factor of 2. For rare earth elements, little information has been found. As a first hypothesis, it has here been assumed that aging is complete after 6 full months and justifies an increase by a factor of 3.4 in the effect concentration established after 0 months, and the correction decreases linearly with time in-between. The breakpoint time of 6 months is quite arbitrary but for instance for lead, no major change in sensitivity occurred after 6 months (Zhang & Van Gestel, 2019). The assumed curve is given in Figure A2.1 which also illustrates the only found information for rare earth elements, from Jiang, Weng, Huang, Jiang, and Wang (2008). Considering the large variation up to a factor of 100 (Smolders et al., 2009), the measured points are not inconsistent with the assumed curve.

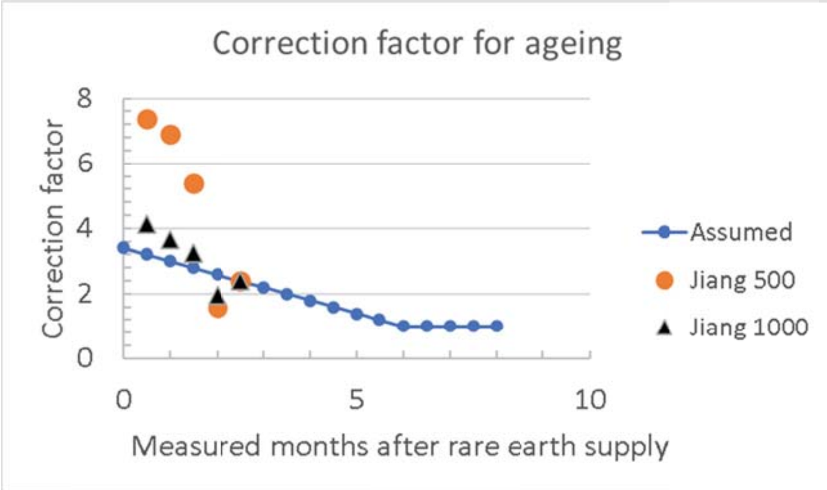


Figure A2.1. Assumed correction factor for aging

*Note*. It should be noted that literature data encompass up to about a hundredfold change in sensitivity. The lowering of sensitivity by a factor of 3.4 after 6 months aging represents the median of a large number of studies (Smolders et al., 2009). The break in the relation is set relatively arbitrarily at 6 months. The two sets of points marked Jiang represent the total soil bacterial count in pots with grass after exposure to 500 mg/kg and 1000 mg/kg lanthanum (Jiang et al., 2008). They denote the change in sensitivity normalized to the assumed relation at 2.5 months which was the longest time studied by Jiang et al. (2008).

*Leaching loss.* Some studies have investigated the loss of metal from the upper soil layer of 15-20 cm concomitant with aging (Oorts, Ghesquiere, & Smolders, 2007; van Gestel et al., 2012). These have concerned freshy prepared soils which may have been less stable than well-established soils. Experience from long-term field studies for many cations suggest typical leaching of about 10% after 6 months from 15 cm tilled soils, and about 30% in extreme cases (Bengtsson, 2015). This leaching is judged too small to warrant consideration, in relation to uncertainties of a factor of 2 or more, and no correction is made in the current assessment.

*Variability.* The concentration ratio of biota/soil can be very fluctuating. For different soils in Japan, Tagami, Uchida, and Zheng (2019) report concentration ratios per million,

- For La in the range 7-500 for rice and 100-18 000 for leafy vegetables, and
- For Ce in the range 2-400 for rice and 100-10 000 for leafy vegetables.

The variability is also noticeable among more than 1100 food samples from China measured by Jiang et al. (2012); here the maximum concentration divided by the median was 3600 for La and 600 for Ce, and in the extreme cases of Dy and Er it reached above 50 000 and 200 000, respectively.

*Statistical parameter choice.* Data on concentration distributions in soil and biota are sometimes including arithmetic means (AM) and other times using geometric means (GM). For a lognormal distribution, AM = 1.65 × $GM^2$ and the arithmetic mean may sometimes be ten times as high as the geometric one, so the difference may be exceptionally important.

The extremely high lanthanide concentration in some foodstuffs may be reflected in the geometric standard deviation for an assumed lognormal distribution. This standard deviation departing from the four lightest lanthanides was in some examples quite high, being:

- 2.52 for the concentrations in about 1200 food samples from China given by Jiang et al. (2012), derived from the $97.5^{th}$ percentile concentration which on average was 140 times the median (AM = 10.5 × GM). The maximum values were about 3400 times higher than the median corresponding to a geometric standard deviation of about 2.25.
- 0.95 for the vegetable segment of Jiang et al. (2012) (about 470 samples from China, AM = 1.5 × GM).
- 1.69-2.19 for wheat grains (Liang et al., 2005) (42 samples from China and 18 samples from other countries, AM = (4.7 – 7.9) × GM).

- 1.23 for cereals (Zhuang et al., 2017) based on an interquartile range of 1.94-2.11 times the median concentration (AM = 2.5 × GM).

*Fractionation.* Concentrations in biota should as a first approximation follow the concentration of lanthanides in soil. However, the concentration in biota divided by soil concentration is often subject to large statistical variations that preclude the definition of any trends with lanthanide atomic number. Uptake in plants may be fractionated in very variable ways to plant parts like root, stems, leaves, and seeds, i.a depending on the species concerned (Liang et al., 2008; Grosjean et al., 2019).

*Lack of threshold data.* For rare earth elements, no thresholds have been found relating to mammals in a soil environment. However, many data from the effects of lanthanides intake are available. These might be translated to threshold soil levels if the transfer relations soil-mammal-diet can be determined.

*A2.2 Terminology*

The following terminology is used, adapted from International Atomic Energy Agency [IAEA] (2010).

- Concentration ratio (dimensionless): For soil to biota transfer, the ratio of the element concentration in the biota (mg/kg dry mass) to that in the soil.

*A2.3 Relative Concentrations Among Lanthanides*

Three conditions are discussed below concerning the relative transfers from soil to biota among lanthanides:

- Aberrations for Ce, Eu, and Yb in relation to the immediate lanthanide neighbor elements.
- Biota showing little variation in transfer among lanthanides.
- Biota for which there are clear trends in transfer among lanthanides.

A2.3.1 Aberrations for Ce, Eu, and Yb

Based on the mentioned atomic properties (Section 2.1.1) it could be expected that there might be differences in organic matter concentrations relative to soil concentrations for light versus heavy lanthanides, and Ce, Eu, and Yb. Any aberrations might be masked by analytical uncertainties for heavier lanthanides due to their low concentration, which is generally less than one-tenth of the Ce concentration. Thus, aberrations might above all be found for Ce (Wyttenbach, Furrer, Schleppi, & Tobler, 1998). Factors influencing the Ce and Eu anomalies have been discussed by Kovaříková, Tomášková, and Soudek (2019).

Some clear anomalies can be demonstrated, where the aberrant concentration divided by the soil concentration is higher than that for the neighboring elements. In many cases, the aberrant plant/soil ratio is up to a factor of 5 higher for Eu. In a few cases, there is also enhancement or depletion for Yb and in a few cases depletion for Ce, as detailed below. The enhancement factor is:

- 4 times for Eu in cereals from a mining area and 2 for a control area (derived from Zhuang et al., 2017); no anomaly could be found for Yb; for the control area Ce was depleted in relation to La and Pr but La was strongly enhanced in cereals in relation to the mean soil concentration.
- 4-4.5 times for Eu in wheat grain derived from data of the soil dressing study of Liang et al. (2005), applying to the control soil and a soil subject to rare earth dressing, but not to the soil with three times higher dressing; the latter result seems to be an outlier or possibly a misprint. In the highly dressed soil, there was also an outlier for the grain/soil concentration ratio for Ce. For the control and slightly dressed soils, there was no anomaly for Ce. For the three soil cases, there was a depletion in the Yb grain/soil concentration ratio to 0.2-0.4 of the Tm and Lu ratios.
- 5 times for cereals and 2-3 times for other plants for Eu (Uchida, Tagami, & Hirai, 2007); no data were given for Yb; there was a small tendency for a depletion of Ce by less than a factor of 2.
- 1.9 times for Eu and 3.0 times for Yb for human excreta derived from Ulusoy and Whitley (2000), normalized to English rural soils (Ross et al., 2007); there was no anomaly for Ce.
- 2.2 times for Eu but no effect for Yb for whole fish collected in an unimpacted reservoir in Washington state, USA (Mayfield & Fairbrother, 2015); there was a small depletion for Ce.

No aberration could be seen for Eu or Yb in the wheat sample study of Liang et al. (2005). That study, however, concerned only 60 samples, 18 of which were from outside China, so there were confounding factors that might have masked any anomalies. No Ce anomaly could be found. For Ce, any effect might have been masked by the high concentrations of La.

Most of the mentioned apparent anomalies should be viewed as hypothetical since the soil used for normalization is not the exact soil from which the organic matter was derived. It is well known that the Ce (Kraemer, Tepe, Pourret, & Bau, 2017) and Eu (McLeod & Krekeler, 2017) anomalies can appear as both positive and negative concentration changes in rock material. Local soils may therefore be aberrant relative to national averages; the only of the enumerated cases where the local soils were used for reference were the dressing cases of Liang et al. (2005). In addition, concentrations tend to be lognormally distributed both for soils and organic matter with typical geometric standard deviations of 1-2. The geometric means of soil and organic matter concentrations are often reported while the arithmetic mean may be several times higher with a possibility for large impact of single samples with extremely high concentrations, a fact that suggests caution in the interpretation, particularly at small sample sizes. An extreme case in point is the maximum concentration of Er in food samples (Jiang et al., 2012) which is at 20 000 times the $90^{th}$ percentile concentration (see *Variability* under Section A2.1).

Since Eu and Yb hold less than a few per cent of the sum concentration in soil, any aberration may not be significant for the health or environmental effects compared to those of the sum of lanthanides. In contrast, Ce accounts for 40-60% of the total lanthanide concentration in soils, and differences in the health and environmental effects of Ce in relation to other lanthanides might lead to important differences in the total lanthanide effects. However, Ce concentrations could be enhanced, depleted or non-anomalous (Section A1.5) so any major influence on ecological effects is unlikely.

A2.3.2 Biota Showing Little Variation in Transfer Among Lanthanides

There was no significant trend with lanthanide atomic number for the assessed quantity divided by the soil concentration for,

- Parsley roots (Kučera, Mizera, Řanda, & Vávrová, 2007)
- Alfalfa, oats, and a range of fruits and nuts that are grown on sandy soil (Napier, Fellows, & Minc, 2014); the elements assessed were La, Ce, Sm, Eu, and Sc
- Food or horticultural waste ashes (El-Ramady, 2008), based on soil concentration for Japan from El-Ramady (2008), max/min = 5.8 and 1.5, respectively.
- Hair and urine of people living in agricultural soil near smelting and mining areas in Hezhang County, China (Meryem, Ji, Gao, Ding, & Li, 2016).
- The concentrations in human rib bone (S. Zaichick, V. Zaichick, Karandashevc, & Nosenkoc, 2011), (max/min = 2.3).

A2.3.3 Biota for Which There Are Clear Trends in Transfer Among Lanthanides

There were clear trends with lanthanide atomic number for the assessed quantity divided by the soil concentration in some cases:

- The geometric mean concentration ratios for wheat seeds (Liang et al., 2005) decreased systematically with the atomic number from 0.005 for La to 0.0005 for Lu with a mean for the lighter lanthanides around 0.001. A similar pattern exists for the factors for wheat, maize, and legume reported by Zhuang et al. (2017). In a follow-up study from Japan (Tagami et al., 2019), the concentration ratio from soil to leafy vegetables showed relatively small variations (max/min = 6.7) but that for brown rice increased systematically by a factor of 20 from La to Lu. The concentration ratios varied between samples by about a factor of 100, so the means were uncertain but increased by the atomic number to about the tenth power.
- Yuan et al. (2018) studied the naturally growing herbaceous plant *Phytolacca americana* L. All tissues were characterized by a light rare earth element enrichment and a heavy element depletion. This trend however applied to the absorption process (from soil to root) and the reverse was true for translocation process (from stem to leaf).

For aquatic food chains, there were large variations:

- For some turtles (Censi et al., 2013) the concentration ratio blood/sea water was relatively independent of the lanthanide element whereas for others, the concentration ratio was almost four times higher for light lanthanides than for the heavy element Lu, and for the scute, the concentration ratio was up to 40 times higher for the intermediate lanthanides Eu and Gd than for Lu.
- Lanthanides of lower atomic mass were consistently more concentrated in sea urchins, freshwater benthos, crabs, soft tissues of mussels, as well as in freshwater mosses; however, while preferential light

lanthanide concentrations occurred in natural benthos samples of bivalves, medium lanthanide concentration in bivalves was preferred in an acid mine drainage exposure experiment (Blinova et al., 2020).

A2.3.4 Summing up Trends in Biota Concentration Ratios

Because of lack of evidence and the soil concentration dominance of light lanthanides over heavy, the best description of overall transfer in relation to effects might be that:

- There is no trend with the atomic number at all for the transfer of lanthanides from soil to human intake.
- Except that aberrations for Ce might be important according to the section below.

*A2.4 The Extent of the Transfer From Soil to Biota*

The concentration ratios of La and Ce from biota to soil span the range 0.000 002-0.018 for rice and leafy vegetables, according to measurements across Japan (Tagami et al., 2019). Some examples of transfer data have been given in Section 3.1. Others are:

- The concentration ratios for radioactive materials (IAEA, 2009) from soil to cereals span almost three orders of magnitude, from 0.000 03 for rice (La and Pm) to 0.014 for wheat grain (Pm).
- There is a tendency for concentration ratios for tropical conditions to be higher that subtropical ones (Velasco & Yuri Ayub, 2009).
- Redling (2006) quotes data by Shan et al. (2003) on transfer of La, Ce, Pr and Nd to wheat shoots (mean concentration ratio 0.004) and roots (0.19). Data by Eriksson (2001) for wheat grain correspond to an arithmetic mean concentration ratio for light lanthanides of 0.000 01, which is less than one-tenth of the results of other studies.
- For a limited number of lanthanides, the concentration ratio was for parsley root 0.02, for lucerne and for wheat chaff 0.002, for wine grapes 0.001, for kale 0.0008 and for wheat and apricot 0.0005 (Kučera et al., 2007).
- Under natural conditions, the mean concentration ratio across lanthanides was for leafy vegetables 0.002 (Tagami et al., 2019), and for fresh vegetables 0.0016 (Jiang et al., 2012).
- Under natural conditions, for pasture plants, the mean concentration ratio across rare earths was for ryegrass 0.02-0.2 dependent on soil type (Liang et al., 2005; for recently added rare earth elements, the concentration ratio for ryegrass was up to 4 times higher, dependent on soil type), and for alfalfa 0.002-0.01 (Napier et al., 2014).
- The concentration ratio for radioactive cerium to fresh fruit was 0.0004 (IAEA, 2009), and for many lanthanides to orange pulp 0.00025 (Cheng et al., 2015) or 0.0009 (Turra, 2010).
- The concentration ratio for cereals for human consumption in a control group (Wang, Zhou, Xiong, Liu, & You, 2020) was 0.000 2 with no clear trend versus atomic number (range 0.000 03-0.000 6) when compared to average Chinese soils (obtained from El-Ramady, 2008).
- Data on the relation between rare earth elements in soil and fish are highly conflicting, with the arithmetic mean of the sum concentration of lanthanides in the dry fish muscle being in the range of 0.002-3 mg/kg based on sediments to biota or water to fish bioaccumulation factors (IAEA, 2009), and measured concentrations in fish (Mayfield & Fairbrother, 2015; Donald & Sardella, 2010).

The conclusions concerning soil to biota transfer are given under Results Section 3.1.

*A2.5 Concentration Ratios for Scandium and Yttrium*

Some concentration ratios for Sc and Y are given below in Table A2.1.

Table A2.1. Examples of concentration ratios for rare earth elements

| Transfer chain and unit | Reference | | Concentration ratio | | |
|---|---|---|---|---|---|
| | Receptor | Soil | Scandium | Yttrium | Lanthanides |
| To human food items China | Jiang, 2012 | Liang, 2005 | 0.00123 | 0.00077 | 0.00116 |
| To plants Canada | McMillan, 2017 | Liang, 2005 | | 0.00046 | 0.00080 |
| To hare, ptarmigan, caribou muscle Canada | McMillan, 2017 | Liang, 2005 | | 0.000008 | 0.000015 |
| To roots of rye and wheat | Shtangeeva, 2004 | Shtangeeva, 2004 | 0.017 | | 0.019 |
| To leaves of rye and wheat | Shtangeeva, 2004 | Shtangeeva, 2004 | 0.0024 | | 0.0031 |
| To food scrap ashes | El-Ramady, 2008 | Salminen, 2005 | 0.34 | 0.40 | 0.40 |
| To animal waste ashes | El-Ramady, 2008 | Salminen, 2005 | 0.54 | 0.46 | 0.64 |
| To horticultural waste ashes | El-Ramady, 2008 | Salminen, 2005 | 0.85 | 0.78 | 0.74 |
| To alfalfa and oats | Napier, 2014 | Napier, 2014 | 0.0025 | | 0.002 |
| To fruits and nuts | Napier, 2014 | Napier, 2014 | 0.0026 | | 0.004 |

*Note*. The references give first author and year.

The concentration ratios are not significantly different between Sc, Y, and lanthanides but the variation is large. Assuming the same ratios for Sc and Y as for the mean of the lanthanides would be consistent with all the table entries within about a factor of 2.

## Appendix 3

## Examples of Effects of Rare Earth Elements

### *A3.1 Variations in Sensitivity Among Rare Earth Elements*

#### A3.1.1 Monocellular Organisms

The findings by Su, Tai, Li, and Ke (2005) suggest that there may be small variations in sensitivity for the rare earth elements. The concentration that resulted in 50% reduction in growth of the exposed alga (*Chlorella autotrophica*) varied only in the range 29.00±0.50 μmol/liter for 12 lanthanides. A later follow-up by the same group (Tai, Zhao, Su, Li, & Stagnitti, 2010) with 13 lanthanides confirmed for a different alga (*Skeletonema costatum*) the small variation, 29.04±0.61 μmol/liter. In that case, the response to the total concentration was the same if lanthanides were mixed. The corresponding concentrations for Sc were 21 μmol/liter and for Y 43 μmol/liter. It appears that the response depends only on the total concentration of the lanthanides, not on the individual lanthanides involved.

For other monocellular organisms, a somewhat larger sensitivity range has been reported. Kurvet et al. (2017) studied the concentrations of La, Ce, Pr, Nd and Gd nitrates that affected one-half of the organisms in monocellular strains of a bacterium (luminescence) and a protozoan (viability). The more complex effect viability was associated with less variability (highest concentration 1.5 times the lowest, relative standard deviation 20%) than the less complex effect luminescence (6 times, 81%). The luminescence effect concentration decreased clearly with atomic number.

Rucki et al. (2021) studied the acute oral toxicity of the well-established mouse fibroblast 3T3 cells using the Neutral Red Uptake cytotoxic assay. The concentrations that led to a 50% reduction in cell growth $IC_{50}$ were calculated and a 50% lethal dose $LD_{50}$ was estimated. Salts of 14 lanthanides and yttrium that were chloride hexa-or heptahydrates except for the thulium anhydrous chloride were used. The calculated $IC_{50}$ had a range of ±12% from the mean with Y 2% above the mean. The estimated $LD_{50}$ in mol per kg body weight had a standard deviation of 17% among the elements. Yttrium had the lowest $LD_{50}$, 18% below the mean.

From pot studies of ammonium oxidation and mineralization of nitrogen by microorganisms, Xu and Wang (2001) concluded that the influence of individual rare earth elements in the mixtures on the two processes could be additive.

#### A3.1.2 Terrestrial Plants

The uptake of rare earth elements to different parts of plants depends on many factors as discussed in depth by Kovaříková et al. (2019). Transfer tends to decrease with increasing atomic mass and decreasing ionic radius. Often, however, the effect concentrations differ by less than a factor of 2, as also the following examples show.

Xiong and Zhang (1997) performed pot experiments where the growth periods were 33-70 days for wheat, soybean, rice, and rape. The ratio of the concentrations of Ce and Nd resulting in a 25% reduction in leaf or root weight to those for La had no systematic trend and a range of 0.6-1.4.

Corn and mungbean root and shoot mass reductions were affected in similar ways by La and Ce (Diathloff, Smith, & Asher, 2008).

Effects on wheat seedlings were generally similar for La and a mixture of rare earth elements at the same concentration, but some effects differed, for instance in the induction of antioxidant enzymes (d'Aquino, de Pinto, Nardi, Morgana, & Tommasi, 2009).

A3.1.3 Invertebrates

The small compost-living nematode *Caenorhabditis elegans* was studied by Xu et al. (2017). For tri-chloride rare earths, the median lethal concentrations were 100 mg/liter for Nd, 157 for Pr, and 106 for Sc. The corresponding numbers were 0.40, 0.63, and 0.70 mmol of rare earth element per liter. There was thus rather small variation in sensitivity to the three rare earth metals. There were some differences in behavioural and neural toxicity between the three chlorides with Sc generally being less toxic than Nd and Pr.

Huang et al. (2020) studied the effects of exposures to La, Ce and Gd on the annelid ringed worm *Enchytraeus crypticus*. The overall uptake rates and ultimate $LC_{50}$ values ($LC_{50\infty}$) were within about 10% of the mean value over the three elements while elimination rate constants were within about 25%.

Rucki et al. (2021) studied the sediment living ringworm *Tubifex tubifex*. The 50% effect concentration of 14 lanthanides and yttrium for inhibition of movement had a range of ±12% on a molar basis for salts that were chloride hexa-or heptahydrates except for the thulium anhydrous chloride. Yttrium fitted well into the toxicity pattern of the lanthanides.

A3.1.4 Mammals

Effects of rare earth nitrates intraperitoneally administered in female mice were early on studied for all 14 non-radioactive lanthanides (Bruce, Hietbrink, & DuBois, 1963). The dose that was lethal to 50% of the mice was in the range 0.73-1.53 of the geometric mean with a relative standard deviation of 23%. A compilation of data for chlorides in male mice encompassing 8 lanthanides (Ramos et al., 2016) showed a corresponding range around the geometric mean of 0.91-1.10 with a relative standard deviation of 6%.

Rucki et al. (2021) used the 3T3 Neutral Red Uptake (NRU) Phototoxicity assay on mouse fibroblast cells to estimate rodent oral intake $LD_{50}$ concentrations for 14 lanthanides and yttrium. The estimated $LD_{50}$ concentration in mg per kg body weight had a range of 0.90-1.18 in relation to its mean. Y fitted well into the toxicity pattern of the lanthanides, also per mol of rare earth element.

*A3.2 Examples of Effects*

A3.2.1 Microorganisms

Tang et al. (2004) report three-year field plot experiments to study the ecological effects of low dosage mixed rare earth elements accumulation on major soil microbial groups in a yellow cinnamon soil. The crop rotation encompassed rice, rape-seed, soybean, wheat, rice, and horse bean. There was no significant reduction in the total number of bacteria, actinomycetes, or fungi below 700 mg/kg of mixed rare earth chlorides, but the composition of the species changed.

Dehydrogenase activity is considered as a suitable indicator of microbial activity in soil (Wolińska & Stępniewska, 2012). The influence of lanthanide applications on dehydrogenase activity was studied in pot experiments by El-Ramady (2008) for fields sown with maize and oilseed rape. Harvest was 66 days after application of lanthanides and sowing. No decrease in dehydrogenase activity could be seen except in maize soil after application of 270 mg/kg of mixed rare earth elements; the mean decrease for maize and rape was 75%. No significant decrease could be seen in soil microbial counts (heterotrophic bacteria + actinomycetes + fungi) after the application of 270 mg/kg of mixed rare earth elements.

After 16 weeks of exposure in rice soil, the microbial biomass decreased by 10% after exposure to about 600 mg/kg of rare earth elements and by 25% after 1140 mg/kg (Zhou, Chen, Cao, Pu, & Peng, 2003).

Chu, Li, Xie, Zhu, and Cao (2001) applied La in a rice potted plant test. The geometric mean of soil microorganism nitrogen, carbon, and carbon dioxide emission were reduced by 25% at 1160 mg La per kg soil after 16 weeks.

A3.2.2 Invertebrates

Huang et al. (2009) studied soil fauna in a plum orchard. Arthropods dominated. There was a clear and strong effect on the number of individuals at apparently relatively low concentrations of lanthanides. However, the description of soil concentrations is brief and not verified, and concentrations are given as mg/kg and mg/L, casting doubts on the real concentrations, so the results are deselected.

From the same university, Huang (2009) looked at the addition of 1000 mg/kg soil of La or Nd in a horticultural vineyard. The number of species was 10-40% higher and the number of individuals 60-80% higher at the lanthanide plots compared with the control. This would suggest that the no-effect concentration would be well above 1000 mg/kg.

Li et al. (2018) exposed 5 species of invertebrates for 3-4 weeks in La spiked soil without vegetation that was equilibrated for at least 2 weeks. Juvenile earthworms were exposed additionally for 4 weeks. Inhibition concentrations that affected the organism reproduction to 25% were respectively 420, 920, 1160, 1190, and 160 mg La per kg dry soil. The authors note that aging had not been complete and that the arthropods had been exposed for a relatively short fraction of their lifetime. For survival, the concentrations were several times higher.

Six community parameters of 19 invertebrate species were studied by Li et al. (2006) (diversity index, evenness, species richness, dominant index, dominant centralization and species number). No parameter was significantly changed from yearlong exposures in a bean field up to 3000 mg mixed rare earth element per kg soil. The study had poor statistical power. A non-significant trend towards a smaller number of individuals at higher rare earth concentrations was found, with a 25% decrease at 4600 mg rare earth element per kg soil.

A3.2.3 Terrestrial Plants

The concentrations of 6 lanthanide chlorides (Pr, Nd, Sm, Tb, Dy, Er) in soil that caused a 25% reduction in plant biomass ($IC_{25}$) were assessed for 2 species by Carpenter et al. (2015). The same group (Thomas et al., 2014) added assessments for 2 further lanthanides (La, Ce) and another rare earth element (Y). Results were also obtained for some of the elements for 3 additional plant species, and for Ce two different values of pH were used. The results were highly variable but exhibited no clear trend with atomic number. The smallest variation among the elements was obtained for the species *Asclepias syriaca L* (minimum $IC_{25}$ = 0.32 of the mean, maximum = 1.29). For the other species, the minimum was 0.10-0.21 of the mean, and the maximum 1.68-2.00. The extremes were obtained at different combinations of species and elements, but Dy entailed the least sensitivity for 4 of the 5 species. Large variation was also obtained for the exposures to Ce at different pH, where the $IC_{25}$ at low pH varied from 0.26 to 1.48 of that at high pH across the plant species.

The mean $IC_{25}$ concentrations across the 9 rare earth chlorides (Y, La, Ce, Pr, Nd, Sm, Tb, Dy, Er) were 212, 156, 370, 805, and 735 mg/kg, respectively for the 5 species. During the approximate 2-month period of the study, aging could hardly be expected to have been fully developed, suggesting an underestimation of the long-term effect concentrations.

Onion bulbs (*Allium cepa* L.) were exposed in 6 months aged soil containing La and Ce up to 200 mg/kg soil (Kotelnikova, Fastovetsa, Rogovaa, Volkova, & Stolbova, 2019). No significant effects on root length were found but for both La and Ce, the mitotic index was significantly reduced at 200 mg/kg but not at 100 mg/kg. The conditions were quite artificial, with exposures only 5 days in test tubes with only 5 g of soil and often low statistical power.

Turra et al. (2015) exposed Rangpur lime (*Citrus Limonia* Osbeck) to lanthanum chloride heptahydrate and measured plant mass and length after 3 weeks. Extrapolation suggests that the 25% plant mass reduction level was about 350 mg La/kg soil. No significant effect on plant height was observed. The substrate was extremely rich in organic matter and not here considered representative of common soils.

Xiong and Zhang (1997) performed pot experiments where the growth periods were 33-70 days. The ratio of the concentrations of Ce and Nd resulting in a 25% reduction in leaf or root weight to those for La had no systematic trend and a range of 0.6-1.4. The mean concentrations resulting in a 25% reduction in leaf weight were 1670 mg/kg for wheat, 1600 mg/kg for soybean, and 2900 mg/kg for rice. For rape, there was an increase in leaf weight at 250 mg/kg and a strong decrease at 500 mg/kg, so the 25% reduction concentration should be around 300 mg/kg.

Zeng, Zhu, Cheng, Xie, and Chu (2006) studied the effects of La on rice. The concentration that reduced the mass of tillers and the height by 25% was 480±40 mg La per kg soil.

Hu, Song, Lan, Lin, and Ren (2009) studied the development of mulberry leaves after La exposures 22-48 days. For many endpoints, there was no significant adverse effect at the maximum concentration of 600 mg La/kg soil. However, a 25% reduction in height and diameter of young sprout was noted after about 250 mg La/kg soil.

Wang et al. (2005) cultured rice and wheat in greenhouse conditions. Exposure to mixed rare earth elements reduced the biomass of maturity rice by 25% at a concentration of 600 mg per kg soil and of maturity wheat at 1 700 mg/kg. The pot experiment was repeated for two years. The description is brief and does not give the rare earth compound or the length of exposure.

Zhang et al. (2001) studied the growth rate of rice, rape, and soybean for red soil, yellow tide soil, and yellow-brown soil exposed to rare earth chloride mixtures. The geometric means across soils of the concentration that reduced the growth rate by 25% were 400 mg rare earth element per kg soil, 400 mg/kg, and 510 mg/kg, respectively. The geometric mean across soils of effect concentrations for emergence was higher: 25% reduction at 1100, 670, and 510 mg/kg respectively. It should be noted that the exposure situation with soil in Petri dishes was rather artificial.

Zhang et al. (2015) studied an *Eucalyptus grandis* × *Eucalyptus urophylla* hybrid in a pot experiment. No effects were found at La concentrations up to 500 mg/kg soil.

A3.2.4 Mammals

Hutcheson, Gray, Venugopal, and Luckey (1975) studied the nutritional safety of La, Sm, Eu, Tb, Dy, Tm, Yb, Sc, and Cr oxides and barium sulfate in two species. The mouse study was conducted for three generations, including reproduction. The mice were not affected by quantities of metals in the feed which were 1 000 times the anticipated levels. To achieve such a concentration in nature, the soil concentration would have had to be 1 000 times the normal one, or about 130 000 mg/kg soil. It is conceivable that analytical and other methods have improved since this early study.

According to Fang et al. (2018), no loss of body weight and food intake occurred for 90 days daily exposure of rats by gavage to 10 mg La per kg body weight. The daily feed intake is 0.078 kg feed per kg body weight, so 10 mg intake would correspond to a concentration in a normal diet of 10/0.078 = 128 mg per kg feed. The typical soil concentration to give this intake would be about 128 000 mg/kg soil if the mice had a standard diet (LabSupply, 2021), and the mouse diet had a similar metal composition as a human diet, which is suggested by the similarity of the composition of other metals to the human dietary composition of Bengtsson (2018). Fang et al. (2018) found a significant effect on body weight and feed intake at 6 times higher concentration, or 60 mg La per kg body weight. However, Cao et al. (2020) found no effect after a shorter 28-day exposure to 129 mg/kg body weight by gavage but a clear effect at the ten-fold concentration. This shorter exposure should be less representative of continuous exposures than the 90-day exposures of Fang et al. (2018).

Adu, Akinmuyisitan, and Gbore (2013) exposed rabbits for 8 weeks to $CeO_2$. There was no effect on weight gain after feeding the rabbits a diet containing 240 mg Ce per kg feed. If rabbits in nature only consumed pasture with a concentration ratio of 0.02, the no-effect level would be 12 000 mg per kg soil.

A3.2.5 Birds

There was no significant influence on growth performance, relative organ weight, and excreta microflora for broiler chickens supplemented for 28 days with 1 500 mg per kg feed of rare earth elements-enriched yeast (Cai, Park, Seong, Yoo, & Kim, 2015). The concentration of the elements La plus Ce was 113 mg per kg feed. With a concentration ratio of 0.02 (Section 3.4), the threshold would be 5 650 mg per kg of soil. Note that this was a short exposure compared to broiler life. In nature, many large birds would have a life expectancy above 10 years.

*A3.3 Effects of Sc and Y*

A3.3.1 Examples of Effects

- Wu, Feng, and Qian (2012) exposed earthworms to natural soil contaminated with yttrium nitrate. The concentration leading to 25% mortality was 350 mg Y element per kg soil.
- Luo et al. (2018) exposed soil in pots to $YCl_3$ for a study of soil microbial community structures after 120 days incubation. The soil organic matter content was not significantly changed at 114 mg Y per kg soil, but a significant decrease was found after 228 mg/kg. There was no vegetation in the pots and the significance of the findings for plant growth is unclear.
- Kastori, Maksimović, Zeremski-Škorić, and Putnik-Delić (2010) quote an early study by Young 1935 which found that 500 mg Y/kg soil had a stimulating effect on the growth of the grass timothy.

- Wu, Zhang, Yang, Liu, and Mo (2006) studied the effects on rat brains of long-term intake of Y in drinking water. A water concentration of 0.534 mg Y per liter water might improve the function of learning and memory while 53,4 mg/L had little effect and 5340 mg/L could strongly restrain both the function of learning-memory functions and growth-development in rats. Similarly, Zhang, Yang, Liu, Zhang, and Xue (2006) found a boost to the immune response (IgG and IgM) at 0.534 mg/L, no response at 53.4 mg/L and strongly impaired immune functions and strong weight loss of spleen and thymus at 5340 mg/L. The clear effect dose at an assumed water intake of 0.05 L/d (Toxicology Excellence for Risk Assessment, 2021) gives 267 mg/d for 0.4 kg body weight or about 700 mg/d per kg. With a food factor of 0.078 kg food intake per day per kg body weight, the food concentration would have to be 9 000 mg element per kg food. A concentration ratio soil-food of 0.02 (Section 3.4) would imply a clear effect from 450 000 mg element per kg soil. No effect was observed at one-hundredth of this corresponding to 4 500 mg/kg.

Also, the ECHA registration dossier for yttrium (ECHA, 2021) contains information suggesting a no observed effect level of 1 000 mg per kg body weight per day, based on oral exposures of Wistar rats of both sexes. This is the same as the level that can be derived from the data of Shin, Kim, and Rim (2019). It can be compared to the effective dose of 700 mg/kg/day of Wu et al. (2006). Neurotoxic effects in rats have been found at about one-thousandth of these concentrations for Y (Feng et al., 2007) and La (Xiao et al., 2020).

A3.3.2 Sc and Y Effects in the Context of Lanthanides

Concentration ratios from soil to plants have been dealt with in Section A2.4. For Y, the concentration ratio was 0.6 and 1.05 of the mean for lanthanides for plants (MacMillan, Chételat, Heath, Mickpegak, & Amyot, 2017) and horticultural waste ashes (El-Ramady, 2008), respectively. For Sc, the corresponding ratio for horticultural waste ashes was 1.15. Considering the uncertainties, the same concentration ratio soil-plants should be applied for Sc and Y as for lanthanides, and data suggest this is accurate to better than a factor of 2.

The discussion below puts Sc and Y in relation to mean lanthanide effect concentrations. The results suggest that within about a factor of 2, organisms have the same sensitivity per mol to Sc and Y as they have to lanthanides.

- In Section A3.1.1, studies on two algae were mentioned where the concentration that resulted in 50% reduction in growth for 12 or 13 lanthanides had small variation around 29 µmol/liter. The corresponding concentrations for Sc were 21 µmol/liter and for Y 43 µmol/liter.

- Ramos et al. (2016) compiled data on $LD_{50}$ for male mice after intraperitoneal administration of rare earth chlorides. For Sc element the $LD_{50}$ was 130 mg/kg body weight whereas it was the range 300-350 mg/kg for other lanthanides. In terms of mmol per kg, the numbers are 2.9 *vs.* 2.0-2.3. It should be noted that all the lanthanides data were obtained by one research team while the Sc data were acquired by another team.

- The effect concentration of chlorides of Sc, Pr, and Nd in wells (48 h) was studied by Xu et al. (2017) for the nematode *Caenorhabditis elegans*. In relation to Pr, the effect concentrations for Sc in terms of mol per unit volume were in the range 0.39-1.3 (mean 0.73) for median lethal concentration after 48 h and 96 h, as well as for 25% reductions of body length, track length and mean speed. The corresponding ratio Nd/Pr had the range 0.25-1.26 (0.89).

- Y was included among the elements tested on plants by Thomas et al. (2014) (compare Section A3.2.3). The concentration in soil that caused 25% reduction in plant biomass ($IC_{25}$) for Y was not significantly different from that for 8 lanthanides tested on 3 species by Thomas et al. (2014) and Carpenter et al. (2015). The results were highly variable but the mean $IC_{25}$ ratio for Y relative to the lanthanides had a range of 1.1-2.9 in terms of mol per kg soil.

- On a molar basis, the 50% lethal effect concentration for *Daphnia* (EC50) for Y was 0.99 of that for Sc (Okamoto 2014).

*A3.4 Effects of Nano-Lanthanides*

Studies of rare earth nanomaterial effects are dominated by research on cerium oxide nanoparticles. No adverse effect on plant yields have been reported for cilantro (500 mg $CeO_2$ per kg soil; Morales et al., 2013); cucumber (1000; Zhao et al., 2013)¸ corn (1000; Zhao et al., 2015), radish (500; Corral-Diaz et al., 2014); wheat (500; Rico et al., 2014; and 400; Du et al., 2015); barley (500; Rico et al., 2015, and 1000; Pošćić, Mattiello, Fellet, Miceli, & Marchiol, 2016); tomato (130; Wang, Ma, Zhang, Pei, & Chen, 2012); soybean (1000; Priester et al., 2012—no effect on total plant but a low weight on pods that singly stands out), the brush *Clarkia unguiculata* (290 dosed each of 8 weeks; Conway, A. L. Beaulieu, N. L. Beaulieu, Mazer, & Keller, 2015) and common bean (500; Majumdar et al., 2015).

Generally, these have had a duration of less than 3 months although one was a life cycle study of 7 months (Du et al., 2015). The studies suggest that $CeO_2$ nanoparticles had no adverse effects on plant yields below Ce element concentrations in the soil of 800 mg per kg soil, or about 2400 mg/kg when corrected for aging. The nutritional content may however be changed. For instance, Rico et al. 2013 showed changes in many nutritional parameters for rice at 500 mg of $CeO_2$ nanoparticles per kg soil.

Broader effects of $CeO_2$ nanoparticles were noted in two studies. Zhang et al. (2017) observed a 25% weight loss in romaine lettuce (*Lactuca sativa* L.) at 744 mg element per kg soil (2300 mg/kg when corrected for aging). Priester et al. (2012) found a decrease in soybean plant stem length at 81 mg Ce per kg soil that almost disappeared at 814 mg/kg.

In a study on rats, $CeO_2$ nanoparticles up to 1000 mg daily per kg body weight were administered by gavage to parental rats for 4 weeks (Lee et al., 2020). No marked toxicities (including neurotoxicity) were observed in any observation parameters in parents or pups.

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**References**

Adamidou, K., Kassoli-Fournaraki, A., Filippidis, A., Christanis, K., Amanatidou, E., Tsikritzis, L., & Patrikaki, O. (2007). Chemical investigation of lignite samples and their ashing products from Kardia lignite field of Ptolemais, Northern Greece. *Fuel, 86*, 2502-2508. https://doi.org/10.1016/j.fuel.2007.02.008

Adeel, M., Lee, J. Y., Zain, M., Rizwan, M., Nawab, A., Ahmad, M. A., ... Xing, B. (2019). Cryptic footprints of rare earth elements on natural resources and living organisms, *Environment International, 127*, 785-800. https://doi.org/10.1016/j.envint.2019.03.022

Adisa, I. O., Pullagurala, V. L. R., Rawat, S., Hernandez-Viezcas, J. A., Dimkpa, C. O., Elmer, W. H., ... Gardea-Torresdey, J. L. (2018). Role of Cerium Compounds in Fusarium Wilt Suppression and Growth Enhancement in Tomato (*Solanum lycopersicum*). *Journal of Agricultural and Food Chemistry,* 66, 5959-5970. https://doi.org/10.1021/acs.jafc.8b01345

Adu, O. A., Akinmuyisitan, I. W., & Gbore, F. A. (2013). Growth performance and blood profile of female rabbits fed dietary cerium oxide. *Journal of Bio-Science, 21*, 69-75. https://doi.org/10.3329/jbs.v21i0.22521

Agathokleous, E., Kitao, M., & Calabrese, E. J. (2019). Hormetic dose responses induced by lanthanum in plants. *Environmental Pollution, 244*, 332-341. https://doi.org/10.1016/j.envpol.2018.10.007

Aide, M. T., & Aide, C. (2012). Rare earth elements: Their importance in understanding soil genesis. *IRSN Soil Science, 2012*, Article ID 783876. https://doi.org/10.5402/2012/783876

Alexy, T., Baskurt, O. K., Nemeth, N., Uyuklu, Wenby, R. B., & Meiselman, H. J. (2011). Effect of lanthanides on red blood cell deformability and response to mechanical stress: Role of lanthanide ionic radius. *Biorheology, 48*, 173-183. https://doi.org/10.3233/BIR-2011-0589

Bengtsson, G. (2015). Metals leak from tilled soil in a century—A review. *Journal of Agricultural Science, 7*(12), 15-49. https://doi.org/10.5539/jas.v7n12p15

Bengtsson, G. (2018). Past, present and future exposures from natural elements. *Proceedings of the global symposium on soil pollution May 2-4, 2018, FAO Headquarters Rome, Italy*. Rome: Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/fileadmin/user_upload/GSP/GSOP18/Bengtsson_Environmental_full.pdf

Bengtsson, G. (2019). Hypothetical thresholds for effects of platinum group elements. *Environment and Pollution, 8*(1), 39. https://doi.org/10.5539/ep.v12n1p39

Blaise, C., Gagné, F., Harwood, M., Quinn, B., & Hanana, H. (2018). Ecotoxicity responses of the freshwater cnidarian Hydra attenuata to 11 rare earth elements, *Ecotoxicology and Environmental Safety, 163*, 486-491. https://doi.org/10.1016/j.ecoenv.2018.07.033

Blinova, I., Muna, M., Heinlaan, M., Lukjanova, A., & Kahru, A. (2020). Potential Hazard of Lanthanides and Lanthanide-Based Nanoparticles to Aquatic Ecosystems: Data Gaps, Challenges and FutureResearch Needs Derived from Bibliometric Analysis. *Nanomaterials, 10*, 328. https://doi.org/10.3390/nano10020328

Bölükbaşı, S. C., Al-sagan, A. A., Ürüşan, H., Erhan, M. K., Durmuş, O., & Kurt, N. (2016). Effects of cerium oxide supplementation to laying hen diets on performance, egg quality, some antioxidant enzymes in serum and lipid oxidation in egg yolk. *Journal of Animal Physiology and Animal Nutrition, 100*, 686-693. https://doi.org/10.1111/jpn.12429

Brown, P. H., Rathjen, A. H., Graham, R. D., & Tribe, D. E. (1990). Rare earth elements in biological systems. In K. A. Jr. Gschneidner & L. Eyring (Eds.), *Handbook on the Physics and Chemistry of Rare Earths* (Vol. 13, pp. 423-453). Elsevier Sciences Publisher B.V., New York. https://doi.org/10.1016/S0168-1273(05)80135-7

Bruce, D. W., Hietbrink, B. E., & DuBois, K. P. (1963). The acute mammalian toxicity of rare earth nitrates and oxides. *Toxicology and Applied Pharmacology, 5*, 750-9. https://doi.org/10.1016/0041-008X(63)90067-X

Cai, L., Park, Y. S., Seong, S. I., Yoo, S. W., & Kim, I. H. (2015). Effects of rare earth elements-enriched yeast on growth performance, nutrient digestibility, meat quality, relative organ weight, and excreta microflora in broiler chickens. *Livestock Science, 172*, 43-49. https://doi.org/10.1016/j.livsci.2014.11.013

Canadian Centre for Occupational Health and Safety. (2021). *Occupational Hygiene-Occupational Exposure Limits*. Retrieved from https://www.ccohs.ca/oshanswers/hsprograms/occ_hygiene/occ_exposure_limits.html

Canadian Council of Ministers of the Environment. (2006). *A protocol for the derivation of environmental and human health soil quality guidelines*. Winnipeg, Canada: Canadian Council of Ministers of the Environment. Retrieved from http://www.ccme.ca/files/Resources/supporting_scientific_documents/sg_protocol_1332_e.pdf

Cao, B., Wu, J., Xu, C., Chen, Y., Xie, Q., Ouyang, L., & Wang J. (2020). The accumulation and metabolism characteristics of rare earth elements in Sprague-Dawley rats. *International Journal of Environmental Research and Public Health, 17*, 1399. https://doi.org/10.3390/ijerph17041399

Carpenter, D., Boutin, C., Allison, J. E., Parsons, L., & Ellis, D. M. (2015). Uptake and effects of six rare earth elements (REEs) on selected native and crop species growing in contaminated soils. *PloS ONE, 10*(6), e0129936. https://doi.org/10.1371/journal.pone.0129936

Censi, P., Zuddas, P., Randazzo, L. A., Saiano, F., Mazzola, S., Aricò, P., … Punturo, R. (2010). Influence of dissolved organic matter on rare earth elements and yttrium distributions in coastal waters. *Chemistry and Ecology, 26*(2), 123-135. https://doi.org/10.1080/02757541003627720

Censi, P., Randazzo, L. A., D'Angelo, S., Saiano, F., Zuddas, P., Mazzola, S., & Cuttitta, A. (2013). Relationship between lanthanide contents in aquatic turtles and environmental exposures. *Chemosphere, 91*, 1130-1135. https://doi.org/10.1016/j.chemosphere.2013.01.017

Center for Computational Toxicology and Exposure. (2021). *EPA's: Species Sensitivity Distribution (SSD) Toolbox* [Software]. The United States Environmental Protection Agency's Center for Computational Toxicology and Exposure. https://doi.org/10.23645/epacomptox.11971392.v1

Cheng, J., Ding, C., Li, X., Zhang, T., & Wang, X. (2015). Rare Earth Element Transfer from Soil to Navel Orange Pulp (*Citrus sinensis* Osbeck cv. Newhall) and the Effects on Internal Fruit Quality. *PLoS ONE, 10*(3), e0120618. https://doi.org/10.1371/journal.pone.0120618

Chu, H., Li, Z., Xie, Z., Zhu, J., & Cao, Z. (2001). Effect of lanthanum on the microflora of red soil. *Journal of the Chinese Rare Earth Society, 19*, 158-161. Retrieved from http://caod.oriprobe.com/articles/3254953/Effects_of_Lanthanum_on_Microbial_Biomass_Carbon__Nitrogen_and__Respi.htm

Conway, J. R., Beaulieu, A. L., Beaulieu, N. L., Mazer, S. J., & Keller, A. A. (2015). Environmental stresses increase photosynthetic disruption by metal oxide nanomaterials in a soil-grown plant. *ACS Nano, 9*(12), 11737-11749. https://doi.org/10.1021/acsnano.5b03091

Cornelis, G., Hund-Rinke, K., Kuhlbusch, T., van den Brink, N., & Nickel, C. (2014). Fate and Bioavailability of Engineered Nanoparticles in Soils: A Review. *Critical Reviews in Environmental Science and Technology, 44*, 2720-2764. https://doi.org/10.1080/10643389.2013.829767

Corral-Diaz, B., Peralta-Videa, J. R., Alvarez-Parrilla, E., Rodrigo-García, J., Morales, M. I., Osuna-Avilad, P., ... Gardea-Torresdey, J. L. (2014). Cerium oxide nanoparticles alter the antioxidant capacity but do not impact tuber ionome in *Raphanus sativus* (L). *Plant Physiology and Biochemistry, 84*, 277-285. https://doi.org/10.1016/j.plaphy.2014.09.018

d'Aquino, L., de Pinto, M. C., Nardi, L., Morgana, M., & Tommasi, F. (2009). Effect of some light rare earth elements on seed germination, seedling growth and antioxidant metabolism in Triticum durum. *Chemosphere, 75*, 900-905. https://doi.org/10.1016/j.chemosphere.2009.01.026

Darrah, T. H., Prutsman-Pfeiffer, J. J., Poreda, R. J., Campbell, M. E., Hauschkac, P. V., & Hannigand, R. E. (2009). Incorporation of excess gadolinium into human bone from medical contrast agents. *Metallomics, 1*, 479-488. https://doi.org/10.1039/b905145g

Diathloff, E., Smith, F. W., & Asher, C. J. (2008). Effects of lanthanum and cerium on the growth and mineral nutrition of corn and mungbean. *Annals of Botany, 101*, 971-982. https://doi.org/10.1093/aob/mcn021

Donald, D. B., & Sardella, G. D. (2010). Mercury and other metals in muscle and ovaries of goldeye (Hiodon alosoides). *Environmental Toxicology and Chemistry, 29*, 373-379. https://doi.org/10.1002/etc.39

Dostal, J. (2017). Rare earth element deposits of alkaline igneous rocks. *Resources, 6*(3), 12. https://doi.org/10.3390/resources6030034

Du, W., Gardea-Torresdey, J. L., Ji, R. Y., Zhu, J., Peralta-Videa, J. R., & Guo, H. (2015). Physiological and biochemical changes imposed by ceo2 nanoparticles on wheat: A life cycle field study. *Environmental Science & Technology, 49*, 11884-11893. https://doi.org/10.1021/acs.est.5b03055

ECHA. (2018). *Substance evaluation conclusion Silver* (Substance Evaluation Conclusion Document EC No. 231-131-3). Helsinki, European Chemicals Agency. Retrieved from https://echa.europa.eu/documents/10162/776ad739-c591-16fd-2e2c-62b9e50169ee

ECHA. (2021). *Registration dossier for yttrium*. Retrieved from https://echa.europa.eu/sv/registration-dossier/-/registered-dossier/22904/7/6/1

EFSA Panel. (2019). Safety of Lancer® (lanthanide citrate) as a zootechnical additive for weaned piglets. Scientific Opinion, EFSA Panel on Additives and Products or Substances used in Animal Feed. *EFSA Journal, 17*(12), e05912. https://doi.org/10.2903/j.efsa.2019.5912

EFSA Scientific Committee. (2016). Update: Use of the benchmark dose approach in risk assessment. *EFSA Journal, 15*, 4658. https://doi.org/10.2903/j.efsa.2017.4658

El-Ramady, H. R. (2008). *A contribution on the bio-actions of rare earth elements* (Doctoral dissertation, Technischen Universität Carolo-Wilhelmina, Braunschweig). Retrieved from https://ojs.openagrar.de/index.php/DissJKI/article/view/1618/8449

El-Ramady, H. R. (2010). Ecotoxicology of rare earth elements: I. Effects on soils and plants. In H. R. El-Ramady, *Ecotoxicology of REEs: Effects of rare earth elements (REEs) on soil and plant and Ecotoxicology of REE-fertilizers* (pp. 1-85). Düsseldorf, VDM Verlag Dr. Müller. Retrieved from https://www.researchgate.net/publication/215596160_Ecotoxicology_of_rare_earth_elements_Ecotoxicology_of_rare_earth_elements_within_soil_and_plant_environments#fullTextFileContent

Emsbo, P., McLaughlin, P. I., Breit, G. N., du Bray, E. A., & Koenig, A. E. (2015). Rare earth elements in sedimentary phosphate deposits: Solution to the global REE crisis? *Gondwana Research, 27*, 776-785. https://doi.org/10.1016/j.gr.2014.10.008

Eriksson, J. (2001). *Levels of 61 trace elements in sewage sludge, manure, fertiliser, precipitation as well as soil and crops* (Report 5148). Stockholm Naturvårdsverkets Förlag. Retrieved from http://www.naturvardsverket.se/Om-Naturvardsverket/Publikationer/ISBN/5100/91-620-5148-2

Fang, H. Q., Yu, Z., Zhi, Y., Fang, J., Li, C. X., Wang, Y. M., ... Jia, X. D. (2018). Subchronic oral toxicity evaluation of lanthanum: A 90-day, repeated dose study in rats. *Biomedical and Environmental Sciences, 31*, 363-375. https://doi.org/10.3967/bes2018.047

Feng, L., He, X., Xiao, H. Li, Z., Li, F., Liu, N., … Zhang, Z. (2007). Ytterbium and trace element distribution in brain and organic tissues of offspring rats after prenatal and postnatal exposure to ytterbium. *Biological Trace Element Research, 117*, 89-104. https://doi.org/10.1007/BF02698086

Folgueras, M. B., Alonso, M., Folgueras, J. R., & Lage, S. (2018). An overview of rare earth elements in sewage sludges and their ashes. *Proceedings, 2*, 1462. https://doi.org/10.3390/proceedings2231462

Gaudet, C. L., Bright, D., Adare, K., & Potter, K. (2002). A rank-based approach to deriving Canadian soil and sediment quality guidelines. In L. Posthuma, G. W. Suter II, & Traas, T. P (Eds.), *Species sensitivity distributions in ecotoxicology* (pp. 255-273). Boca Raton, Lewis Publishers. https://doi.org/10.1201/9781420032314.ch13

Ge, Y., Priester, J. H., Van De Werfhors, L. C., Walker, S. L., Nisbet, R. M., An, Y. J., … Holden, P. A. (2014). Soybean plants modify metal oxide nanoparticle effects on soil bacterial communities. *Environmental Science & Technology, 48*, 13489-13496. https://doi.org/10.1021/es5031646

Gonzaléz, V., Vignati, D. A. L., Pons, M., Montarges-Pelletier, E., Bojic, C., & Giamberini, L. (2015). Lanthanide ecotoxicity: First attempt to measure environmental risk for aquatic organisms. *Environmental Pollution, 199*, 139-147. https://doi.org/10.1016/j.envpol.2015.01.020

Grosjean, N., Le Jean., M., Berthelot, C., Chalot, M., Gross, E. M., & Blaudez, D. (2019). Accumulation and fractionation of rare earth elements are conserved traits in the *Phytolacca* genus. *Scientific Reports, 9*, 18458. https://doi.org/10.1038/s41598-019-54238-3

Guo, W., Nazim, H., Liang, Z., & Yang, D. (2016). Magnesium deficiency in plants: An urgent problem. *Crop Journal, 4*(2), 83-91. https://doi.org/10.1016/j.cj.2015.11.003

Gwenzi, W., Mangori, L., Danha, C., Chaukura, N., Dunjana, N., & Sanganyado, E. (2018). Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. *Science of the Total Environment, 636*, 299-313. https://doi.org/10.1016/j.scitotenv.2018.04.235

Hamilton, J. G. (1944). *Metabolism of fission products* (Document MDCC-1142). Oak Ridge, Technical Information Division. https://ufdc.ufl.edu/AA00009282/00001/1x

Harju, L., Rajander, J., Saarela, K.-E., Lill, J.-O., Heselius, S.-J., & Lindroos, A. (2004). Losses of elements during dry ashing of biological materials. *Proceedings of the. 10th International Conference on Particle Induced X-ray Emission and its Analytical Applications, June 4-8, 2004, Portorož, Slovenia*. Retrieved from http://users.abo.fi/jlill/0945.pdf

Hatje, V., Bruland, K. W., & Russel Flegal, R. (2016). Increases in Anthropogenic Gadolinium Anomalies and Rare Earth Element Concentrations in San Francisco Bay over a 20 Year Record. *Environmental Science & Technology, 50*(8). https://doi.org/10.1021/acs.est.5b04322

He, M. L., Ranz, D., & Rambeck, W. A. (2001). Study on the performance enhancing effect of rare earth elements in growing and fattening pigs. *Animal Physiology and Animal Nutrition, 85*, 263-270. https://doi.org/10.1046/j.1439-0396.2001.00327.x

Health Canada. (2007). Average dietary intakes (ug/kg bw/day) of trace elements for Canadians in different age/sex groups for total diet study. *Results for several cities and years until 2007*. Retrieved from https://www.canada.ca/en/health-canada/services/food-nutrition/food-nutrition-surveillance/canadian-total-diet-study/dietary-intakes-contaminants-other-chemicals-different-sex-groups-canadians.html

Hu, Y.-M., Shen, X.-Y., & Zhao, Q. (2006). The eco-toxicity effect of agricultural rare earth elements on soil creature. *Jinhua Institute of Vocational and Technical Sciences, 6*(6), 53-56. Retrieved from http://en.cnki.com.cn/Article_en/CJFDTotal-JHZY200606014.htm

Hu, Z., Song, S., Lan, W., Lin, C., & Ren, L. (2009). Research on the influence of rare earth element La on the growth and quality of mulberry leaf. *Journal of Anhui Agricultural Science, 37*, 3401-3403. Retrieved from http://caod.oriprobe.com/articles/15627903/Research_on_the_Influence_of_Rare_Earth_Element_La_on_the_Growth_and_Q.htm

Huang, B., Zou, Y., Bi, S., Wu, H., Li, G., & Peng, J. (2009). Effects of rare earth elements on the composition of the soil fauna community in a plum orchard. *Acta Scientiae Circumstantiae, 29*, 1849-1857. Retrieved from http://caod.oriprobe.com/articles/24306497/Effects_of_rare_earth_elements_on_the_composition_of_the_soil_fauna_co.htm

Huang, Q. (2009). Effects of rare earth elements on community structure of arthropod in graperies. *Anhui Agricultural Science Bulletin, 15*, 37-41. Retrieved from https://caod.oriprobe.com/articles/17819107/Efflects_of_Rare_Earth_Elements_on_Community_Structure_of_Arthropod_in.htm

Huang, S.-F., Li, Z.-Y., Wang, X.-Q., & Hu, F.-F. (2010). Cerium caused life span shortening and oxidative stress resistance in *Drosophila melanogaster. Ecotoxicology and Environmental Safety, 73*(1), 89-93. https://doi.org/10.1016/j.ecoenv.2009.09.017

Huang, X., He, E., Qiu, H., Zhang, L., Tang, Y., Zhao, C., … Qiu, R. (2020). Do toxicokinetic and toxicodynamic processes hold the same for light and heavy rare earth elements in terrestrial organism

Enchytraeus crypticus? *Environmental Pollution, 262*, 114234. https://doi.org/10.1016/j.envpol.2020.114234

Hutcheson, D. P., Gray, D. H., Venugopal, B., & Luckey, T. D. (1975). Safety of heavy metals as nutritional markers. *Environmental Quality and Safety, Suppl. 1*, 74-80, 114-5. Retrieved from https://pubmed.ncbi.nlm.nih.gov/808414

IAEA. (2009). *Quantification of radionuclide transfer in terrestrial and freshwater environments for radiological assessments* (IAEA-TECDOC-1616, p. 622). Vienna, International Atomic Energy Agency. Retrieved from https://www-pub.iaea.org/MTCD/Publications/PDF/te_1616_web.pdf

IAEA. (2010). *Handbook of parameter values for the prediction of radionuclide transfer in terrestrial and freshwater environments* (Technical Reports Series No. 472, p. 194). Vienna, International Atomic Energy Agency. Retrieved from https://www.iaea.org/publications/8201/handbook-of-parameter-values-for-the-prediction-of-radionuclide-transfer-in-terrestrial-and-freshwater-environments

IFA. (2021). *Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallsversicherung*. GESTIS International Limit Values. Retrieved from http://limitvalue.ifa.dguv.de

Jenkins, W., Perone, P., Walker, K., Bhagavathula, N., Aslam, M. N., DaSilva, M., ... Varani, J. (2011). Fibroblast Response to Lanthanoid Metal Ion Stimulation: Potential Contribution to Fibrotic Tissue Injury. *Biological Trace Element Research, 144*(1-3), 621-635. https://doi.org/10.1007/s12011-011-9041-x

Jiang, D. G., Yang, J., Zhang, S., & Yang, D. J. (2012). A survey of 16 rare earth elements in the major foods in China. *Biomedical and Environmental Sciences, 25*(3), 267-271. https://doi.org/10.3967/0895-3988.2012.03.003

Jiang, Z., Weng, B., Huang, Y., Jiang, Z., & Wang, X. (2008). Effects of lanthanum on soil microorganism. *Journal of The Chinese Rare Earth Society, 26*(4), 498-502. Retrieved from https://en.cnki.com.cn/Article_en/CJFDTOTAL-XTXB200804022.htm

Kastori, R. R., Maksimović, I. V., Zeremski-Škorić , T. M., & Putnik-Delić, M. I. (2010). Rare earth elements—Yttrium and higher plants. *Zbornik Matice Srpske za Prirodne Nauke, 118*, 87-98. https://doi.org/10.2298/ZMSPN1018087K

Knapp, C. W., Callan, A. C., Aitken, B., Shearn, R., Koenders, A., & Hinwood, A. (2017). Relationship between antibiotic resistance genes and metals in residential soil samples from Western Australia. *Environ Sci Pollut Res, 24*, 2484-2494. https://doi.org/10.1007/s11356-016-7997-y

Knapp, C. W., McCluskey, S. M., Singh, B. K., Campbell, C. D., Hudson, G., & Graham, D. W. (2011). Antibiotic resistance gene abundances correlate with metal and geochemical conditions in archived Scottish soils. *PLoS ONE, 6*(11), e27300. https://doi.org/10.1371/journal.pone.0027300

Kotelnikova, A., Fastovetsa, I., Rogovaa, O., Volkova, D. S., & Stolbova, V. (2019). Toxicity assay of lanthanum and cerium in solutions and soil. *Ecotoxicology and Environmental Safety, 167*, 20-28. https://doi.org/10.1016/j.ecoenv.2018.09.117

Kovaříková, M., Tomášková, I., & Soudek, P. (2019). Rare earth elements in plants. *Biologia Plantarum, 63*, 20-32. https://doi.org/10.32615/bp.2019.003

Kraemer, D., Tepe, N., Pourret, O., & Bau, M. (2017). Negative cerium anomalies in manganese (hydr)oxide precipitates due to cerium oxidation in the presence of dissolved siderophores. *Geochimica et Cosmochimica Acta, 196*, 197-208. https://doi.org/10.1016/j.gca.2016.09.018

Kučera, J., Mizera, J., Řanda, Z., & Vávrová, M. (2007). Pollution of agricultural crops with lanthanides, thorium and uranium studied by instrumental and radiochemical neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry, 271*, 581-587. https://doi.org/10.1007/s10967-007-0310-2

Kulaksız, S., & Bau, M. (2011). Rare earth elements in the Rhine River, Germany: First case of anthropogenic lanthanum as a dissolved microcontaminant in the hydrosphere. *Environment International, 37*(5), 973-979. https://doi.org/10.1016/j.envint.2011.02.018

Kurvet, I., Juganson, K., Vija, H., Sihtme, M., Blinova, I., Syvertsen-Wiig, G., & Kahru, A. (2017). Toxicity of nine (doped) rare earth metal oxides and respective individual metals to aquatic microorganisms *Vibrio fischeri* and *Tetrahymena thermophila*. *Materials, 10*, 754.https://doi.org/10.3390/ma10070754

LabSupply. (2021). *Laboratory roden diet 5001*. Fort Worth, LabSupply. Retrieved from https://www.labsupplytx.com/wp-content/uploads/2012/10/5001.pdf

Laskowski, R. (1995). Some good reasons to ban the use of NOEC, LOEC and related concepts in ecotoxicology. *OIKOS, 73*, 140-144. https://doi.org/10.2307/3545738

Laudal, D. A., Benson, S. A., Palo, D., & Addleman, R. S. (2018), Rare earth elements in North Dakota lignite coal and lignite-related materials. *Journal of Energy Resources Technology, 140*(6), 062205. https://doi.org/10.1115/1.4039738

Lee, J., Jeong, J.-S., Kim, S. Y., Lee, S.-J., Shin, Y.-J., Im, W.-J., … Yu, W.-J. (2020) Safety assessment of cerium oxide nanoparticles: combined repeated-dose toxicity with reproductive/developmental toxicity screening and biodistribution in rats, *Nanotoxicology, 14*, 696-710. https://doi.org/10.1080/17435390.2020.1751322

Li, G., Jiang, J., Chen, J., Zou, Y., & Zhang, X. (2006). Effects of rare earth elements on soil fauna community structure and their ecotoxicity to *Holotrichia parallela*. *Chinese Journal of Applied Ecology, 17*(1), 159-162. Retrieved from https://pubmed.ncbi.nlm.nih.gov/16689254

Li, J., Hong, M., Yin, X., & Liu, J. (2010). Effects of the accumulation of the rare earth elements on soil macrofauna community. *Journal of Rare Earths, 28*, 957. https://doi.org/10.1016/S1002-0721(09)60233-7

Li, J., Verweij, R. A., & van Gestel, C. A. M. (2018). Lanthanum toxicity to five different species of soil invertebrates in relation to availability in soil. *Chemosphere, 193*, 412-420. https://doi.org/10.1016/j.chemosphere.2017.11.040

Liang, T., Ding, S., Song, W., Chong, Z., Zhang, C., & Li, H. (2008). A review of fractionations of rare earth elements in plants. *Journal of Rare Earths, 26*, 7-15. https://doi.org/10.1016/S1002-0721(08)60027-7

Liang, T., Zhang, S., Wang, L., Kung, H.-T., Wang, Y., Hu, A., & Ding, S. (2005). Environmental biogeochemical behaviors of rare earth elements in soil-plant systems. *Environmental Geochemistry and Health, 27*, 301-311. https://doi.org/10.1007/s10653-004-5734-9

Liu, S., Wang, L., & Zhang, S. (1997). Effect of long-term foliage-dressing rare earth elements (REEs) on their distribution, accumulation and translocation in soil-spring wheat system. *Chinese Journal of Applied Ecology, 8*(1), 55-58. Retrieved from http://en.cnki.com.cn/Article_en/CJFDTotal-YYSB199701010.htm

Luo, C., Deng, Y., Liang, J., Zhu, S., Wei, Z., Guo, X., & Luo, X. (2018). Exogenous rare earth element-yttrium deteriorated soil microbial community structure. *Journal of Rare Earths, 36*, 430-439. https://doi.org/10.1016/j.jre.2017.10.003

Ma, Y., Kuang, L., He, X., Bai, W., Ding, Y., Zhang, Z., … Chai, Z. (2010). Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere, 78*, 273-279. https://doi.org/10.1016/j.chemosphere.2009.10.050

Ma, Y., Zhang, P., Zhang, Z., He, X., Li, Y., Zhang, J., … Chai, Z. (2015). Origin of the different phytotoxicity and biotransformation of cerium and lanthanum oxide nanoparticles in cucumber. *Nanotoxicology, 9*(2), 262-270. https://doi.org/10.3109/17435390.2014.921344

MacMillan, G. A., Chételat, J., Heath, J., Mickpegak, R., & Amyot, M. (2017). Rare earth elements (REE) in freshwater, marine, and terrestrial ecosystems in the eastern Canadian Arctic. *Environmental Science: Processes & Impacts, 19*, 1336-1345. https://doi.org/10.1039/C7EM00082K

Mader, P., Száková, J., & Miholová, D. (1998). Classical dry ashing of biological and agricultural materials. Part II. Losses of analytes due to their retention in an insoluble residue. *Analusis, 26*, 121-129. https://doi.org/10.1051/analusis:1998121

Majumdar, S., Almeida, I. C., Arigi, E. A., Choi, H., VerBerkmoes, N. C., Trujillo-Reyes, J., … Gardea-Torresdey, J. L. (2015). Environmental effects of nanoceria on seed production of common bean (*Phaseolus vulgaris*): A proteomic analysis. *Environmental Science & Technology, 49*(22), 13283e13293. https://doi.org/10.1021/acs.est.5b03452

Mayfield, D. B., & Fairbrother, A. (2015). Examination of rare earth element concentration patterns in freshwater fish tissues. *Chemosphere, 120*, 68-74. https://doi.org/10.1016/j.chemosphere.2014.06.010

McLeod, C., & Krekeler, M. P. S. (2017). Sources of extraterrestrial rare earth elements: To the moon and beyond. *Resources, 6*(3), 40. https://doi.org/10.3390/resources6030040

Meryem, B., Ji, H., Gao, Y., Ding, H., & Li, C. (2016). Distribution of rare earth elements in agricultural soil and human body (scalp hair and urine) near smelting and mining areas of Hezhang, China. *Journal of Rare Earths, 34*(11), 1156. https://doi.org/10.1016/S1002-0721(16)60148-5

Ministry of the Environment, Finland. (2007). *Government decree on the assessment of soil contamination and remediation needs* (214/2007). Retrieved from https://www.finlex.fi/en/laki/kaannokset/2007/en20070214.pdf

Morales, M. I., Rico, C. M., Hernandez-Viezcas, J. A., Nunez, J. E., Barrios, A. C., Flores-Marges, J. P., ... Gardea-Torresdey, J. L. (2013). Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum* L.) plants grown in organic soil. *Journal of Agricultural and Food Chemistry, 61*, 6224-6230. https://doi.org/10.1021/jf401628v

Nakamura, Y., Tsumura, Y., Tonogai, Y., Shibata, T., & Ito, Y. (1997). Differences in Behavior among the Chlorides of Seven Rare Earth Elements Administered Intravenously to Rats, *Fundamental and Applied Toxicology, 37*(2), 106-116. https://doi.org/10.1006/faat.1997.2322.

Napier, B. A., Fellows, R. J., & Minc, L. D. (2014). *Transfer factors for contaminant uptake by fruit and nut trees* (Report NUREG/CR-7174, PNNL-22975, p. 93). Richland, WA, Pacific Northwest National Laboratory. https://doi.org/10.2172/1149245

Norris, W. P., Lisco, H., & Brues, A. (1956). The radiotoxicity of cerium and yttrium. In G. C. Kyker & E. B. Anderson (Eds.), *Rare Earths in Biochemical and Medical Research* (Report ORINS-12, pp. 102-115). U.S. Atomic Energy Commission. Retrieved from https://www.osti.gov/biblio/4348549-rare-earths-biochemical-medical-research-conference-sponsored-medical-division-oak-ridge-institute-nuclear-studies-october

OECD. (2021). *eChemPortal. Organisation for Economic Co-operation and Development*. Retrieved January 31, 2021, from https://www.echemportal.org/echemportal/substance-search

Okamoto, A., Yamamuro, M., & Tatarazako, N. (2014). Acute toxicity of 50 metals to Daphnia magna. *Journal of Applied Toxicology, 35*(7), 824-830. https://doi.org/10.1002/jat.3078

Oorts, K. (2020). *Threshold calculator for metals in soil. A global tool for terrestrial risk assessment* (p. 43). Wondelgem, Belgium, Arche Consulting. Retrieved from https://www.arche-consulting.be/wp-content/uploads/2020/07/Background-document-threshold-calculator-for-metals-in-soil-v.3.pdf

Oorts, K., Ghesquiere, U., & Smolders, E. (2007). Leaching and aging decrease nickel toxicity to soil microbial processes in soils freshly spiked with nickel chloride. *Environmental Toxicology and Chemistry, 26*, 1130-1138. https://doi.org/10.1897/06-533R.1

Özbayoglu, G. (2011). Partitioning of major and trace elements of a Turkish lignite with size and density. *Physicochemical Problems of Mineral Processing, 47*, 51-60. http://www.journalssystem.com/ppmp/Partitioning-of-major-and-trace-elements-of-a-Turkish-lignite-with-size-and-density,79105,0,2.html

Pošćić, F., Mattiello, A., Fellet, G., Miceli, F., & Marchiol, L. (2016). Effects of cerium and titanium oxide nanoparticles in soil on the nutrient composition of barley (*Hordeum vulgare* L.) kernels. *International Journal of Environmental Research and Public Health, 13*, 577. https://doi.org/10.3390/ijerph13060577

Posthuma, L., & De Zwart, D. (2014). Species Sensitivity Distributions. In P. Wechsler (Ed.), *Encyclopedia of Toxicology* (3rd ed., pp. 363-368). Cambridge, Massachusetts, Elsevier Inc., Academic Press. https://doi.org/10.1016/B978-0-12-386454-3.00580-7

Posthuma, L., van Gils, J., Zijp, M. C., van de Meent, D., & de Zwart, D. (2019). Species sensitivity distributions for use in environmental protection, assessment, and management of aquatic ecosystems for 12 386 chemicals. *Environmental Toxicology and Chemistry, Hazard/Risk Assessment, 38*, 905-917. https://doi.org/10.1002/etc.4373

Priester, J. H., Ge, Y., Mielke, R. E., Horst, A. M., Cole Moritz, S., Espinosa, K., … Holden, P. A. (2012). Soybean susceptibility to manufactured nanomaterials with evidence for food quality and soil fertility interruption. *PNAS, 109*(37), E2451-E2456. https://doi.org/10.1073/pnas.1205431109

Ramos, S. J., Dinali, G. S., Oliveira, C., Martins, G. C., Moreira, C. G., Siqueira, J. O., & Guilherme, L. R. G. (2016). Rare earth elements in the soil environment. *Current Pollution Reports, 2*, 28-50. https://doi.org/10.1007/s40726-016-0026-4

Redling, K. (2006). *Rare earth elements in agriculture with emphasis on animal husbandry* (Doctoral Dissertation, Ludwig-Maximilians-Universität München). https://doi.org/10.5282/edoc.5936

Richardson, J. B., Görres, J. H., & Sizmur, T. (2020). Synthesis of earthworm trace metal uptake and bioaccumulation data: Role of soil concentration, earthworm ecophysiology, and experimental design. *Environmental Pollution, 262*, 114126. https://doi.org/10.1016/j.envpol.2020.114126

Rico, C. M., Barrios, A. C, Tan, W., Rubenecia, R., Lee, S. C., Varela-Ramirez, A., … Gardea-Torresdey, J. L. (2015). Physiological and biochemical response of soil-grown barley (*Hordeum vulgare* L.) to cerium oxide nanoparticles. *Environmental Science and Pollution Research, 22*, 10551-10558. https://doi.org/10.1007/s11356-015-4243-y

Rico, C. M., Lee, S. C., Rubenecia, R., Mukherjee, A., Hong, J., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2014). Cerium oxide nanoparticles impact yield and modify nutritional parameters in wheat (*Triticum aestivum* L.). *Journal of Agricultural and Food Chemistry, 62*, 9669-0675. https://doi.org/10.1021/jf503526r

Rico, C. M., Hong, J., Morales, M. I., Zhao, L., Barrios, A. C., Zhang, J. Y., … Gardea-Torresdey, J. L. (2013). Effect of cerium oxide nanoparticles on rice: A study involving the antioxidant defense system and i*n vivo* fluorescence imaging. *Environmental Science & Technology, 47*, 5635-5642. https://doi.org/10.1021/es401032m

Rim, K. T. (2016). Effects of rare earth elements on the environment and human health: A literature review. *Toxicology and Environmental Health Sciences, 8*, 189-200. https://doi.org/10.1007/s13530-016-0276-y

Ross, S. M., Wood, M. D., Copplestone, D., Warriner, M., & Crook, P. (2007). Environmental concentrations of heavy metals in UK soil and herbage. *UK Soil and Herbage Pollutant Survey* (UKSHS Report No. 7, p. 123). Bristol, Environment Agency. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/291161/scho0607bmta-e-e.pdf

Rucki, M., Kejlova, K., Vlkova, A., Jirova, D., Dvorakova, M., Svobodova, I., … Heinonen, T. (2021). Evaluation of toxicity profiles of rare earth elements salts (lanthanides). *Journal of Rare Earths, 39*, 225-232. https://doi.org/10.1016/j.jre.2020.02.011

Rudnick, R. L., & Gao, S. (2003). 3.01 Composition of the Continental Crust. In H. D. Holland and K. K. Turekian (Eds), *Treatise on Geochemistry, 3*, 1-64. https://doi.org/10.1016/B0-08-043751-6/03016-4

Sadeghi, M., & Andersson, M. (2015). Sllsynta jordartsmetaller i Sverige, förekomst och utbredning i berg och jord. *The rare earth element distribution over Sweden* (SGU-Rapport 2015:21, p. 98). Uppsala, Geological Survey of Sweden. https://apps.sgu.se/geolagret/?Query=serie=%27SGURapporter%27

Salminen, R., Batista, M. J., Bidovec, M., Demetriades, A., De Vivo, B., De Vos, W., … Tarvainen, T. (2005). Part 1-Background Information, Methodology and Maps. *Geochemical Atlas of Europe*. Espoo, Geological Survey of Finland. Retrieved from http://weppi.gtk.fi/publ/foregsatlas/index.php

Shin, S.-H., Kim, H.-O., & Rim, K.-T. (2019). Worker safety in the rare earth elements recycling process from the review of toxicity and issues. *Safety and Health at Work, 10*, 409-419. https://doi.org/10.1016/j.shaw.2019.08.005

Shtangeeva, I., Ayrault, S., & Jain, J. (2004). Scandium bioaccumulation and its effect on uptake of macro- and trace elements during initial phases of plant growth. *Soil Science and Plant Nutrition, 50*, 877-883. https://doi.org/10.1080/00380768.2004.10408549

Smolders, E., Oorts, K., Van Sprang, P., Schoeters, I., Janssen, C. R., McGrath, S. P., & Mclaughlin, M. J. (2009). Toxicity of trace metals in soil as affected by soil type and aging after contamination: Using calibrated bioavailability models to set ecological soil standards. *Environmental Toxicology and Chemistry, 28*, 1633-1642. https://doi.org/10.1897/08-592.1

Su, D., Tai, P., Li, P., & Ke, X. (2005). Toxic effects of lanthanides on *Chlorella autotrophica*. *Chinese Journal of Ecology, 24*, 382-384. Retrieved from http://caod.oriprobe.com/articles/9036753/Toxic_effects_of_lanthanides_on_Chlorella_autotrophica.htm

Suanon, F., Sun, Q., Yang, Y., Chi, Q., Mulla, S. I., Mama, D., & Yu, C.-P. (2017). Assessment of the occurrence, spatiotemporal variations and geoaccumulation of fifty-two inorganic elements in sewage sludge: A sludge management revisit. *Scientific Reports, 7*, 5698. https://doi.org/10.1038/s41598-017-05879-9

Suter II, G. L., Traas, T. P., & Posthuma, L. (2002). Issues and practices in the derivation and use of species sensitivity distributions. In L. Posthuma, G. W. Suter II, & Traas, T. P (Eds.), *Species sensitivity distributions in ecotoxicology* (pp. 255-273). Boca Raton, Lewis Publishers. https://doi.org/10.1201/9781420032314.sec4

Swedish Environmental Protection Agency. (2016). *Naturvårdsverkets generella riktvärden för förorenad mark*. Stockholm, Sweden: Naturvårdsverket. Retrieved from http://www.naturvardsverket.se/upload/stod-i-miljoarbetet/vagledning/fororenade-omraden/berakning-riktvarden/generella-riktvarden-20160707.pdf

Tagami, K., Uchida, S., & Zheng, J. (2019). Soil to crops transfer of rare earth elements and some other metals. In K. Bessho, H. Matsamura, T. Miura, K. Tagami, & K. Takamiyua (Eds.), *Proceedings of the 18th Workshop on Environmental Radioactivity* (pp. 157-162). KEK, Tsukuba, Japan. Retrieved from https://iss.ndl.go.jp/books/R100000002-I028698805-00

Tai, P., Zhao, Q., Su, D., Li, P., & Stagnitti, F. (2010). Biological toxicity of lanthanide elements on algae. *Chemosphere, 80*, 1031-1035. https://doi.org/10.1016/j.chemosphere.2010.05.030

Tang, X., Sun, Y., Xia, M., Wen, C. Q., & Zhang, Z. (2004). Ecological effects of low dosage mixed rare earth elements accumulation on major soil microbial groups in a yellow cinnamon soil. *Chinese Journal of Applied Ecology, 15*, 2137-2141. Retrieved from https://www.x-mol.com/paper/1212978049316626434?adv

Thomas, P. J., Carpenter, D., Boutin, C., & Allison, J. E. (2014). Rare earth elements (REEs): Effects on germination and growth of selected crop and native plant species. *Chemosphere, 96*, 57-66. https://doi.org/10.1016/j.chemosphere.2013.07.020

Tóth. G., Hermann, T., Da Silva, M. R., & Montanarella, L. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. *Environment International, 88*, 299-309. https://doi.org/10.1016/j.envint.2015.12.017

Toxicology Excellence for Risk Assessment. (2021). *Rat/Mouse default values*. Retrieved from https://tera.org/Tools/ratmousevalues.pdf

Turra, C. (2010). *Impact of rare earth elements in citrus agroecosystem* (Doctoral dissertation, Centro de Energia Nuclear na Agricultura, University of Sao Paulo, Brazil). https://doi.org/10.11606/T.64.2010.tde-26092013-155801

Turra, C., De Nadai Fernandes, E. A., Arruda Bacchi, M., Adrián Sarriés, G., Barbosa Júnior, F., Teixeira Creste, A. L., & Lai Reyes, A. E. (2015). Effects of lanthanum on citrus plant. *International Journal of New Technology and Research, 1*, 48-50. Retrieved from https://www.ijntr.org/download_data/IJNTR01070026.pdf

Uchida, S., Tagami, K., & Hirai, I. (2007). Transfer factors of stable elements and naturally occurring radionuclides (1) Upland field crops collected in Japan. *Journal of Nuclear Science and Technology, 44*, 628-640. https://doi.org/10.1080/18811248.2007.9711851

Ulusoy, U., & Whitley, J. E. (2000). Profiles of faecal output of rare earth elements and stable isotopic tracers of iron and zinc after oral administration. *British Journal of Nutrition, 84*, 605-617. https://doi.org/10.1017/S000711450000194X

USEPA. (2012). *Rare earth elements: A review of production, processing, recycling, and associated environmental issues* (EPA/600/R-12/572). Cincinnati, Office of Research and Development. Retrieved from https://nepis.epa.gov/Adobe/PDF/P100EUBC.pdf

USGS. (2021). *Rare earths statistics and information*. Retrieved from https://www.usgs.gov/centers/nmic/rare-earths-statistics-and-information

van Gestel, C. A. M.. McGrath, S. P., Smolders, E., Diez Ortiz, M., Borgman, E., Verweij, R. A., … Oorts, K. (2012). Effect of long-term equilibration on the toxicity of molybdenum to soil organisms. *Environmental Pollution, 162*, 1-7. https://doi.org/10.1016/j.envpol.2011.10.013

Velasco, H., & Juri Ayub, J. (2009). Root uptake: Tropical and sub-tropical environments. *Quantification of radionuclide transfer in terrestrial and freshwater environments for radiological assessments* (IAEA-TECDOC-1616, p. 622). Vienna, International Atomic Energy Agency. Retrieved from https://www-pub.iaea.org/MTCD/Publications/PDF/te_1616_web.pdf

Wang, L., & Liang, T. (2015). Geochemical fractions of rare earth elements in soil around a mine tailing in Baotou, China. *Scientific Reports, 5*, 12483. https://doi.org/10.1038/srep12483

Wang, L., Hu, A., Zhou, Q., Tao, L., Wang, X., & Yan, X. (2006). Accumulation and fractionation of rare earth elements in soil-rice system. *Journal of the Chinese Rare Earth Society, 24*, 91-97. Retrieved from http://caod.oriprobe.com/articles/10067736/Accumulation_and_Fractionation_of_Rare_Earth_Elements_in_Soil_Rice_Sys.htm

Wang, L., Liang, T., Hu, A., Ding, S., Zhang, C., & Yan, X. (2005). Accumulation and fractionation of rare earth elements in soil rice systems. *Journal of Rare Earths, 23*, 747-751. Retrieved from http://caod.oriprobe.com/articles/14662713/Accumulation_and_Fractionation_of_Rare_Earth_Eleme.htm

Wang, Q., Ma, X., Zhang, W., Pei, H., & Chen, Y. (2012). The impact of cerium oxide nanoparticles on tomato (*Solanum lycopersicum* L.) and its implications for food safety. *Metallomics, 4*, 1105-1112. https://doi.org/10.1039/C2MT20149F

Wang, W.-X. (2013). Dietary toxicity of metals in aquatic animals: Recent studies and perspectives. *Chinese Science Bulletin, 58*(2), 203-213. https://doi.org/10.1007/s11434-012-5413-7

Wang, Y., Zhou, H., Xiong, L., Liu, C., & You, X. (2020). Residual levels of rare earth elements in cereal and their health risk assessment from mining area in Jiangxi, South China. *Journal of Food and Nutrition Research, 8*(1), 58-62. https://doi.org/10.12691/jfnr-8-1-8

Western Australia Department of Environment and Conservation. (2010). Assessment levels for Soil, Sediment and Water. *Contaminated Sites Management Series*. Wanneroo: Western Australia Department of Environment and Conservation. Retrieved from https://www.der.wa.gov.au/images/documents/your-environment/contaminated-sites/guidelines/2009641_-_assessment_levels_for_soil_sediment_and_water_-_web.pdf

Wolińska, A., & Stępniewska, Z. (2012). Dehydrogenase activity in the soil environment. In R. A. Canuto (Ed.), *Dehydrogenases*. IntechOpen. https://doi.org/10.5772/2903

Wu, J., Feng, X.-J., & Qian, X.-Y. (2012). Rare earth yttrium: its acute toxicity to earthworm and bioaccumulation in earthworm. *Environmental Science& Technology, 35*, 46-50. Retrieved from https://www.ixueshu.com/document/e1e2d6e83f5086a8318947a18e7f9386.html

Wu, M.-Y., Zhang, P., Yang, W., Liu, J., & Mo, W. (2006). Effect of long-term intake of $Y^{3+}$ in drinking water on learning-memory functions and growth-development of rats. *Journal of Hygiene Research, 35*, 283-285. Retrieved from http://caod.oriprobe.com/articles/10522718/Effect_of_long_term_intake_of_Y3__in_drinking_water_on_learning_memeor.htm

Wyttenbach, A., Furrer, V., Schleppi, P., & Tobler, L. (1998). Rare earth elements in soil and in soil-grown plants. *Plant and Soil, 199*, 267-273. https://doi.org/10.1023/A:1004331826160

Xiao, X., Yong, L., Liu, D., Yang, H., Liang, C., Jia, X., Liu, Z., & Song, Y. (2020). Effects of in utero exposure to lanthanum on neurological behavior in rat offspring. *Neurotoxicology and Teratology, 77*, 106854. https://doi.org/10.1016/j.ntt.2019.106854

Xiong, J., & Zhang, Z. (1997). Maximum threshold of the rare earth concentration and crop growth. *Chinese Journal of Ecology, 16*, 1-7. Retrieved from http://www.cje.net.cn/EN/volumn/volumn_537.shtml

Xu, T., Zhang, M., Hu, J., Li, Z., Wu, T., Bao, J., … He, D. (2017). Behavioral deficits and neural damage of *Caenorhabditis elegans* induced by three rare earth elements. *Chemosphere, 181*, 55-62. https://doi.org/10.1016/j.chemosphere.2017.04.068

Xu, X., & Wang, Z. (2001). Effects of lanthanum and mixtures of rare earths on ammonium oxidation and mineralization of nitrogen in soil. *European Journal of Soil Science, 52*, 323-329. https://doi.org/10.1046/j.1365-2389.2001.00385.x

Yu, Y., Chi, H.-F., Liu, J.-R., Hu, G.-R., Yu, R.-L., Huang, H.-B., & Lin, C.-Q. (2020). Provenance and bioaccessibility of rare earth elements in atmospheric particles in areas impacted by the optoelectronic industry. *Environmental Pollution, 263*(Part A), 114349. https://doi.org/10.1016/j.envpol.2020.114349

Yuan, M., Liu, C., Liu, W.-S., Guo, M.-N., Morel, J. L., Huot, H., … Qiu, R.-L. (2018). Accumulation and fractionation of rare earth elements (REEs) in the naturally grown *Phytolacca americana* L. in southern China. *International Journal of Phytoremediation, 20*, 415-423. https://doi.org/10.1080/15226514.2017.1365336

Zaichick, S., Zaichick, V., Karandashevc, V., & Nosenkoc, S. (2011). Accumulation of rare earth elements in human bone within the lifespan. *Metallomics, 3*, 186-194. https://doi.org/10.1039/c0mt00069h

Zeng, Q., Zhu, J. G., Cheng, H. L., Xie, Z. B., & Chu, H. Y. (2006). Phytotoxicity of lanthanum in rice in haplic acrisols and cambisols. *Ecotoxicology and Environmental Safety, 64*, 226-233. https://doi.org/10.1016/j.ecoenv.2005.03.016

Zhang, L., & Van Gestel, C. A. M. (2019). Effect of ageing and chemical form on the bioavailability and toxicity of Pb to the survival and reproduction of the soil invertebrate *Enchytraeus crypticus*. *Science of the Total Environment, 664*, 975-983. https://doi.org/10.1016/j.scitotenv.2019.02.054

Zhang, P., Yang, W.-D., Liu, J.-S., Zhang, X. L., & Xue, Y. (2006). Effect of long-term intake of $Y^{3+}$ in drinking water on immune function of rats. *Journal of Hygiene Research, 35*, 279-281. https://doi.org/10.1016/S1002-0721(06)60126-9

Zhang, P., Ma, Y., Liu, S., Wang, G., Zhang, J., He, X., … Zhang, Z. (2017). Phytotoxicity, uptake and transformation of nano-$CeO_2$ in sand cultured romaine lettuce. *Environmental Pollution, 220*(Part B), 1400-1408. https://doi.org/10.1016/j.envpol.2016.10.094

Zhang, S. R., Li, L., Xu, X. X., Li, T., Gong, G. S., Deng, O. P., & Pu, Y. L. (2015). Lanthanum tolerance and accumulation characteristics of two Eucalyptus species. *Ecological Engineering, 77*, 114-118. https://doi.org/10.1016/j.ecoleng.2015.01.018

Zhang, Z., Chang, J., Wang, C., Chai, S., Han, X., & Li, R. (2001). Effect of rare earth elements on the seedling ratio of crops. *Chinese Journal of Applied Ecology, 12*, 395-397. Retrieved from http://caod.oriprobe.com/articles/3434725/Effect_of_rare_earth_elements_on_the_seedling_ratio_of_crop.htm

Zhao, L., Sun, Y., Hernandez-Viezcas, J. A., Hong, J., Majumdar, S., Niu, G., & Gardea-Torresdey, J. L. (2015). Monitoring the environmental effects of CeO2 and ZnO nanoparticles through the life cycle of corn (*Zea mays*) plants and in situ μ-XRF mapping of nutrients in kernels. *Environmental Science & Technology, 49*, 2921-2928. https://doi.org/10.1021/es5060226

Zhao, L., Sun, Y., Hernandez-Viezcas, J. A., Servin, A. D., Hong, J., Niu, G., … Gardea-Torresdey, J. L. (2013). Influence of $CeO_2$ and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: A life cycle study. *Journal of Agricultural and Food Chemistry, 61*, 11945-11951. https://doi.org/10.1021/jf404328e

Zhou, F., Chen, F., Cao, J., Pu, L., & Peng, B. (2003). Effects of rare earth elements on microbial characteristics and temporal availability in rice soil. *Journal of the Chinese Rare Earth Society, 21*, 589-592. Retrieved from http://caod.oriprobe.com/articles/6224453/effects_of_rare_earth_elements_on_microbial_characteristics_and_tempor.htm

Zhu, W. F., Xu, S. Q., Zhang, H., Shao, P. P., Wu, D. S., Yang, W. J., & Feng, J. (1996). Investigation on the intelligence quotient of children in the areas with high REE background (I)—REE bioeffects in the REE-high areas of southern Jiangxi Province. *Chinese Science Bulletin, 41*, 1977-1981. Retrieved from https://engine.scichina.com/doi/10.1360/sb1996-41-23-1977

Zhuang, M., Wang, L., Wu, G., Wang, K., Jiang, X., Liu, T., … Chu, Z. (2017). Health risk assessment of rare earth elements in cereals from mining area in Shandong, *China. Scientific Reports, 7*, 9772. https://doi.org/10.1038/s41598-017-10256-7

Životić, D., Cvetković, O., Vulić, P., Gržetić, I., Simić, V., Ilijević, K., … Trifunović, S. (2019). Distribution of major and trace elements in the Kovin lignite (Serbia). *Geologia Croatica, 72*(1), 51-79. https://doi.org/10.4154/gc.2019.06

## Abbreviations

$IC_{XX}$: XX% of the maximal inhibitory concentration, representing the point in which a compound of interest produces complete inhibition of a biological or biochemical function.

$LD_{50}$: Median lethal dose, the dose required to kill half the members of a tested population after a specified test duration.

TOC: Total organic carbon.

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# Effectual Gold Nanoprobe Sensor for Screening Cow Milk Adulteration in Goat Milk—Comparison With Conventional PCR

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**Abstract**

Different methods have been used to detect milk adulteration, but in recent years the use of nanomaterials has been introduced as an interesting tool, due to their optical properties. A gold nanoparticle (AuNP) probe application was developed in order to evaluate milk adulteration. The methodology relies on the colorimetric differentiation and the participation profiles of the hybridization DNA sequence with the AuNPs. Various concentrations, from 0.01 (traces) to 50%, of cows' milk in goats' milk samples were prepared for DNA extraction, further identification with the AuNPs and comparison with a conventional PCR. Also, a total of 40 dairy products from goat milk, commonly consumed in Greece, were tested. Negative and goat reaction mixtures showed a purplish coloured solution with a peak at > 570 nm, while samples containing bovine DNA had an absorbance closer to the characteristic peak of the AuNPs at 520-525 nm. Presence of bovine milk was detected even at traces level, achieving a detection level comparable to those achieved by conventional PCR. The use of AuNPs in milk products, provides a low-cost and easy-to-perform method and offers the possibility to detect fraudulent practices in various food matrices.

**Keywords:** food authentication, goat milk adulteration, gold nanoparticles

## 1. Introduction

Food adulteration has always been a major problem of the food industry. Substitution of expensive materials by other ones with lower commercial value or addition of ingredients non-related with the nature of the product, are practices that have been reported and which can cause lack of confidence in the Food Industry (EU Science Hub, 2016).

Natural milk is considered as an important nutritive food, as it is a good source of carbohydrates, fats, proteins, minerals and vitamins (Neumann et al., 2002). Its beneficial effects for mothers, infants, children and elderly people originate from its easy digestibility and absorption, while milk proteins are a significant source of amino acids needed for proper growth (Afzal et al., 2011).

Bovine milk is commonly used for adulteration of other milk kinds because of its predominant production and its lower cost (Borkova & Snaselova, 2005). The consumption of adulterated milk may affect people with allergies to cow milk components (Cheng et al., 2006; Haenlein, 2004; Pesic et al., 2011), especially since milk proteins, are potential allergens even at low concentrations (Sampson, 2003; Rance et al., 2005). Nowadays, the goat milk consumption is increasing in the occidental countries, so this kind of adulteration practices may have a serious impact in public health. Previous studies have shown that adulteration of dairy products with cow milk is a common practice, especially in developing countries (Darwish et al., 2009; Golinelli et al., 2014; Salih & Yang, 2017).

Due to the aforementioned social and health impact of milk adulteration, there is a vast number of publications about different methods for its detection. Over the time, new methods were introduced, but still the majority of methodologies to identify dairy products' authenticity is based on the study of the major milk proteins (Stanciuc & Rapeanu, 2010). The techniques used to estimate these differences include non-immunological methods, analysis of fatty acid composition by gas chromatography or the detection of specific protein components by HPLC, SDS-PAGE (polyacrylamide gel electrophoresis) (Lee et al., 2004; Mayer, 2005), and isoelectric focusing (Amigo et al., 1991; Kim & Jimeez-Flores, 1993; Chen et al., 2004; Enne et al., 2005). All of these methods have the disadvantage of being time consuming and uneconomic. Immunological approaches use an enzyme-linked immunosorbent assay (Anguita et al., 1996; Ritcher et al., 1997; Hurley et al., 2004; Zelenakova & Golian, 2008); some false positive and false negative results remain to be overcome. PCR (polymerase chain reaction) provides an alternative way of identifying the additions of foreign milk or dairy products to the original milk by analysis of mitochondrial DNA (Bania et al., 2001; Maudet & Taberlet, 2001; Bottero et al., 2003). PCR is the most widely employed method to identify milk adulteration (Poonia et al., 2016; Das et al., 2016) due to its high level of sensitivity and its excellent repeatability. Researchers have reported that they were able, to detect as low as 0,5% (Darwish et al., 2009) and 0,1% (Lopez-Calleja et al., 2004) cow milk in milk of other species.

Over the last years, new strategies for the identification of the origin of food have been introduced, which are related to the development of precise nanoparticle-based probes and are competent for detecting DNA target sequences (Ali et al., 2011, 2012, 2014; Houhoula et al., 2017). Nanotechnology and, especially gold nanoparticles (AuNPs) chemistry, offers innovative opportunities for quick and easy analysis of authenticity, since it is capable to evidence even low level of adulteration, thanks to their particular optical properties (Zeng et al., 2011). AuNPs, have been used effectively as colorimetric sensors for visual identification and they are suggested as a cheaper and simple alternative to other molecular methods since they can to work in different food matrices (Ali et al., 2011, 2012, 2014; Houhoula et al., 2017). The aim of the present study is to demonstrate that functionalized AuNPs can be used for the development of a simple DNA detection procedure for the identification of cow milk adulteration in goat milk, and to compare its sensitivity with the most used PCR method.

## 2. Method

### *2.1 Selection and Preparation of Samples*

Cow milk (*Bos taurus*) samples and goat milk (*Capra hircus*) samples were acquired directly from animals of a local dairy farm into collection tubes, and were immediately refrigerated. Upon arrival to the laboratory, they were stored at -18 °C until use.

A total of 40 milk and dairy products were collected from local super markets in the area of Athens (Greece). The products included 25 packaged goat milk products and 15 goat cheeses of different brands.

Spiked samples were prepared with different concentrations of cow milk in goat milk (0.01% (traces), 1%, 2%, 5%, 10%, 20% and 50%) and were used as positive controls in order to find the detection limit. Pure goat, sheep, donkey milk and water were used as negative controls.

### *2.2 Preparation of Gold Nanoparticle Probes*

Gold colloid nanoparticles (AuNPs) of 20 nm size, were purchased from BBI Solutions (Cardiff, UK). The AuNPs were conjugated with specific oligonucleotides for cow mitochondrial DNA: sense primer GCCATATACTCTCCTTGGTGACA and antisense primer GTAGGCTTGGGAATAGTACGA (Invitrogen™, USA). The oligonucleotides were modified with 10xdATP in the 5′-end of the primer and thiolated (Cheng et al., 2006; Lahiff et al., 2001; Kusec et al., 2017). The AuNPs were conjugated with the oligonucleotides by adding 1 ml of AuNPs into 4 nmol of the oligonucleotides using a previously described protocol by Hill and Mirkin (2006). The solution was initially incubated overnight with the AuNPs using an orbital shaker at room temperature and then phosphate buffer 9 mM (pH 7) and SDS solution 0.1% (w/v) were added in order to prevent aggregation. The total salting buffer needed to reach the final concentration of 0.3 M NaCl and it was added in six doses over the next 48 hours. After centrifugation, the precipitate was washed twice with 500 µl of 10 mM PBS (pH 7.4), 150 mM NaCl 0.1% SDS. The prepared AuNPs solution was stored in glass vials kept in dark place, at room temperature.

In order, to test the possibility to work only with one primer, part of the nanoprobes were prepared by conjugating only with the sense primer under the same conditions as described above.

The obtained functionalized AuNPs solution was measured with a Spectrophotometer Epoch of BioTek®. The maximum absorbance was at 520-530 nm indicating a proper conjugation of the AuNPs with the oligonucleotides.

### *2.3 DNA Isolation*

DNA extraction from 1.5mL of centrifuged (10 minutes at 12000 × g), different milk products, was performed using the NucleoSpin Food® kit (Macherey-Nagel, GmbH & Co. KG, Germany), according to the manufacturer's instructions with a modification of adding an overnight incubation with the Lysis Buffer and the Proteinase K at 65 °C instead of a 30 min incubation. The extracted DNA was quantified spectrophotometrically at 260 nm.

### *2.4 PCR Amplification and Electrophoresis*

PCR was performed in 50 μL final volume solution using the Master Mix (KAPA BIOSYSTEMS), according to a previously published protocol by Maskova and Paulickova (2006). The program of the PCR was as follows: an Initial denaturation: 94 °C, 10 min; 40 cycles with the following step-cycle profile: denaturation 94 °C, 30 s; annealing 60 °C, 30 s; extension 72 °C, 30 s; Final extension 72 °C, 7 min. The chosen primers amplify cow DNA fragments of the size of 274 bp: forward primer: 5′-GAC CTC CCA GCT CCA TCA AAC ATC TCA TCT TGA TGA AA-3′; reverse primer: 5′-CTA GAA AAG TGT AAG ACC CGT AAT ATA AG-3′. PCR products were separated in 2% agarose gel, stained with ethidium bromide (0.5 μg/ml) and documented under UV illumination using MiniBIS Pro device (DNR Bio-Imaging Systems Ltd., Israel).

### *2.5 Direct Hybridization and Color Detection in Milk Samples of AuNPs*

Hybridization on the extracted DNA from the isolates with the AuNPs thiolated with the oligonucleotides solution was performed by adding 20 μl of eluted DNA (20 μl of water or pure goat, sheep and donkey milk as Negative controls). The first step was 5 min at 95 °C, made as the denaturation step. Following, the hybridization step included 5 min at 55 °C after the addition of 20 μl Au-NPs-oligonucleotides solution and 10 μl of phosphate buffer prepared as described by Hill & Mirkin, 2006. The aggregation step followed, included the addition of 8 μl HCl 1 M. When the color remained pink, the sample was positive (presence of cow DNA), because the Au-NPs-oligonucleotides hybridized the target DNA sequence and they did not aggregate. When the color turned to purple, the sample was considered negative (no cow DNA), because the AuNPs had not found any DNA sequence to hybridize and they aggregated, causing the color change. Positive and negative controls were used for comparison in all cases. The results could be confirmed by UV-Vis spectroscopic analysis.

### *2.6 Direct Application on Food Samples*

In order to obtain an indication of the method's performance on milk and dairy samples, the method was applied with the optimized assay for the detection of adulteration of cow milk in goat milk as referred above. The results were evaluated directly visually and were confirmed further with an absorption spectrum. Also, the samples were compared to those obtained by PCR assays.

## 3. Results and Discussion

The developed method is based on the specific target hybridization of the AuNPs with a specific DNA sequence. In general, colloidal solutions of AuNPs with diameters ranging from 5 to 20 nm show a pink-red color (Figure 1), due to their optical absorption peak at around 520-525 nm, caused by the collective excitation of the free conduction band electrons of the dispersed particles, known as the surface plasmon resonance (Houhoula et al., 2017; Bohren & Huffman, 1983; Li & Rothberg, 2004).

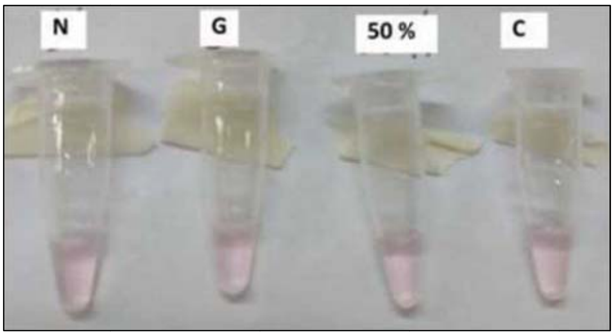


Figure 1. Samples with the characteristic light pink color previous to the addition of HCl: (1) Negative control-$H_2O$; (2) Negative control 100% Goat milk; (3) Goat milk adulterated with 50% cow milk; (4) Positive control 100% cow milk

The aggregation of the AuNPs displaces the absorption peak to a longer wavelength (>570nm) and the color of the colloidal solution turns into purple, as a result of the coupling on the surface plasmons of the particles in the aggregates (Sato et al., 2003). Therefore, the degree of aggregation of AuNPs in suspension determines the color of the gold colloid (Quinten and Kreibig, 1986) and this aggregation can be simply induced with the addition of hydrochloric acid (Houhoula et al., 2017). In the presence of the complementary target, the functionalized AuNPs hybridize with the cow DNA and do not aggregate. Therefore, after the addition of 8 µl of HCl the reaction mixture remains with its original pink coloration in the cases of cow DNA presence, whereas the mixture changes to purple, due to the aggregation of the AuNPs in the case of cow DNA absence (Figure 2).

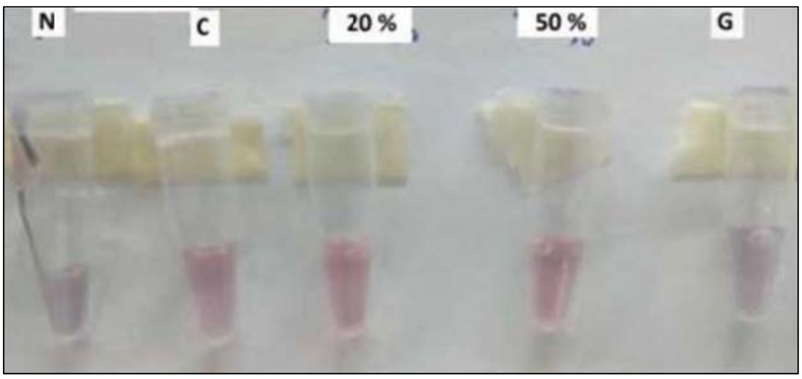


Figure 2. Positive samples exhibit a darker color than the original pink, but still perfectly distinguishable from the purple color of negative samples: (1) Negative control $H_2O$; (2) Positive control 100% cow milk; (3) Goat milk adulterated with 20% cow milk; (4) Goat milk adulterated with 50% cow milk; (5) Negative control 100% Goat milk

The results were compared with those obtained by PCR assay (Figure 3).

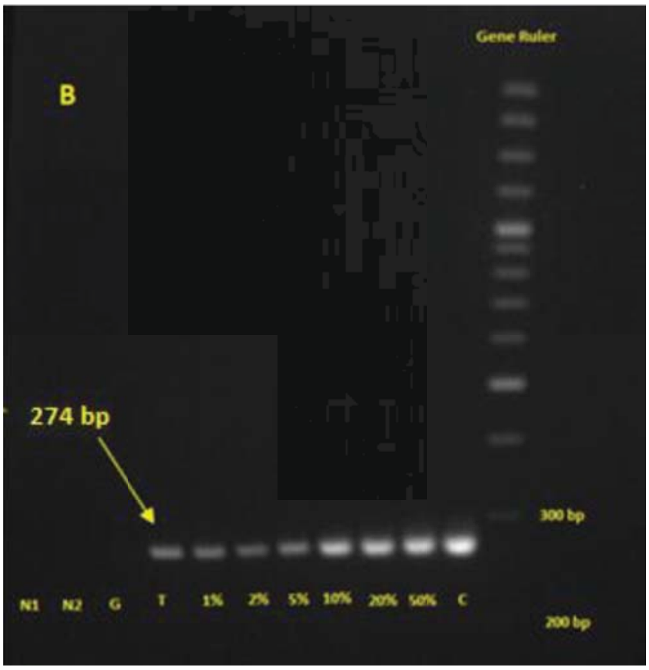


Figure 3. Electrophoretic analysis of the PCR products obtained from DNA extracts of different dilutions of cow milk in goat milk, (1) and (2) Negative control $H_2O$ and Sheep milk, respectively; (3) Negative control 100% goat milk; (4) Goat milk adulterated with traces of cow milk; (5) Goat milk adulterated with 1% cow milk; (6) Goat milk adulterated with 2% cow milk; (7) Goat milk adulterated with 5% cow milk; (8) Goat milk adulterated with 100% cow milk; (9) Goat milk adulterated with 50% cow milk; (10) Positive control 100% Cow milk

The PCR tests used, were very sensitive, specific, and reproducible. PCR tests showed a minimum detection limit of 0.01% (traces) indicating that the analytical assay detected cow milk, even when it was present in small amounts. All the negative controls (water, goat, sheep and donkey milk) didn't give an amplification in the PCR. The sensitivity and the specificity of the method was 100%. The detection limit is very similar to that described by Lopez-Calleja et al. (2005) and Mafra et al. (2007), who reported detection limit of 0.1% cow's milk. Other authors detected amounts of cow milk as low as 1% (Maškova & Paulikova, 2006) and 0.5% (Bottero et al., 2005; Feligini et al., 2005).

The results of this biosensor were the same when functionalized with both primers and when working only with the forward primer. Similar results were reported by Houhoula et al. (2017) using AUNP's for the identification of horse adulteration in meat products. The studied technique is a useful screening test to detect the presence of cow milk in goat milk, even at traces levels. All the positive samples (100%) were found to have the characteristic absorbance peak at 520 nm (Figure 4) with gradual decrease in absorbance relative to the concentration of genomic DNA present in the test sample (Figure 5).

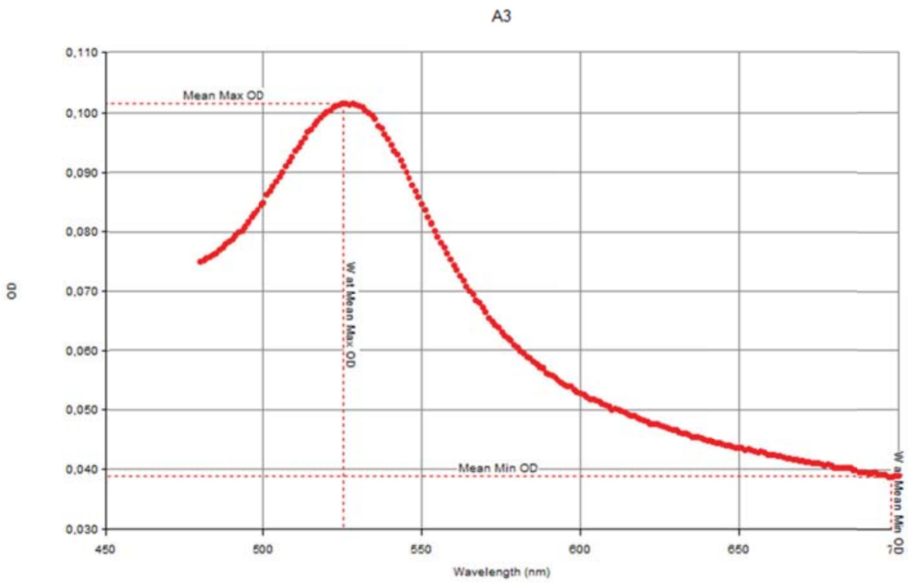


Figure 4. The wavelength at maximum absorbance 520 nm for Cow milk (Positive control)

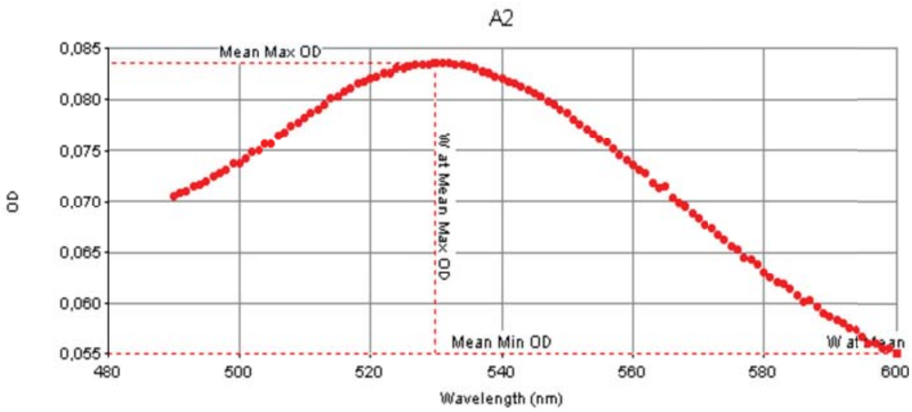


Figure 5. The wavelength at maximum absorbance 530 nm for Goat milk adulterated with 2% cow milk

Whereas the negative controls (water, goat, sheep and donkey milk) had a purple colour with the absorbance peak shifted to longer wavelength (Figure 6). The specificity was 100% (true negative rate). An interesting feature of this assay is that the positive samples even when preserved overnight at room temperature, continue to retain their colour suggesting the long-time stability of AuNP-oligo probe hybridization with the target sequence. This feature is particularly useful for prolonged read-out capability required for high-throughput applications. Therefore, it was confirmed that following this protocol it is possible to find even traces of cow milk in goat milk. The method was repeatable and produced the same results all five times it was performed. The proposed method is a fast, reliable, and cheap for the detection of adulteration of goat milk. The visual results can be further confirmed inexpensively by reliable absorption spectroscopy which incurs only the instrumental cost and cuvettes.

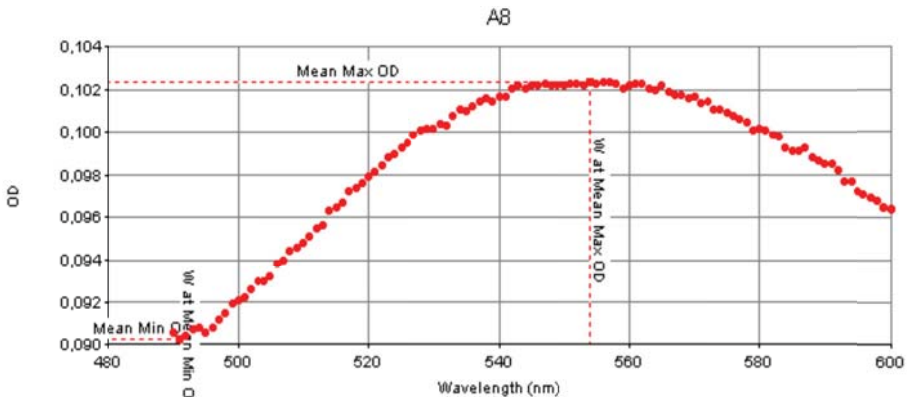


Figure 6. The wavelength at maximum absorbance 554 nm for Goat milk (Negative control)

Forty (40) samples of commercial goat milk and dairy products were analyzed by the PCR assay. In all of them, the PCR assays amplified the 274-bp fragment expected for cow derived-material. The former fragment indicates the addition of cow milk, considered a twenty percent (20%) at least adulterant component, in thirty six (36) commercial samples. This means that 90% of the products tested were adulterated with cow milk. In specific, all the 25 packaged goat milk products (100%) and 11 of the goat cheeses (73%) were adulterated with cow milk. The rest four (4) samples were found adulterated at a level of approximately 1-5% again with cow milk.

The same 40 samples were tested with the AuNPs assay method. All the samples gave positive and comparable to the conventional PCR method results (100% sensitivity, true positive rate). All the 25 packaged goat milk products (100%) and the 11 goat cheeses (73%) had the the characteristic absorbance peak at 520 nm and the color remained pink. The four samples that were found positive by PCR with an adulteration of 1-5% had an absorbance peak at 530 nm and the color remained pink.

The method provided a semiquantitative information of the target DNA. On the other hand, UV-vis spectroscopy is available in most laboratories and can authenticate the visually identified results of colloidal gold (Verma et al., 2015).

The evaluation of the specificity and the repeatability of the AuNPs method, indicate that it can detect—in a reliable and highly specific manner—a broad spectrum of adulterated samples without cross reactions. The performance of the proposed method proved comparable to that of PCR regarding both the sensitivity (100% concordance of positive results) and specificity (100% concordance of negative results).

## 4. Conclusions

The proposed method is a rapid (less than 20 min), reliable, and cheap method (4 euros/reaction) for the selective detection of target DNA sequences. It does not need any instrument or surface modification chemistry and directly detects target DNA in non-amplified mixed genomic DNA. The procedure is very simple and relies on the colour change of 20-nm AuNPs following salt addition. The visual indications are solid and can be further confirmed by an inexpensive, widely available, and reliable absorption spectroscopy which incurs only the instrumental cost and cuvettes. The use of absorption spectroscopy increases sensitivity and eliminates any sort of colour-blindness error or ambiguity in visual detection by producing well-defined bands of aggregated and non-aggregated colloidal particles.

## References

Afzal, A., Mahmood, M. S., Hussain, I., & Akhtar, M. (2011). Adulteration and microbiological quality of milk (A Review). *Pakistan Journal of Nutrition, 10*(12), 1195-1202. https://doi.org/10.3923/pjn.2011.1195.1202

Ali, M. E., Hashim, U., Mustafa, S., Che Man, Y. B., & Islam, K. N. (2012). Gold Nanoparticle Sensor for the visual detection of pork adulteration in meatball formulation. *Journal of Nanomaterials, 7*, Article ID 103607. https://doi.org/10.1155/2012/103607

Ali, M. E., Hashim, U., Mustafa, S., Che Man, Y. B., Adam, T., & Humayun, Q. (2014). Nanobiosensor for the detection and quantification of pork adulteration in meatball formulation. *Journal of Experimental Nanoscience, 9*(2), 152-160. https://doi.org/10.1080/17458080.2011.640946

Ali, M. E., Hashim, U., Mustafa, S., Che Man, Y. B., Yusop, M. H., Bari, M. F., … Hasan, M. F. (2011). Nanoparticle sensor for label free detection of swine DNA in mixed biological samples. *Nanotechnology, 22*(19), 195503. https://doi.org/10.1088/0957-4484/22/19/195503

Bohren, C. F., & Huffman, D. R. (1983). *Absorption and Scattering of Light by Small Particles*. John Wiley & Sons, NY.

Borkova, M., & Snaselova, J. (2005). Possibilities of Different Animal Milk Detection in Milk. *Czech Journal of Food Science, 23*(2), 41-50. https://doi.org/10.17221/3371-CJFS

Bottero, M. T., Civera, T., Nucera, D., Rosati, S., Sacchi, P., & Turi, R. M. (2003). A multiplex polymerase chain reaction for the identification of cows', goats' and sheep's milk in dairy products. *International Dairy Journal, 13*, 277-282. https://doi.org/10.1016/S0958-6946(02)00170-X

Cheng, Y. H., Chen, S. D., & Weng, C. F. (2006). Investigation of goats' milk adulteration with cows' milk by PCR. *Asian-Australasian Journal of Animal Sciences, 19*, 1503-1507. https://doi.org/10.5713/ajas.2006.1503

Darwish, S. F., Allam, H. A., & Amin, A. S. (2009). Evaluation of PCR Assay for Detection of Cow's Milk in Water Buffalo's Milk. *World Applied Sciences Journal, 7*(4), 461-467.

Das, S., Goswami, B., & Biswas, K. (2016). Milk Adulteration and Detection: A Review. *Sensor Letters, 14*(1), 4-18. https://doi.org/10.1166/sl.2016.3580

Enne G., Elez, D., Fondrini, F., Bonizzi, I., Feligini, M., & Aleandri, R. (2005). High-performance liquid chromatography of governing liquid to detect illegal bovine milk's addition in water buffalo Mozzarella: Comparison with results from raw milk and cheese matrix. *Journal of Chromatography A, 1094*(1-2), 169-178. https://doi.org/10.1016/j.chroma.2005.09.004

EU Science Hub. (2016). *European Commission—New monthly report on food fraud and authenticity*. Retrieved March 31, 2020, from https://ec.europa.eu/irc/en/science-undate/new-monthly-report-food-fraud-and-authenticity

Feligini, M., Bonizzi, I., Curic, C. V., Parma, P., Greppi, G. F., & Enne, G. (2005). Detection of Adulteration in Italian Mozzarella Cheese Using Mitochondrial DNA Templates as Biomarkers. *Food Technology and Biotechnology, 43*(1), 91-95.

Golinelli, L. P., Carvalho, A. C., Casaes, R. S., Lopes, C. S., Deliza, R., Paschoalin, V. M., & Silva, J. T. (2014). Sensory analysis and species-specific PCR detect bovine milk adulteration of frescal (fresh) goat cheese. *Journal of Dairy Science, 97*(11), 6693-6699. https://doi.org/10.3168/jds.2014-7990

Haenlein, G. F. W. (2004). Goat milk in human nutrition. *Small Ruminant Research, 51*, 155-163. https://doi.org/10.1016/j.smallrumres.2003.08.010

Hill, H. D., & Mirkin, C. A. (2006). The bio-barcode assay for the detection of protein and nucleic acid targets using DTT-induced ligand exchange. *Nature Protocols, 1*(1), 324-36. https://doi.org/10.1038/nprot.2006.51

Houhoula, D. P., Kouzilou, M., Tzogias, C., Kyrana, V., Sflomos, C., Tsaknis, J., & Lougovois, V. (2017). Effectual Gold Nanoprobe Sensor for Screening Horse Adulteration in Meat Products. *Journal of Food Research, 6*(4), 34-39. https://doi.org/10.5539/jfr.v6n4p34

Hurley, I. P., Coleman, R. C., Ireland, H. E., & Williams, J. H. H. (2004). Measurement of bovine IgG by indirect competitive ELISA as a means of detecting milk adulteration. *Journal of Dairy Science, 87*(3), 215-221. https://doi.org/10.3168/jds.S0022-0302(04)73195-1

Kusec, I. D., Samac, D., Margeta, V., Radisic , Z., Vincek, D., & Kusec, G. (2017). Efficiency of PCR-RFLP and Species-specific PCR for the Identification of Meat Origin in Dry Sausages. *Czech Journal of Food Sciences, 35*(5), 386-391. https://doi.org/10.17221/243/2016-CJFS

Lahiff, S., Glennon, M., O'Brien, L., Lyng, J., Smith, T., Maher, M., & Shilton, N. (2001). Species-specific PCR for the identification of ovine, porcine and chicken species in meta and bone meal (MBM). *Molecular and Cellular Probes, 15*(1), 27-35. https://doi.org/10.1006/mcpr.2000.0336

Lopez-Calleja, I., Gonzalez, I., Fajardo, V., Rodriguez, M. A., Hernandez, P. E., Garcia, T., & Martin, R. (2004). Rapid detection of cows' milk in sheeps' and goats' milk by a species-specific polymerase chain reaction technique. *Journal of Dairy Science, 87*(9), 2839-2845. https://doi.org/10.3168/jds.S0022-0302(04)73412-8

Maskova, E., & Paulickova, I. (2006). PCR-based detection of cow's milk in goat and sheep cheeses marketed in the czech republic. *Czech Journal of Food Science, 24*(3), 127-132. https://doi.org/10.17221/3307-CJFS

Mayer, H. K. (2005). Milk species identification in cheese varieties using electrophoretic, chromatographic and PCR techniques. *International Dairy Journal, 15*(6-9), 595-604. https://doi.org/10.1016/j.idairyj.2004.10.012

Neumann, C. G., Harris, D. M., & Rogers, L. M. (2002). Contribution of animal source foods in improving diet quality and function in children in the developing world. *Nutrition Research, 22*(1-2), 193-220. https://doi.org/10.1016/S0271-5317(01)00374-8

Pesic, M., Barac, M., Vrvic, M., Ristic, N., Macej, O., & Stanojevic, S. (2011). Qualitative and quantitative analysis of bovine milk adulteration in caprine and ovine milks using native-PAGE. *Food Chemistry, 125*(4), 1443-1449. https://doi.org/10.1016/j.foodchem.2010.10.045

Poonia, A., Jha, A., Sharma, R., Singh, H. B., Rai, A. K., & Sharma, N. (2016). Detection of adulteration in milk: A review. *International Journal of Dairy Technology, 70*(1), 23-42. https://doi.org/10.1111/1471-0307.12274

Quinten, M., & Kreibig, U. (1986). Optical properties of aggregates of small metal particles. *Surface Science, 172*(3), 557-577. https://doi.org/10.1016/0039-6028(86)90501-7

Rance, F., Grandmottet, X., & Grandjean, H. (2005). Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. *Clinical Experimental Allergy, 35*(2), 165-172. https://doi.org/10.1111/j.1365-2222.2005.02162.x

Salih, M. A. M., & Yang, S. (2017). Common milk adulteration in developing countries cases study in China and Sudan: A Review. *Journal of Advances in Dairy Research, 5*(4). https://doi.org/10.4172/2329-888X.1000192

Sampson, H. A. (2003). Food Allergy. *Journal of Allergy and Clinical Immunology, 111*(2), 540-547. https://doi.org/10.1067/mai.2003.134

Sato, K., Hosokawa, K., & Maeda, M. (2003). Rapid aggregation of gold nanoparticles induced by non-cross linking DNA hybridization. *Journal of the American Chemical Society, 125*(27), 8102-8103. https://doi.org/10.1021/ja034876s

Stanciuc, N., & Rapeanu, G. (2010). Identification of adulterated sheep and goat cheeses marketed in Romania by immunocromatographic assay. *Food and Agricultural Immunology, 21*(2), 157-164. https://doi.org/10.1080/09540100903508683

Verma, M. S, Rogowski, J. L., Jones, L., Frank, X., & Gu, F. X. (2015). Colorimetric biosensing of pathogens using gold nanoparticles. *Biotechnology Advances, 33*(6), 666-680. https://doi.org/10.1016/j.biotechadv.2015.03.003

Zelenakova, L., & Golian, J. (2008). *Application of ELISA tests for detection of milk and cheese adulteration*. Sci. Monograph, SPU Nitra 98.

Zeng, S., Yong, K. T., Roy, I., Dinh, X. Q., Yu, X., & Luan, F. A. (2011). A Review on functionalized gold nanoparticles for bio-sensing applications. *Plasmonics, 6*, 491-506. https://doi.org/10.1007/s11468-011-9228-1

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# Previous and Current Crop Effects on Early-Season Root Growth and Growing Season's Soil Moisture Under Dryland Agriculture in Temperate Climate

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**Abstract**

Understanding the root growth and changes in soil moisture content during the growing season for dryland agriculture crops can improve crop production. It was hypothesized that early-season root growth might be influenced by previous crop and current crops, and soil moisture content and depletion pattern during the growing season and residual soil moisture may be affected by the crop type. A study was conducted on the early-season root growth of canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), and flax (*Linum usitatissimum* L.) in 2015; and changes in soil water content during the 2013, 2014, and 2015 growing seasons under canola, flax, wheat, barley (*Hordeum vulgare* L.), and pea (*Pisum sativum* L.). Early-season root growth of the canola and flax crops was better on wheat than canola stubble, while for wheat it was similar on the stubbles of both wheat and canola. Soil moisture depletion started relatively earlier under the barley and wheat and later under the flax compared to the canola and pea crops. Flax continued to deplete soil moisture for a longer period than the other crops. With some exceptions, all crops could deplete soil moisture to a similar level (down to about 15% or somewhat lower) by the end of their growing seasons. Generally, almost equal amounts of residual soil moisture remained after the different crops.

**Keywords:** canola, cereals, root length, area, volume, flax, pea, soil moisture

## 1. Introduction

Dryland agriculture is practiced in the study area. The water used by crops comes from rain during the growing season, plus the stored soil moisture from rain/snow before seeding and depletion from the root zone (soil moisture in the soil profile at seeding—harvesting of crops). Under such conditions, the temporal and spatial changes in the soil moisture during the growing season of crops depend on root characteristics and their distribution in the soil profile.

The ability of plants to obtain water and mineral nutrients from the soil is related to their capacity to develop their root systems. Gan et al. (2011) suggested that a crop's root system can compensate by increasing or relocating maximal root growth to higher soil moisture regions, helping maintain plant growth under dry soil conditions (Rendig & Taylor, 1989). Jalota et al. (1980) observed more root growth of wheat with higher moisture content in the soil profile at seeding. The root length density of crops during the dry season tends to decrease mid-season at shallow soil depths, whereas it continues to increase throughout the growing season at deeper soil depths (Moroke et al., 2005). These changes parallel the trend in maximum soil moisture depletion from successively deeper layers as the season progresses.

Understanding soil moisture depletion by crops during the growing season and its residual level in the soil profile at crop harvest can help choose appropriate subsequent crops. To enhance the sustainability of dryland cropping systems, the sequence of crops is arranged to grow low water-using crops followed by high water-using crops so that excess soil water unused by the previous crop is available to the next crop (Unger et al., 2006; Lenssen et al., 2014). Stratified soil moisture depletion in crop rotations may improve the overall water use efficiency (Bunting & Kassam, 1988; Gregory, 1989; Roder et al., 1989). Lenssen et al. (2014, 2018) and Schlegel et al. (2017) found greater soil water storage and water use efficiency with diversified crop rotations than monocropping. Schlegel et al. (2019) stated that crop rotations could efficiently use soil water and enhance dryland crop yields compared to monocropping.

There has been considerable research on the root growth and soil moisture changes for various crops under several climatic, agronomic and soil conditions. However, limited research is available on early-season root growth based on the influence of previous and current crops, and on pattern of soil moisture depletion during the growing season and its residual level for different crops under similar rainfed agriculture conditions that prevail in the study area. We hypothesized that a crop grown in the rotation might have better early-season root growth compared to the same crop grown under monoculture, because some negative factors associated with monoculture are alleviated. We also hypothesized that soil moisture content and depletion pattern during the growing season and residual soil moisture might be affected by crop type. Thus, the early-season root characteristics of crops and the depletion of soil moisture by the crops during the growing season were monitored in a field study.

## 2. Materials and Methods

### 2.1 Site, Soil, and Weather

The field experiment was located south of Donnelly in a field with the legal land location of NW7-77-20W5 (GPS: 55°39′38.43″ N, 117°6′10.64″ W), southeast Peace Region, Alberta, Canada. The soil at the site was a clay loam Luvisol (Soil Classification Working Group 1998). The 0-15 cm soil depth had 48 g $kg^{-1}$ organic matter, 6.1 pH (water), and 16.4 cmol (+) $kg^{-1}$ CEC. Spring soil moisture (SSM) and monthly precipitation during the growing season data were obtained from the weather station at Ballater in Alberta, located 5 km from the site (Table 1).

Table 1. Spring soil moisture (SSM), monthly precipitation total during crop growing seasons, and their 30-yr average (Normal); from the Ballater weather station, Alberta, Canada

| | SSM and monthly precipitation total (mm) | | | |
|---|---|---|---|---|
| | 2013 | 2014 | 2015 | Normal |
| SSM | 60.0 | 60.4 | 50.0 | 75.0 |
| May (121-151 Julian days) | 19.6 | 21.1 | 19.4 | 42.2 |
| June (152-181 Julian days) | 101.8 | 58.1 | 34.4 | 74.2 |
| July (182-212 Julian days) | 65.4 | 30.4 | 28.6 | 66.5 |
| Aug. (213-243 Julian days) | 13.6 | 2.6 | 44.5 | 55.8 |
| **May to Aug. total** | **200.4** | **112.2** | **126.9** | **237.7** |

### 2.2 Experimental Details

A study on crop rotation was conducted from 2009 to 2015. A randomized complete block design was used to compare ten crop rotations that included canola, wheat, pea, barley, and flax, plus the monocultures of canola and wheat. The details on treatments, agronomic practices, and crop yield results have been presented by Gill (2018). The current research measured periodic soil moisture content during the 2013, 2014, and 2015 crop growing seasons and root growth in the early-season of 2015. The relevant procedures for these measurements are described below.

The crops were seeded on May 14, 21, and 11 in 2013, 2014, and 2015, which corresponded to the 134, 141, and 131 Julian day, respectively. All crops were harvested on Sept. 14 (257 Julian day) in 2013 and on Sept. 6 (249 Julian day) in 2014. In 2015, crop harvest occurred on Sept. 10 (253 Julian day, wheat and pea), Sept. 19 (262 Julian day, barley), Sept. 28 (271 Julian day, canola), and Oct. 8 (281 Julian day, flax).

For root measurements, selected treatments to compare the effects of 2014 canola and wheat (termed as previous crops) on the early-season root growth of canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), and flax (*Linum usitatissimum* L.) in 2015 (subsequent crops). Ten intact plants (shoots and roots) plus surrounding soil from representative areas of plots under wheat, canola, and flax crops were collected after 37 days of seeding, on June 17, 2015 (168 Julian day). The soil and roots were soaked in water for several hours. This was followed by gently shaking under water and repeated rinsing to remove the soil. The remaining debris was carefully picked off using tweezers. Then the shoots of five representative plants were cut so that all roots were still attached to the base. These representative plant's roots were spread in trays and scanned using a WinRHIZO Regular V.2013, XL Rhizo plus STD4800 scanner imaging machine (www.regentinstruments.com). WinRHIZO assigns root lengths to predefined diameter classes and counts the tips. Then the scanner provides estimates of the length, surface area and volume measurements for different diameter sized root classes, *i.e.*, for the 0 < 0.5, 0.5 < 1.0, 1.0 < 1.5, 1.5 < 2.0, 2.0 < 2.5, 2.5 < 3.0, 3.0 < 3.5, 3.5 < 4.0, 4.0 < 4.5, > 4.5 mm diameter roots. The roots and shoots of the five plants were dried to determine their dry masses. For presentation, data for roots of different sizes were grouped into thin (< 0.5 mm), medium (> 0.5 < 1.5 mm), and thick (> 1.5 mm) categories (Tables 2 and 3). The data were subjected to the Paired T-test to determine the effect of previous wheat and canola crops on root and shoot measurements of canola, wheat, and flax crops. Differences were considered significant at $p < 0.5$.

Soil moisture measurements were done for all the crops being grown in different treatments of experiment during the given growing season. Thus soil moisture could be monitored only under four crops (barley, canola, pea, and wheat) in 2013, two crops (canola and wheat) in 2014, and four crops (canola, flax, pea, and wheat) in 2015. Soil moisture was measured at depths of 0-10, 10-20, 20-30, and 30-40 cm under the wheat, canola, flax, barley (*Hordeum vulgare* L.), and pea (*Pisum sativum* L.). A profile probe (PR2-UM-3.0) and a moisture meter (HH2 version 4.0) of the Delta T Devices Ltd, 2008, 130 Low Road, Burwell, Cambridge, CB25 0EJ (www.delta-t.co.uk) were used to measure soil moisture at different depths. The profile probe has a sealed polycarbonate rod (~2.5 cm diameter) with paired stainless steel electronic sensors at fixed intervals along its length. When power is applied, each pair of sensors generates a simple analog DC voltage (100 MHz) that transmits an electromagnetic field extending about 10 cm in the soil. The moisture meter was used to apply power to the profile probe sensors, measure the output signal voltage returned, and convert it to soil moisture units (volumetric) using a linearization table and soil-specific parameters. The signal's strength is related to the permittivity of soil, predominantly dependent on water (≈ 81 permittivity compared to ≈ 4 for soil and ≈ 1 for air). Specified fiberglass access tubes (2.5 cm diameter) were installed at the start of each growing season to insert the profile probe for readings at different soil depths. The soil moisture values and standard deviations for the 0-20 cm (average for the 0-10 and 10-20 cm) and 20-40 cm (average for the 20-30 and 30-40 cm) depths are presented in Figures 1, 2, and 3.

## 3. Results

### *3.1 Early-Season Root Growth*

#### 3.1.1 Previous Crop Effects

The total length, surface area, volume, and number of tips for canola roots showed significantly greater values on wheat than on canola stubble (Table 2). Similarly, the values for the thin, medium, and thick canola roots were also significantly greater on wheat than on canola stubble; except for the length, surface area, and volume of thick roots and the number of tips for the medium roots. Flax roots length, surface area, volume, and the number of tips also tended to be greater on wheat than on canola stubble, both for the different sizes and total values, but the differences were not statistically significant. Unlike the canola and flax roots, the wheat roots length, surface area, volume, and the number of tips for the thin, medium, and thick roots and their total values were not consistently influenced by the previous crops of canola and wheat.

The root and shoot masses of canola and flax tended to be greater on the wheat than on the canola stubble, with significant differences for the shoot mass of canola (Table 3). The stubble type did not influence the root and shoot masses of wheat.

Table 2. Early-season length, surface area, volume, and the number of tips for the thin (< 0.5 mm), medium (> 0.5 < 1.5 mm), and thick (> 1.5 mm) roots (for five plants) of canola, wheat, and flax in 2015, as influenced by the previous crops of canola and wheat in a field study, Alberta, Canada

| Roots size | Canola | | | Wheat | | | Flax | | |
|---|---|---|---|---|---|---|---|---|---|
| | Canola | Wheat | Std[a] | Canola | Wheat | Std[a] | Canola | Wheat | Std[a] |
| *Length, mm* | | | | | | | | | |
| Thin | 405 | 653 | 71.4* | 765 | 807 | 217 NS | 650 | 758 | 290 NS |
| Medium | 57.2 | 101.7 | 26.2* | 176 | 162 | 50.8 NS | 69.8 | 107.6 | 42.3 NS |
| Thick | 33.3 | 39.1 | 8.6 NS | 36.1 | 34.5 | 15.0 NS | 6.14 | 11.22 | 5.00 NS |
| **Total** | **495** | **794** | **94.1*** | **977** | **1004** | **275 NS** | **730** | **883** | **337 NS** |
| *Surface area, cm²* | | | | | | | | | |
| Thin | 21.3 | 35.0 | 4.2* | 55.8 | 58.8 | 14.3 NS | 47.85 | 58.39 | 25.8 NS |
| Medium | 18.3 | 30.7 | 4.7* | 41.2 | 37.8 | 13.2 NS | 16.27 | 24.19 | 8.40 NS |
| Thick | 29.3 | 41.3 | 11.0 NS | 36.2 | 33.5 | 13.3 NS | 6.14 | 11.22 | 5.00 NS |
| **Total** | **68.9** | **107.3** | **11.9*** | **133.2** | **130.1** | **38.2 NS** | **80.1** | **107.0** | **42.8 NS** |
| *Volume, cm³* | | | | | | | | | |
| Thin | 0.125 | 0.209 | 0.032* | 0.391 | 0.412 | 0.100 NS | 0.327 | 0.419 | 0.202 NS |
| Medium | 0.298 | 0.500 | 0.075* | 0.848 | 0.769 | 0.300 NS | 0.337 | 0.480 | 0.141 NS |
| Thick | 2.284 | 4.119 | 1.17NS | 3.633 | 3.386 | 1.229 NS | 0.293 | 0.609 | 0.300 NS |
| **Total** | **2.71** | **4.83** | **1.16*** | **4.872** | **4.567** | **1.577 NS** | **0.700** | **1.034** | **0.400 NS** |
| *Number of tips* | | | | | | | | | |
| Thin | 1931 | 3132 | 701* | 1885 | 1725 | 603 NS | 1242 | 1378 | 243 NS |
| Medium | 12.8 | 19.8 | 8.4 NS | 46.2 | 41.3 | 8.9 NS | 13.75 | 26.25 | 4.20 NS |
| Thick | 4.75 | 3.50 | 1.00* | 4.25 | 5.33 | 3.40 NS | 4.25 | 4.50 | 1.30* |
| **Total** | **1949** | **3156** | **702*** | **1936** | **1772** | **614 NS** | **1260** | **1409** | **244 NS** |

*Note*. [a] The NS and * refers to the effects of previous crop being not significant and significant at the $p < 0.5$, respectively.

Table 3. Dry masses of roots and shoots (for five plants) of canola, wheat, and flax as influenced by the previous crops of canola and wheat

| Mass (g/5 plants) | Canola | | | Wheat | | | Flax | | |
|---|---|---|---|---|---|---|---|---|---|
| | Canola | Wheat | Std[a] | Canola | Wheat | Std[a] | Canola | Wheat | Std[a] |
| Roots | 0.368 | 0.484 | 0.159 NS | 0.409 | 0.414 | 0.098 NS | 0.099 | 0.162 | 0.060 * |
| Shoots | 2.560 | 3.610 | 0.624* | 1.447 | 1.430 | 0.415 NS | 0.354 | 0.514 | 0.228 NS |

*Note*. [a] The NS and * refers to the effect of previous crop being not significant and significant at the $p < 0.5$, respectively.

### 3.1.2 Root Size Distribution of Crops

Thin roots comprised 78 to 87% of the total root length of the canola, wheat, and flax, with much lower values for the medium (9.6 to 18.0%) and thick (0.8 to 6.7%) roots (Table 2). Almost all the tips (97 to 99%) for the canola, wheat, and flax roots were on the thin roots. The thin roots also had the highest surface area for the wheat (42-45%) and flax (55-60%) crops, followed by medium and thick roots in decreasing order. In contrast, the canola root's surface area was highest for the thick (34.1-38.6%) and lowest for the medium (26.6-28.7%). Unlike the length, surface area, and number of tips, the thick canola and wheat roots had the highest volume, followed by the medium and thin roots in decreasing order. The flax roots volume values were evenly distributed in the thin, medium, and thick classes.

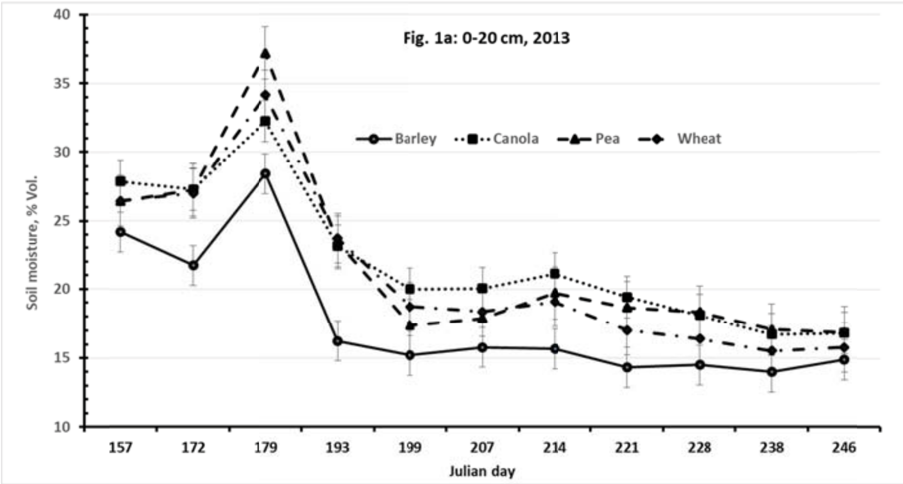
### *3.2 Soil Moisture Content and Depletion Under Different Crops*

Under dryland agriculture practiced in the study area, crops are sown in May and harvested during the fall, followed by a cold winter with no crop until sowing during the following May. Stored soil moisture plus rain and depletion of soil moisture during the growing season provide water for crops. Rain and snow during winter increase the moisture content of the soil. With rare exceptions, soil moisture depletion occurs during the growing period of crops. Crop yield suffers in years with low rainfall, when soil moisture content drops to a low level and the crops cannot extract water.

3.2.1 2013 Season

The season started with 75% of the average spring soil moisture (SSM) and received 84% of the average rainfall during the growing season (Table 1). For the crops seeded on 134 Julian day, the first measurement on 157 Julian day showed that the barley plots had slightly less soil moisture in the 0-20 cm soil than the plots under other crops (Figure 1a). The earlier growth of barley roots compared to other crops may have depleted the soil moisture. Between the 157 and 172 Julian days, soil moisture was depleted under barley but not under other crops, further increasing soil moisture differences under barley and other crops. Greater than average rain (Table 1) increased soil moisture content under all the crops on the 179 Julian day compared to the 172 Julian day. After the 179 Julian day, rapid soil moisture depletion was noticed until the 193 Julian day under barley and until the 199 Julian day under the other crops. From the 199 Julian day until the last measurement on the 246 Julian day (near harvest of crops), minimal soil moisture depletion was noticed under all the crops. During most of the growing season, the soil moisture content was lower under barley than the other crops. Amongst the canola, pea, and wheat crops, the differences in soil moisture content and depletion at the various measurement times were small and not consistent.

For the 20-40 cm depth, the soil moisture content between the 157 and 179 Julian day increased (Figure 1b), likely a result of little water use by the crops from this soil layer and above average rain during this period (Table 1). Between the 179 and 193 Julian days, some soil moisture depletion was observed with no apparent differences in soil moisture content under the different crops. Following the 193 Julian day, depletion of soil moisture content followed different trends for the crops. Under pea, depletion in the soil moisture content was faster than other crops between the 193 and 221 Julian days, and very little change occurred after the 221 Julian day. Under canola, a steady depletion of soil moisture was observed from 193 to 246 Julian days. Like canola, a steady but slightly lesser depletion of the soil moisture content was observed between the 193 and 246 Julian days under wheat. The lowest depletion rate in soil moisture during the 193 to 246 Julian day period was observed under barley compared to other crops. Thus higher soil moisture content was under the barley than other crops on the 207 to 246 Julian day measurements.



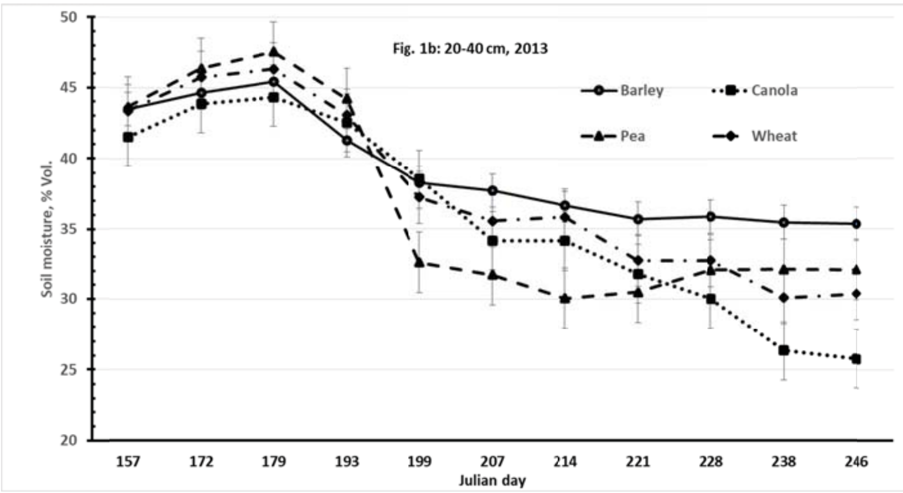




Figure 1. Soil moisture content in the 0-20 and 20-40 cm layers under the barley, canola, pea, and wheat crops at different Julian days during 2013 in a field study, Alberta, Canada

As a result of the different temporal trends of soil moisture depletion from the 20-40 cm soil under the various crops, their rankings in soil moisture content during the measurements period changed as the growing season progressed. Soil moisture content was higher under the barley than other crops from the 207 to 246 Julian days, apparently due to lower depletion from this soil layer by barley compared to other crops. Comparison between the other crops showed that the lowest level of soil moisture content was under pea at the 199, 207, and 214 Julian days and under canola from the 228 Julian day onward. Soil moisture content was lower under canola than wheat on the 207, 214, and 221 Julian days, while the opposite was observed on the 238 and 246 Julian days. Similarly, soil moisture content was lower under pea than canola on the 199, 207, 214, and 221 Julian days, and the opposite was observed on the 228, 238, and 246 Julian days.

The 2013 results indicated that barley depleted soil moisture to a lower level than the other crops from the 0-20 cm soil. From the 20-40 cm soil, barley depleted less soil moisture than other crops, and canola depleted soil moisture to a lower level than other crops.

3.2.2 2014 Season

The growing season started with 81% of the normal SSM but had only 47% of the normal precipitation in the growing season with lower than the normal amount received each month (Table 1).

Between the 142 to 155 Julian days, rain (Table 1) and very little water use during emergence and early growth of the crops seeded on the 141 Julian day increased soil moisture content in the 0-20 cm soil (Figure 2a). A decline in soil moisture content started earlier under wheat (from 155 Julian day) than under canola (from 167 Julian day). Soil moisture depletion continued until the 189 Julian day under both crops. Compared to canola, the depletion of soil moisture content under wheat was faster until the 175 Julian day and slower between 175 and

189 Julian days. By the 189 Julian day, a similar level of soil moisture content was reached under both crops and did not change much after that, probably due to the inability to extract water from dry soil. So both crops depleted soil moisture content to a similar level or utilized equal amounts of soil water from the 0-20 cm depth.

For the 20-40 cm soil in 2014, a slight increase in soil water content was noticed under both crops in the initial stages (Figure 2b), due to the reasons described for the 0-20 soil. Like the 0-20 soil, the decline in soil moisture content started earlier under wheat (from 167 Julian day) than under canola (from 175 Julian day). A higher rate of soil moisture depletion occurred under both crops until the 189 Julian day, with a much slower change after that. From the 167 to 230 Julian day, the soil moisture content was lower under wheat than canola.

The 2014 results indicated an earlier start of soil moisture use by wheat than canola. The better capability of wheat than canola to use soil moisture from the 20-40 cm soil was not consistent with the 2013 results.

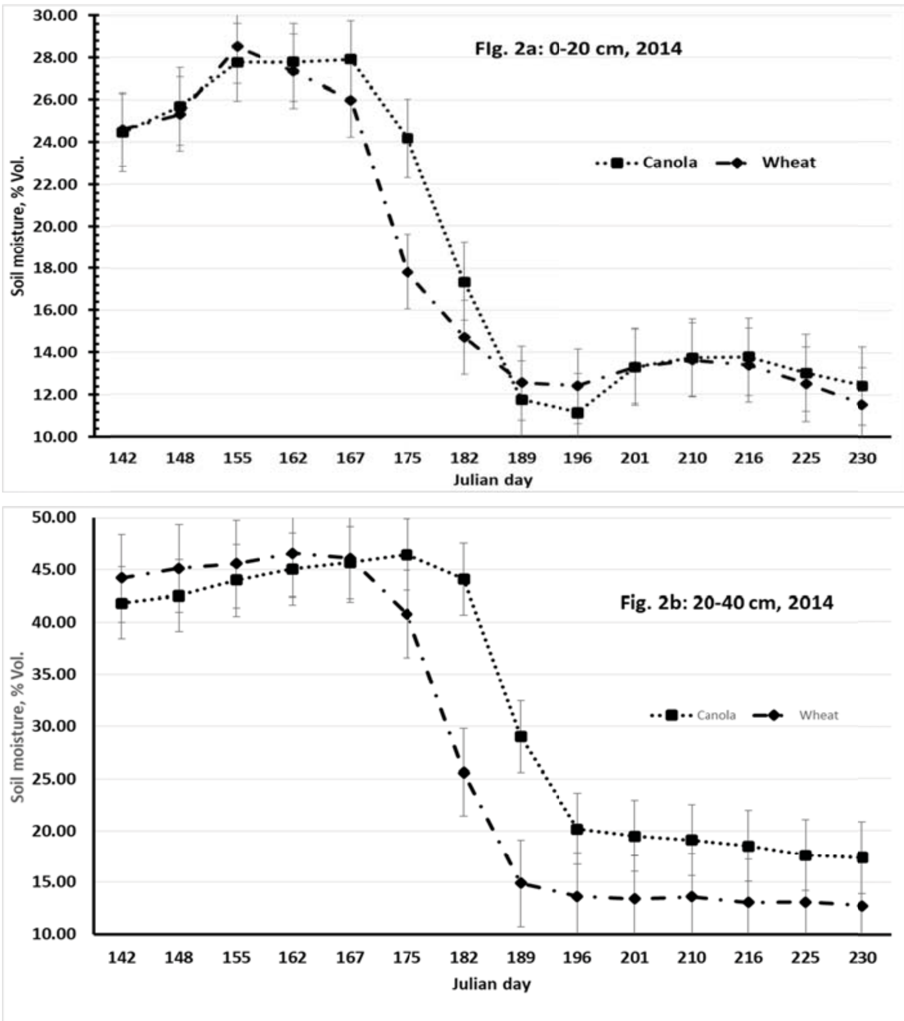



Figure 2. Soil moisture content in the 0-20 and 20-40 cm layers under the canola and wheat crops at different Julian days during 2014 in a field study, Alberta, Canada

3.2.3 2105 Season

Only 68% of the normal SSM was observed at the start of the season, and 53% of normal precipitation was received during the growing season (Table 1). Frequent small amounts of rain occurred during late July and early Aug. (observation based on daily rain events).

Soil moisture depletion from the 0-20 cm soil started after the 147 Julian day under wheat while it began after the 155 Julian day under the other crops (Figure 3a). Compared to other crops, the soil moisture depletion between the 155 and 194 Julian days was faster under wheat and slower under flax. Between the 194 and 225

Julian days, slight soil moisture depletion continued under flax. However, a gain in soil moisture was noticed under the other crops, apparently due to frequent rain events during late July and early August. Soil moisture depletion occurred under all crops between the 225 and 232 Julian days.

From the 155 to 202 Julian day, the soil moisture content in the 0-20 soil was lower under wheat than other crops (Figure 3a). On the opposite side, the soil moisture content was higher under flax than other crops at the 183, 194, and 202 Julian days. Between the 194 and 225 Julian days, the soil moisture content increased under canola, pea, and wheat crops, probably resulting from more rain than water utilization. However, the soil moisture content under flax continued to decline, apparently due to more water use by flax relative to rain during this period. This discrepancy between the flax and other crops was apparently due to the more extended growth period of flax than the other crops.

For the 20-40 cm soil, there was little or no depletion of soil moisture content until the 162 Julian day (Figure 3b). After 162 Julian day, the soil moisture content depleted slowly and consistently under flax until the last observation on 232 Julian day. A noticeable observation on the soil moisture content in the 20-40 cm soil was its higher level under flax than other crops at the 183, 194, and 202 Julian days, indicating delayed growth of flax roots in this soil layer. Under the other crops, soil moisture declined rapidly between the 162 and 194 Julian days, followed by no depletion after that. After the 194 Julian day, the canola, pea, and wheat crops were either unable to extract water at that low soil moisture level or their water need was fulfilled by frequent rain during this period.

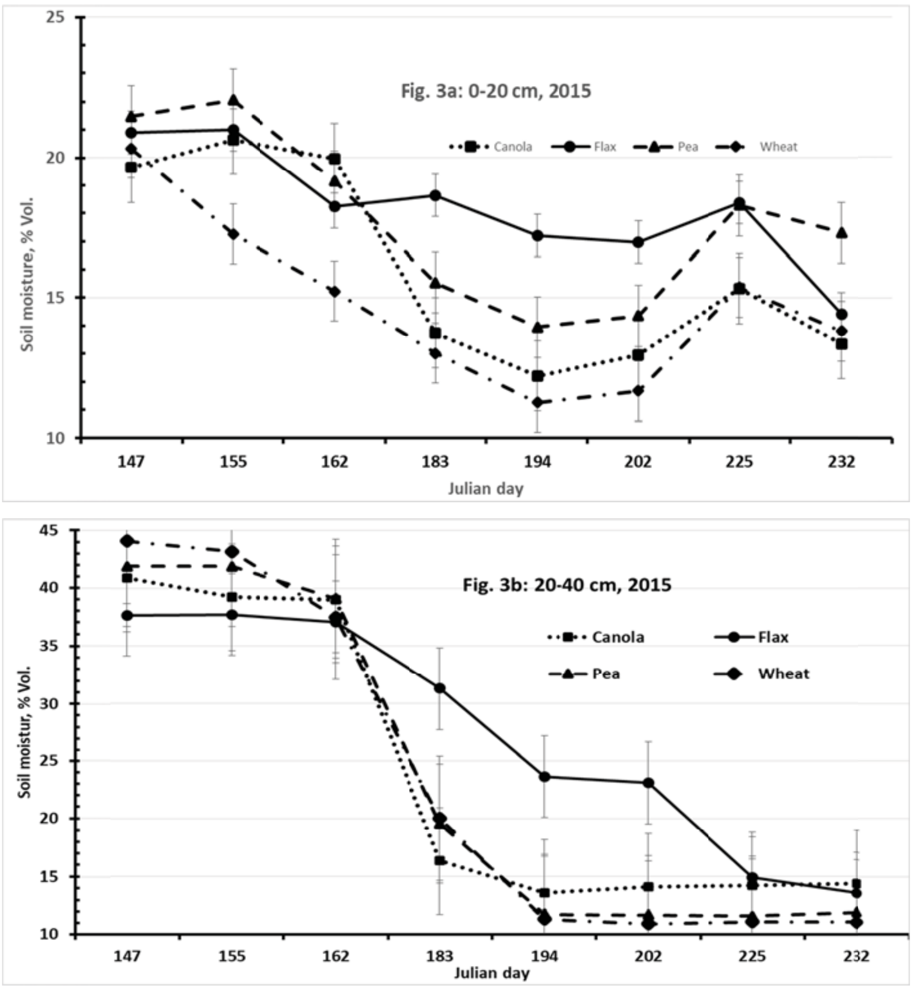



Figure 3. Soil moisture content in the 0-20 and 20-40 cm layers under the flax, canola, pea, and wheat crops at different Julian days during 2015 in a field study, Alberta, Canada

## 4. Discussion

### *4.1 Early-Season Root Growth*

The roots of a crop play vital role of linking the plant to soil and thereby soil to the atmosphere. Thus roots influence soil moisture and nutrient use efficiency by crops. Root growth is modified by soil factors such as temperature, fertility, aeration, and structure; and plant factors like vigor and ability of shoot to supply carbohydrates and other nutrients to developing roots. The root growth pattern may depend on the soil moisture content and depletion during the crop growing season (Ju et al., 2015).

Compared to canola stubble, the beneficial effects of wheat stubble on the early-season root and shoot growth of canola and flax (Tables 2 and 3) is supported by earlier research. Monreal et al. (2011) reported early-season arbuscular mycorrhizal fungi (AMF) colonization, lateral root area, and root biomass of flax to be greater when wheat rather than canola was the preceding crop. The establishment, early-season biomass, phosphorus accumulation, and seed yield of flax were greater on wheat than canola stubble (Grant et al., 2009). Grant et al. (2005) stated that spring wheat readily forms AMF association while canola does not, and the stubble and roots left by the wheat crop can create a favorable environment in the rhizosphere by increasing pore volume and nutrients for root proliferation of the subsequent crops.

The canola and flax root growth being better under the stubble of wheat than canola are supported by beneficial effects on canola yield from a 1 or 2 years break from canola in crop rotations (Gill, 2018). Similarly, Johnston et al. (2005) found that seeding canola two years in a row resulted in the lowest yield compared with canola in rotations, and O'Donovan et al. (2014) reported higher canola yield after a legume than after canola. Kutcher et al. (2013) speculated that lower canola yield on canola stubble might have been a reflection of less available soil N due to the increased demand from canola compared with other crops.

In other studies, the root vigor of crops was improved by rotations (Nickel et al., 1995), as were the root-related nutrient and water uptake functions (Copeland & Crookston, 1992; Copeland et al., 1993). Cresswell and Kirkegaard (1995) observed that a canola crop did not improve a subsequent wheat crop's rooting depth. In contrast, Williams and Weil (2004) observed soybean (*Glycine max* L. Merr.) roots growing through compacted soil using channels made by roots of a previous canola crop. This suggests that a prior crop specie's benefit may depend on the following crop species and environmental conditions.

Similar early-season growth of wheat roots on the wheat and canola stubble in the present study indicates that some other crop rotation-related factors were preventing wheat stubble benefits. Gill (2018) reported the wheat yield on canola and wheat stubble was not consistently different. Evidence of more wheat root growth following maize (*Zea mays* L.) crop than rice (*Oryza sativa* L.) exists in literature (Sur et al., 1981). Other crop sequences research in western Canada indicates that more diverse rotations tend to have fewer pest problems and lower production risk than rotations based heavily on either cereal or broadleaf crops (Bailey et al., 2000; Johnston et al., 2005; Kutcher et al., 2011).

### *4.2 Soil Moisture Content and Depletion*

Soil moisture data in 2013, 2014, and 2015 indicated an earlier start of soil moisture depletion by the cereals than other crops (Figures 1, 2, and 3). Compared to other crops, soil moisture depletion under flax started later and then continued slowly and steadily for a more extended period. Similar to our results, wheat used water faster than pulse and oilseed crops (Gan et al., 2008). It is not only the total root system that affects the soil water depletion, but the depth-wise distribution of roots also influences soil water depletion (Fan et al., 2016).

By end of the growing season, all crops depleted soil moisture down to about 15% or lower, except the 0-20 cm soil under pea in 2015; and the 20-40 cm soil under all crops in 2013 and under canola in 2014. Thus in most cases, there was almost an equal amount of residual soil moisture after different crops, indicating an only limited effect of crops under the study conditions. No depletion of soil moisture during later plant growth periods suggested a lack of available soil moisture, probably limited crop yield during the 2014 and 2015 seasons.

Similar to our results, the rate of root growth and water depletion was significantly faster under sunflower (*Helianthus annuus*) than sorghum (*Sorghum bicolor*), but sorghum reached maturity later in the growing season and water depletion was approximately the same for sunflower and sorghum (Moroke et al., 2005). They also observed that soil water depletion was significantly greater under sorghum than cowpea (*Vigna unguiculata*). Thus, the residual water content at the end of the growing season was greater under cowpea than under sorghum. Cowpea may exert a lower suction, hence lesser water uptake than sorghum and sunflower (Bunting & Kassam, 1988). Comparisons of water depletion by Merrill et al. (2003) demonstrated more soil water content after dry pea than sunflower. However, the soil water gains following a pulse crop seemed to be partially offset by lower soil moisture gain during winter fallow.

Less soil moisture depletion from the 20-40 cm soil compared to the 0-20 cm soil during the initial period of crop growth and increase in the later season indicated the delayed establishment of roots in the deeper soil. Root systems of crops have the ability to increase or relocate maximal root growth to regions with greater water content in the soil profile, thereby maintaining plant growth under dry conditions (Rendig & Taylor, 1989). Moroke et al. (2005) observed that the root length densities of sorghum and sunflower near the soil surface increased rapidly after planting but then declined, they increased throughout the growing season in deeper soil, and the soil water depletion corresponded to root length density of the crops. The root length density of crops during an extremely dry season tended to decrease during mid-season at shallow soil depths, whereas it continued to increase throughout the growing season at deeper soil depths (Moroke et al., 2005). Fan et al. (2016) stated that it is not only the total root system that affects the soil water depletion, but soil water depletion is according to the depth-wise distribution of roots. These statements support our result of more soil moisture depletion from deeper soil as the season progressed.

Different residual amounts of soil moisture after crops may have positive and negative aspects. Positively, more depletion capability means better water use efficiency and more rain infiltration, with less water runoff and soil erosion in years with adequate rain. Negatively, if the crop root zone soil is not fully charged before the start of the next crop, the subsequent crop can suffer from water stress (likely in dry areas).

## 5. Conclusions

Wheat stubble was beneficial compared to canola stubble in making the soil environment congenial to promote early-season root growth of the subsequent canola and flax crops. Similar growth of wheat roots in wheat and canola stubble indicated that some other monoculture-related factors were preventing the benefits of wheat stubble. About 80% of the total length for canola, wheat, and flax roots came from the thin roots. About 98% of the tips were on the thin roots. Root measurements data showed that an earlier crop could affect the early-season root growth of crops, and the effect may vary with the previous as well as the current crop type.

The 2013, 2014, and 2015 soil moisture depletion data indicated a relatively earlier start under barley and wheat and a delayed start under flax compared to canola and pea crops. The flax continued to deplete soil moisture for an extended period. With a few exceptions, all crops could deplete soil moisture to a similar level (down to about 15% or lower) by the end of the growing season. Thus, there was almost equal amount of residual soil moisture after the different crops and only a limited effect of the crops on the residual soil moisture content. The soil moisture availability probably limited crop production during the 2014 and 2015 seasons. Under the area's dryland agriculture conditions, the crop type could alter the soil moisture depletion pattern during the growing season, but had limited influence on the residual soil moisture at crop harvest. These findings indicated only limited influence of previous crops on the soil moisture availability for subsequent crops.

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## References

Bailey, K. L., Johnston, A. M., Kutcher, H. R., Gossen, B. D., & Morrall, R. A. A. (2000). Managing crop losses from foliar diseases with fungicides, rotation, and tillage in the Saskatchewan Parkland. *Can. J. Plant Sci., 80*, 169-175. https://doi.org/10.4141/P99-069

Bunting, A. H., & Kassam, A. H. (1988). Principles of crop water use, dry matter production, and dry matter partitioning that govern choices of crops and systems. In F. R. Bidinger & C. Johansen (Eds.), *Drought research priorities for dryland tropics* (pp. 43-61). ICRISAT, Pantacheru, AP, India.

Copeland, P. J., & Crookston, R. K. (1992). Crop sequence affects nutrient composition of corn and soybean grown under high fertility. *Agron. J., 84*, 503-509. https://doi.org/10.2134/agronj1992.00021962008400030028x

Copeland, P. J., Allmaras, R. R., Crookston, R. K., & Nelson, W. W. (1993). Corn-soybean rotation effects on soil water depletion. *Agron. J., 85*, 203-210. https://doi.org/10.2134/agronj1993.00021962008500020008x

Cresswell, H. P., & Kirkegaard, J. A. (1995). Subsoil amelioration by plant roots—the process and the evidence. *Aust. J. Soil Res., 33*, 221-239. https://doi.org/10.1071/SR9950221

Fan, J., McConkey, B., Wang, H., & Janzen, H. (2016). Root distribution by depth for temperate agricultural crops. *Field Crop Research, 189*, 68-74. https://doi.org/10.1016/j.fcr.2016.02.013

Gan, Y., Campbell, C. A., Liu, L., Basnyat, P., & McDonald, C. L. (2008). Water use and distribution profile under pulse and oilseed crops in semiarid northern high latitude areas. *Agricultural Water Management, 96*, 337-348. https://doi.org/10.1016/j.agwat.2008.08.012

Gan, Y., Liu, L., Cutforth, H., Wang, X., & Ford, G. (2011). Vertical distribution profiles and temporal growth patterns of roots in selected oilseeds, pulses and spring wheat. *Crop and Pasture Sci., 62*, 457-466. https://doi.org/10.1071/CP10406

Gill, K. S. (2018).Crop rotations compared with continuous canola and wheat for crop production and fertilizer use over 6 yr. *Can. J. Plant Sci., 98*, 1-11. https://doi.org/10.1139/cjps-2017-0292

Grant, C. A., Bittman, S., Monreal, M., Plenchete, C., & Monreal, C. (2005). Soil and fertilizer phosphorus: effects on plant P supply and mycorrhizal development. *Canadian J. Plant Sc., 85*, 3-14. https://doi.org/10.4141/P03-182

Grant, C. A., Monreal, M. A., Irvine, R. B., Mohr, R. M., McLaren, D. L., & Khakbazan, M. (2009). Crop response to current and previous season applications of phosphorus as affected by crop sequence and tillage. *Can. J. Soil Sci., 89*, 49-66. https://doi.org/10.4141/CJPS07178

Gregory, P. J. (1989). Crop water use efficiency. In C. E. Whitman, J. F. Parr, R. I. Papendick, et al. (Eds.), *Soil, water and crop/livestock management systems: Rainfed agriculture in the Near East Region* (pp. 162-172). USAID, USDA, and ICARDA, Washington, DC.

Jalota, S. K., Prihar, S. S., Sandhu, B. S., & Khera, K. L. (1980). Yield, water use and root distribution of wheat as affected by presowing and postsowing irrigation. *Agricultural Water Management, 2*, 289-297. https://doi.org/10.1016/0378-3774(80)90029-3

Johnston, A. M., Kutcher, H. R., & Bailey, K. L. (2005). Impact of crop sequence decisions in the Saskatchewan Parkland. *Can. J. Plant Sci., 85*, 95-102. https://doi.org/10.4141/P04-090

Ju, C., Buresh, R. J., Wang, Z., Zhang, H., Liu, L., Yang, J., & Zhang, J. (2015). Root and shoot traits for rice varieties with higher grain yield and higher nitrogen use efficiency at lower nitrogen rates application. *Field Crop Research, 175*, 47-55. https://doi.org/10.1016/j.fcr.2015.02.007

Kutcher, H. R., Brandt, S. A., Smith, E. G., Ulrich, D., Malhi, S. S., & Johnston, A. M. (2013). Blackleg disease of canola mitigated by resistant cultivars and four-year crop rotations in western Canada. *Can. J. Plant Pathol., 35*, 209-221. https://doi.org/10.1080/07060661.2013.775600

Kutcher, H. R., Johnston, A. M., Bailey, K. L., & Malhi, S. S. (2011). Managing crop losses from plant diseases with foliar fungicides, rotation and tillage on a Black Chernozem in Saskatchewan, Canada. *Field Crops Res., 124*, 205-212. https://doi.org/10.1016/j.fcr.2011.05.018

Lenssen, A. W., Sainju, U. M., Iversen, W. M., Allen, B. L., & Evans, R. G. (2014). Diversification, tillage, and management influence spring wheat yield and water use. *Agron. J., 106*, 1445-1454. https://doi.org/10.2134/agronj14.0119

Lenssen, A. W., Sainju, U. M., Jabro, J. D., Allen, B. L., & Stevens, W. B. (2018). Dryland pea productivity and soil water responses to tillage, crop rotation, and weed management practice. *Agron. J., 110*, 1843-1853. https://doi.org/10.2134/agronj2018.03.0182

Merrill, S. D., Tanaka, D. L., Krupinsky, J. M., & Ries, R. E. (2003). Soil water depletion and coverage by residue under sunflower compared to other diverse crops. *Proceedings of the 25th Sunflower research Workshop, Jan. 16-17, 2003, National Sunflower Association, Fargo, ND*.

Monreal, M. A., Grant, C. A., Irvine, R. B., Mohr, R. M., McLaren, D. L., & Khakbazan, M. (2011). Crop management effect on arbuscular mycorrhizae and root growth of flax. *Can. J. Plant Sci., 91*, 315-324. https://doi.org/10.4141/CJPS10136

Moroke, T. S. (2002). *Root distribution, water extraction patterns, and crop water use efficiency of selected dryland crops under differing tillage systems* (Ph.D. Diss., Texas A&M Univ., College Station, TX).

Moroke, T. S., Schwartz, R. C., Brown, K. W., &. Juo, A. S. R. (2005). Soil water depletion and root distribution of three dryland crops. *Soil Sci. Soc. Am. J., 69*, 197-205. https://doi.org/10.2136/sssaj2005.0197

Nickel, S. E., Crookston, R. K., & Russelle, M. P. (1995). Root growth and distribution are affected by corn-soybean cropping sequence. *Agron. J., 87*, 895-902. https://doi.org/10.2134/agronj1995.00021962008700050020x

O'Donovan, J. T., Grant, C. A., Blackshaw, R. E., Harker, K. N., Johnson, E. N., Gan, Y., … Smith, E. G. (2014). Rotational effects of legumes and non-legumes on hybrid canola and malting barley. *Agron. J., 106*, 1921-1932. https://doi.org/10.2134/agronj14.0236

Rendig, V. V., & Taylor, H. M. (1989). *Principles of soil-plant interrelationships*. McGraw-Hill Publ. Co., New York.

Roder, W., Mason, S. C., Clegg, M. D., & Kniep, K. R. (1989). Yield-soil relationships in sorghum-soybean cropping systems with different fertilizer regimes. *Agron. J., 81*, 470-475. https://doi.org/10.2134/agronj1989.00021962008100030015x

Schlegel, A. J., Assefa, Y., Haag, L. A., Thompson, C. R., Holman, J. D., & Stone, L. R. (2017). Yield and soil water in three dryland wheat and grain sorghum rotations. *Agron. J., 109,* 227-238. https://doi.org/10.2134/agronj2016.07.0387

Schlegel, A. J., Assefa, Y., Haag, L. A., Thompson, C. R., Holman, J. D., & Stone, L. R. (2019). Soil water and water use in long-term dryland crop rotations. *Agron. J., 111*, 1-10. https://doi.org/10.2134/agronj2018.09.0623

Soil Classification Working Group. (1998). The Canadian system of soil classification. *Agriculture and Agri-Food Canada* (Publication 1646, Revised). National Research Council of Canada, Ottawa, ON.

Sur, H. S., Prihar, S. S., & Jalota, S. K. (1981). Effect of rice-wheat and maize-wheat relation on water transmission and wheat root development on a sandy loam of the Punjab, India. *Soil and Till. Res., 1*, 361-371. https://doi.org/10.1016/0167-1987(80)90038-0

Unger, P. W., Payne, W. A., & Peterson, G. A. (2006). Water conservation and efficient use. In G. A. Peterson, P. W. Unger, & W. A. Payne (Eds.), *Dryland Agriculture* (2nd ed.). *Agron. Monograph 23* (pp. 39-85). ASA, CSSA, and SSSA, Madison, WI. https://doi.org/10.2134/agronmonogr23.2ed.c3

Williams, S. M., & Weil, R. R. (2004). Crop cover root channels may alleviate soil compaction effects on soybean crop. *Soil Sci. Soc. Am. J., 68*, 1403-1409. https://doi.org/10.2136/sssaj2004.1403

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# Backcross Aiming at the Resistance of Passion Fruit to Soil Pathogen

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**Abstract**

Brazil is the world's largest producer of passion fruit, however, the crop suffers from serious phytosanitary problems, as well as those caused by soil fungi. Thus, the objective of the present work was to estimate the genetic parameters and to select genotypes resistant to *Fusarium solani species complex*—FSSC in a segregating population from the first generation of backcross among *P. edulis* and interspecific hybrids, aiming at advancing generation in the genetic improvement program of passion fruit. The Interspecific Hybrid was used (IH) UNEMAT 142 resistant to colon rot, for generation advancement and to cultivate BRS Sol do Cerrado (*Passiflora edulis* Sims). In order to evaluate the resistance of the 27 genotypes of the first generation of backcrosses, inoculation with the FSUNEMAT 40 (*F. solani*) inoculum was performed. To estimate the components of variances, the method of maximum restricted likelihood (REML) was used and to select the best genotypes by the non-addicted linear prediction (BLUP). The variables that showed the highest heritability values were the survival period and the area under the lesion length expansion curve. The three families of backcrosses presented genotypes resistant to the fungus *F. solani*, however, by the methodology of mixed models REML/BLUP, only the genotypes BC1-22/1, BC1-22/2, BC1-22/3, BC1-22/4, BC1-22/6, BC1-22/7, BC1-113/3, BC1-113/7 and BC1-113/8, were selected to advance the generation of the UNEMAT passion fruit breeding program, therefore, they presented among the ten placed, mainly for the variable survival period (SP).

**Keywords:** *Passiflora* spp., plant breeding, *Fusarium solani species complex*—FSSC, REML/BLUP

## 1. Introduction

The genus *Passiflora* L. is originally from South America with concentration and distribution in Brazil, Paraguay and northern Argentina, although in some reports it is listed as native to Venezuela and Colombia (Cervi, 1997; Meletti & Maia, 1999; Dhawan et al., 2004). Brazil is the world's largest producer of passion fruit, with a production of 593.429 t (IBGE, 2020), with passion fruit (*Passiflora edulis* Sims) accounting for more than 90% of this production. However, production and productivity have been hindered by several phytosanitary problems, reducing the time of economic exploitation or even preventing their cultivation in some regions.

Among the diseases, the colon rot, caused by *Fusarium solani species complex*—FSSC (Fischer & Resende, 2016). The symptoms of this disease are characterized by sudden wilting, collapse and death of the plants at any stage of development (Fischer & Resende, 2016). Thus, the occurrence of colon rot drastically reduces the productivity and longevity of the crop and is responsible for constant migrations of the passion fruit plantations. This fact is aggravated by the fact that *F. solani* produces chlamydospores, which are structures that allow these pathogens to survive in the soil for several years (Fischer & Resende, 2016).

There is no efficient control form for this disease, and the development of resistant cultivars would be a viable alternative, since there is no resistant passion fruit cultivar that has been registered so far. Thus, the only way to combat these problems is the transfer of resistance genes, found in wild species. In view of this, the genetic improvement research group of passion fruit at the State University of Mato Grosso (UNEMAT) has been conducting research aimed at obtaining cultivars resistant to soil pathogens, thus contributing to the genetic progress of the culture. This program started with the selection of wild *Passiflora* species resistant to *F. solani* and *F. oxysporum* f.sp. *passiflorae* (Preisigke et al., 2015, 2017). Subsequently, interspecific hybrids resistant to

*F. solani* were obtained and currently, genotypes of the first generation of backcross are being developed (Marostega et al., 2020).

For genetic progress in breeding programs it is of great importance to estimate the genetic parameters, for this, the methodology of the mixed models REML/BLUP (maximum restricted likelihood/best non-impartial linear forecast) has been highly indicated due to its potential in estimating the gain genetic and better precision in the selection process in relation to selection indices or phenotypic selection (Freitas et al., 2013). Many authors (Santos et al., 2015; Silva et al., 2016, 2017), used the mixed REML/BLUP models to estimate the genetic parameters in the passion fruit culture.

Therefore, the objective of the work was to estimate genetic parameters and select genotypes resistant to colon rot in a segregating population from the first generation of backcrossing between *P. edulis* and interspecific hybrids, aiming at advancing generation in the passion fruit breeding program.

## 2. Material and Methods

The work was carried out at the Plant Breeding Laboratory, at the State University of Mato Grosso (UNEMAT), Cáceres campus, 16°11′42″ south latitude and 57°40′51″ west longitude, with temperature annual average of 26.24 °C, total annual precipitation of 1,333 mm and altitude of 118 m. The municipality integrates the mesoregion of the Center-South of Mato Grosso and the microregion of the Upper Pantanal, 215 km from the capital, where the climate is hot and humid tropical, with dry winter (Neves et al., 2011).

It was selected from the working collection of the Active Germplasm Bank of UNEMAT, the Inter-specific Hybrid (HI) UNEMAT 142, to be the pollen grain donor. This HI is the result of the cross between the resistant species *P. nitida* and the cultivar BRS Sol do Cerrado (*P. edulis*), it has resistance to *F. oxysporum* f. sp. *passiflorae* and *F. solani* and was described by Marostega et al. (2020), as one of the genotypes selected to advance generation for resistance to soil fungi.

### *2.1 Obtaining the Genotypes of the First Generation of Backcross (BC1s)*

In order to recover the agronomic characteristics of the recurrent parent (*P. edulis*), a crossover between IH and *P. edulis* (BRS Sol do Cerrado) was carried out, obtaining the families of complete brothers (FCB) from the first generation of backcross (BC1).

The backcrosses were performed with three plants of *P. edulis* cultivar BRS Sol do Cerrado (plants 22, 28 and 113), and the transfer of pollen to the stigma was performed with the aid of forceps, carefully rubbing the anther about the stigma of each flower protected in pre-anthesis with a paper bag. The flowers were then labeled and, five days after pollination, the setting was verified.

The seeds of BC1s, were sown in trays containing vermiculite substrate, kept in a screen covered with 50% shade. Subsequently, the seedlings were transplanted into 500 ml plastic cups, and kept on a 50% covered shade screen until inoculation.

### *2.2 Inoculation of F. solani*

The inoculation of *F. solani* was performed with the FSUNEMAT 40 inoculum, the most aggressive (Marostega et al., 2019). First, the preserved segments were transferred to Petri dishes containing PDA culture medium (potato-dextrose-agar) and maintained at 25 °C with a 12 h photoperiod for seven days in the BOD.

The inoculation was performed with a mycelium disk of the pathogen of five millimeters in diameter and fixed with PVC plastic over a wound of three millimeters in diameter in the neck of the plant, at a height of two centimeters from the soil. Removing the PVC plastic five days after inoculation (DAI), according to the methodology of Fischer et al. (2005).

27 genotypes of BC1s were evaluated, with nine genotypes from each family of complete siblings (BC1-22, BC1-28 and BC1-113). The evaluation of the resistance of the genotypes to *F. solani* was quantified through 10 resistance variables as described by Preisigke et al. (2015), which are:

- SP = Survival period;
- NDP = Number of dead plants;
- LL = Length of the lesion;
- WL = width of the lesion;
- NPL-50% = Number of plants in which the lesion reached less than 50% of the circumference;
- PILA 50% = Period of inoculation until the lesion reaches 50% of the circumference;

- PILA 100% = Period of inoculation until the lesion reaches 100% of the circumference;
- AULAEC = Area under the lesion area expansion curve;
- AULLEC = Area under the lesion length expansion curve;
- AULWEC = Area under the lesion width expansion curve.

The evaluations were carried out after five days of inoculation, being carried out every two days until completing 33 days or until the death of the plants. The lesions were measured for their length and width of the necrotic area, with the aid of a digital caliper. The area of the lesion (AL, $mm^2$) was estimated considering the formula for calculating the area of an ellipse ($\pi \cdot L \cdot W/4$), where L is the length of the lesion and W is the width of the lesion.

To estimate the components of variances, the method of maximum restricted likelihood (REML) was used and to select the best genotypes by the non-addicted linear prediction (BLUP) (Resende, 2002; Alves & Resende, 2008).

REML/BLUP analyzes were performed using the Selegen-Reml/Blup program (Resende, 2016), which follows the statistical model $y = Xr + Zg + Wp + e$, where, $y$ is the vector of observations, $r$ is the vector of the effects of repetition (assumed to be fixed) added to the general average, $g$ is the vector of the individual genotypic effects (assumed to be random), $p$ is the vector of the effects of plots (random) and $e$, the vector of errors or residues (random). The capital letters represent the incidence matrices for the said effects. The statistical model used was the 147 of the Selegen program.

The following components of variance (REML) were estimated:

$\sigma^2_g$: genotypic variance between genotypes, equivalent to 1/2 of the additive genetic variance plus 1/4 of the dominance genetic variance, ignoring epistasis;

$\sigma^2_f$: individual phenotypic variance;

$h^2_a$: individual heritability in the narrow sense, obtained by ignoring the fraction (1/4) of the genetic dominance variance;

$h^2_{mp}$: heritability of the average of the genotypes, assuming complete survival and

Acprog: accuracy of genotype selection.

For the selection of the genotypes with the greatest genetic gains by the BLUP method (*Best Linear Unbiased Prediction*), the variables used were those in which the accuracy of the selection of the genotypes (Acprog), was above 0.50. In addition, the variables were separated into two groups: a group with the variables in which the increase in the average gives resistance to the fungus, such as SP: which is the survival period of the plants and PILA 100%: Period of inoculation until the lesion reaches 100% of circumference. The second group is formed with the variables in which the decrease in the average is what gives the resistance as the NDP: Number of dead plants, AULAEC: area under the expansion curve of the lesion area, AULLEC: area below the expansion curve of the lesion length and AULWEC: area below the lesion width expansion curve.

## 3. Results and Discussion

### *3.1 Estimation of Genetic Parameters*

In general, the highest values of genotypic variance ($\sigma^2_g$), were for the variables SP and AULLEC, with 25.71 and 20.44% respectively, in relation to the phenotypic variance ($\sigma^2_f$) (Table 1), that is, the genotypes evaluated showed considerable genetic variability for these characteristics. According to Cruz and Carneiro (2006), knowledge of the genotypic variation for breeding programs is extremely important, as it indicates the extent of genetic variation of a variable, with the use of breeding technologies. The variables that presented lower values of $\sigma^2_g$, are NPL-50% and WL, with 0.04 and 0.06% respectively, in relation to $\sigma^2_f$, this indicates that these variables had a high environmental influence and should not be used to indicate genotypes promising.

Table 1. Estimates of the components genotypic variance between genotypes ($\sigma^2_g$), individual phenotypic variance ($\sigma^2_f$), individual heritability in the strict sense ($h^2_a$), heritability of the genotype mean ($h^2_{mp}$) and accuracy of the selection of genotypes (Acprog) obtained by REML procedure, for the 10 variables evaluated in twenty-seven backcross genotypes resistant to the fungus *F. solani*

| **Genetics** \ **Parameters** | $\sigma^2_g$ | $\sigma^2_f$ | $h^2_a$ % | $h^2_{mp}$ % | $Ac_{prog}$ |
|---|---|---|---|---|---|
| **SP** | 38.2997 | 148.9340 | **51.43** | 75.12 | **0.8667** |
| **NDP** | 0.0185 | 0.2345 | 15.81 | 37.55 | 0.6127 |
| **LL** | 0.0008 | 0.2101 | 0.82 | 1.87 | 0.1369 |
| **WL** | 0.0960 | 153.2657 | 0.12 | 0.55 | 0.0746 |
| **NPL-50%** | 0.0092 | 20.7320 | 0.08 | 0.39 | 0.0628 |
| **PILA 50%** | 0.6756 | 164.7772 | 0.82 | 1.87 | 0.1369 |
| **PILA 100%** | 6.2659 | 166.1458 | 7.54 | 25.89 | 0.5088 |
| **AULAEC** | 24978.1116 | 566973.4560 | 8.81 | 28.91 | 0.5377 |
| **AULLEC** | 2448.3118 | 11974.5827 | **40.89** | 69.47 | **0.8335** |
| **AULWEC** | 239.9045 | 5197.5914 | 9.23 | 30.13 | 0.5489 |

*Note*. SP: survival period; NDP: Number of dead plants; LL: Length of the lesion; WL: Width of the lesion; NPL-50%: Number of plants in which the lesion reached less than 50% of the circumference; PILA 50%: Period of inoculation until the lesion reaches 50% of the circumference; PILA 100%: Period of inoculation until the lesion reaches 100% of the circumference; AULAEC: area below the lesion area expansion curve; AULLEC: area below the lesion length expansion curve; AULWEC: area under the lesion width expansion curve.

The values of individual heritability in the strict sense ranged from 0.08 to 51.43%, with the lowest values observed for the characteristics NPL 50% (0.08) and WL (0.12). The variables with the highest values were SP (51.43) and AULLEC (40.89).

The low values of heritability are due to the low value of the genetic variation associated with the high phenotypic variation, indicating a high environmental influence for these characteristics. However, according to Santos et al. (2015), even with low heritability variables, with the mixed models favorable genetic gains are expected, therefore, the use of these models in the present study is justified.

Contrasting result to that found by Freitas et al. (2015), who assessed the heritability for the AUDPC variable regarding CABMV resistance in a segregating population of passion fruit (Cowpea aphid-borne mosaic virus), found high heritability (94%).

According to Silva et al. (2017), the accuracy value takes into account precision of the real value of the genetic variance based on the observed phenotypic variance. Low accuracy values indicate that possibly these variables are highly influenced by the environment and that the data for these variables are less reliable. In the present study, variables with accuracy values classified as high ($0.70 \leq Acprog \leq 0.89$) were found for the variables SP and AULLEC, with values of 0.8667 and 0.8335, respectively. Variables classified as moderate ($0.50 \leq Acprog \leq 0.69$), for the variables NDP, PILA 100%, AULAEC and AULWEC (Table 1) and the rest of the variables were classified with low accuracy values ($Acprog \leq 0.49$) (Resende & Duarte, 2007).

3.1.1 Individual Selection and Earnings Estimates

From a total of 27 evaluated genotypes, the 10 best were selected for each of the analyzed variables, with genetic gains being predicted and the new averages estimated (Table 2).

Table 2. Ranking of the twenty-seven genotypes with the highest estimates of genetic gain and new predicted averages, estimated via BLUP, in passion fruit genotypes from the first generation of backcross

| Ord. | Genotypes | SP | | Genotypes | NDP | | Genotypes | PILA 100% | |
|---|---|---|---|---|---|---|---|---|---|
| | | Gain (%) | New media | | Gain (%) | New media | | Gain (%) | New media |
| 1 | **BC1-22/6** | 13.90 | 30.97 | **BC1-22/6** | 0.0000 | 0.6667 | **BC1-22/5** | 2.3391 | 23.7094 |
| 2 | **BC1-22/1** | 11.83 | 28.90 | **BC1-22/7** | 0.0073 | 0.6740 | **BC1-22/6** | 2.3391 | 23.7094 |
| 3 | **BC1-22/2** | 11.14 | 28.21 | **BC1-22/3** | 0.0139 | 0.6806 | **BC1-22/7** | 2.2386 | 23.6090 |
| 4 | **BC1-22/3** | 10.80 | 27.87 | **BC1-22/2** | 0.0198 | 0.6865 | **BC1-22/8** | 2.1884 | 23.5587 |
| 5 | **BC1-22/7** | 10.29 | 27.37 | **BC1-22/1** | 0.0263 | 0.6929 | **BC1-22/1** | 2.0843 | 23.4547 |
| 6 | **BC1-113/3** | 9.72 | 26.80 | **BC1-113/3** | 0.0332 | 0.6999 | **BC1-22/2** | 2.0149 | 23.3853 |
| 7 | **BC1-22/8** | 9.24 | 26.31 | **BC1-113/8** | 0.0388 | 0.7055 | **BC1-22/3** | 1.9654 | 23.3357 |
| 8 | **BC1-113/7** | 8.71 | 25.78 | **BC1-113/7** | 0.0441 | 0.7108 | **BC1-22/4** | 1.8329 | 23.2033 |
| 9 | **BC1-113/8** | 8.29 | 25.37 | **BC1-22/5** | 0.0500 | 0.7166 | **BC1-22/9** | 1.6841 | 23.0545 |
| 10 | **BC1-22/4** | 7.77 | 24.84 | **BC1-22/4** | 0.0555 | 0.7222 | **BC1-28/6** | 1.5598 | 22.9301 |

*Note*. SP: survival period; NDP: Number of dead plants and PILA 100%: Period of inoculation until the lesion reaches 100% of the circumference.

Table 2. Continued

| Ord. | Genotypes | AULAEC | | Genotypes | AULLEC | | Genotypes | AULWEC | |
|---|---|---|---|---|---|---|---|---|---|
| | | Gain (%) | New media | | Gain (%) | New media | | Gain (%) | New media |
| 1 | **BC1-28/4** | 0.0000 | 44.6868 | **BC1-28/4** | 0.0000 | 6.9660 | **BC1-28/4** | 0.0000 | 3.6209 |
| 2 | **BC1-28/5** | 0.0165 | 44.7034 | **BC1-28/7** | 0.0406 | 7.0066 | **BC1-28/1** | 0.0076 | 3.6286 |
| 3 | **BC1-28/8** | 0.0327 | 44.7196 | **BC1-28/3** | 0.0802 | 7.0462 | **BC1-28/3** | 0.0146 | 3.6355 |
| 4 | **BC1-28/1** | 0.0478 | 44.7346 | **BC1-28/1** | 0.1221 | 7.0881 | **BC1-28/5** | 0.0222 | 3.6431 |
| 5 | **BC1-28/3** | 0.0641 | 44.7509 | **BC1-28/5** | 0.1676 | 7.1336 | **BC1-28/7** | 0.0298 | 3.6507 |
| 6 | **BC1-28/6** | 0.0819 | 44.7687 | **BC1-28/8** | 0.2152 | 7.1811 | **BC1-28/2** | 0.0380 | 3.6589 |
| 7 | **BC1-28/2** | 0.1006 | 44.7874 | **BC1-28/2** | 0.2578 | 7.2237 | **BC1-28/6** | 0.0458 | 3.6667 |
| 8 | **BC1-28/7** | 0.1204 | 44.8072 | **BC1-28/6** | 0.3033 | 7.2693 | **BC1-28/9** | 0.0544 | 3.6753 |
| 9 | **BC1-28/9** | 0.1413 | 44.8281 | **BC1-28/9** | 0.3503 | 7.3163 | **BC1-28/8** | 0.0627 | 3.6836 |
| 10 | **BC1-22/5** | 0.1600 | 44.8468 | **BC1-113/4** | 0.3874 | 7.3534 | **BC1-22/5** | 0.0719 | 3.6929 |

*Note*. AULAEC: area below the lesion area expansion curve; AULLEC: area below the lesion length expansion curve and AULWEC: area below the lesion width expansion curve.

In the selection of genotypes by the BLUP procedure, it was observed that the genetic gains obtained for the SP variable ranged from 13.9 to 7.77%, with the ten selected genotypes being: BC1-22/6, BC1-22/1, BC1-22/2, BC1-22/3, BC1-22/7, BC1-22/8, BC1-22/4, BC1-113/3, BC1-113/7 and BC1-113/8. A similar result was found for the NDP variable, where most of the genotypes selected for the SP characteristic were also selected for the NDP characteristic, with the exception of the BC1-22/8 genotype, which in its place was selected the BC1-22/5 genotype. The genetic gain values for this variable were from 0.00 to 0.0555%.

For the variable PILA 100%, the selected genotypes were similar to those found for the variables SP and NDP, however, in addition to the genotypes BC1-22/5, BC1-22/6, BC1-22/7, BC1-22/8, BC1-22/1, BC1-22/2, BC1-22/3 and BC1-22/4, which have already been selected, in this variable the genotypes BC1-22/9 and BC1-28/6 were also selected. The genetic gains ranged from 2.33 to 1.55%.

However, for the variables related to the area under the lesion area expansion curve, lesion length and lesion width (AULAEC, AULLEC and AULWEC), the best ranked genotypes were BC1-28/1, BC1-28/2, BC1-28/3, BC1-28/4, BC1-28/5, BC1-28/6, BC1-28/7 and BC1-28/9. These variables are directly related to the CC and WL variables, and these genotypes only showed low values of CC and WL because they also presented low values of SP, for example, the genotypes BC1-28/1, BC1-28/3 and BC1 -28/7, had only five days of survival (SP), therefore, even with a low value of CC and WL, they were unable to survive the damage caused by the fungus *F. solani*, therefore, they are not selected to continue the program of improvement.

Thus, the characteristics SP, NDP and PILA 100% are the ones that best distinguish resistant genotypes, therefore, only the genotypes selected based on these variables, in this study, will be part of the advancement of the breeding program aiming resistance to rot the lap.

By the BLUP procedure, in general, most of the selected genotypes come from family 22, indicating that the parent involved in this crossing is efficient in transferring the resistance gene to the offspring. However, it was possible to select genotypes from all families. Thus, the importance of carrying out individual selection, since there is a very large variation within families at this stage of the program.

## 4. Conclusion

The variables that showed the highest heritability values were the survival period and the area under the lesion length expansion curve.

The three families of backcrosses presented genotypes resistant to the fungus *F. solani*, however, by the methodology of mixed models REML/BLUP, only the genotypes BC1-22/1, BC1-22/2, BC1-22/3, BC1-22/4, BC1-22/6, BC1-22/7, BC1-113/3, BC1-113/7 and BC1-113/8, were selected to advance the generation of the UNEMAT passion fruit breeding program, therefore, they presented among the ten placed, mainly for the variable survival period (SP).

## References

Alves, F. M., & Resende, M. D. V. (2008). Avaliação genética de indivíduos e progênies de cupuaçuzeiro no estado do Pará e estimativas de parâmetros genéticos. *Revista Brasileira de Fruticultura, 30*, 696-701. https://doi.org/10.1590/S0100-29452008000300023

Bernardo, R. (2002). Genotype × environment interaction. In R. Bernardo (Ed.), *Breeding for quantitative traits in plants* (pp. 147-171). Stemma Press, Woodbury.

Cervi, A. C. (1997). Passifloraceae do Brasil: Estudo do gênero *Passiflora* L., subgênero Passiflora. *Fontqueria, 45*, 1-92.

Cruz, C. D. (2016). Genes Software—Extended and integrated with the R, Matlab and Selegen. *Acta Scientiarum, 38*(4), 547-552. https://doi.org/10.4025/actasciagron.v38i3.32629

Cruz, C. D., & Carneiro, P. C. S. (2006). *Biometric models applied to genetic improvement* (2nd ed.). Imprensa Universitária, Viçosa.

Dhawan, K., Dhawan, S., & Sharma, A. (2004). Passiflora: A review update. *J. Ethnopharmacol., 94*, 1-23. https://doi.org/10.1016/j.jep.2004.02.023

Fischer, I. H., Lourenco, S. A., Martins, M. C., Kimati, H., & Amorim, L. (2005). Seleção de plantas resistentes e de fungicidas para o controle da podridão do colo do maracujazeiro causada por *Nectria haematococca*. *Fitopatologia Brasileira, 30*, 250-258. https://doi.org/10.1590/S0100-41582005000300006

Fischer, I. H., & Resende, J. A. M. (2016). Doenças do maracujazeiro. In L. Amorim, J. A. M. Rezende, A. Bergamin Filho & L. E. A. Camargo (Eds.), *Manual de Fitopatologia: Doenças das plantas Cultivadas* (Vol. 5, pp. 535-544). São Paulo: Agronômica Ceres.

Freitas, I. L. D. J., Amaral Junior, A. T., Viana, A. P., Pena, G. F., Cabral, P. S., Vittorazzi, C., & Silva, T. R. D. C. (2013). Genetic gain evaluated with selection indices and with REML/Blup in popcorn. *Pesq. Agrop. Bras., 48*, 1464-1471. https://doi.org/10.1590/S0100-204X2013001100007

Freitas, J. C. O., Viana, A. P., Santos, E. A., Paiva, C. L., Rodrigues, R., Souza, M. M., & Eiras, M. (2015). Genetic basis of the resistance of a passion fruit segregant population to Cowpea Aphid-Borne Mosaic Virus (CABMV). *Tropical Plant Pathology, 40*, 291-297. https://doi.org/10.1007/s40858-015-0048-2

IBGE. (2020). *Diretoria de Pesquisa, Coordenação de Agropecuária, Produção Agrícola Municipal 2019*. Retrieved January 29, 2020, from https://sidra.ibge.gov.br/tabela/5457#resultado

Lopes, S. C. (1991). Citogenética do maracujá, *Passiflora* spp. In A. R. São José (Eds.), *A cultura do maracujá no Brasil* (pp. 201-209). Jaboticabal: FUNEP.

Marostega, T. N., Lara, L. P., Oliveira, D. S., Chimello, A. M., Gilio, T. A. S., Preisigke, S. C., … Neves, L. G. (2019). Molecular and Aggressiveness Characterization of Isolates of *Fusarium solani* and *Fusarium oxysporum* f.sp. *passiflorae* Associated to Passion Fruit Wilting. *Journal of Agricultural Science, 11*, 01-14. https://doi.org/10.5539/jas.v11n3p407

Marostega, T. N., Preisigke, S. C., Chimello, A. M., Gilio, T. A. S., Araujo, K. L., Barelli, M. A. A., & Neves, L. G. (2020). Genetic resistance to fusariosis and base rot in interspecific F1 progenies of passion fruit (*Passiflora* spp.). *Research, Society and Development, 9*, 1-25. https://doi.org/10.33448/rsd-v9i11.10519

Meletti, L. M., & Maia, M. L. (1999). Maracujá: Produção e comercialização. *Boletim Técnico 181* (pp. 1-64). IAC: Campinas.

Neves, L. G., Bruckner, C. H., Picanço, M. C., Sobrinho, S. P., Araújo, K. L., Luz, P. B., … Krause, W. (2013). Genetic correlation between agronomically important traits in yellow passion fruit. *Amer J Plant Sci, 4*, 2112-2117. https://doi.org/10.4236/ajps.2013.411262

Neves, S. M. A. S., Nunes, M. C. M., & Neves, R. J. (2011). Caracterização das condições climáticas de Cáceres/MT Brasil, no período de 1971 a 2009: Subsídio às atividades agropecuárias e turísticas municipais. *Boletim Goiano Geográfico, 31*, 55-68. https://doi.org/10.5216/bgg.v31i2.16845

Preisigke, S. C., Martini, F. V., Rossi, A. A. B., Serafim, M. E., Barelli, M. A. A., Luz, P. B., … Neves, L. G. (2015). Genetic variability of *Passiflora* spp. against collar rot disease. *Aust. J. Crop. Sci., 9*, 69-74.

Preisigke, S. C., Silva, L. P., Serafim, M. E., Bruckner, C. H., Araújo, K. L., & Neves, L. G. (2017). Seleção precoce de espécies de *Passiflora* resistente a fusariose. *Summa Phytopathologica, 43*, 321-325. https://doi.org/10.1590/0100-5405/175390

Resende, M. D. V. (2002). *Genética biométrica e estatística no melhoramento de plantas perenes* (p. 975). Brasília: Embrapa Informação Tecnológica.

Resende, M. D. V. (2016). Software Selegen-REML/BLUP: A useful tool for plant breeding. *Crop Breed. Appl. Biotechnol., 16*, 330-339. https://doi.org/10.1590/1984-70332016v16n4a49

Resende, M. D. V., & Duarte, J. B. (2007). Precisão e controle de qualidade em experimentos de avaliação de cultivares. *Pesquisa Agropecuária Brasileira, 37*, 182-194.

Santos, E. A., Viana, A. P., Freitas, J. C. O., Rodrigues, D. L., Tavares, R. F., Paiva, C. L., & Souza, M. M. (2015). Genotype selection by REML/BLUP methodology in a segregating population from an interspecific *Passiflora* spp. crossing. *Euphytica, 204*, 1-11. https://doi.org/10.1007/s10681-015-1367-6

Silva, F. H. L., Viana, A. P., Santos, E. A., Freitas, J. C. O., Rodrigues, D. L., & Amaral Junior, A. T. (2017). Prediction of genetic gains by selection indexes and REML/BLUP methodology in a population of sour passion fruit under recurrent selection. *Acta Scientiarum Agronomy, 39*. https://doi.org/10.4025/actasciagron.v39i2.32554

Silva, F. H. L., Muñoz, P. R., Vincent, C. I., & Viana, A. P. (2016). Generating relevant information for breeding *Passiflora edulis*: Genetic parameters and population structure. *Euphytica, 208*, 609-619. https://doi.org/10.1007/s10681-015-1616-8

Viana, A. P., Pereira, T. N. S., Pereira, M. G., Amaral Júnior, A. T., Souza, M. M., & Maldonado, J. F. M. (2004). Genetic parameters in populations of yellow passion fruit. *Revista Ceres, 51*, 541-551. https://doi.org/10.1590/S1516-89132009000300002

Wijeratnam, S. W. (2016). Passion Fruit. *Encyclopedia of Food and Health, 23*, 231-235. https://doi.org/10.1016/B978-0-12-384947-2.00521-3

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# Homeopathy in the Rust Severity and Growth of *Malva sylvestris* L.

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**Abstract**

The production of medicinal plants which have an association with biotrophic fungi requires non-residual and favorable methods to the host with tolerance to the presence of phytopathogens. The objective of this work was to evaluate the effect of homeopathic preparations on the rust severity and the growth of *Malva sylvestris* plants. *M. sylvestris* seedlings were prepared in 600 ml containers with commercial substrate. The seedlings were arranged in pots at 26 days of age and outlined in two experiments. The treatments consisted of *Amonnium carbonicum* (Am. carb.), *Atropa belladonna* (Bell.), *Calcarea carbonica* (Calc. carb.), *Silicea terra* (Sil.) and *Sulfur* (Sulf.), all at 30CH (centesimal Hahnemannian dilution order). The last two dynamizations (29 and 30CH) were prepared in distilled water for all treatments. Control plants were treated with water. Natural inoculation of the plants with *Puccinia malvacearum* occurred in the first experiment, and the applications of homeopathic preparations were carried out every seven days for five weeks. Four evaluations of rust severity, diameter, height and number of leaves were conducted. Next, *M. sylvestris* seedlings were transplanted into pots with 5 liters of substrate in the second experiment and the growth curve of the plant was observed in relation to the diameter and height variables. Am. Carb. reduced 18.29% of the rust severity in relation to the control plants. Sil. 30CH contributed to an increase in stem diameter. There was no interference in the plants' height by homeopathic preparations. The application of homeopathies in *M. sylvestris* can contribute to their production, reducing the rust intensity considered in the crop cycle and can assist in the plant growth without leaving residues which can harm pollinators and hyperparasites.

**Keywords:** high dynamic dilutions, *Puccinia malvacearum*, homeopathic preparations

## 1. Introduction

*Malva* spp. species evolutionarily developed in close interaction with the *Puccinia malvacearum* Bertero ex Mont. biotrophic fungus. This relationship is demonstrated by the increase in the number of cells with mucilage in the area with pustules and the passage of *P. malvacearum* through these cells without hindrance or degeneration (Classen, Amelunxen, & Blaschek, 2001). The bioactivity of *Malva* spp. is related to mucilage production (Tomoda et al., 1989; Classen & Blaschek, 1998). According to Mitchell and Roberts (1973), polysaccharides such as arabitol and trehalose can be stored in leaves infected with *P. malvacearum* in *Althea rosea* at pustule sites and trehalose in teliospores, which shows the evolutionary advantages of the intimate host and pathogen relationship.

The supply of *M. sylvestris* leaves and mericarps on the market is restricted by rust symptoms so that they cannot have more than one brown teliospore web of the *P. malvacearum* fungus per $cm^2$ (Brasil, 2000). On the other hand, in accordance with IN No. 17/2014 of the Ministry of Agriculture and Livestock (MAPA) and the National Policy on medicinal and phytotherapic plants of the Ministry of Health, medicinal plants cannot be handled with pesticides (Brasil, 2014, 2016).

The risk of residues of active ingredients and heavy pesticide metals is related to the concentration of these ingredients in the plant's drying process and alteration of the chemical composition of the plants in processing and extracting the herbal medicine (Carvalho, Costa, & Carnelossi, 2010). In view of this problem, high dynamized dilutions, or homeopathic preparations, have been studied as an adequate measure in treating medicinal plants, since they do not leave chemical residues in fresh or processed products (Casali et al., 2010).

The choice of using homeopathies in agricultural production is provided for in the Organic Law, however, it is not exclusive to these systems (Brasil, 2014). Pulido et al. (2017) demonstrated an increase in broccoli (*Brassica oleraceae* L. var. itálica) biomass through the use of *Sulfur* 6CH, *Silicia terra* 6CH, *Carbo vegetalis* 30CH and *Sulfur* 30CH. Leonel and Barros (2013) reported using homeopathy to manage coffee rust (*Hemileia vastatrix*), in which they used four leaf sprays with a complex of basic pharmaceutical preparations (*Silicea* 6CH, *Sulfur* 6CH, and *Arsenicum album* 6CH) and isotherapic rust pustules (30CH) during a period of cultivation (December-may), with a positive effect on stains and defoliation. Homeopathic preparations of propolis, *Sulfur* and *Ferrum sulphuricum* applied to tomato plants were effective in reducing the intensity of the *Alternaria solani* phytoparasite and interfering with tomato (*Solanum lycopersicum* L.) (sin.: *Lycopersicon esculentum* Mill.) growth (Toledo, Stangarlim, & Bonato, 2015). The mode of action of homeopathies in plants is still being investigated, but there are studies where the activation of mechanisms of resistance of plants to diseases has been observed (Carneiro & Teixeira, 2018).

Keeping in mind that the application of high dynamized dilutions can alter pathological processes established in plants and/or prevent them, the objective of this work was to evaluate the effect of homeopathic preparations on the rust severity and the growth of *M. sylvestris* plants.

## 2. Method

### *2.1 Plant Location and Production*

The study was conducted in semi-controlled conditions (greenhouse) in a residential-urban area in the municipality of Caçador, SC, in the period 2018, with support from the Epagri-Lages Homeopathy and Plant Health Laboratory and the UNIARP-Caçador Phytopathology Laboratory.

Malva seedlings were produced from *Malva sylvestris* seeds introduced in the garden of the Municipality of Caçador. The seedlings were kept in containers of expanded polystyrene (EPS) pots with 600 mL of Agrinobre® TMX substrate. The plants were kept until the 26th day in a greenhouse with sprinkler irrigation for 5 minutes, three times a day. The plants were then divided from that date into two experiments: (a) evaluation of the Area Under the Disease Progress Curve (AUDPC) and growth of *M. sylvestris*; and (b) evaluation of the height and diameter growth curve estimate of *M. sylvestris*.

### *2.2 Exp. 1 Evaluation of the Area Under the Disease Progress Curve (AUDPC) and Growth of M. sylvestris*

The plants at 26 days remained in the EPS pots and in the greenhouse with sprinkler irrigation 10 minutes three times a day and were submitted to natural inoculation. The greenhouse was maintained with natural ventilation at the top so that the *P. malvacearum* spores from the *M. sylvestris* plants kept outside the greenhouse could be carried by the wind inwards to proceed with natural inoculation, being performed on a day with heavy rain and wind. The sprinkling time increased to 15 minutes and the number of irrigations was changed to four times a day for a period of 24 days. Rust symptoms appeared on the leaves one week after the natural inoculation, and all leaves were evaluated according to grades assigned as follows: grade 0 = 0%; grade 1 ≥ 0 < 5%; grade 2 ≥ 5 < 15%; grade 3 ≥ 15 < 25%; grade 4 ≥ 25%. The experimental design was a randomized block with five replications and a double-blind procedure, with the treatments for both the applicator of the preparations and the evaluator of the treatments being unknown. Each repetition consisted of one container.

The weekly treatments started one week after being considered a natural infection by applying the homeopathic preparations of *Amonnium carbonicum* (Am. Carb.), *Atropa belladonna* (Bell.), *Calcarea carbonica* (Calc. Carb.), *Silicea terra* (Sil.) and *Sulfur* (Sulf.) in the thirtieth centesimal Hahnemannian dilution (30CH). The choice of homeopathic preparations was made according to the characteristics of plant and rust symptoms, with analogy between the symptoms presented in the homeopathic matter medical. The symptoms used for repertorization were: eruptions, red; eruptions, pustules; thrist, large quantities, for; food and drinks, sugar desire; inflamation; light-desire for. The control was carried out with distilled water. The dynamizations were prepared according to FHB (Brasil, 2011a). The last two dilutions for preparing homeopathies for each potency/treatment were done in distilled water. The adopted homeopathic nomenclature, name and abbreviation were based on that described by Soares (2005). The treatments were applied once a week with 2±0.2 mL per plant, so that at least one drop touched each leaf and the rest was applied to the plant's stem for a period of five weeks. Evaluations were performed by measuring the growth in height and diameter by the difference between the first and last evaluation divided by the number of days. The evaluation of the area affected by rust of all leaves was performed weekly by the diagrammatic scale in the period of 4 weeks. Afterwards, the disease severity index per plant, McKinney index (McKinney, 1923), Equation 1 was calculated which was subsequently used to calculate AUDPC (Campbell & Madden, 1990), using four evaluations for the construction of the curve.

$$ID = 100 \times \sum_{i=1}^{k} \frac{y_i \cdot f_i}{n \cdot y_k} \quad (1)$$

where, $y_i$ represents the degree of the scale, f represents the number of plants with infection level $y_i$, n represents the number of plants evaluated, and $y_k$ represents the maximum degree of infection.

*2.3 Exp. 2 Evaluation of the Height and Diameter Growth Curve Estimate of M. sylvestris*

Seedlings at 26 days were standardized and transplanted to pots with 5 liters of Agrinobre® TMX substrate, kept in a shelter with a white screen and drip irrigated four times a day for five minutes. After 13 days, the plants received the same homeopathic treatments described above for a total of six applications. Plants were evaluated for diameter and height growth variables at 7, 14, 21, 28, 49 and 56 days after treatments. The experimental design was a randomized block with five replications and double blind, with the repetition being formed by a vase.

*2.4 Statistical Analyses*

Data analyses of the diameter, height and AUDPC variables of the treatments were performed using the classic analysis of variance, with the presupposition of normality being verified through the Shapiro-Wilk test and the homoscedasticity through the Bartlett test. Tukey's contrasts and the procedure for testing linear hypotheses described by Hothorn, Bretz, and Westfall (2008) were used to compare treatment levels.

The number of leaves variable was analyzed using the Poisson model with a superdispersion parameter (quasipoisson) as it is counting, and the deviance analysis was performed using the F test.

The growth curve for the height and diameter variables of the *M. sylvestris* treated with Am. Carb., Bell., Calc. carb., Sil., and Sulf. homeopathic preparations and control were adjusted by linear and logistic models, considering the treatments and the different evaluation dates (days) as descriptive variables. The Akaike Information Criterion (AIC) was used for selection among them. The comparison between treatments was performed using the confidence intervals of the parameters of the selected model.

All analyzes were performed using the R software program (R Core Team, 2017) considering 5% significance.

## 3. Results and Discussion

The use of Am. Carb in the 30CH provided a reduction of 18.29% in the AUDPC of rust compared to the control (Table 1).

Table 1. Area Under Disease Progress Curve and *Malva sylvestris* growth treated with homeopathic preparations, Caçador-SC, Brazil, 2018

| Treatments | AUDPC | Diameter (mm $d^{-1}$) | Height (cm $d^{-1}$) | Leaves (nº.) |
|---|---|---|---|---|
| *Sulfur* 30CH | 871.50±25.69 c | 0.10±0.02 $^{ns}$ | 0.52±0.04 $^{ns}$ | 4.8±0.66 $^{ns}$ |
| *Calcarea carbonica* 30CH | 805.00±23.49 bc | 0.08±0.02 | 0.46±0.03 | 4.6±0.25 |
| Control | 818.13±11.42 bc | 0.09±0.01 | 0.48±0.05 | 4.6±0.24 |
| *Silicea* 30CH | 780.50±33.28 b | 0.09±0.02 | 0.46±0.06 | 4.2±0.37 |
| *Atropa Belladonna* 30CH | 759.50±14.06 b | 0.10±0.02 | 0.54±0.03 | 4.6±0.25 |
| *Amonnium carbonicum* 30CH | 668.50±14.00 a | 0.09±0.01 | 0.44±0.03 | 4.2±0.37 |

*Note.* AUDPC: Area Under Disease Progress Curve; 30CH: centesimal Hahnemannian dilution order; Averages followed by the same letter in the column do not differ significantly from each other, considering the Tukey test ($p \leq 0.05$); ns: there was no significant effect of the treatments ($p \leq 0.05$); Control: water; data from four evaluations, 28-day period.

Am. carb is associated with rust symptoms on the leaves, as in the case of Bell., by analogy to the presence of dark red spore mass. According to Tyler (1992), Calc. carb. is Bell's chronic state and may also present weakness, late development and worsens in cold and wet weather (Tyler, 1992; Vannier & Poirier, 1987). By analogy to medical matter, Am. Carb. is indicated in cases of weakness when skin rashes worsen due to wet and rainy weather. It is a suitable remedy for the female organism, fainting, vertigo and considered antipsoric by Hahnemann (Hahnemann, 2018; Tyler, 1992). Therefore, there was a certain similarity correspondence in *M. sylvestris* with the difficulty of the plant to remain upright many times, marked weakness after flowering and fruiting, with signs of *P. malvacearum* on the leaves resembling eruptions, with humid and rainy weather favoring infection, and showy flowers with feminine characteristics.

Plants treated with Sulfur showed higher mean AUDPC values when compared to the average values obtained for Am. Carb., Sil. and Bel., but did not differ from the values observed for Calc. carb and the control. The rust

AUDPC was 23.82% lower when using Am. Carb than when using Sulfur. The responses in the body in relation to applying homeopathies are dependent on the vital force of individuals and the similarity between the symptoms generated by homeopathy and those presented by the individual (Hahnemann, 2013). There is a report on the efficiency of Sil 30CH application in grape buds for rust control, observing the occurrence of 7% severity in the treated ones, while there was 100% severity in the buds in the water control treatment (Souza, Collet, & Bonato, 2005). The use of Sil. 12CH in high humidity conditions favorable to chrysanthemum mortality provided 100% plant survival, while 82% of them withered and died in the control treatment (Singhania & Singhania, 2014).

*M. sylvestris* growth in experiment 2 was affected by the homeopathic application. Table 2 shows that the confidence interval asymptote of the logistic model for the diameter of plants treated with Sil. was from 14.77 mm to 15.94 mm, and differs from the range of control plants which are in 13.18 mm to 14.65 mm. The Sulf., Calc-carb., Am. Carb. and Bel. homeopathies did not differ from the control, as the confidence intervals for the parameters overlap (Table 2).

Table 2. Estimates of the confidence intervals of the logistic model parameters adjusted according to homeopathy treatments for the diameter variable. Caçador, SC, Brazil, 2018

| Treatments | Asymptote | | x median | | Scale | |
|---|---|---|---|---|---|---|
| | Limit (mm) | | Limit (cm) | | Limit (cm) | |
| | Lower | Upper | Lower | Upper | Lower | Upper |
| *Sulfur* 30CH | 14.30 | 16.19 | 5.71 | 10.70 | 6.42 | 13.36 |
| *Calcarea carbonica* 30CH | 14.09 | 15.10 | 6.24 | 8.96 | 6.19 | 9.63 |
| Control | 13.18 | 14.65 | 5.67 | 9.92 | 5.79 | 11.30 |
| *Silicea* 30CH | 14.77 | 15.94 | 5.56 | 8.83 | 7.36 | 11.79 |
| *Atropa belladonna* 30CH | 14.44 | 15.62 | 6.95 | 9.97 | 6.59 | 10.50 |
| *Amonnium carbonicum* 30CH | 13.92 | 15.41 | 6.50 | 10.35 | 5.69 | 10.59 |

It can be seen in Figure 1 that the estimate of the Sil diameter curve is superior to that of the control plants. The diameter of the other treatments (Sulf., Calc. Carb., Bell. And Am. Carb.) is represented in the intermediate curve between the control and Sil., but there is no difference between them and the control.

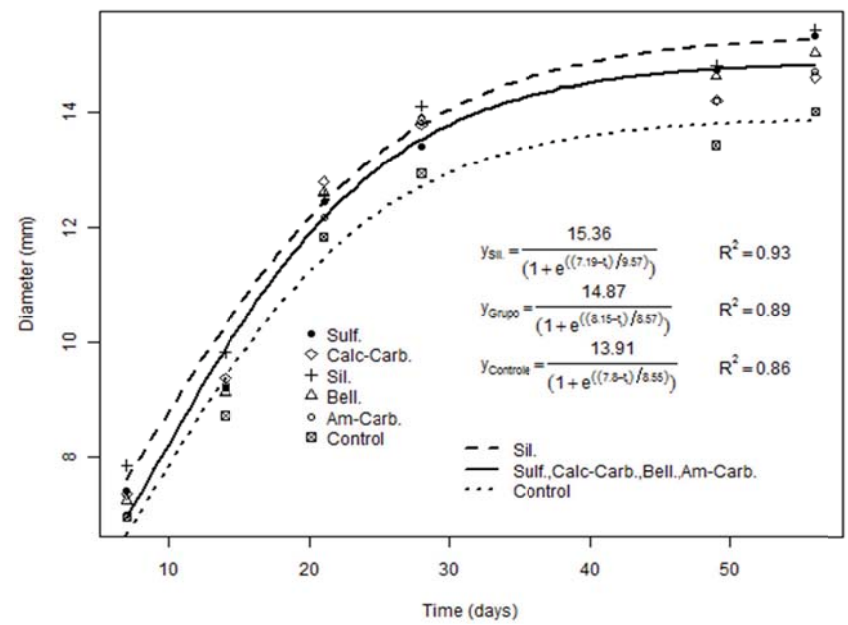


Figure 1. Estimates logistic model according to homeopathy treatments for the diameter variable. Caçador, SC, Brazil, 2018

Signs of weakness are reported in Sil's medical material, which is not due to deficient nutrition, but to deficient assimilation, which is influenced by the moon, worsening in the new and full moon (Tyler, 1992). The better assimilation of nutrients and water capture may explain the increase in diameter observed when Sil was applied. The influence of treatments with homeopathic preparations on plant growth and protection has been reported by Swarowsky et al. (2014) using Cina in managing *Meloidogyne incognita*, without nematostatic and nematicidal effect. In the case of *M. sylvestris* and *P. malvacearum* association, the best assimilation of nutrients can assist in mucilage production and plant survival.

The homeopathic applications did not affect plant height. In this case, there was an overlapping of the intervals of all the logistic model parameters between treatments (Table 3).

Table 3. Estimates of the confidence intervals of the logistic model of homeopathy treatments for the height parameter. Caçador, SC, Brazil, 2018

| Treatments | Asymptote | | x median | | Scale | |
|---|---|---|---|---|---|---|
| | Limit (mm) | | Limit (cm) | | Limit (cm) | |
| | Lower | Upper | Lower | Upper | Lower | Upper |
| *Sulfur* 30CH | 14.30 | 16.19 | 5.71 | 10.70 | 6.42 | 13.36 |
| *Calcarea carbonica* 30CH | 14.09 | 15.10 | 6.24 | 8.96 | 6.19 | 9.63 |
| Control | 13.18 | 14.65 | 5.67 | 9.92 | 5.79 | 11.30 |
| *Silicea* 30CH | 14.77 | 15.94 | 5.56 | 8.83 | 7.36 | 11.79 |
| *Atropa belladonna* 30CH | 14.44 | 15.62 | 6.95 | 9.97 | 6.59 | 10.50 |
| *Amonnium carbonicum* 30CH | 13.92 | 15.41 | 6.50 | 10.35 | 5.69 | 10.59 |

Figure 2 shows the general growth curve for the height of *M. sylvestris*. Excessive height growth of *M. sylvestris* can favor the plant tipping. The plants were at the beginning of flowering and slowing the growth rate at 56 days after the first treatment, corresponding to 95 days since sowing.

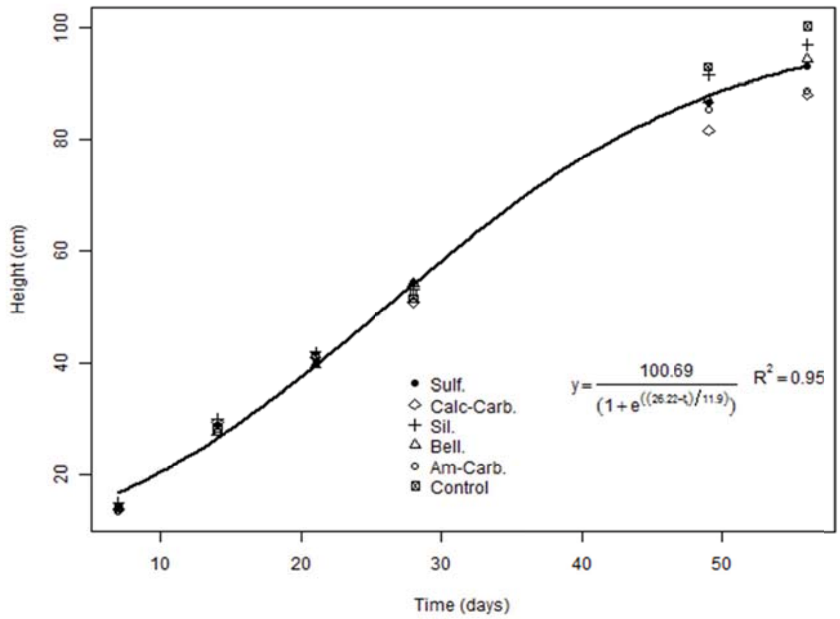


Figure 2. Estimation of the cumulative height growth curve from the data means adjusted to the logistic model

## 4. Conclusion

It was observed that Ammonium carbonic was effective in reducing 18.29% of the rust severity expressed by the AUDPC in relation to the control with water. In comparing Am. Carb. with the other tested homeopathic treatments, it was observed that the AUDPC was 23.82% lower than that of Sulf. and differed from the others. *M.*

*sylvestris* growth was affected by the use of Sil. 30CH due to the increase in diameter compared to the control with water.

Am. Carb 30CH is recommended for managing the *Malva sylvestris* and *Puccinia malvacearum* interaction. Considering that the plant's resistance is overcome as the fungus develops specialized structures in the host forming a matrix of nutrient exchange, the use of Sil. 30CH can assist in assimilating nutrients and favor plant growth.

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**References**

Brasil, Ministério da Agricultura, Pecuária e do Abastecimento. (2014). Altera Instrução Normativa n° 46, de 6 de outubro de 2011 que estabelece o regulamento técnico para os sistemas orgânicos de produção animal e vegetal, bem como as listas de substâncias permitidas para uso nos sistemas orgânicos de produção animal e vegetal (Instrução Normativa n° 17, de 18 de Junho de 2014). *Diário Oficial [da] República Federativa do Brasil* (Section 1, pp. 32-36). Poder Executivo, Brasília, DF.

Brasil, Ministério da Saúde. (2016). *Política e Programa Nacional de Plantas Medicinais e Fitoterápicos*. Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica, Brasília.

Brasil. (2000). *Farmacopeia Brasileira* (4th ed.). São Paulo, SP: Atheneu Editora.

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. New York, NY: Wiley-Interscience Publication.

Carneiro, S. M. T. P. G., & Teixeira, M. Z. (2018). Homeopatia e controle de doenças de plantas e seus patógenos. *Scientia Agraria, 17*(3), 250-262. Retrieved from http://e-revista.unioeste.br/index.php/scientiaagraria/article/view/17994/13430

Carvalho, L. M., Costa, J. A. M. da, & Carnelossi, A. G. (2010). *Qualidade em plantas medicinais*. Embrapa Tabuleiros Costeiros, Aracajú, SE. Retrieved from http://www.cpatc.embrapa.br/publicacoes_2010/doc_162.pdf

Casali, V. W. D., Castro, D. M., Armond, C., Fonseca, M. C. M., Andrade, F. M. C. de, Duarte, E. S. M., & Arruda, V. M. (2010). Benefícios da homeopatia no cultivo de plantas medicinais. *Informe Agropecuário, Belo Horizonte, 31*(255), 79-84. Retrieved from http://www.epamig.br/download/informe-agropecuario-255-plantas-medicinais-e-aromaticas-2010

Classen, B., & Blaschek, W. (1998). High molecular weight acidic Polysaccharides from *Malva sylvestris* and *Alcea rosea*. *Planta Medica, 64*, 640-644. https://doi.org/10.1055/s-2006-957538

Classen, B., Amelunxen, F., & Blaschek, W. (2001). Ultrastructural observations on the rust fungus *Puccinia malvacearum* in *Malva sylvestris* ssp. Mauritiana. *Plant Biology, 3*, 437-442. https://doi.org/10.1055/s-2001-16456

Hahnemann, S. (2013). *Exposição da Doutrina Homeopática, ou, Organon da Arte de Curar* (5th ed.). São Paulo, SP: GEHSP "Benoit Mure".

Hahnemann, S. (2018). *Doenças crônicas: Matéria medicas dos medicamentos antipsóricos*. Tradução Tarcísio Freitas Bazílio. Retrieved from https://bentomure.com.br/_files/200000094-e4bbce5b68/medicamentos_antipsoricos.pdf

Leonel, A. H., & Barros, B. H. R. (2013). Utilização de preparados homeopáticos para controle da ferrugem do café (*Hemileia vastatrix*) na região da Alta Mogiana. *Caderno de Agroecologia*. Congresso Brasileiro de Agroecologia, 8, Porto Alegre, Brasil.

McKinney H. H. (1923). Influence of soil, temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *Journal of Agricultural Research, 26*, 195-217.

Mitchell, D. T., & Roberts, S. M. (1973). Carbohydrate composition of tissues infected by the autoecious rusts, *Puccinia pelargonii-zonalis* and *P. malvacearum*. *Physyological Plant Pathology, 3*, 481-488. https://doi.org/10.1016/0048-4059(73)90058-1

Pulido, E., Boff, P., Duarte, T., & Boff, M. I. (2017). High dilution preparations for organic production system of broccoli. *Agronomía Colombiana, 35*, 53-58. https://doi.org/10.15446/agron.colomb.v35n1.58586

R Core Team. (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.R-project.org

Singhania, P. K., & Singhania, A. (2014). Homoepathy in agriculture. In G. Rahmann & U. Aksoy (Eds.), *Proceedings of the 4th ISOFAR Scientific Conference*. Building Organic Bridges at the Organic World Congress, 4, Istanbul, Turkey. Retrieved from https://orgprints.org/23727/1/23727_MM.pdf

Souza, A. F., Collet, M. A., & Bonato, C. M. (2005). Efeito de soluções homeopáticas no controle da ferrugem (*Phakopsora euvitis*) em Videira. *Arquivos da Apadec, 9*(2), 27-30. Retrieved from http://periodicos.uem.br/ojs/index.php/ArqMudi/article/view/20684

Swarowsky, R. A., Kunh, O. J., & Estevez, R. L. (2014). Influence of high dilutions of cina for the control of *Meloidogyne incognita* in tomato plants. *American Journal of Plant Sciences, 5*, 3695-3701. https://doi.org/10.4236/ajps.2014.525385

Toledo, M. V., Stangarlin, J. R., & Bonato, C. M. (2015). Controle da pinta preta e efeito sobre variáveis de crescimento em tomateiro por preparados homeopáticos. *Summa Phytopathologica, 41*, 126-132. https://doi.org/10.1590/0100-5405/1944

Tomoda, M., Gonda R., Shimizu, N., & Yamada, H. (1989). Plant. Mucilages. XLII. An anti-complentary mucilage from leaves of *Malva sylvestris* var. mauritiana. *Chemical and Pharmaceutical Bulletin, 37*, 3029-3032. https://doi.org/10.1248/cpb.37.3029

Tyler, M. L. (1992). *Retratos de medicamentos homeopáticos.* São Paulo, SP: Editora Santos.

Vannier, L., & Poirier, J. (1987). *Tratado de matéria médica homeopática* (9th ed.). São Paulo, SP: Andrei.

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# A Preliminary Study of Nutritional Quality of Five Drought Tolerant Millets

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**Abstract**

Seeds from greenhouse-grown plants of five food millet crops—[barnyard millet (*Echinochloa frumentacea* Link.), finger millet (*Eleusine coracana* Gaertn.), kodo millet (*Paspalum scrobiculatum* L.), little millet (*Panicum sumatrense* Roth ex Roem. & Schult.), and proso millet (*Panicum miliaceum* L.)] contained 10.4, 5.5, 7.2, 1.3, and 17.1 percent protein; 69, 29, 239, 105, and 32 Fe (mg/100 g); and 33, 22, 23, 31, and 37 (mg/100 g) Zn, respectively. Concentrations (g/100 g) of oil in seeds varied from 1.32 for finger millet to 3.58 for little millet. The oil concentrations for barnyard, kodo, and proso millets were 1.59, 1.64, and 3.36 g/100 g, respectively. Predominant fatty acid in the oil in the seed of these millets was C18:2 followed by C18:1, and C16:0. Concentration of omega-3 fatty acid (a heart-healthy oil) was 1.06, 0.62, 1.01, 0.91, and 3.11 g/100 g in barnyard, kodo, little, proso, and finger millet, respectively. Oils from seeds of these millet crops were essentially free from concentration of anti-nutritive fatty acid C22:1 (Erucic acid). Concentrations (g/100 g) of total sugars varied from 0.96 for barnyard millet to 2.09 for finger millet. The total sugar concentrations for kodo, little, and proso millets were 1.81, 1.95, and 1.99 g/100 g, respectively. Fructose, glucose, sucrose, maltose, raffinose, and stachyose concentrations varied from 0.05 to 0.47, 0.44 to 0.85, 0.44 to 0.98, 0.02 to 0.33, 0.005 to 0.083, and 0.002 to 0.053, respectively for barnyard, kodo, little, proso, and finger millet, respectively. Results indicated that these millet crops have potential as sources of healthy food and it might be worthwhile to further study their production potential.

**Keywords:** barnyard millet, finger millet, kodo millet, little millet, proso millet, protein concentration, oil concentration, fatty acids, sugars, new crops

## 1. Introduction

Increasing world population and droughts are a concern for global food supplies. A report by United Nations (https://www.un.org/en/sections/issues-depth/population/index.html) states that “In 1950, five years after the founding of the United Nations, world population was estimated at around 2.6 billion people. It reached 5 billion in 1987and 6 billion in 1999. In October 2011, the global population was estimated to be 7 billion. The world’s population is expected to increase by 2 billion persons in the next 30 years, from 7.7 billion currently to 9.7 billion in 2050 and could peak at nearly 11 billion around 2100.

Given that drought stress may reduce more than 50% of the average crop yield and can further limit agriculture productivity, special attention must be given to agricultural research, extension services and development in order to attain the required agricultural yield and productive gains. To counter the effect of future food crises and drought stress, we have to design and grow crops that are rich in proteins, fibers and essential nutrients to overcome malnutrition and food-related diseases (Dubey et al., 2019).

To improve abiotic stress, particularly drought, tolerance of cereals is of extreme importance as cereals including wheat and barley are the main constituents of the world food supply (Sinclair, 2011). However, several minor crops, such as millets, are potential candidates for supplying nutritious food. A publication of the Millet Network of India—Deccan Development Society—FIAN, India (Anonymous, 2007) indicates that millets need very little water for their production. Compared to irrigated commodity crops, millets require just around 25% of the rainfall regime and grow well on skeletal soils that are less than 15 cm deep and can be a boom for dryland areas. Millets are known to have excellent nutrition content being three to five times nutritionally superior to the widely promoted rice and wheat in terms of proteins, minerals and vitamins. This publication lists barnyard millet, finger millet, kodo millet, little millet, and proso millet as "climate change compliant crops". Climate change portends less rain, more heat, reduced water availability and increased malnutrition.

The current study was conducted as a first step towards introduction/establishment of several millets in the mid-Atlantic region of the United States of America. The specific objective was to characterize nutritional quality of locally-grown barnyard millet, finger millet, kodo millet, little millet, and proso millet.

## 2. Materials and Methods

### *2.1 Plant Material*

We conducted a preliminary experiment by growing five millet crops (barnyard millet, kodo millet, little millet, proso millet, and finger millet) to characterize their growth especially their nutritional quality. Seeds for planting were purchased from a grocery store.

### *2.2 Production*

All five millets were grown in a greenhouse located on Randolph Farm of Virginia State University (37°15′N and 077°30.8′W) using a completely randomized design. Each millet (approximately 20-30 seeds per pot) was planted in two 30 cm tall plastic pots filled with growing material (Promix-BX, https://www.pthorticulture.com/en/products/pro-mix-bx/). The seeds were spread on top of the growing material and then covered lightly. All pots were placed in water trays and were bottom watered. These plantings were done on April 1, 2019. Greenhouse temperature was maintained from 75 to 90 °F. Upon germination, the pots were thinned to 2-3 plants per pot. Seeds were harvested as they natured starting about 120 days after planting.

### *2.3 Analysis of Seed Composition for Concentrations of Fatty Acids and Sugars*

Seeds from all five millets were used for nutritional quality analyses. Mineral concentrations, including nitrogen (N), in seed were determined according to AOAC methods (AOAC, 2016) by Waypoint Analytical Laboratory (Richmond, Virginia, USA). Total protein concentration was calculated by multiplying N content with protein factor 6.25.

The oil concentration was determined in the Common Laboratory of Agricultural Research Station of Virginia State University. The oil was extracted from ground millet seeds (5 g) three times at room temperature by homogenization for 2 min in 20 mL hexane/isopropanol (3:2, v/v) with a Biospec Model 985-370 Tissue Homogenizer (Biospec Products, Inc. Racine, WI, USA) and centrifuged at 4000 g for 5 min, as described by Hamama et al. (2003). The three extractions were combined and the hexane-lipid layer was separated from the combined extract after shaking with 10 mL of 1% solution of equal amounts of $CaCl_2$ and NaCl in 50% methanol. The hexane lipid layer was removed by aspiration and dried over anhydrous Na2SO4. The oil percentage (g/100 g dry basis) was determined gravimetrically after drying under vacuum at 40 °C and stored under nitrogen at -10 °C until analysis.

Sugars were extracted from ground seed samples of each millet (1 g) and analyzed by HPLC following the methods optimized by Johansen et al. (1996). Sugars in the extracts were identified by comparing their retention times with standard sugars. For quantification, trehalose was used as internal standard and the sugar concentration was expressed as g/100 g meal (Bhardwaj & Hamama, 2016).

### *2.4 Data Analysis*

All data were analyzed using version 9.1 of SAS (SAS Institute, Inc., 2014) using ANOVA with 5 % level of significance.

## 3. Results and Discussion

Our study is an attempt to address issues related to climate change and increasing droughts. Climate change, global warming, and related increase in droughts are already having comparable effects on the efficiency in food production as well as on its quality worldwide (FAO, 2019). The climate change and droughts are expected to significantly affect the duration and quality of the growing season and with increasing drought could cause damage to crops and food production (Gomez-Zavaglia et al., 2020). It has been suggested that mitigation of drought-related problems could be achieved via developing new agricultural practices, introducing other crops and animal varieties, and applying the principles of integrated pest management. Several strategies for crop and livestock have been devised to increase their resilience to extreme climate. Therefore, crops can be managed by breeding for drought and temperature tolerance and selecting appropriate cultivars (Sofi et al., 2019).

All five millets included in our study germinated and established a stand. The best germination and stand establishment was observed for finger millet (about 80 percent) whereas corresponding values for other four millets were about 30 to 50 percent. All five millets produced some seed—finger millet produced the most seed. Seeds were harvested from all millets starting about 120 days after planting. All seeds of each millet from all pots were bulked and used or chemical composition analyses. The experiment was successfully completed.

Table 1. Composition of seeds of barnyard, kodo, little, proso, and finger millet grown in greenhouse during 2019

| Trait | Barnyard | Kodo | Little | Proso | Finger |
|---|---|---|---|---|---|
| Protein (%) | 10.4 | 7.25 | 1.3 | 17.1 | 5.50 |
| Oil (%) | 1.59 | 1.64 | 3.58 | 3.36 | 1.32 |
| P (%) | 0.20 | 0.16 | 0.28 | 0.33 | 0.27 |
| K (%) | 0.21 | 0.16 | 0.22 | 0.29 | 0.58 |
| Ca (%) | 0.05 | 0.04 | 0.05 | 0.04 | 0.32 |
| Mg (%) | 0.11 | 0.08 | 0.18 | 0.17 | 0.18 |
| S (%) | 0.14 | 0.12 | 0.15 | 0.18 | 0.12 |
| Al (mg/100 g) | 9.00 | 41.0 | 12.0 | 8.00 | 9.00 |
| B (mg/100 g) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Cu (mg/100 g) | 7.00 | 11.0 | 8.00 | 11.0 | 9.00 |
| Fe (mg/100 g) | 69.0 | 239 | 105 | 32.0 | 29.0 |
| Mn (mg/100 g) | 23.0 | 15.0 | 14.0 | 15.0 | 349 |
| Na (mg/100 g) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Zn (mg/100 g) | 33.0 | 23.0 | 31.0 | 37.0 | 22.0 |
| *Saturated fatty acids (percentage of total fatty acids)* | | | | | |
| C14:0 | 0.08 | 0.08 | 0.04 | 0.03 | 0.06 |
| C15:0 | 0.04 | 0.02 | 0.02 | 0.02 | 0.07 |
| C16:0 | 17.40 | 16.95 | 15.63 | 7.53 | 23.46 |
| C18:0 | 4.68 | 3.20 | 5.52 | 1.85 | 2.25 |
| C20:0 | 0.89 | 1.19 | 0.95 | 0.81 | 0.37 |
| C22:0 | 0.26 | 0.46 | 0.24 | 0.57 | 0.20 |
| C24:0 | 0.04 | 0.05 | 0.01 | 0.01 | 0.01 |
| TS[1] | 23.4 | 21.9 | 22.4 | 10.8 | 26.4 |
| *Unsaturated fatty acids (percentage of total fatty acids)* | | | | | |
| C15:1 | 0.01 | 0.02 | 0.01 | 0.02 | 0.04 |
| C16:1 | 0.32 | 0.16 | 0.26 | 0.14 | 0.40 |
| C18:1 | 29.45 | 38.83 | 35.55 | 25.16 | 46.98 |
| C18:2 | 45.23 | 37.75 | 40.12 | 61.96 | 24.48 |
| C18:3 | 1.06 | 0.62 | 1.01 | 0.91 | 3.11 |
| C20:1 | 0.33 | 0.53 | 0.40 | 0.72 | 0.38 |
| C20:2 | 0.06 | 0.03 | 0.01 | 0.04 | 0.01 |
| C20:3 | 0.02 | 0.04 | 0.03 | 0.06 | 0.05 |
| C20:5 | 0.06 | 0.01 | 0.06 | 0.02 | 0.06 |
| C22:1 | 0.03 | 0.03 | 0.08 | 0.05 | 0.04 |
| C24:1 | 0.06 | 0.07 | 0.09 | 0.13 | 0.05 |
| TUS[1] | 76.6 | 78.1 | 77.6 | 89.2 | 75.6 |
| *Sugars (percentage based on dry weight basis)* | | | | | |
| Fructose | 0.048 | 0.04 | 0.473 | 0.131 | 0.347 |
| Glucose | 0.445 | 0.838 | 0.606 | 0.523 | 0.680 |
| Sucrose | 0.439 | 0.569 | 0.705 | 0.930 | 0.978 |
| Maltose | 0.024 | 0.176 | 0.113 | 0.329 | 0.042 |
| Raffinose | 0.005 | 0.083 | 0.002 | 0.050 | 0.026 |
| Stachyose | 0.002 | 0.047 | 0.053 | 0.027 | 0.021 |
| Total sugars | 0.962 | 1.806 | 1.951 | 1.991 | 2.095 |

*Note*. [1]: TS = total saturated fatty acids; TUS = total unsaturated fatty acids.

Composition of seeds of five millet crops under study are presented in Table 1. Seeds of proso millet contained most protein (17.1 percent) whereas seeds of little millet contained only 1.3 percent protein. Protein concentration in barnyard, kodo, and finger millet contained 10.4, 7.25, and 5.50 percent protein. Kodo millet seeds were observed to considerable concentrations of aluminum and iron (41.0 and 239 mg/100 g, respectively whereas proso millet seeds contained considerable concentration of zinc and finger millet contained considerable concentration of magnesium (349 mg/100 g). Seeds of all five millets contained negligible concentrations of sodium. Fe concentrations (mg/100 g) were 69, 29, 239, 105, and 32; and Zn concentrations (mg/100 g) were 33, 22, 23, 31, and 37, respectively for barnyard, finger, kodo, little, and proso millets.

Barnyard, kodo, little, and finger millet seeds from India have been reported to contain 7 to 13, 6 to 11, 8 to 11, 5 to 6 percent protein, respectively (Chandel et al., 2014). In comparison, protein concentrations from our study were, generally, similar to those from India except for little millet which only contained 1.3 percent protein. Similarly, Zn concentration in our study was comparable to those from India for these millets (36 to 40, 20 to 24, 30 to 33, and 21 to 28 mg/100 g). However, seeds of millets in our study contained considerable higher concentrations of Fe in comparison to those from India reported to be 20 to 45, 20 to 24, 30 to 33, and 21 to 28 mg/100 g, respectively (Chandel et al., 2014).

Seeds of all five millet crops contained some oil. The oil concentration varied from 1.32 (finger millet) to 3.58 (little millet) percent. Oil concentrations in seeds of barnyard, kodo, and proso millets were 1.59, 1.64, and 3.36 percent, respectively. Predominant saturated fatty acids in seeds of all five millets were C16:0, C18:0, C20:0, and C22:0 in descending order. Predominant unsaturated fatty acids in seeds of barnyard, little, and proso millet was C18:2 whereas that in kodo and finger millet seeds was C18:1. Concentration of heart-healthy Omega-3 fatty acid (percentage of total fatty acids) in seeds of five millets varied from 0.62 (kodo millet) to 3.11 (finger millet). Concentrations of this fatty acid in barnyard, little, and proso millets were 1.06, 1.01, and 0.91 percent, respectively. Duke and Ayensu (1985) reported that finger millet, generally, contains 1.5 percent fat in comparison to 1.32 percent in our study. Heuze and Tran (2015) reported the oil content in millet seeds as 1.9 percent in finger millet, 1.5 percent in kodo millet, 1.5 percent in little millet, 5.6 percent in proso millet, and 6 percent in barnyard millet.

Sugar concentration (percentage on dry weight basis) in seeds of varied from 0.96 (barnyard millet) to 2.09 (finger millet). Sugar concentrations in kodo, little, and proso millet seeds were 1.81, 1.95, and 1.99, respectively. Two predominant sugars in seeds of five millet crops in this study were glucose and sucrose. Glucose and sucrose concentrations in barnyard, kodo, little, proso, and finger millet seeds, respectively, were 0.44, 0.84, 0.61, 0.52, and 0.68; 0.44, 0.57, 0.70, 0.93, and 0.98. Heuze and Tran (2015) reported sugar concentrations in barnyard, kodo, little, an proso millet seeds as 1.9, 1.4, 1.4, and 1.9 percent, respectively. Concentrations in seeds of finger millet from our study were, generally, lower than those reported by Wankhede et al. (1979).

Our results are preliminary and would need to be substantiated and expanded to include other seed constituents. However, a preliminary review indicates that the five millet crops under study have potential to provide healthy food.

## 4. Conclusions

Results of this preliminary study indicate that barnyard, kodo, little, proso, and finger millets have potential as sources of healthy food. As these crops are previously well known as drought tolerant, it may be desirable to further study their production potential in the mid-Atlantic region of the United States of America. We are encouraged by the observation of Ugare et al. (2014) indicating that most millets are nutritionally superior to cereals and can yield food and forage in a short duration and at low inputs even under adverse climatic conditions.

## Disclaimer

This study was conducted as a student training project for the first two authors. Use of any trade names or vendors does not imply approval to the exclusion of other products or vendors that may also be suitable.

## References

Anonymous. (2007). *Millets: Future of Food and Farming*. Retrieved from https://milletindia.org/wp-content/uploads/2015/07/Milletsfutureoffoodandfarming.pdf

AOAC (Association of Official Analytical Chemists). (2016). *Official Methods of Analysis* (20th ed.). AOAC, Arlington, VA. Retrieved from http://www.aoac.org/aoac_prod_imis/AOAC/AOAC_Member/PUBSCF/OMACF/OMAP_M.aspx

Bhardwaj, H. L., & Hamama, A. A. (2016). Cultivar, planting date, and row spacing effects on Mungbean seed composition. *Journal of Agricultural Science, 8*, 26-32. https://doi.org/10.5539/jas.v8n10p26

Chandel, G., Meena, R. K., Dubey, M., & Kumar, M. (2014). Nutritional properties of minor millets: Neglected cereals with potentials to combat malnutrition. *Current Science, 107*, 1109-1111. Retrieved from https://www.jstor.org/stable/24105623

Dubey, A., Kumar, A., Abd Allah, E. F., Hashem, A., & Khan, M. L. (2019). Growing more with less: Breeding and developing drought resilient soybean to improve food security. *Ecological Indicators, 105*, 425-437 https://doi.org/10.1016/j.ecolind.2018.03.003

Duke, J. A., & Ayensu, E. S. (1985). *Medicinal Plants of China*. Reference Publications, Inc.

FAO (Food and Agriculture Organization of the United Nations). (2019). The state of food security and nutrition in the world. *Safeguarding against economic slowdowns and downturns*. FAO, Rome.

Gomez-Zavaglia, A., Mejuto, J. C., & Simal-Gandara, J. (2020). Mitigation of emerging implications of climate change on food production systems. *Food Research International, 134*, 109256. https://doi.org/10.1016/j.foodres.2020.109256

Hamama, A. A., Bhardwaj, H. L., & Starner, D. E. (2003). Genotype and growing location effects on phytosterols in canola oil. *Journal of AOCS, 80*, 1121-1126. https://doi.org/10.1007/s11746-003-0829-3

Heuzé, V., & Tran, G. (2015). *Finger millet (Eleusine coracana), grain*. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. Retrieved May 11, 2015, from https://feedipedia.org/node/721

Johansen, H. N., Gilts, V., & Knudsen, K. E. N. (1996). Influence of Extraction Solvent and Temperature on the Quantitative Determination of Oligosaccharides from Plant Materials by High-Performance Liquid Chromatography. *J. Agric. Food Chem., 44*, 1470-1474. https://doi.org/10.1021/jf950482b

SAS. (2014). *SAS for Windows Version 9.4*. SAS Institute, Cary, N.C. Retrieved from http://www.sas.com/en_us/software/sas9.html

Sinclair, T. R. (2011). Challenges in breeding for yield increase for drought. *Trends in Plant Science, 16*(6), 289-293. https://doi.org/10.1016/j.tplants.2011.02.008

Sofi, P. A., Ara, A., Gull, M., & Rehman, K. (2019). Canopy temperature depression as an effective physiological trait for drought screening. *Drought—Detection and Solutions*. Gabrijel Ondrasek, Intechopen. https://doi.org/10.5772/intechopen.85966

Ugare, R., Chimmad, B., Naik, R., Bharati, P., & Itagi, S. (2014). Glycemic index and significance of barnyard millet (*Echinochloa frumentacae*) in type II diabetics. *J Food Sci Technol, 51*(2), 392-395. https://doi.org/10.1007/s13197-011-0516-8

Wankhede, D.B., A. Shehnaj, and M.R. R. Rao. 1979. Carbohydrate composition of finger millet (*Eleusine coracana*) and foxtail millet (setaria italica). *Qualitas Plantarum, 28*, 293-303. https://doi.org/10.1007/BF01095511

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# Screening of African Yam Bean Accessions for Imbibition and Seed Physiological Quality

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**Abstract**

African yam bean is a nutritionally-important but neglected food crop with several health benefits. But its large scale cultivation and consumption are still limited by lack of systematic genetic improvement and breeding programme for the crop. Preliminary studies have established that the inherent field establishment potential of African yam bean is low. Hence the need to screen available accessions to identify those with outstanding seed physiological qualities for selection as parental materials for further improvement. Thirty-four African yam bean accessions were collected from different farmers in five states of Southeastern Nigeria. Seed physiological quality (viability and vigour) was assessed using hundred seed weight, water imbibition, standard germination, accelerated ageing and conductivity tests. The mean squares due to varietal effect were significant ($p < 0.05$) for germination index and highly significant ($p < 0.01$) for all other traits. Accessions ENAGag, ENAGmg 1 and, ENAGmg 2 had high standard (> 80%) and accelerated ageing (> 70%) germination percentages. Only 11 of the accessions had an accelerated ageing germination percentage of above 50%. All but one of the 34 accessions had conductivity values less than 5.00 $\mu Scm^{-1}$ $g^{-1}$. On the average, African yam bean seeds require up to 76.9% moisture of their initial weight over a period of approximately 53 hours prior for the commencement of germination process. Accessions ENAGag, ENAGmg 1, ENAGmg 2, ENNKob, ENNSog, IMISis, IMOKeo 1 and IMOKeo 3 were outstanding and are therefore potential candidates for genetic improvement of seed quality in African yam bean.

**Keywords:** accelerated aging, African yam bean, conductivity, germination, imbibition

## 1. Introduction

African yam bean [*Sphenostylis stenocarpa* (ex. A. Rich.) Harms] is an underutilized indigenous legume consumed for its nutritious grains and tubers (Potter & Doyle, 1992). It is cultivated throughout West Africa countries particularly, Cameroon, Cote d'Ivore, Ghana, Nigeria and Togo (Potter, 1992). It is a perennial climbing species whose morphotypes may be prostrate or erect and about 1-3 m in height. Its leaves are trifoliate, 2.7 to 13 cm long and 0.2 to 5.5 cm broad (Nnamani et al., 2017). The edible seeds have high amino acid content (lysine and methionine) which has been reported to be higher than those of pigeon pea, cowpea, and Bambara groundnut (Uguru & Madukaife, 2001). It is commonly cultivated by traditional farmers along the South West and South East regions of Nigeria (Nnamani et al., 2017). The propagation of African yam bean (AYB) is predominantly through seeds and despite its great nutritional potentials, the consumption and cultivation of the legume is still limited by lack of systematic genetic improvement studies on the crop. While 55% of farmers cultivating AYB in South East Nigeria reported poor availability of seeds, 60% reported poor seed quality as constraints to the extensive cultivation of African yam bean (Nnamani et al., 2017).

Seed viability and vigour constitute the physiological component of seed quality. Seed viability is the ability of the seed to germinate under optimal conditions, while seed vigor denotes inherent ability of seeds to emerge uniformly even under adverse environmental conditions in the field (Kandasamy et al., 2020). Low-vigor seeds emerge less uniformly than high-vigor seeds (Egli & Rucker, 2012), and delay in emergence affects the subsequent stages of growth such as the seedling's competitive ability for light, water, and nutrients. These irregularities in crop emergence and development within a population result in decreased yield (Finch-Savage, 1995). Higher plant biomass and yield have been attributed to seeds with high physiological potential (Höfs, Schoeman, & Vaissayrel, 2004; Egli & Rucker, 2012). The extent that reserved food in seeds supports seedling establishment is not fully understood, nor is the variation in different varieties and the sizes of their seeds within the same variety/hybrids, since the small and large seeds tend to have different amounts and compositions of nutritional reserves (Eggert & von Wirén, 2015). Venuto, Redfearn, Pitman, & Alison, (2002) showed that seed weight significantly influences germination, seedling vigor and seasonal yield performance of annual ryegrass cultivars.

Vigour tests are efficient in identifying less advanced stages of seed deterioration and they facilitate decision making in seed production enterprises, especially in establishing storage, commercialization, and quality control policies (Medeiros, Silva, Capobiango, Fialho, & Dias, 2019). The main objective of vigor tests is to identify possible differences in the physiological potential of seed lots which have similar germination percentages (Marcos-Filho, 2015). The use of tests based on different principles could be combined to present more reliable response with respect to the physiological potentials of the seed lots. Among vigor tests, accelerated ageing and electrical conductivity tests are considered the most sensitive tests for categorizing seed lots (Medeiros et al., 2019). The accelerated ageing test provides valuable information regarding storage and field emergence potentials of seedlings (Marcos-Filho, 2015), while the electrical conductivity test is useful for speedy assessment of the integrity of cellular membranes in seeds (Silva, Lopes, Marcos-Filho, & Vieira, 2012). It is known that degeneration of cell membranes is the first event in the seed deterioration process. The greater the conductivity value, the lower the seed germination and vigor, due to loss of cell membrane integrity (Medeiros et al., 2019). Therefore, the tests results reflect the physiological potential of seeds, and inferences can be made regarding the storage potential of diverse seed lots (Binotti et al., 2008).

The vigour of AYB seeds is known to be inherently low (Olisa, Ajayi, & Akande, 2010a). But this inference was based on a limited number of accessions. Hence this study with the aim to screen 34 available accessions of African yam bean for viability cum vigour characteristics with the view of identifying accessions with outstanding seed physiological qualities for selection in breeding as well as conservation programmes.

## 2. Materials and Methods

### *2.1 Seed Collection*

Thirty four African yam bean accessions were collected from different AYB stakeholders (farmers' gene bank and vendors) in five states of Southeastern Nigeria (Table 1). The study area was Southeastern Nigeria, comprising of Abia, Anambra, Ebonyi, Enugu and Imo States (Figure 1).

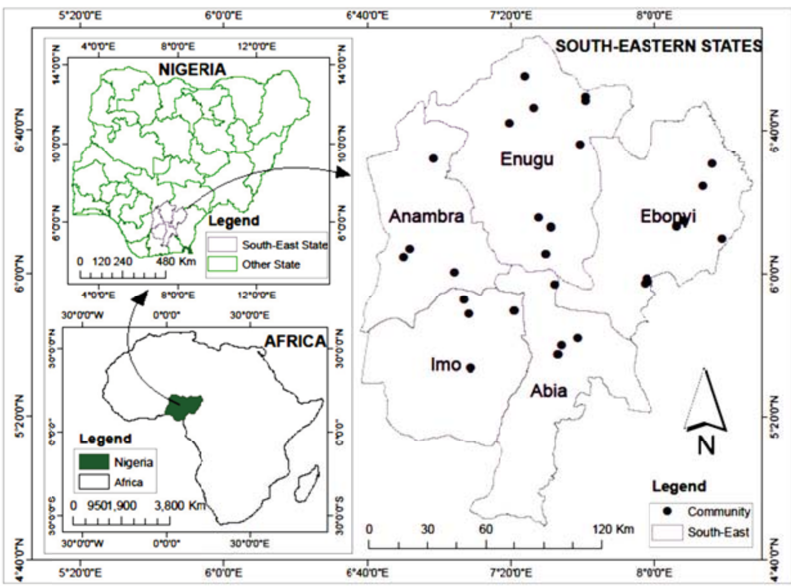


Figure 1. Southeastern states of Nigeria where samples of African yam bean (*Sphenostylis stenocarpa*) accessions were collected. Source (Nnamani et al., 2017)

Table 1. Details of the Africa yam bean accessions collected within the Southeastern part of Nigeria

| S/N | Sample Code | State | LGA | Community | Longitude | Latitude | Source |
|---|---|---|---|---|---|---|---|
| 1 | ABBEuz | ABIA | Bende | Ngwu Uzoakoli | 7°55.07′E | 5°62.02′N | Farmer |
| 2 | ABBEuz 1 | ABIA | Bende | Ngwu Uzoakoli | 7°55.23′E | 5°62.22′N | Farmer |
| 3 | ABUNum 2 | ABIA | Umuahia North | Ubani Makt | 7°56.78′E | 5°66.23′N | Vendor |
| 4 | ABBEng | ABIA | Bende | Ngwu | 7°55.08′E | 5°62.02′N | Farmer |
| 5 | ABBEnu 1 | ABIA | Bende | Court | 7°64.26′E | 5°69.57′N | Farmer |
| 6 | ANAGan | ANAMBRA | Aghamelum | Anaku | - | - | Vendor |
| 7 | ANAGug | ANAMBRA | Aguata | Uga | - | - | Farmer |
| 8 | ANINum | ANAMBRA | Idemili North | Umuoji | - | - | Farmer |
| 9 | ANISnn | ANAMBRA | Idemili South | Nnobi | - | - | Market |
| 10 | EBANam | EBONYI | Afipko North | Amata | 7°97.1′E | 5°96.57′N | Farmer |
| 11 | EBANao 1 | EBONYI | Afipko North | Apku Oha | 7°96.34′E | 5°97. 97′N | Farmer |
| 12 | EBANau | EBONYI | Afipko North | Apku Ugo | 7°95.47′E | 5°95.47′N | Farmer |
| 13 | EBIKea | EBONYI | Ikwo | Eleke Achara | 8°13.56′E | 6°24.33′N | Farmer |
| 14 | EBIKok | EBONYI | Ikwo | Okputomo | 8°10.43′E | 6°22.13′N | Farmer |
| 15 | EBISlo | EBONYI | Ishielu | Labassa Okpoto | 7°51.82′E | 6°21.69′N | Farmer |
| 16 | EBIZib | EBONYI | Izzi | Iboko | 8°22.59′E | 6°40.68′N | Farmer |
| 17 | EBIZig | EBONYI | Izzi | Igboagu | - | - | Farmer |
| 18 | EBIZwa | EBONYI | Izzi | Waka | 8°31.5′E | 6°16.44′N | Farmer |
| 19 | ENAGag | ENUGU | Agwu | Agbaogugu | 7°46.07′E | 6°25.72′N | Farmer |
| 20 | ENAGmg 1 | ENUGU | Agwu | Mgbowo | 7°49.57′E | 6°09.46′N | Farmer |
| 21 | ENAGmg 2 | ENUGU | Agwu | Mgbowo | 7°49.57′E | 6°09.46′N | Farmer |
| 22 | ENANam 2 | ENUGU | Aninri | Amoro | 7°53.7′E | 5°95.1′N | Farmer |
| 23 | ENISib | ENUGU | Igboeze South | Ibagwa | 7°39.84′E | 6°91.63′N | Farmer |
| 24 | ENIWea | ENUGU | Igbetiti West | Eke Aku | 7°32.68′E | 6°70.29′N | Farmer |
| 25 | ENNEub | ENUGU | Nkanu East | Ubahu | 7°67.93′E | 6°80.52′N | Vendor |
| 26 | ENNKob | ENUGU | Nkanu East | Obe | 7°67.94′E | 6°82.56′N | Farmer |
| 27 | ENNSeh | ENUGU | Nsukka | Ehandagu | 7°65.24′E | 6°59.90′N | Farmer |
| 28 | ENNSog | ENUGU | Nsukka | Ogbaozara | 7°43.96′E | 6°77.26′N | Farmer |
| 29 | IMIKum | IMO | Ikeduru | Umudi | - | - | Vendor |
| 30 | IMINak | IMO | Ideato North | Akuokwa | - | Farmer | Farmer |
| 31 | IMISis | IMO | Ideato South | Isiekenesi | - | - | Vendor |
| 32 | IMOKeo 1 | IMO | Okigwe | Eke Okigwe | 7°34.95′E | 5°82.56′N | Farmer |
| 33 | IMOKeo 2 | IMO | Okigwe | Eke Okigwe | 7°34.96′E | 5°82.57′N | Farmer |
| 34 | IMOKeo 3 | IMO | Okigwe | Eke Okigwe | 7°34.96′E | 5°82.57′N | Farmer |

Source: Field survey, 2016.

### *2.2 Hundred Seed Weight*

One hundred seeds in three (3) replicates of each accession were counted and weighed using a sensitive measuring scale.

### *2.3 Standard Germination Test*

This test was conducted following International Seed Testing Association (ISTA) 2018 rules (ISTA, 2018). Fifty seeds in four replicates were planted on moistened sterilized riverbed sand for 10 days in plastic bowls and covered with transparent polythene sheets to maintain humidity. Counts were taken daily from the 3rd to the 10th day after planting (DAP) (Olisa et al., 2010a). Germination was assessed as a percentage of seeds producing normal seedlings as defined by ISTA (International Seed Testing Association) rules:

$$\text{Germination percentage (GPCT)} = \frac{100 \times \text{Number of seedlings emerged 10DAP}}{\text{Total number of seeds planted}} \quad (1)$$

Germination index (GI): This was calculated as proposed by Fakorede and Agbana (1983) as,

$$\text{Germination Index} = \frac{\Sigma\ [(\text{number of plants emerged in a day})\ (\text{DAP})]}{\text{Total Number of plants emerged by the } 10^{\text{th}} \text{ day}} \quad (2)$$

*2.4 Accelerated Ageing Test*

Fifty seeds in three replicates from each of the accessions were weighed and placed in wire mesh in accelerated ageing boxes containing 40 ml of distilled water. The seeds were aged by placing them in an accelerated ageing chamber at 43°C for 72 h at the end of which the seeds were re-weighed. The amount of water imbibed during the ageing (WIA) process was calculated as the difference between the final and initial weights (g) of the seeds and expressed as a percentage of initial weight. Standard germination test was then conducted with the aged seeds as described above to achieve the accelerated ageing germination percentage (AAT). Accelerated ageing germination index (AAI) was calculated by substituting the germination counts after accelerated ageing into the formula used earlier to obtain germination index.

*2.5 Conductivity Test*

Three replicates of 50 pre-weighed seeds from each accession were soaked for 24 h in 100 ml distilled water in 200 ml conical flasks covered with aluminium foils to prevent contamination. The conductivity of water was also measured using a flask with 100 ml distilled water without seeds. Conductivity was measured using Jenway 4510 (Bibby Scientific Ltd., Staffordshire, UK) conductivity meter. The results were expressed as $\mu Scm^{-1}\ g^{-1}$:

$$\text{Conductivity (COND)} = \frac{\text{Conductivity } (\mu S) \text{ for each flask} - \text{Conductivity of distilled water}}{\text{Initial weight (g) of seed sample}} \quad (3)$$

*2.6 Vigour Index*

Vigour index was calculated as an average of the percentage difference across the treatments in each of the tests (Awosanmi, 2010). The higher the VI, the more stable the genotype.

$$VI = [(GPCT + AAT + COND\%)/3] \quad (4)$$

Where, GPCT = Germination percentage, AAT = Accelerated ageing germination percentage, COND = conductivity.

Conductivity values were converted to % as follows:

$$COND\% = [100 \times (30 - COND)/30] \quad (5)$$

Given that lower conductivity values indicate higher quality, the factor 30 was used based on interpretation of conductivity values in relation to field emergence as suggested by Hampton and TeKrony (1995). Therefore, seed lots with conductivity values greater than 30 $\mu Scm^{-1}\ g^{-1}$ were regarded as not suitable for sowing especially under adverse weather conditions (Awosanmi, 2010).

*2.7 Water Imbibition Test*

This was carried out by planting 10 pre-weighed individual seeds from each accession in pre-numbered positions on a paper towel in a covered transparent plastic box. This was replicated 3 times. The seeds were removed at intervals of 6 h, weighed and returned to continue imbibition until sprouting or the emergence of the radicle. The duration of imbibition (DIM) was the period in hours from the planting to the time 50% of the seeds per replicate emerged radicle. The amount of water imbibed was then calculated and expressed in percentage (WIP) as the difference between the initial and final weights according to Olisa, Ajayi, and Akande (2010b).

*2.7 Statistical Analyses*

Analysis of variance was carried out using statistical analysis system (SAS) and the means were separated using least significant difference (LSD) at 1 and 5% levels of probability. Correlation analysis was also carried out to evaluate the relationship among the tests.

## 3. Results

There were significant accession effects for all the traits: hundred seed weight, germination percentage, germination index, water imbibed during ageing, accelerated ageing germination percentage, accelerated ageing germination index, conductivity, vigour index, water imbibition percentage and duration of imbibition (Table 2). The coefficient of variation was less than 20 for 7 out of the 10 traits assessed, the exceptions being germination percentage, accelerated ageing water imbibed and accelerated ageing test. Also, the coefficients of determination ($R^2$) values were high (> 70%) for hundred seed weight, germination percentage, accelerated ageing germination percentage, conductivity, vigour index, water imbibition percentage and duration of imbibition, and moderate (50-69%) for germination index, water imbibed during ageing and accelerated ageing germination index (Table 2).

Table 2. Mean square values from the Analysis of variance of the 34 African yam bean accessions subjected to seed physiological tests.

| SV | df | HSW | GPCT | GI | WIA | AAT | AAI | COND | VI | WIP | DIMB |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rep | 2 | 0.36 | 538.16 | 0.58 | 50.44 | 265.53 | 0.18 | 0.13 | 169.17** | 18.32 | 1.06 |
| Acc | 33 | 143.69** | 2315.17** | 0.81* | 112.86** | 1878.15** | 0.71** | 2.84** | 813.95** | 67.24** | 158.56** |
| Error | 66 | 2.54 | 173.23 | 0.41 | 27.38 | 106.50 | 0.24 | 0.13 | 28.66 | 10.62 | 0.70 |
| Mean | | 33.29 | 64.31 | 5.07 | 22.80 | 34.82 | 4.93 | 3.18 | 62.85 | 76.90 | 52.76 |
| CV | | 4.79 | 20.46 | 12.64 | 22.95 | 29.63 | 9.89 | 11.39 | 8.52 | 4.24 | 1.58 |
| $R^2$ | | 96.58** | 87.14** | 51.09* | 67.91** | 89.89** | 60.73** | 91.59** | 93.50** | 76.30** | 99.13** |

*Note*. SV: Sources of variation; df: degrees of freedom; Rep: Replication; Acc: Accession; HSW: Hundred Seed weight (g); GPCT: Germination percentage (%); GI: Germination index; WIA: Water imbibed during accelerated ageing (%); AAT: Accelerated ageing germination percentage (%); AAI: Accelerated ageing germination index; COND: Conductivity per gram of seed ($\mu Scm^{-1}$ $g^{-1}$); VI: Vigour index; WIP: Percentage water imbibed (%); DIM: Duration of imbibition (hrs).

Significant differences existed in the seed physiological quality of the AYB seeds collected from the 5 states in Southeast, Nigeria. Seeds collected from Enugu State had outstanding seed physiological traits in terms of hundred seed weight, highest germination percentage, accelerated ageing germination percentage and highest vigour index. While seeds from Anambra State performed poorly among the other states (Table 3).

Table 3. Mean values of the performance of the 34 African yam bean accessions for seed physiological tests across the states of collection

| States | HSW | GPCT | GI | WIA | AAT | AAI | COND | VI | WIP | DIM |
|---|---|---|---|---|---|---|---|---|---|---|
| Abia | 35.62 | 68.53 | 5.42 | 19.68 | 31.20 | 5.36 | 2.65 | 63.63 | 74.48 | 60.80 |
| Anambra | 30.64 | 43.00 | 5.65 | 19.70 | 21.33 | 5.50 | 4.27 | 50.04 | 75.98 | 56.00 |
| Ebonyi | 29.79 | 57.85 | 4.91 | 24.85 | 20.96 | 4.75 | 3.36 | 55.88 | 79.47 | 47.33 |
| Enugu | 37.50 | 72.47 | 4.95 | 21.80 | 49.47 | 4.77 | 3.03 | 70.62 | 75.65 | 52.80 |
| Imo | 31.37 | 71.11 | 4.93 | 26.01 | 43.22 | 4.78 | 2.88 | 68.25 | 77.76 | 52.00 |
| $LSD_{0.05}$ | 2.60 | 21.46 | 1.04 | 8.53 | 16.82 | 0.80 | 0.59 | 8.73 | 5.31 | 1.36 |

*Note*. HSW: Hundred Seed weight (g); GPCT: Germination percentage (%); GI: Germination index; WIA: Water imbibed during accelerated aging (%); AAT: Accelerated ageing germination percentage (%); AAI: Accelerated aging germination index; COND: Conductivity per gram of seed ($\mu Scm^{-1}$ $g^{-1}$); VI: Vigour index; WIP: Percentage water imbibed (%); DIM: Duration of imbibition (hrs).

The accessions differed widely in HSW, ranging from 22.70 to 52.83 g. Out of the 34 accessions evaluated, 11 had HSW < 30 g, 13 were 30-35 g, 7 were 36-40 g, and 3 had HSW > 40 g (Table 4). 33 of the 34 accessions had germination percentages ranging from 2.67 to 98%. Only 9 accessions had germination percentage that was less than 50% while for more than half of the accessions, germination percentage was above 70%. Unlike seed weight (HSW) and viability (GPCT), germination index, GI, was less variable, ranging from 3.98-6.61 days with a mean of 5.07 days. The range of the amount of water imbibed varied widely. While ENIWea absorbed 130% and EBIZig and EBIZib up to 100% of the initial weight, ENAGmg series absorbed less than 50% of their respective weights (Table 4). Accessions ENAGag, ENAGmg 1, ENAGmg 2 also had germination percentages greater than 80% combined with germination percentage after accelerated ageing test (AAT) of above 70%. Although ABBEuz, EBIKea, EBIZib, EBIZwa, ENNEub and IMOKeo 2 accessions had very high mean values for germination percentage (> 80%), the percentage of germination after being subjected to accelerated ageing test drastically reduced to beyond half of the initial germination percentage.

Table 4. Mean values of the performance of the 34 African yam bean accessions when subjected to seed physiological tests

| States | Accession | HSW | GPCT | GI | WIA | AAT | AAI | COND | VI | WIP | DIM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Abia | ABBE uz | 34.97 | 85.33 | 5.60 | 21.02 | 25.33 | 5.01 | 3.22 | 66.64 | 76.77 | 66.00 |
| Abia | ABBEng | 38.89 | 76.67 | 5.12 | 20.23 | 56.67 | 5.49 | 2.45 | 75.05 | 73.53 | 58.00 |
| Abia | ABBEnu 1 | 35.01 | 58.00 | 4.98 | 22.35 | 19.33 | 6.17 | 2.47 | 56.37 | 72.27 | 66.00 |
| Abia | ABBEuz 1 | 36.76 | 64.67 | 5.32 | 19.30 | 36.00 | 5.63 | 1.87 | 64.81 | 73.02 | 60.00 |
| Abia | ABUNum 2 | 32.47 | 58.00 | 6.08 | 15.50 | 18.67 | 4.50 | 3.24 | 55.29 | 76.83 | 54.00 |
| Anambra | ANAGan | 31.38 | 26.00 | 6.61 | 21.91 | 30.00 | 4.97 | 3.21 | 48.43 | 77.71 | 54.00 |
| Anambra | ANAGug | 33.68 | 84.00 | 5.02 | 19.90 | 22.00 | 5.43 | 3.20 | 65.11 | 75.70 | 50.00 |
| Anambra | ANINum | 27.56 | 62.00 | 5.30 | 22.46 | 33.33 | 6.09 | 3.26 | 61.49 | 77.82 | 54.00 |
| Anambra | ANISnn | 29.92 | 0.00 | - | 14.54 | 0.00 | - | 7.39 | 25.12 | 72.71 | 66.00 |
| Ebonyi | EBANam | 32.81 | 49.33 | 4.84 | 25.26 | 7.33 | 4.79 | 4.72 | 46.98 | 81.49 | 42.00 |
| Ebonyi | EBANao 1 | 32.91 | 66.00 | 4.86 | 25.80 | 10.67 | 5.27 | 3.32 | 55.20 | 77.96 | 54.00 |
| Ebonyi | EBANau | 30.33 | 2.67 | 5.25 | 27.95 | 1.33 | 5.00 | 4.64 | 29.51 | 80.25 | 48.00 |
| Ebonyi | EBIKea | 25.20 | 90.67 | 4.81 | 35.75 | 35.33 | 4.92 | 2.65 | 72.39 | 88.98 | 48.00 |
| Ebonyi | EBIKok | 36.81 | 20.67 | 6.23 | 21.66 | 0.00 | - | 3.49 | 36.34 | 75.62 | 54.00 |
| Ebonyi | EBISlo | 29.85 | 34.00 | 3.98 | 21.73 | 29.33 | 4.70 | 2.47 | 51.71 | 78.27 | 42.00 |
| Ebonyi | EBIZib | 26.60 | 90.67 | 4.50 | 26.60 | 34.67 | 5.04 | 3.42 | 71.31 | 78.73 | 48.00 |
| Ebonyi | EBIZig | 24.47 | 80.00 | 5.04 | 24.60 | 51.33 | 4.45 | 2.55 | 74.27 | 73.98 | 42.00 |
| Ebonyi | EBIZwa | 29.13 | 86.67 | 4.74 | 14.33 | 18.67 | 3.88 | 2.93 | 65.19 | 79.97 | 48.00 |
| Enugu | ENAGag | 50.87 | 97.33 | 4.82 | 21.97 | 70.00 | 4.30 | 3.81 | 84.88 | 64.10 | 54.00 |
| Enugu | ENAGmg 1 | 52.83 | 86.00 | 4.81 | 17.33 | 91.33 | 4.81 | 3.17 | 88.92 | 66.53 | 60.00 |
| Enugu | ENAGmg 2 | 48.88 | 93.33 | 4.77 | 18.56 | 74.67 | 4.98 | 3.62 | 85.31 | 70.51 | 60.00 |
| Enugu | ENANam 2 | 31.35 | 98.00 | 5.02 | 15.03 | 50.67 | 5.02 | 2.73 | 79.86 | 81.49 | 42.00 |
| Enugu | ENISib | 25.60 | 26.67 | 5.13 | 27.82 | 15.33 | 4.61 | 2.95 | 44.06 | 82.55 | 48.00 |
| Enugu | ENIWea | 29.33 | 56.00 | 5.10 | 37.92 | 29.33 | 4.22 | 2.91 | 58.55 | 74.95 | 54.00 |
| Enugu | ENNEub | 32.20 | 80.67 | 4.87 | 20.66 | 4.67 | 5.00 | 2.85 | 58.62 | 80.32 | 54.00 |
| Enugu | ENNKob | 36.60 | 89.33 | 5.36 | 20.25 | 72.00 | 5.12 | 2.17 | 84.70 | 74.21 | 66.00 |
| Enugu | ENNSeh | 32.86 | 33.33 | 4.79 | 20.01 | 21.33 | 4.90 | 3.54 | 47.63 | 79.62 | 42.00 |
| Enugu | ENNSog | 34.53 | 64.00 | 4.79 | 18.49 | 65.33 | 4.71 | 2.51 | 73.66 | 82.20 | 48.00 |
| Imo | IMIKum | 34.28 | 32.67 | 4.42 | 29.91 | 18.00 | 4.56 | 3.95 | 45.83 | 78.64 | 48.00 |
| Imo | IMINak | 36.55 | 60.67 | 4.47 | 18.75 | 11.33 | 4.39 | 2.74 | 54.29 | 79.91 | 48.00 |
| Imo | IMISis | 24.28 | 96.67 | 5.24 | 34.29 | 70.67 | 5.03 | 2.52 | 86.31 | 81.76 | 54.00 |
| Imo | IMOKeo 1 | 22.70 | 73.33 | 4.88 | 35.37 | 68.00 | 4.62 | 3.06 | 77.05 | 74.54 | 48.00 |
| Imo | IMOKeo 2 | 33.47 | 91.33 | 4.96 | 18.16 | 31.33 | 5.09 | 2.61 | 71.33 | 75.36 | 54.00 |
| Imo | IMOKeo 3 | 36.91 | 72.00 | 5.59 | 19.89 | 60.00 | 4.97 | 2.38 | 74.69 | 76.38 | 60.00 |
| | $LSD_{0.05}$ | 2.60 | 21.46 | 1.04 | 8.53 | 16.82 | 0.80 | 0.59 | 8.73 | 5.31 | 1.36 |

Only 11 of the accessions had an accelerated ageing germination percentage of above 50%. The accelerated ageing germination values for the accessions varied from 0% to 91.33% (Table 4). Accessions ENAGmg 1 and ENNSog revealed a higher accelerated ageing germination percentage values than their standard germination percentage values, while other accessions had lesser values. Also, the accelerated ageing index values ranged from 3.88-6.17 days similar to the germination index values. All conductivity values for the 34 accessions were less than 5.00 $\mu Scm^{-1}$ $g^{-1}$, except for ANISnn which had a value of 7.39 $\mu Scm^{-1}$ $g^{-1}$. Conversely, accession ANISnn had the least vigour index value, while others had vigour index values ranging from 29.51 to 88.92. The water imbibed percentage varied from 64.10% (ENAGag) to 88.98% (EBIKea), while the duration in which the water was imbibed varied from 42 hrs to 66 hrs. Half of the accessions had VI above 65% (88.92-65.11) and the other half below, ranging from 25.12-64.81. The first 7 accessions with the highest VI were from Enugu and Imo States (Table 4).

From the correlation result (Table 5), a highly significant ($p < 0.01$) negative correlation existed between hundred seed weight and accelerated ageing water imbibed, hundred seed weight and percentage water imbibed, germination percentage and conductivity, conductivity and vigour index, and between percentage water imbibed and duration of imbibition. Whereas a highly significant ($p < 0.01$) positive correlation existed between germination percentage and accelerated ageing germination percentage, germination percentage and vigour index, accelerated ageing germination percentage and vigour index. Also, a significant ($p < 0.05$) positive correlation existed between hundred seed weight and the duration of imbibition, germination index and duration of imbibition, accelerated ageing index and duration of imbibition. Furthermore, relationship between accelerated ageing germination percentage and conductivity, and between accelerated ageing germination percentage and percentage water imbibed were significant ($p < 0.05$) but negative.

Table 5. Correlation analysis of the seed physiological quality tests of the 34 African yam bean accessions (n = 32)

| | HSW | GPCT | GI | WIA | AAT | AAI | COND | VI | WIP |
|---|---|---|---|---|---|---|---|---|---|
| GPCT | 0.21 | | | | | | | | |
| GI | -0.02 | -0.22 | | | | | | | |
| WIA | -0.47** | -0.04 | -0.12 | | | | | | |
| AAT | 0.38* | 0.63** | -0.09 | 0.03 | | | | | |
| AAI | 0.03 | 0.03 | 0.19 | -0.10 | -0.02 | | | | |
| COND | 0.02 | -0.54** | -0.01 | -0.09 | -0.41* | -0.19 | | | |
| VI | 0.31 | 0.91** | -0.18 | -0.004 | 0.88** | 0.01 | -0.58** | | |
| WIP | -0.69** | -0.14 | -0.07 | 0.30 | -0.40* | -0.08 | -0.11 | -0.28 | |
| DIM | 0.42* | 0.10 | 0.40* | -0.23 | 0.17 | 0.44* | 0.06 | 0.14 | -0.53** |

*Note*. HSW: Hundred Seed weight (g); GPCT: Germination percentage (%); GI: Germination index; WIA: Water imbibed during accelerated aging (%); AAT: Accelerated ageing germination percentage (%); AAI: Accelerated aging germination index; COND: Conductivity per gram of seed ($\mu Scm^{-1}$ $g^{-1}$); VI: Vigour index; WIP: Percentage water imbibed (%); DIM: Duration of imbibition (hrs).

## 3. Discussion

The accessions exhibited a widely divergent variability for both physical and physiological traits. The wide variability in the performance of the African yam bean accessions across the seed physical and physiological quality tests corroborates the report of Adewale and Odoh (2013) that Nigeria is among the centers of diversity of African yam bean. Olasoji, Akande, and Owolade, (2011) also reported genetic variability for seed quality among 10 accessions of AYB studied. The variation was observed in their seed sizes, amount and rate of water imbibed in the process of germination, viability status and speed of germination, ability to withstand stress or adverse conditions and the integrity of their seed coats. Accessions ENAGag, ENAGmg 1 and ENAGmg 2 had comparative larger seeds than other accessions and showed similarities in their performances across the tests indicating that they were of a similar genetic background. Seed size is an important physical indicator of seed quality that affects seed germination, emergence, seedling survival, plant growth and performance of the crop in the field (Adebisi et al., 2013; Makinde, Oyekale, & Daramola, 2020). The outstanding performances of large-seeded accessions can be attributed to their higher reserve content. Shahi, Bargahi, and Bargali, (2015) reported that distinct seed sizes have different levels of starch and other energy reserves which may be an important factor to improve the expression of germination and initial growth of seedlings. The seed size of the accessions also varied across their location of collection. Although, it is believed that seed sizes may vary widely between the crop species and the germination and growth environment, generally, large seeds have a higher seedling survival rate, larger biomass and adaptability than small seeds, under adverse conditions (Ambika, Manonmani, & Somasundaram, 2014).

The failure of accession ANISnn to germinate under stress and ideal conditions suggests that the embryo must have been dead despite that other features of the seed remain intact and dormancy is not associated with African yam bean (Olisa et al., 2010a). This loss of physiological quality could have been as a result of the inherent low vigour of African yam bean seeds upon which is superimposed a poor storage environment. Accessions EBANau and EBIKok were also losing their germination potential, revealing a gradual decline in viability and vigour owing to physiological deterioration. Physiological seed deterioration is a phenomenon which is not visually apparent. The inability of seeds to maintain vigour for a long period poses storage problems and can cause the specie/crop to go into extinction (Finch-Savage & Bassel, 2016).

Most of the accessions had very low vigour as revealed by their low germination after being subjected to accelerated ageing. This corroborates the fact that African yam bean seeds are inherently low in seed vigour (Olisa et al., 2010a). The significant positive relationship between standard germination percentage and accelerated ageing germination percentage suggests that, accessions with higher germination under stress conditions will also exhibit higher germination under normal conditions, while poor germination under normal conditions will lead to poor performance under stress conditions. However, that some accessions like ENAGag, ENAGmg 1, ENAGmg 2, ENNKob, ENNSog, IMISis, IMOKeo 1 and IMOKeo 3 had higher and comparable standard germination and accelerated ageing germination percentages suggests that there exist a pool of high seed vigour potential among the African yam bean accessions that can be improved upon. High seed vigor enhances the ability to obtain optimal plant densities and high crop yields (Milosevic, Vujakovic, & Karagic, 2010). The germination index and accelerated ageing index values indicated that African yam bean seed germination occurs between averages of 4-7 days, whether under ideal or stressed conditions. These two test procedures (standard germination and accelerated ageing tests) alongside conductivity test were the key determinants of the vigour index of the accessions. The consistently low conductivity values for all the accessions could be as a result of their hard seed coat which could in turn reduce seed exudates. Tungate, Susko, and Rufty (2002) attributed low germination rate to the prevention of water and respiratory gases from penetrating into the seed by hard seed coats during imbibition. However, the considerably low conductivity values also indicated that the seeds were suitable for sowing. According to Hampton and TeKrony (1995) conductivity values less than 30 $\mu Scm^{-1}$ $g^{-1}$ indicate that the seeds are suitable for sowing, even under adverse conditions.

Even though the amount and duration of water imbibed did not follow any definite pattern nor correlate with the number of seeds that germinated, their persistent correlation with hundred seed weight under ideal conditions (WIP) and under stress (WIA) revealed that imbibition is strongly influenced by or dependent on the size or weight of the seed. Olisa et al. (2010b) stated that the duration of each phase of imbibition in African yam bean depended on seed properties such as size, content of hydratable substances, seed coat permeability and oxygen uptake. However, the speed of germination under both ideal and stress conditions in this study were influenced by the duration of water imbibed. That is, a delay in the number of days of the seed to germinate extended also the period during which water is imbibed prior to germination, without necessarily increasing the overall amount of water imbibed. On the average, African yam bean seeds require moisture of up to 76.9% of their initial weight over a period of approximately 53 hours prior for the commencement of germination.

## 4. Conclusion

Seed size is an important trait in African yam bean that could be improved upon for the selection of landraces of high seed physiological potentials. Conductivity test was not very effective in determining the variability in seed vigour. Seed germination in AYB can be easily assessed 7 days after planting. Accessions ENAGag, ENAGmg 1, ENAGmg 2, ENNKob, ENNSog, IMISis, IMOKeo 1 and IMOKeo 3 were outstanding and are therefore recommended for genetic improvement of seed quality in African yam bean.

## References

Adebisi, M. A., Kehinde, T. O., Salau, A. W., Okesola, L. A., Porbeni, J. B. O., Esuruoso, A. O., & Oyekale, K. O. (2013). Influence of different seed size fractions on seed germination, seedling emergence and seed yield characters in tropical soybean (*Glycine max* L. Merrill). *International Journal of Agricultural Research, 8*(1), 26-33. https://doi.org/10.3923/ijar.2013.26.33

Adewale, B. D., & Odoh, N. C. (2013). A review on genetic resources, diversity and agronomy of African Yam Bean (*Sphenostylis stenocarpa* (Hochst. Ex A. Rich.) Harms): A potential future food crop. *Sustainable Agriculture Research, 2*, 3-43. https://doi.org/10.5539/sar.v2n1p32

Ambika, S., Manonmani, V., & Somasundaram, G. (2014). Review on effect of seed size on seedling vigour and seed yield. *Research Journal of Seed Science, 7*(2), 31-38. https://doi.org/10.3923/rjss.2014.31.38

Awosanmi, F. E. (2010). *Effect of genotype and drought on seed vigour in maize (Zea mays L.)* (p. 67, Thesis, Obafemi Awolowo University, Ile-Ife, Nigeria).

Binotti, F. F. S., Haga, K. I., Cardoso, E. D., Alves, C. Z., De Sá, M. E., & Arf, O. (2008). Effect of the accelerated aging period on the electrical conductivity test and on the physiological quality of bean seeds. *Acta Scientiarum Agronomy, 30*(2), 247-254. https://doi.org/10.4025/actasciagron.v30i2.1736

Eggert, K., & von Wirén, N. (2015). The role of boron nutrition in seed vigour of oilseed rape (*Brassica napus* L.). *Plant and Soil, 402*, 63-76. https://doi.org/10.1007/s11104-015-2765-1

Egli, D. B., & Rucker, M. (2012). Seed Vigor and the Uniformity of Emergence of Corn Seedlings. *Crop Science, 52*, 2774-2782. https://doi.org/10.2135/cropsci2012.01.0064

Fakorede, M. A. B., & Agbana S. B. (1983). Heterotic effects and association of seedling vigour with mature plant characteristics and grain yield in some tropical maize cultivars. *Maydica, 27*, 327-338.

Finch-Savage, W. E. (1995). Influence of seed quality on crop establishment, growth and yield. In A. S. Basra (Ed.), *Seed Quality: Basic Mechanisms and Agricultural Implications* (pp. 361-384). Food Products Press: New York, NY, USA. https://doi.org/10.4324/9781003075226-11

Finch-Savage, W. E., & Bassel, G. W. (2016). Seed vigour and crop establishment: Extending performance beyond adaptation. *Journal of Experimental Botany, 67*(3), 567-591. https://doi.org/10.1093/jxb/erv490

Hampton, J. G., & TeKrony, D. M. (1995). *Handbook of Seed vigour Test Methods*. International Seed Testing Association, Zurich.

Hofs, J. L. Schoeman, A., & Vaissayrel, M. (2004). Effect of Bt cotton on arthropod biodiversity in South African Cotton fields. *Communications in Agricultural and Applied Biological Sciences, 69*(3), 191-194.

ISTA (International Seed Testing Association). (2018). *International Rules for Seed Testing*. International Seed Testing Association, Zurich.

Kandasamy, S., Weerasuriya, N., Gritsiouk, D., Patterson, G., Saldias, S., Ali, S., & Lazarovits, G. (2020). Size Variability in Seed Lot Impact Seed Nutritional Balance, Seedling Vigor, Microbial Composition and Plant Performance of Common Corn Hybrids. *Agronomy, 10*(2), 1-17. https://doi.org/10.3390/agronomy10020157

Makinde, A. I., Oyekale, K. O., & Daramola, D. S. (2020). Impact of seed size on the seedling vigour, dry matter yield and oil content of Jatropha (*Jatropha curcas* L.). *Journal of Agricultural Science, 12*(3), 197-203. https://doi.org/10.5539/jas.v12n3p197

Marcos-Filho, J. (2015). Seed vigor testing: An overview of the past, present and future perspective. *Scientia Agricola, 72*(4), 363-374. https://doi.org/10.1590/0103-9016-2015-0007

Medeiros A. D., Silva, L. J., Capobiango, N. P., Fialho, C. A., & Dias, D. C. F. S. (2019). Assessing the physiological quality of common bean seeds using the Vigor-S® system and its relation to the accelerated aging test. *Journal of Seed Science, 41*(2), 187-195. https://doi.org/10.1590/2317-1545v41n2211401

Milosevic, M., Vujakovic, M., & Karagic, D. (2010). Vigour tests as indicators of seed viability. *Genetika, 42*, 103-118. https://doi.org/10.2298/GENSR1001103M

Nnamani, C. V., Ajayi, S. A., Oselebe, H. O., Atkinson, C. J., Igboabuchi, A. N., & Ezigbo, E. C. (2017). *Sphenostylis stenocarpa* (ex. A. Rich.) Harms. a Fading Genetic Resource in a Changing Climate: Prerequisite for Conservation and Sustainability. *Plants, 6*(30), 1-16. https://doi.org/10.3390/plants6030030

Olasoji, J. O., Akande, S. R., & Owolade, O. F. (2011). Genetic variability in seed quality of African yam beans (*Sphenostylis stenocarpa* Hochst. Ex A. Rich Harms). *African Journal of Agricultural Research, 6*(27), 5848-5853. https://doi.org/10.5897/AJAR11.1311

Olisa, B. S., Ajayi, S. A., & Akande, S. R. (2010a). Physiological Quality of Seeds of Promising African Yam Bean (*Sphenostylis stenocarpa* (Hochst. Ex A. Rich) Harms) and Pigeon Pea (*Cajanus cajan* L. Mill sp.) Landraces. *Research Journal of Seed Science, 3*, 93-101. https://doi.org/10.3923/rjss.2010.93.101

Olisa, B. S., Ajayi, S. A., & Akande, S. R. (2010b). Imbibition and Response of Pigeon Pea (*Cajanus cajan* L. Mill sp.) and African Yam Bean (*Sphenostylis stenocarpa* (Hochst. ex A. Rich) Harms) Seeds to Scarification. *Research Journal of Seed Science, 3*, 150-159. https://doi.org/10.3923/rjss.2010.150.159

Potter, D. (1992). Economic Botany of Sphenostylis stenocarpa (Leguminosae). *Economic Botany, 46*, 262-275. https://doi.org/10.1007/BF02866625

Potter, D., & Doyle, J. J. (1992). Origin of African yam bean (*Sphenostylis stenocarpa*, Leguminosae): Evidence from morphology, isozymes, chloroplast DNA and Linguistics. *Economic Botany, 46*, 276-292. https://doi.org/10.1007/BF02866626

Shahi, C. V., Bargahi, K., & Bargali, S. S. (2015). How seed size and water stress affect the seed germination and seedling growth in wheat varieties? *Current Agriculture Research Journal, 3*(1), 60-68. https://doi.org/10.12944/CARJ.3.1.08

Silva, C. B., Lopes, M. M., Marcos-Filho, J., & Vieira, R. D. (2012). Automated system of seedling image analysis (SVIS) and electrical conductivity to assess sun hemp seed vigor. *Revista Brasileira de Sementes, 34*(1), 55-60. https://doi.org/10.1590/S0101-31222012000100007

Tungate, K. D., Susko, D. J., & Rufty, T. W. (2002). Reproduction and offspring competitiveness of *Senna obtusifolia* are influenced by nutrient availability. *New Phytologist, 154*, 661-669. https://doi.org/10.1046/j.1469-8137.2002.00407.x

Uguru, M. I., & Madukaife, S. O. (2001). Studies on the variability in agronomic and nutritive characteristics of African yam bean (*Sphenostylis stenocarpa* Hochst ex. A. Rich. Harms). *Plant Production and Research Journal, 6*, 10-19.

Venuto, B. C., Redfearn, D. D., Pitman, W. D., & Alison, M. W. (2002). Seed variation among annual ryegrass cultivars in south-eastern USA and the relationship with seedling vigour and forage production. *Grass and Forage Science, 57*, 305-311. https://doi.org/10.1046/j.1365-2494.2002.00328.x

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# Impact of Recapitalisation and Development Programme on Performance of Land Reform Beneficiary Farmers in KwaZulu-Natal, South Africa

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**Abstract**

Providing appropriate post-settlement support to farmers is crucial for sustainable development of smallholder agriculture in South Africa. In unravelling this, the South Africa's Recapitalization and Development Programme (RADP) was initiated. Hence, this study analysed the impact of RADP on performance of land reform beneficiary farmers in KwaZulu-Natal, South Africa. A multistage sampling procedure was used to select (n = 264) respondents for the study. Accounting for endogeneity issues in RADP assessments and its impact on the performance of land reform farmers, an endogenous switching regression model (ESRM) was employed. In the same vein, a doubly-robust inverse probability weighted regression adjustment was used as credible remedy for potentially biased estimates of ATT and POM of endogenous treatment model. The main findings revealed that tax compliance, secondary organization, legal entity, farm potential income at acquisition, farmers receiving third party assistance and strategic partnership were statistically significant in influencing the participation of farmers in RADP. Mentorship remains an extremely challenging element in post-settlement. However, through the strategic partnership of RADP farmers had likelihood to improve the farm and increase farm income. The results of the suggest that the RADP can contribute to a deep process of change and empowerment of farmers. In the same vein, strategic partnership of RADP is likely to improve the farmers' performance. Therefore, there is a need to strongly improve mentorship and strategic partnership programme to encourage participation of land reform farmers in the support programmes.

**Keywords:** land reform, RADP, ESRM, IPWRA, South Africa

## 1. Introduction

Effective use of land reforms as a tool for poverty alleviation crucially depends on how the beneficiaries are guided through for efficient use of the land they have acquired for productive purposes (Zhang et al., 2019; van Noordwijk, 2019). Continued support through engaging them by developing an enabling environment, institutional and individual capcities are important. In the absence of susch support the full benefits of the land reform programs may not be realized. The case of South Africa is typical.

Post-settlement support in South Africa is a programme of improving and broadening post-settlement support services to land reform farms (Anseeuw et al., 2015; Nthai, 2020). There is a necessity to strengthen farmer support to ensure cohesion function of government organization. Hence, it must be explicitly facilitated that post-settlement must not remain the duties of government, but numerous interested organizations in land reform are critical in playing a role in farmer support development. The mandate of post-settlement is to ensure beneficiaries make use of economic development through sustainable livelihood and poverty eradication (Mafora, 2014).

Existing post-settlement programmes in land reform in were replaced with (RADP) in 2013, including settlement support grants for beneficiaries of land restitution (Anseeuw et al., 2015). The significance of Recapitalization and Development Programme (RADP) is premised on the fact that most of land reforms have been failing because of insufficient and ineffective post-settlement support and are in 'distress', and consequently in need of additional recap funds (University of the Western Cape, 2016; Staal, 2019). Therefore, the Department of Rural Development and Land Reform (DRDLR) initiated a farmer support programme, RADP, to enhance the involvement of a range of institutions, especially local government, in the post settlement stage of land reform to assist farmers with RADP (DRDLR, 2014).

RADP is the programme implemented after Comprehensive Agricultural Support Programme (CASP) with an aim to revive under-performing farms by supporting with; capital to improve farm production, machineries, infrastructure and access to mentorship in order to gain skills and knowledge on how to sustainably manage the farm. DRDLR (2013) suggested that the programme also aims to increase farm income, enhance food security and improve livelihoods of the beneficiaries. Additionally, RADP found to replace all previous forms of funding for land reform in 2013, including settlement support grants for beneficiaries awarded land through restitution, claimed by the University of the Western Cape (2016).

In the spectrum of land redistribution across countries, interested stakeholders in land reform in Southern Africa revised land reform against the experiences of the recent land crisis in the region. This led to initiation of negotiated transfer land redistribution program pursued in the foundation of four questions: What has been the experience with land redistribution in the region over the last decade or so? What has been the impact on people's livelihoods? How are redistribution programmes expected to develop in future? What might be the role of donors in the process (Adams & Howell, 2001)? Byamugisha (2014) argued that land reform is an imperative aspect of social and economic transformation in South Africa, as a means of both redressing past injustices and alleviating the pressing problems of poverty and inequality. Hence, given the significance of the agricultural sector in economic growth, poverty alleviation and employment, Weideman (2003) suggested that it is crucial that land reform contributes to increased (or at least sustained) levels of agricultural production.

Martin (2000) revealed that a paradigm shift from the initial poverty-reduction objective of land restitution programme and towards land redistribution for 'productive' objective brought a heavy criticism on the grounds of Zimbabwe that farms were being compulsorily acquired and distributed to politicians, military employees and officials and used as a pillar to underpin political support. However, the stakeholders recommended that infrastructure and settler support, particularly in the form of 'starter packs' must be provided for proposed beneficiaries. This approach of establishing commercial native farmers was once observed in Chile, in 1967 the government invested land reform with the major goal of increasing farm production and productivity of the agricultural industry and also established polices promoting production at farm level (Binswanger-Mkhize, 2014; Tilley, 2008; Janvry & Sadoulet, 2002; Heit, 2003) in Sibisi (2015).

The large-scale farmer model in land reform is a very efficient model around the world and is the fundamental of land reform success and with great contribution to agricultural growth. Global experience shows that this model is successful from very small and labour-intensive operations to very large, mechanized operations. Rogier et al. (2006) emphasized that larger farmers generally have easier access to cheaper credit. This enables them to quickly respond to the market, especially when the market demands agricultural products with high investment costs. According to Kahn (2007) in Sibisi (2015) suggested that repossession of land is meaningless without appropriate post-settlement support. The problem remains in that in across countries land reform is failing because of insufficiency and late delivery of farmer support/post-settlement services, argued by Hans and Mkhize (2014). Across the world, land and post-settlement support are provided by different stakeholders, which proves impossible to coordinate. As indicated above, the stakeholder arrangements for the delivery of farmer support services to proposed land reform farmers are largely dysfunctional and the services provided have been neither adequate nor appropriate (Byamugisha, 2014). Sibisi (2015) criticized the collaboration of stakeholders and lack of scope of post-settlement support required to be implemented which results in poor after-care support. Locally and internationally, poor post-settlement support has appeared to be a hindrance to achieving success.

As a result, Sibisi (2015) finds the RADP to be the best programme because it focuses on the whole farm development but not limited to farmer capacity development. Maka and Aliber (2019) supported that one outstanding characteristic of the RADP is that it connects proposed farmers with mentors or strategic partners, significantly as a condition of receiving financial support. Phatudi-Mphahlele (2016) proposed that the mention link of stakeholders is with an aim to invest infrastructure and provide with mentorship and strategic partners as to ensure the growth and development of the farmer and farm. However, there is dearth of information on impact of RADP on net farm income of land reform beneficiaries in KwaZulu-Natal (KZN) Province of South Africa. It is against this backdrop that this study sought to unravel the impact of RADP participation on net farm income of beneficiaries of the land reform to support the public policy makers' job and informing the society in KZN.

## 3. Materials and Methods

### *3.1 Study Region and Data*

The study was conducted in KwaZulu-Natal to analyze the impact of RADP on performance of land reform farmers. In the study area, respondents (n = 264) successfully participated in the study both non-benefited and benefited RADP beneficiaries. The factors being assessed were adopted from the literature, seeing as the elements required and increases the likelihood to participate in RADP and also contribute in farm income generation. Probability sampling was adopted with an aim to estimate the probability of a participant's being included in the sample (Taherdoost, 2016). A survey was conducted using a structured questionnaire that was directed to land reform beneficiaries in the study sites. Multistage sample tool was employed to guarantee that non-RADP and RADP beneficiaries are given each adequately represented within the entire sample (Crossman, 2020). Hence, it was done to remove bias from selection procedure and result in representative of sample (Dudovskiy, 2018).

The use of structured interview was independently during one-on-one interviews because respondents were segregated and in-order to acquire in-depth data relevant to factors contribute in participating in RADP and improving farm income which is examined to be contributing to farm development and sustainable livelihood. It was this also this reason the research study employed probability sampling to assess factors influencing participation of farmers in RADP and factors contribute in increase farm income. The interview was conducted using isiZulu so that farmers can best respondent with an understanding. Data collection was conducted to a total of 264 questionnaires (n = 264) and were successfully completed.

During primary data collection, the letter of consent was read clearly, attached to each survey for land reform farmers, to encourage that the respondent fully comprehended the nature of the research. This was done and signed by the respondents prior to their involvement to make sure that participants agreed to the condition of the research study. Hence, a mentioned letter of consent informed respondent prior that the participant true identification would not be presented, but indication using numbers and class name like land reform farmer were used to ensure protection of their identification. An inferential analysis was employed with an aim to analyze the data to show the relationship between multiple variables to generalize results and make predictions (Lutabingwa, 2007). An Endogenous switching regression model was adopted to show the level of significance of variables or the relationship between two variables influencing participation of farmers in RADP and the variables that contribute in the likelihood of farm income of RADP farmers and non-RADP farmers to find the impact of recapitalization and development programme on performance of land reform farmers. The data was captured in a computerized manner using STATA.

### *3.2 Econometric Estimation Strategy*

Following Lokshin and Sajaia (2004), Khanal et al. (2018), and Aravindakshan et al. (2018), an endogenous switching regression model (ESRM) was employed for this study. This approach, however, estimated the impact of RADP participation on the net farm income of farmers using RADP participation as a dummy variable, which might yield biased and inconsistent estimates because participation is potentially endogenous (Ojo & Baiyegunhi, 2020a). This model consists of two parts; endogeneity due to self-selection using a probit selection model (Note 1) was corrected for in the first part of the model, in which farmers were partitioned (divided) into participants and non-participants of RADP programme. Following Abdulai and Huffman (2014), a RADP participation is normally chosen by a farmer if the net benefits derived by participating in it are higher than the benefits derived by not participating in it ($P_{Y1} \geq P_{Y2}$), where, $P_{Y1}$ is the net benefit that farmer *i* derives from RADP participation and $P_{Y2}$ is the net benefit of not participating in it. The net benefits derived by RADP participation were not known to the researcher. However, the characteristics of farmers were observed during the survey period, with $Y_i^*$ representing the net benefits derived from RADP participation that was not observed, but could be expressed as a function of the observed attributes.

$$Y_i^* = \beta F_i + \varepsilon_i \tag{1}$$

$$Y_i = 1 \text{ if } Y_i^* > 0 \text{ and 0 if otherwise}$$

where, $Y_i^*$ is a variable that was not observed (or latent) for RADP participation, while $Y$ is the observable counterpart (equal to 1 if the farmer participated it, and 0 if otherwise).

In the second stage, the outcome equations on the impact of the RADP participation on net farm income was estimated using a production function, expressed in Equation (2) as:

$$P = f(Y, \beta, F) + \varepsilon \tag{2}$$

where, $P$ is the log form of net farm income; $Y$ is the RADP participation; $\beta$ is a vector of parameters to be estimated; and $F$ is a set of covariates used in the model.

$$\text{Regime 1 (participants): } P_{1i} = \lambda_1 H_i + v_{1i} \tag{3a}$$

$$\text{Regime 2 (non-participants): } P_{2i} = \lambda_2 H_i + v_{2i} \tag{3b}$$

where, $P_{1i}$ and $P_{2i}$ are the logs of the rice yields in regimes 1 and 2, respectively; $H_i$ is a matrix of covariates that are, hypothetically, the determinants of net farm income and $v_{1i}$ and $v_{2i}$ are the stochastic error terms. The stochastic error terms were assumed to have a trivariate normal distribution, with a zero mean and non-singular covariance matrix, as expressed in Equation (4):

$$\operatorname{cov}(\varepsilon_i v_1 v_2)\begin{vmatrix} \sigma_1^2 & \sigma_{12} & \sigma_{1\varepsilon} \\ \sigma_{12} & \sigma_2^2 & \sigma_{2\varepsilon} \\ \sigma_{1\varepsilon} & \sigma_{2\varepsilon} & \sigma^2 \end{vmatrix} \tag{4}$$

where, $\sigma_1^2 = \text{var}(v_1)$; $\sigma_2^2 = \text{var}(v_2)$; $\sigma^2 = \text{var}(\varepsilon_1)$; $\sigma_{12} = \text{cov}(v_1, v_2)$; $\sigma_{1\varepsilon} = \text{cov}(v_1, \varepsilon_i)$; $\sigma_{2\varepsilon} = \text{cov}(v_2, \varepsilon_i)$; $\sigma^2$ represents the variance of the error term in the selection equation; while $\sigma_1^2$, $\sigma_2^2$ indicate the variance of the stochastic error term in the generated equation.

According to Maddala (1983), when latent characteristics are related to selection bias, the structure of the error might arise because the error term, $\varepsilon_i$, of the selection Equation (2) is correlated with the error terms, $v_{1i}$ and $v_{2i}$, of the generated Equations (3a) and (3b), with the expected values of $v_{1i}$ and $v_{2i}$ being conditional on sample selection being non-zero.

$$E(v_{1i} \mid Y_i = 1) = E(v_{1i} \mid \varepsilon_i > -F_i\beta) = \sigma_{1\varepsilon}\left[\frac{\theta(F_i\beta/\sigma)}{\phi(F_i\beta/\sigma)}\right] \equiv \beta_{1\varepsilon}\gamma_1 \tag{5a}$$

$$E(v_{2i} \mid Y_i = 0) = E(v_{2i} \mid \varepsilon_i \le -F_i\beta) = \sigma_{2\varepsilon}\left[\frac{-\theta(F_i\beta/\sigma)}{1-\phi(F_i\beta/\sigma)}\right] \equiv \beta_{2\varepsilon}\gamma_2 \tag{5b}$$

where, $\theta$ and $\varphi$ are the PDF and CDF of the standard normal distribution, respectively. The ratio of $\theta$ and $\varphi$ were evaluated at $\beta F_i$, as represented by $\gamma_1$ and $\gamma_2$ in Equations (5a) and (5b). This ratio is the inverse mills ratio (IMR), which indicates the selection bias terms. The IMR shows the correlation between the RADP participation and net farm income of smallholder farmers. Previous studies used the two-stage endogenous switching model (Fuglie & Bosch, 1995). A probit model of the selection equation was estimated in the first stage, and the IMRs $\gamma_1$ and $\gamma_2$ were predicted as indicated in Equations (5a) and (5b). The second stage involved adding the derived IMRs to Equations (3a) and (3b), respectively, with the following sets of equations being formed:

$$P_{1i} = \lambda_1 H_i + \beta_{1\varepsilon}\gamma_1 + \varphi_{1i} Y_{1i} + \psi_1 \tag{6a}$$

$$P_{2i} = \lambda_2 H_i + \beta_{2\varepsilon}\gamma_2 + \varphi_{2i} Y_{2i} + \psi_2 \tag{6b}$$

The coefficient of the variables $\gamma_1$ and $\gamma_2$ gave parameter estimates of the covariance terms $\beta_{1\varepsilon}$ and $\beta_{2\varepsilon}$, respectively. Through estimating variables $\gamma_1$ and $\gamma_2$, the standard errors of the two-stage estimates could not be calculated using the residuals $\psi_1$ and $\psi_2$. Heteroskedastic errors are always confounded with methods where IMRs are manually inserted from probit equations into the generated equations. A full information maximum likelihood (FIML), as proposed by Lokshin and Sajaia (2004), represents an efficient method for analysing endogenous switching regression models. The FIML simultaneously fits the selection equation and the generated equations (Equation (1) and Equations (3a) and (3b), respectively) to yield consistent standard errors. In turn, this makes$\gamma_1$ and $\gamma_2$ in Equations (6a) and (6b), respectively, homoscedastic. The log likelihood function of the FIML for the switching regression model employed in this study followed that proposed by Lokshin and Sajaia (2004):

$$LnY_i = \sum_{i=1}^{N} \left\| \begin{matrix} Y_i t_i \left[ \ln Q(\frac{F_i\beta + \sigma_{1\varepsilon}(P_{1i} - H_{1i}\lambda / \varphi_1)}{\sqrt{1-\alpha_{1i}^2}}) + \ln(q(P_{1i} - H_{1i}\lambda / \varphi_1)) \right] + \\ (1-Y_1)t_i \left[ \frac{\ln(1 - Q(F_i\beta + \sigma_{2\varepsilon}(P_{2i} - H_{2i}\lambda / \varphi_2)))}{\sqrt{1-\alpha_{2\varepsilon}^2}} + \ln(q(P_{2i} - H_{2i}\lambda / \varphi_2)) \right] \end{matrix} \right\| \tag{7}$$

According to Fuglie and Bosch (1995), the signs of the correlation coefficients $\alpha_{i\varepsilon}$ and $\alpha_{2\varepsilon}$ have economic meanings. If $\alpha_{i\varepsilon}$ and $\alpha_{2\varepsilon}$ have alternate signs, RADP participation based on their comparative advantages. For instance, farmers who participated would have above-average net farm income, while those who did not participate would have below-average net farm income. However, if the coefficient has the same sign, participants would have above-average net farm income whether they participated or not but would be better off if they participated. In comparison, non- participant would have below-average net farm income in either case but would be better off if they decided not to participate. As posited by Khanal et al. (2018), and Ojo et al. (2019) the current study shows how an endogenous switching treatment regression model determines counterfactual effects and the effects of participation. The counterfactual effect is the net farm income by the participants that would have been derived if the characteristics of the net farm income had been the same as the characteristics of the net farm income of non-participants, and vice versa. The change to the net farm income of farmers as a result of participation in RADP was estimated as the difference between Equations 3a and 3b, which were termed the average treatment effects on the treated (ATT):

$$ATT = E(P_{1i} - P_{2i} \mid Y_i = 1) = H_i(\lambda_1 - \lambda_2) + (\sigma_{1\pi} - \sigma_{2\pi})\gamma_1 \tag{8}$$

In Equation (3), $E(P_{1i} \mid Y_i = 1) = \lambda_1 H_i - \sigma_{1\mu}\gamma_1$ represents the expected outcome for the participants, had they participated, while $E(P_{2i} \mid Y_i = 1) = \lambda_2 H_i - \sigma_{2\mu}\gamma_1$ represents the expected net farm income for farming households that participated had they chosen not to participate in RADP programme.

## 4. Results and Discussion

### *4.1 Descriptive Statistics*

Table 1 presents the description of variables and their units of measurement. A thorough search from pieces of literature shows that farmers' socioeconomic, farm-specific and policy or institutional variables influence participation in RADP programme among smallholder farmers. For instance, it is expected that a farmer with a higher educational level understands farm management practices and that can enhance productivity and efficiency of resource use. As posited by Myeni et al. (2019), Ojo and Baiyegunhi (2020b) farmers with satisfactory level of education are capable to process, interpret, analyze and respond to innovations for adoptions for sustainable agricultural management practices. Hence, this is line with the study of Ukhanal et al. (2018) who stated that farmers with a higher level of education are more likely to have better access to information to implement better farming strategies. Hence, the study hypothesized a positive effect of the number of years in formal education on participation in RADP programme.

Table 1. Descriptive statistics of the sampled farmers

| Variable | Description | Mean | SD |
|---|---|---|---|
| Ln Income | Log of Income of the farmers (Rands) | 12.23 | 1.76 |
| Gender | 1 = if farmer is male | 0.58 | 0.50 |
| Age | Age of the farmer in years | 49.72 | 12.71 |
| Marital status | 1 = if farmer is married | 0.81 | 0.68 |
| Formal education | 1 = if farmer had access to formal education | 0.69 | 0.67 |
| Off-farm income | 1 = if farmer engaged in off-farm economic activities | 0.64 | 0.47 |
| Household size | The number of persons in a household (count) | 4.65 | 1.24 |
| Farming experience | Number of years in farming | 10.9 | 3.87 |
| Access to extension | 1 = if farmer had access to extension services | 0.58 | 0.47 |
| Access to credit | 1 = if farmer had access to credit | 0.58 | 0.49 |
| Legal entity | 1 = if farmer had access to legal entity | 0.48 | 0.40 |
| Farm potential income at acquisition | Amount of income at acquisition (Rands) | 14.24 | 1.13 |
| Tax compliance | 1 = if farmer is tax compliant | 0.37 | 0.48 |
| Mentorship | 1 = if farmer had access to mentorship | 0.17 | 0.37 |
| Strategic partnership | 1 = if farmer had access to partnership | 0.54 | 0.50 |
| Project contract signed | 1 = If farmer signed the contract | 0.69 | 0.50 |
| Farm- based organizations (FBO) | 1 = if farmer belongs to FBO | 0.34 | 0.47 |

Generally, the average age of respondents is about 49 years and about 80% of the sampled farmers are married. While about 64% of farmers were engaged in off-farm economic activities, the average number of persons in a household is about five. Similarly, the number of years in crop farming (proxied for experience) is expected to have positive effects on participation in RADP programme and its impact on net farm income. This is because, with more years of farming, farmers understand the agricultural production environment and process market information, which subsequently increases the likelihood of participating in on participation in RADP programme. About 70% of the respondents had a signed contractual agreement. These findings confirmed that the majority of South African PLAS land reform farmers do have readily available market with contract agreements but have no command or bargaining power since that the majority do not grade produce before selling. Therefore, a crucial role needs to be played by key stakeholders in agricultural development is to capacitate farming in marketing.

Table 2. Full information maximum likelihood (FIML) estimates of the endogenous switching regression model (ESRM)

| Variables | Participation in RADP Programme | | | Farm income | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | RADP Beneficiaries | | | Non-RADP Beneficiaries | | |
| | Coef. | Std. Err. | P-value | Coef. | Std. Err. | P-value | Coef. | Std. Err. | P-value |
| Age | 0.060 | 0.027 | **0.029**** | 0.026 | 0.023 | 0.251 | 0.010 | 0.015 | 0.496 |
| Farm potential income at acquisition | 0.391 | 0.229 | **0.088*** | 0.307 | 0.226 | 0.175 | 0.118 | 0.196 | 0.547 |
| Access to non-farm income | -0.064 | 0.535 | 0.905 | 0.185 | 0.487 | 0.703 | -0.587 | 0.410 | 0.152 |
| Strategic partnership | 1.243 | 0.631 | **0.049**** | 1.534 | 0.666 | **0.021**** | 1.700 | 0.899 | **0.059*** |
| Secondary education | -0.052 | 0.446 | 0.907 | 0.324 | 0.428 | 0.449 | 0.450 | 0.394 | 0.254 |
| Legal entity | 0.127 | 0.868 | 0.883 | 0.560 | 1.473 | 0.704 | 1.097 | 0.469 | **0.019**** |
| Mentorship | 0.188 | 0.580 | 0.746 | 0.741 | 0.492 | 0.132 | -0.533 | 0.787 | 0.498 |
| Farmer's receiving 3rd party assistance | 0.321 | 0.643 | 0.618 | -0.928 | 0.839 | 0.269 | 1.253 | 0.750 | **0.095*** |
| Tax compliance | 1.655 | 0.527 | **0.002***** | 1.881 | 0.735 | **0.010**** | 0.726 | 0.613 | 0.236 |
| Gender | 0.381 | 0.494 | 0.441 | | | | | | |
| Project contract signed | 0.466 | 0.462 | 0.313 | | | | | | |
| Farming experience | -0.020 | 0.022 | 0.354 | | | | | | |
| Farmers organizations/Associations | 1.006 | 0.454 | **0.027**** | | | | | | |
| Constants | -11.165 | 4.152 | **0.007***** | 5.031 | 4.205 | 0.232 | 8.788 | 3.087 | **0.004***** |
| **/lns1** | **0.234** | **0.107** | | | | | | | |
| **/lns2** | **0.280** | **0.109** | | | | | | | |
| **/r1** | **0.167** | **0.465** | | | | | | | |
| **/r2** | **0.576** | **0.552** | | | | | | | |
| **sigma_1** | **1.263** | **0.135** | | | | | | | |
| **sigma_2** | **1.324** | **0.144** | | | | | | | |
| **rho_1** | **0.165** | **0.452** | | | | | | | |
| **rho_2** | **0.520** | **0.403** | | | | | | | |
| **LR test of indep** | **10.80** | | | | | | | | |
| **Prob > chi2** | **0.001** | | | | | | | | |
| **Loglikelihood** | **-202.364** | | | | | | | | |
| **Wald chi2 (14)** | **15.74** | | | | | | | | |
| **Prob > chi2** | **0.072** | | | | | | | | |

*Note*. ***, **, and * represent significance level at 1%, 5%, and 10%, respectively.

### *4.2 Results From Full Information Maximum Likelihood (FIML) Estimation of the Endogenous Switching Regression Model (ESRM)*

The result is subjected to a more rigorous estimation method by employing the full information maximum likelihood (FIML) ESRM (Table 2). The FIML ESR model involves a selection equation and separate outcome equations for RADP beneficiaries and non-RADP beneficiaries, which are estimated simultaneously with factors influencing participation in RADP

The results are based on the factors influencing the participation of land reform beneficiaries in RADP and farm income estimates for both participating RADP beneficiaries and non-RADP beneficiaries using the endogenous switching regression model (ESR), and the results are presented in Table 2 above. The covariance terms (Constants) for RADP Participation and non-RADP beneficiaries equations are both statistically significant at the 1% level. The statistical significance of the covariance terms implies that the application of the ESR in the empirical estimation is suitable. The results of the ESRM estimation are presented in Table 2, with the second column showing the factors influencing participation of farmers in RADP. The results showed that the coefficients of age, farm potential income at acquisition, strategic partnership, and tax compliance were statistically significant in influencing the participation in RADP.

The age variable had a significantly positive influence on the participation in RADP. These results show that there was 6% of probability of "middle age" beneficiaries are significant to influence the participation in RADP. The result implies that participation in RADP increase the likelihood of older beneficiaries. The mean beneficiaries are getting older as indicated by their mean age of 50 years which is below 62, the average age of farmers in south Africa, (Sihlobo, 2015; Thinda et al., 2020). Hence, the more farmers get old the less is the production, workforce and income. This is in line with the study of Mahembe (2001) who also found a strong correlation between the age of an enterprise and its risk profile in their study on literature review on small and medium enterprises' access to credit and support in South Africa.

The coefficient of farm potential income at acquisition had a significantly positive influence on participation of beneficiaries in RADP. This implies that the participation on RADP increase the likelihood of the farm potential income at acquisition. This is not unconnected with the regulations of Department Rural Development and Land Reform (DRDLR) (2014), with an aim to revitalize poor performing and with a low farm potential income at acquisition through RADP programme. Hence, this is in line with the study of Nenngwekhulu (2019) who also found a positive relationship between RADP and farm income in their study on financial analysis of the RADP in South Africa.

The coefficient of strategic partnership had a significantly positive influence on the participation of land reform beneficiaries in RADP. The result implies that participation of land reform beneficiaries in RADP increase the likelihood of working with strategic partners. This is not unconnected with the regulations of Department Rural Development and Land Reform (DRDLR) (2014), that farmers must be in profit & risk sharing based shareholding mechanisms with strategic partner(s) for farm sustainability. This is in line with the study of Sibisi, (2015) who also a positive relationship between strategic partners and land reform farmers in their study on importance and role of stakeholders involved in support services of land reform in South Africa.

The coefficient of tax compliance had a significantly positive influence on participation of beneficiaries in RADP. The result implies that the participation in RECAP programme increases the likelihood of being tax compliant. This is not unconnected with the regulations of Department Rural Development and Land Reform (DRDLR) (2014), that all beneficiaries must comply with South African Revenue Services (SARS) requirements and a tax clearance certificate must be provided to the DRDLR on an annual basis. This is in line with the study of De Janvry et al., (2015) who also found a positive relationship between tax compliance and migration in their study on delinking land rights from land use in Mexico.

### *4.3 Impact of RADP on Performance of Land Reform Farmers on Farmers' Net Farm Income*

The estimates of the second stage of the ESRM on the impact of recapitalization and development programme on performance of land reform farmers of participation on RADP on the net farm income of beneficiaries (RADP and non-RADP) are presented in the third and fourth columns of Table 2. The coefficients of strategic partnership, legal entity, farmer receiving third party assistance and tax compliance were statistically significant in explaining differences in the net farm income of RADP and non-RADP beneficiaries in land reform. For the non-RADP beneficiaries, the coefficients of strategic partnership, legal entity and farmer's receiving third party assistance were statistically significant in explaining differences in the net farm income of land reform farmers.

The strategic partnership had a significantly positive influence in explaining variation in the net farm income of both RADP and non-RADP beneficiaries of land reform. Thus, this is not unconnected with the regulations of Department Rural Development and Land Reform (DRDLR) (2014), that farmers must be in profit & risk sharing based shareholding mechanisms with strategic partner(s) for farm sustainability. The result on both RADP Beneficiaries and Non-RADP Beneficiaries implies that the net farm income increases the likelihood for intervention of strategic partners. Furthermore, indicates that on Non-RADP beneficiaries the farm income is more likely to be increased by the engagement with strategic partners since never benefited from RADP. This is in line with the study of Sibisi (2015) who also a positive relationship between strategic partners and land reform farmers in their study on Agricultural extension and post-settlement support of land reform beneficiaries in South Africa: the case of Ixopo in the province of KwaZulu-Natal.

The establishment legal entities (co-operatives, CPA, and private companies) had a significantly positive influence in explaining variation in the net farm income of just the non-RADP beneficiaries of land reform. The result implies that Non-RADP beneficiaries increases the likelihood of possessing legal entities in other to increase farm income as a result of structured entities with roles and responsibilities. This is in line with the study of Ojo and Baiyegunhi, (2020a) who found that rice farmers being in cooperatives had a significantly positive influence in explaining the variation in net farm income in the study of perception and economic impact of climate change on rice production in South-West, Nigeria. Furthermore, Ntlou (2016) stated that the group formation of beneficiaries should be taken into deliberation that group members' interests may differ, even though they all want to farm.

The farmer's receiving third party assistance through farmer-to-farmer approach and consultations had a significantly positive influence in explaining variation in the net farm income of just the non-RADP beneficiaries of land reform. The result implies that Non-RADP beneficiaries since RADP is not made available to them the likelihood to increase the net farm income is seen on the engagement with other farmers and consultants. This is in line with the study of Abdulai and Huffman (2014) who also found a positive relationship between extension agents and access technology in their study on the adoption and impact of soil and water conservation technology in USA.

The tax compliance had a significantly positive influence in explaining variation in the net farm income of just the RADP beneficiaries of land reform. The result implies that the participation in RADP increases the likelihood of being tax compliant and increase the likelihood of positive net farm income. This is not unconnected with the regulations of Department Rural Development and Land Reform (DRDLR) (2014), that all beneficiaries must comply with South African Revenue Services (SARS) requirements and a tax clearance certificate must be provided to the DRDLR on an annual basis. This is in line with the study of De Janvry et al. (2015) who also found a positive relationship between tax compliance and migration in their study on delinking land rights from land use in Mexico.

#### 4.3.1 Treatment Effects for the RADP Participation—Endogenous Switching Regression Treatment Effect

This study estimated endogenous switching regression with the inclusion of RADP beneficiary among the smallholder farmers for treating the endogeneity problem as presented in Table 3.

Table 3. Treatment effects for the RADP participation—endogenous switching regression treatment effect

| **Treatment effects** | **Coefficient** | **Std.** |
|---|---|---|
| Average treatment effect (ATE) | 18.24*** | 4.71 |
| Average treatment on the treated (ATT) | 14.66*** | 1.71 |

The results show a positive and significant effect on participation in RADP . A simple considerable difference in the average income between the participants and non-participants of participation in RADP in impact evaluation studies is misleading as they usually fail to control for potential differences in the characteristics between the two groups. The estimate from the endogenous switching regression model can also be inadequate even if not misleading though it accounts for endogeneity. This is because direct coefficients from the model cannot be considered as ATT since the issue of missing data (counterfactual scenario) has not been accounted for. To account for this, the study turned to the results of the causal effects of the participation in RADP on farmers' net income using ATE and ATT, where the switching regression with endogenous treatment was used and then complement it with IPWRA as a robustness check. Hence, the estimates from the endogenous switching regression are discussed first. ATE and ATT were estimated after fitting the endogenous regression with endogenous treatment effects (Note 2). As indicated in Table 3, the estimated potential outcome means (ATE) of participation in RADP programme on net farm income by farm households is about 18.24 and statistically significant at 1%. The ATE estimate suggests that an average farm household in the study area will make about R18 more net farmincome if he participates in RADP programme. In the same vein, the conditional treatment effects which measure the ATT of participating in RADP on the net farm income adopted is about 14.66 and also statistically significant at 1%. Thus, the average farm household participating in RADP would realize about R15 more of net farm income than it would if it did not participate in RADP programme.

#### 4.3.2 Treatment Effects for the RADP Participation—Doubly-Robust Inverse Probability Weighted Regression Adjustment

The ex-post estimates of the causal effects of the adoption of SWC on rice productivity of smallholder farmers from the IPWRA are presented in Table 4.

Table 4. Treatment effects for RADP participation—inverse-probability-weighted regression adjustment

| **Treatment effects** | **Coefficient** | **Std. Err.** |
|---|---|---|
| Average treatment effect (ATE) | 1.641*** | 0.556 |
| Average treatment on the treated (ATT) | 5.708*** | 1.247 |
| Potential-outcome mean (POM) | 17.304*** | 0.766 |

*Note*. The bootstrap replications were changed from 100-1,000 but no significant change occurred, hence 500 replications were used to bootstrap the standard errors.

From Table 4, the ATE and POM are approximately two (2) and seventeen (17), respectively. Thus, the average net farm income if all of our sampled farmers were to participate in RADP would be two times more the average of seventeen that would occur if none of the farmers had participated in RADP. Likewise, RADP participants treated group realized 5.7 more net farm income than they would have if they did not participate RADP programme.

The results from the two estimation techniques indicate that participation in RADP programme significantly increases the net farm income. The results of the average causal effects reported in Tables 3 and 4 indicate that the magnitudes of the estimates of the outcome variables are divergent between endogenous switching regression and IPWRA. This divergence in the results of both may be due to differences in unobserved heterogeneity among smallholder farmers (Danso-Abbeam & Baiyegunhi, 2018). The positive impact of RADP participation on performance of smallholder farmers agrees with the studies of Worku et al. (2020), and Martey et al. (2020) in Eastern Africa and Ghana, respectively. The results of the study suggest that the participation of smallholder farmers in RADP programme increases the propensity of improved net farm income as compared to those who did not participate in RADP programme (Ojo et al., 2019, 2020a). The implication of these results reflects the important role of RADP for improved agricultural production.

## 5. Conclusion

Based on the results reported above we find that the factors such as age of the farmer, farm potential income at acquisition, strategic partnership, and tax compliance significantly influence on the participation of farmers in RADP. Programmatic interventions to increase the participation of the farmers - benefiting from land reforms- in the RADP needs to identify opportunities that can increase income farmers. Strategic partnership is significant factor in explaining differences in the net farm income of RADP and non-RADP beneficiaries in land reform. For the non-RADP beneficiaries, the factors—strategic partnership, legal entity and farmer's receiving third party assistance were statistically significant in explaining differences in the net farm income. And for RADP beneficiaries, strategic partnership and tax compliance were statistically significant in explaining differences in the net farm income. This indicates that the magnitudes of the estimates of the outcome variables are divergent between endogenous switching regression and IPWRA. This divergence in the results of both may be due to differences in unobserved heterogeneity among land reform beneficiaries. However, there are still more interventions required both from private and public sector to enhance the performance of post-settlement support to improve farmers livelihood and farm development. Hence, through the strategic partnership of RADP farmers had likelihood to improve the farm and increase farm income. The study's results suggest that the RADP can contribute to a deep process of change and empowerment of farmers. In the same vein, strategic partnership of RADP is likely to improve the farmers' performance. Therefore, there is a need to strongly improve mentorship and strategic partnership programme to encourage participation of land reform farmers in the support programmes.

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## References

Abdulai, A., & Huffman, W. (2014). The Adoption and Impact of Soil and Water Conservation Technology: An Endogenous Switching Regression Application. *Board of Regents of the University of Wisconsin System, 90*(1), 26-43. https://doi.org/10.3368/le.90.1.26

Adams, M. & Howell, J. (2001). Redistributive Land Reform in Southern Africa. In J. Farrington & A. Wyles (Eds.), *Natural Resource Perspective*. London: Overseas Development Institute. Retrieved from http://dlc.dlib.indiana.edu/dlc/bitstream/handle/10535/3779/64-redistributive-land-reform-southern-africa.pdf?sequence=1

Anseeuw, W., Liebenberg, F., & Kirsten, J. (2015). Agrarian reform in South Africa: Objectives, evolutions and results at national level. *South Africa's Agrarian Question*, 28-51. Retrieved from https://hal.archives-ouvertes.fr/hal-01376094/file/South_Africa%27s_Agrarian_Question_-_eBook.pdf#page=55

Aravindakshan, S., Rossi, F., Amjath-Babu, T. S., Veettil, P. C., & Krupnik, T. J. (2018). Application of a bias-corrected meta-frontier approach and an endogenous switching regression to analyze the technical efficiency of conservation tillage for wheat in South Asia. *Journal of Productivity Analysis, 49*(2-3), 153-171. https://doi.org/10.1007/s11123-018-0525-y

Byamugisha, F. (2014). *Agricultural Land Redistribution and Land Administration in Sub-Saharan Africa: Case Studies of Recent Reforms*. Washington, DC: The World Bank. https://doi.org/10.1596/978-1-4648-0188-4

Crossman, A. (2020). *Understanding stratified samples and how to make them*. Retrieved January 29, 2021, from https://www.thoughco.com/stratified-sampling-3026731

De Janvry, Alian, Emerick, K., Gonzalez-Navarro, M., & Sadoulet, E. (2015). Delinking Land Rights from Use: Certification and Migration in Mexico. *American Economic Review, 105*(10), 3125-49. https://doi.org/10.1257/aer.20130853

DRDLR. (2013). *Three Years Review of Recapitalization and Development Programme: September 2013*. Pretoria: Department of Rural Development and Land Reform.

DRDLR. (2014). *Revised Policy for the Recapitalisation and Development Programme of the Department of Rural Development and Land Reform*. Pretoria: Department of Rural Development and Land Reform.

Dudovskiy, J. (2018). *The Ultimate Guide to Writing a Dissertation in Business Studies: A Step-by-Step Assistance*. Retrieved December 16, 2019, from https://research-methodology.net/about-us/ebook

Fuglie, K. O., & Bosch, D. J. (1995). Economic and environmental implications of soil nitrogen testing: A switching-regression analysis. *American Journal of Agricultural Economics, 77*(4), 891-900. https://doi.org/10.2307/1243812

Khanal, P., Wagle, B. H., Upadhaya, S., Ghimire, P., & Acharya, S. (2019). Perceived Climate Change Impacts and Adaptation Strategy of Indigenous Community (Chepangs) in Rural Mid-hills of Nepal. *Forestry: Journal of Institute of Forestry, 16*, 48-61. https://doi.org/10.3126/forestry.v16i0.28353

Lokshin, M., & Sajaia, Z. (2004). Maximum likelihood estimation of endogenous switching regression models. *The Stata Journal, 4*(3), 282-289. https://doi.org/10.1177/1536867X0400400306

Lutabingwa, J. (2007). Data Analysis in Quantitative Research. *Journal of Public Administration, 42*(6), 528-548.

Maddala, G. S. (1983). Methods of estimation for models of markets with bounded price variation. *International Economic Review*, 361-378. https://doi.org/10.2307/2648751

Mafora, M. (2014). *Socio-economic Analysis of Land Reform Projects at Elias Motsoaledi Local Municipality in the Limpopo Province of South Africa: Comparing the degree of contribution to food security* (Masters Dissertation, University of South Africa, South Africa).

Mahembe, E. (2001). *Literature review on small and medium enterprises' access to credit and support in South Africa*. South Africa, Pretoria: National Credit Regulator. Retrieved from https://www.smallbusinessinstitute.co.za/wp-content/uploads/2019/12/NCRLiterature-Review-on-SME-Access-to-Credit-in-South-Africa_Final-Report_NCR_Dec-2011.pdf

Maka, L., & Aliber, M. (2019). The role of mentors in land reform projects supported through the recapitalisation and development programme: findings from Buffalo city metropolitan municipality, South Africa. *South Africa Journal Agricultural Extension, 47*(2), 37-45. https://doi.org/10.17159/2413-3221/2019/v47n2a501

Martey, E., Etwire, P. M., & Kuwornu, J. K. (2020). Economic impacts of smallholder farmers' adoption of drought-tolerant maize varieties. *Land Use Policy, 94*, 104524. https://doi.org/10.1016/j.landusepol.2020.104524

Martin, A. (2000). *Breaking Ground: Development Aid for Land Reform*. London, UK: Overseas Development Institute.

Nenngwekhulu, N. (2019). *Financial analysis of the Recapitalisation and Development Programme in South Africa* (Doctoral Dissertation, University of Pretoria, South Africa).

Nenngwekhulu, N. (2019). *Financial analysis of the Recapitalisation and Development Programme in South Africa* (Masters Dissertation, University of Pretoria, South Africa).

Nthai, M. M. (2020). Funding Rural Development in Post-Apartheid South Africa's Land Reform Programme. *African Perspectives on Reshaping Rural Development* (pp. 118-139). IGI Global. https://doi.org/10.4018/978-1-7998-2306-3.ch006

Ntlou, T. (2016). *Potential for economic sustainability of land reform projects benefiting from the Recapitalisation and Development Programme in South Africa* (Masters Dissertation, University of Pretoria, South Africa).

Ojo, T. O., & Baiyegunhi, L. J. S. (2020). Determinants of climate change adaptation strategies and its impact on the net farm income of rice farmers in south-west Nigeria. *Land Use Policy*, 103946. https://doi.org/10.1016/j.landusepol.2019.04.007

Ojo, T. O., Baiyegunhi, L. J. S., & Salami, A. O. (2019). Impact of Credit Demand on the Productivity of Rice Farmers in South West Nigeria. *Journal of Economics and Behavioral Studies, 11*(1), 166-180. https://doi.org/10.22610/jebs.v11i1(J).2757

Phatudi-Mphahlele, M. (2016). *Analysis of Socio-economic Impact of Comprehensive Agricultural Support Programme on Agrarian Reform Farmers of Sedibeng District Municipality in Gauteng Province South Africa* (Masters Dissertation, University of South Africa, South Africa).

Sibisi, N. (2015). *Agricultural Extension and Post-Settlement Support of Land Reform Beneficiaries in South Africa: The Case of Ixopo in the province of Kwazulu-Natal* (Masters Dissertation, University of Kwazulu-Natal, South Africa).

Sihlobo, W. (2015). *The youth are the agriculture's future*. Retrieved December 16, 2019, from https://mg.co.za/article/2015-06-18-the-youth-are-agricultures-future/?amp=1

Staal, B. M. (2019). *Land reform in South Africa: A case study of the Witzenberg pals initiative* (Doctoral Dissertation, Stellenbosch University, Stellenbosch, South Africa).

Taherdoost, H. (2016). Sampling Methods in Research Methodology; How to Choose a Sampling Technique for Research. *International Journal of Academic Research in Management, 5*(2), 18-27. https://doi.org/10.2139/ssrn.3205035

Thinda, K. T., Ogundeji, A. A., Belle, J. A., & Ojo, T. O. (2020). Understanding the adoption of climate change adaptation strategies among smallholder farmers: Evidence from land reform beneficiaries in South Africa. *Land Use Policy, 99*, 104858. https://doi.org/10.1016/j.landusepol.2020.104858

University of the Western Cape. (2016). *Diagnostic Report on Land Reform in South Africa: Commissioned Report for High Level Panel on the Assessment of Key Legislation and the Acceleration of Fundamental Change, an Initiative of the Parliament of South Africa*. University of the Western Cape, South Africa.

Uzunoz, M., & Akcay, Y. (2012). A Case Study of Probit Model Analysis of Factors Affecting Consumption of Packed and Unpacked Milk in Turkey. *Economics Research International, 2012*, Article ID 732583. https://doi.org/10.1155/2012/732583

Van den Brink, R., Glen, T., Hans, B., Bruce, J., & Byamugisha, F. (2006). *Consensus, Confusion, and Controversy: Selected Land Reform Issues in Sub-saharan Africa*. Washington, DC: The World Bank. https://doi.org/10.1596/978-0-8213-6440-6

van Noordwijk, M. (2019). Integrated natural resource management as pathway to poverty reduction: Innovating practices, institutions and policies. *Agricultural Systems, 172*, 60-71. https://doi.org/10.1016/j.agsy.2017.10.008

Weideman, M. (2003). *Land reform, Equity and Growth in South Africa: A Comparative Analysis* (PhD Thesis, University of Witwatersrand, Johannesburg, South Africa).

Worku, M., De Groote, H., Munyua, B., Makumbi, D., Owino, F., Crossa, J., … Mutinda, C. (2020). On-farm performance and farmers' participatory assessment of new stress-tolerant maize hybrids in Eastern Africa. *Field Crops Research, 246*, 107693. https://doi.org/10.1016/j.fcr.2019.107693

Zhang, J., Brown, C., Qiao, G., & Zhang, B. (2019). Effect of eco-compensation schemes on household income structures and herder satisfaction: lessons from the grassland Ecosystem Subsidy and Award Scheme in Inner Mongolia. *Ecological Economics, 159*, 46-53. https://doi.org/10.1016/j.ecolecon.2019.01.006

**Notes**

Note 1. For this study, the decision of farmers to RADP participation in response to an improved net farm income was a dummy variable, taking the value 1 as a participant and 0 as a non-participant.

Note 2. ATE and ATT were estimated as a post-estimation after fitting the Stata command teffects for endogenous switching regression with endogenous treatment. The ATE estimated after teffects is the potential outcome means while ATT is the conditional treatment effect.

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# Genetic Evaluation and Correlation Analysis Among Various Quantitative Traits in Maize Single-Cross Hybrids Under Diverse Environments

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**Abstract**

Genetic variation abundance, high genetic advance coupled with high heritability estimates presents the most suitable condition for selection. Ninety-five hybrids generated from elite and new inbred lines crossed using half diallel mating design were evaluated under diverse environments. The objectives were to estimate genetic variances, heritability of traits and genetic advance and to determine correlations of grain yield and its component characters in maize hybrids. Analysis of variance revealed significant differences among genotypes for all traits studied except for ear rots. Estimates of phenotypic coefficient of variation were slightly higher than genotypic coefficient of variation for all traits suggesting low influence of environment in the expression of these traits. High heritability and genetic estimates were recorded for grain yield (79%; 30.27%), plant height (85%; 102.42%) and ear height (86%; 117.15%) whilst high heritability and low genetic advance were observed for anthesis date (87%; 5.8%), texture (75%; 8%) and ear position (71%; 0.23%). Correlation between environments using grain yield data revealed existence of a very strong positive correlation between CIMMYT2 and RARS2 suggesting that the sites have the same discriminating effect. Correlation among traits revealed that grain yield had significant ($P < 0.05$) positive correlation with plant height and ear height. Similarly, plant height had significant and positive correlation with ear height while ear position was positively correlated to ear height. Path analysis showed that plant height, ears per plant and ear position had positive direct effects on grain, while anthesis date, ear height, ear position, grain moisture content at harvest and texture indirectly influenced grain yield. These characters' contribution to grain yield is important and the strong association with grain yield implied that these can be used as secondary traits to indirectly select for grain yield performance in this set of germplasm across all the environments.

**Keywords:** maize, single-cross hybrids, direct selection, genetic advance, heritability, path analysis

## 1. Introduction

Maize (*Zea mays* L.) plays a crucial role in food security and livelihoods of people in the sub-Saharan Africa (SSA) (Kassie et al., 2017). Maize yield in SSA average is 2.1 tons per hectare which is much lower compared to world average of 5.6 tons per hectare (FAOSTAT, 2017). Abiotic stresses such as heat, drought and poor soils with low nitrogen hinder maize productivity in the region (Makumbi et al., 2018; Das et al., 2019). In the current study the focus was on estimating genetic parameters of hybrids evaluated under diverse environments. Genetic parameters such as genetic variability and heritability estimates are crucial in improving selection efficiency and in determining breeding progress. Several authors have reported existence of genetic variability and high heritability estimates among tested maize genotypes (Meena et al., 2016; Jilo et al., 2018; Bartaula et al., 2019). Heritability is a measure of phenotypic variance attributable to genetic causes with predictive function in plant breeding (Meena et al., 2016). Traits with high heritabilities can be easily fixed with simple selection and breeding efficiency can be enhanced (Bello et al., 2012). Environmental changes and complexity of yield and its components makes it difficult to explore genetic variability in maize. Indirect selection for yield through selecting traits closely related to yield may be the most efficient technique in yield improvement (Musundire et al., 2019). Selection of best genotypes using grain yield alone under stress conditions is not effective but can be improved via improvement of yield component traits such as anthesis date, anthesis silking interval, plant height, ears per plant among others (Mhike et al., 2012).

Correlation coefficients elaborate the degree of trait association among important quantitative traits and inter relationships of characters under study (Kumar & Babu, 2015; Malik et al., 2005). Several authors reported high positive and significant correlation coefficients of plant height and ear height with grain yield (Raghu et al., 2011; Seshu et al., 2013: Alhussein et al., 2017). Path coefficient analysis reveals whether the association of traits with yield is caused by direct effects or indirect effects. Plant height showed positive indirect effects to ear height (Jakhar et al., 2017). Genetic correlation and path coefficients estimates are both essential in establishing direct, indirect and total causal effects of yield components. Although these techniques have been extensively studied by many authors there is no set rule on how much each yield component contributes to a particular maize plant population (Malik et al., 2005). The purpose of this study was to estimate genetic parameters such as; genetic variation, heritability, expected genetic advance and trait association of quantitative characters for future hybrid selection.

## 2. Method

### *2.1 Germplasm and Test Locations*

Six elite CIMMYT maize inbred lines (CMLs) and nine new lines were crossed to generate single-cross hybrids. CML311, CML312, CML393 are subtropical inbred lines and CML539, CML543, CML566 are mid-altitude elite CIMMYT lines. The new lines were developed by line breeding department breeders and their prominent traits are high grain yield and improved heat and drought stress tolerance and are still under testing. Experimental material comprised of 105 single-cross hybrids: 95 $F_1$ hybrids generated from a 15 × 15 half-diallel mating design. Ten commercial checks were used in the study as controls. The commercial checks used are hybrids available in the market (CML312/CML444, SC649, PAN7M81, SC653, SC608, SC719, SC633, SC608, SC727, and CZH15429). The trials were conducted in Zimbabwe and Zambia during the 2017/18 and 2018/19 seasons at: CIMMYT Harare (CIMMYT = low N; CIMMYT2 = optimal), Rattray Arnold Research station (RARS = low N; RARS2 = optimal), Chiredzi Research station (CZ = heat stress; CZ2 = drought stress), ART farm = optimal, Mpongwe south (MS = optimal) and Lusaka west (LW = low N). Test locations description is shown in Table 1.

Table 1. Test locations description

| Site | Environment | Longitude | Latitude | Altitude (masl) | Annual rainfall (mm) | Annual temp Range (°C) |
|---|---|---|---|---|---|---|
| **ART** | Optimal | 31°03′E | 17°49′S | 1480 | 830 | 13-28.5 |
| **CIMMYT** | Managed Low N | 31°2′E | 17°5′S | 1483 | 1000 | 10-37 |
| **CIMMYT2** | Optimal | 31°2′E | 17°5′S | 1483 | 1000 | 11-37 |
| **RARS** | Managed Low N | 31°14′E | 17°14′S | 1300 | 918 | 12.8-28.6 |
| **Chiredzi (CZ)** | Heat stress | 31°17′E | 21°58′S | 425 | 450 | 22-31 |
| **Chiredzi (CZ2)** | Managed Drought | 31°17′E | 21°58′S | 425 | 450 | 23-31 |
| **RARS2** | Optimal | 31°14′E | 17°14′S | 1300 | 918 | 12.8-28.6 |
| **Lusaka West (LW)** | Managed Low N | 28°04′E | 15°24′S | oli1216 | 1000 | 14.2-28.9 |
| **Mpongwe South (MS)** | Optimal | 28°03′E | 13°32′S | 1206 | 1200 | 19.95-25.3 |

*Note*. masl = metres above sea level; MDS = managed drought stress; RARS = Rattray Arnold research station; ART = Agriculture Research Trust.

### *2.2 Experimental Design and Crop Management*

Field trials and agronomic management practices were done according to Badu-Apraku et al. (2012). A total of 105 genotypes (*i.e.*, 95 SXs + 10 checks) were evaluated and hybrid trials were laid out at each of the 9 sites using an alpha (0.1) lattice design (Patterson et al., 1978). At each site, hybrids were replicated twice, and each replicate nested 21 incomplete blocks, with a block size of five. Each hybrid was planted in a one-row plot, 4m long, with an inter-row spacing of 0.75 m and an intra-row spacing of 0.25 m. Two seeds were sown per each planting station, and later thinned to one plant per station at three weeks after crop emergence (WACE), in order to obtain a plant density of approximately 53 333 plants $ha^{-1}$. To eliminate variation due to border effects, commercial maize hybrids of similar vigor were used as borders (Davis et al., 1981). The hybrids were subjected to four different types of management regimes: optimal, managed drought, low nitrogen stress (Low N) and managed heat stress conditions.

*2.3 Variables Measured*

Observations were collected on 13 traits using standard procedures followed by CIMMYT (1985). Plant height (PH) was measured as distance between base of a plant and to the auricle of flag leaf, anthesis date (AD) as number of days to 50% pollen shedding from day of planting; anthesis silking interval (ASI) as the difference between anthesis days and silking days, ear height (EH) as distance between ground level and the base of the primary ear, ears per plant (EPP) as number of ears per plot as a fraction of number of plants/prolificacy, ear position (EPO) as the ratio of ear height to plant height, stem lodging (SL) as a percentage of plant per plot that had their stem broken, root lodging (RL) as a percentage of plant per plot which had their stems inclined by at least 45 degrees, ear rots (ER) as number of rotten cobs per plot, moisture content (MC) as a percentage grain moisture content at harvest, grain yield in tones $ha^{-1}$ (GYD) as grain mass per plot adjusted to 12.5% moisture content. Cob husk cover (HC) and grain texture (TEX) were also recorded for the maize hybrids.

*2.4 Statistical Analysis*

Variance components were calculated using DeltaGen software (Jahufer & Luo, 2018). Genotypic and phenotypic coefficients of variation were calculated as suggested by Singh and Chaudhary (1985):

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{\sigma^2 g}}{x} \times 100 \quad (1)$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{\sigma^2 p}}{x} \times 100 \quad (2)$$

GCV and PCV values obtained were categorized as low (0-10%), moderate (10-20%) and high (> 21%) according to Siva-Subramanian and Menon (1973).

Broad sense heritability for traits was estimated as the ratio of genotypic variance to phenotypic variance as a percentage (Singh & Chaudhary, 1985) as follows:

$$\text{Heritability (H)} = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e} \times 100 \quad (3)$$

where, $\sigma^2 g$ is genotypic variance, $\sigma^2 p$ is phenotypic variance $\sigma^2 e$ is environmental variance, $\sigma^2 p$ is $\sigma^2 g + \sigma^2 e$.

The heritability estimates were classified into 3 groups according to Elrod and Stanfield (1949); low (0-20%), moderate (20-50%) and high (> 50).

Genetic advance percentage of mean (GAM) was calculated using the formula suggested by Shuklar et al. (2006):

$$\text{Genetic advance of mean (GAM)} = \frac{K\sigma^2 p}{\mu} \times 100 \quad (4)$$

where, K is standardised selection differential constant (2.06) at 5% selection intensity; $\sigma^2 p$ as phenotypic variance and $\mu$ is the mean.

Correlations and path analysis were conducted using the package "agricolae" (De Mendiburu, 2009) in R software (R Core Team, 2020). Genotypic and phenotypic correlation coefficients were estimated as suggested by Kwon and Torrie (1964). Direct and indirect effects were estimated using path analysis as suggested by Dewey and Lu (1959) and developed by Wright (1921). Path coefficients were obtained by solving a set of simultaneous equations as follows:

$$r_{ny} = P_{ny} + r_{n2}P_{2y} + r_{n3}P_{3y} \quad (5)$$

where, $r_{ny}$ is the correlation between one component and yield; $P_{ny}$ is path coefficient between that character and yield; $r_{n2}$ represents correlation between that character and each of the other components in turn.

Path coefficients values were categorized the coefficient values according to Lenka and Mishra (1973) as: negligible (0.00-0.09), low (0.10-0.19), moderate (0.20-0.29) and high (0.30-0.99).

## 3. Results

### *3.1 Variations*

The traits analyzed in this study include: grain yield (GYD), anthesis date (AD), ear height (EH), plant height (PH), stem lodging (SL), root lodging (RL), husk cover (HC), moisture content (MC), anthesis silking interval (ASI), ear position (EPO), texture (TEX) and ear per plant (EPP) were significant at ($P < 0.05$) except for ear rots (ER). The coefficients of variation (CV) for most traits were low ranging from 6.21 to 28.2 except for stem lodging, husk cover and ear rots where the CVs were high; 187.9, 148.3 and 150.1 respectively. Grain yield ranged from 0.13 to 15 tons per hectare, plant height from 110 cm to 360 cm, anthesis silking interval from-4.5 to 8.5 days. Phenotypic and genotypic coefficients of variation for most traits were very low except for ear rots (29.95; 51.42), stem lodging (45.5; 68.4), root lodging (45.91; 72.71), husk cover (47.46; 61.5) and anthesis silking interval (28.73; 37.07). Phenotypic coefficients of variation (PCV) estimates were greater than genotypic coefficient of variation (GCV) for all traits studied.

### *3.2 Heritability and Genetic Advance Estimates*

Heritability ranged from 20% to 87% with higher estimates recorded for plant height (85%), anthesis date (87%), ear height (86%), grain yield (79%), texture (75%) and ear position (71%) and anthesis silking interval (55%). Moderate estimates were observed for husk cover (49%), stem logging (36%), root logging (28%), ears per plant (29%) and moisture content at harvest (30%) whilst for ear rot heritability was the lowest (20%). Genetic advance estimates as a percentage of mean ranged from 0.23 to 401.48. High genetic advance as mean percentage estimates (GAM) were recorded for traits such as plant height, ear height, root lodging, stem lodging, grain yield, anthesis silking interval, husk cover and ear rot. Moderate GAM estimate was observed for moisture content at harvest and low estimates were recorded for anthesis date, ear position, grain texture and ears per plant.

Table 2. Summary statistics for grain yield and its component characters measured in 105 maize hybrids

| Trait | Mean | Min | Max | P-Value | CV | VG | GCV (%) | PCV (%) | H (%) | GAM (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Grain yield** | 5.23t/ha | 0.13t/ha | 15.3t/ha | *** | 28.2 | 0.67 | 15.61 | 16.76 | 79 | 30.27 |
| **Anthesis Date** | 74.88day | 56day | 98.5day | *** | 6.21 | 1.89 | 1.84 | 1.94 | 87 | 5.80 |
| **ASI** | 1.38day | -4.5day | 8.5day | *** | 8.41 | 0.16 | 28.73 | 37.07 | 55 | 39.14 |
| **Plant height** | 228.01cm | 110cm | 360cm | *** | 7.15 | 99.13 | 4.37 | 4.67 | 85 | 102.41 |
| **Ear height** | 109.98cm | 37cm | 163.2cm | *** | 10.59 | 55.12 | 6.75 | 7.19 | 86 | 117.15 |
| **Ear position** | 0.48 | 0.25 | 0.72 | *** | 9.03 | 0 | 4.16 | 4.85 | 71 | 0.23 |
| **Root lodging** | 2.92% | 0% | 63.75% | * | 22.93 | 1.8 | 45.91 | 72.71 | 28 | 318.33 |
| **Stem lodging** | 4.17% | 0% | 77.47% | ** | 187.9 | 3.59 | 45.5 | 68.4 | 36 | 401.48 |
| **Ears per plant** | 0.86 | 0 | 1.55 | * | 16.58 | 0 | 4.12 | 6.54 | 29 | 0.76 |
| **Husk cover** | 4.73% | 0% | 65% | *** | 148.3 | 5.04 | 47.46 | 61.5 | 49 | 368.5 |
| **Ear rots** | 5.78% | 0% | 98.75% | ns | 150.1 | 3 | 29.95 | 51.42 | 20 | 314.91 |
| **Texture** | 2.76 | 1.5 | 4.5 | *** | 17.28 | 0.1 | 11.29 | 11.85 | 75 | 8.00 |
| **Moisture** | 13.05% | 3.35% | 38.6% | * | 20.28 | 0.26 | 3.9 | 6.18 | 30 | 10.26 |

*Note.* CV = coefficient of variation; GCV = genotypic coefficients of variation; PCV = phenotypic coefficients of variation; VG = genotypic variance; H = heritability; GAM = genetic advance estimates as a percentage of mean; *, **, *** significant at 0.05, 0.01 and 0.001, respectively.

### *3.3 Environmental Correlations*

Phenotypic and genotypic environmental correlations of nine sites for grain yield are shown in Table 3. Significant positive correlations were observed between some of the environments. Highest positive phenotypic correlations for grain yield were observed between CZ and CIMMYT2 (0.61) and the lowest correlations for grain yield were recorded between LW and ART (0.14). Highest positive genotypic correlation was observed between RARS2 and CIMMYT2 (0.86) followed by RARS2 and CZ2 (0.81) whilst the lowest correlation was between CIMMYT and CZ (0.33).

Table 3. Environmental correlations using grain yield (t/ha) data for 105 maize hybrids

| Env | | CZ2 | CIMMYT | RARS | LW | ART | CIMMYT2 | RARS2 | MS |
|---|---|---|---|---|---|---|---|---|---|
| **CZ** | P | 0.44** | 0.17 | 0.29*** | 0.18 | 0.22** | 0.61** | 0.50*** | 0.54** |
| | G | 0.65 | 0.33 | 0.43 | 0.40 | 0.49 | 0.85*** | 0.66 | 0.72*** |
| **CZ2** | P | | 0.17 | 0.43* | 0.22*** | 0.29 | 0.50** | 0.57** | 0.51** |
| | G | | 0.36 | 0.70*** | 0.53 | 0.70** | 0.75** | 0.81** | 0.72** |
| **CIMMYT** | P | | | 0.34*** | 0.15 | 0.21* | 0.20*** | 0.33** | 0.31** |
| | G | | | 0.71** | 0.46 | 0.66 | 0.39 | 0.60 | 0.57 |
| **RARS** | P | | | | 0.26** | 0.19 | 0.29* | 0.40*** | 0.42*** |
| | G | | | | 0.65 | 0.47 | 0.45 | 0.57 | 0.61 |
| **LW** | P | | | | | 0.14 | 0.32** | 0.22 | 0.29** |
| | G | | | | | 0.51 | 0.72*** | 0.46 | 0.61 |
| **ART** | P | | | | | | 0.33** | 0.34 | 0.23* |
| | G | | | | | | 0.74 | 0.74* | 0.50 |
| **CIMMYT2** | P | | | | | | | 0.65 | 0.48** |
| | G | | | | | | | 0.86** | 0.63 |
| **RARS 2** | P | | | | | | | | 0.59*** |
| | G | | | | | | | | 0.75** |

*Note.* *, **, *** significant at 0.05, 0.01 and 0.001 respectively; P = phenotypic correlation; G = genotypic correlation; CIMMYT Harare (CIMMYT = low N; CIMMYT2 = optimal), Rattray Arnold Research station (RARS = low N; RARS2 = optimal), Chiredzi Research station (CZ = heat stress; CZ2 = drought stress), ART farm = optimal, Mpongwe south (MS = optimal) and Lusaka west (LW = low N).

### *3.4 Trait Correlations*

Correlation analysis was done for different management levels and results had similar trends, hence the use of a combined analysis (Data not shown). Significant positive and negative correlations between grain yield and agronomic traits were recorded (Table 4). Grain yield was significantly and positively correlated to anthesis days, plant height, ear height, ear position, ears per plant, texture, moisture content and was negatively correlated to anthesis silking interval and ear rots. Anthesis silking interval was negatively correlated to plant height, ear height, ear position, ears per plant and moisture content. Anthesis date was positively correlated to plant height, ear height, ear position, root lodging, texture and moisture content and negatively correlated to anthesis silking interval.

Table 4. Correlation coefficients of grain yield and its component traits for 105 single cross maize hybrids

| | AD | ASI | PH | EH | EPO | RL | SL | EPP | HC | ER | TEX | MC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GYG** | 0.50*** | -0.69* | 0.82** | 0.84*** | 0.57*** | 0.13 | 0.04 | 0.31 | -0.03 | -0.24*** | 0.21*** | 0.54* |
| **AD** | | -0.33*** | 0.46* | 0.55** | 0.44** | 0.19* | 0.19 | 0.04 | -0.17 | -0.02 | 0.24** | 0.44** |
| **ASI** | | | -0.64** | -0.60** | -0.28* | -0.11 | -0.02 | -0.25** | -0.12 | 0.09 | -0.19 | -0.27** |
| **PH** | | | | 0.88*** | 0.43** | 0.06 | -0.05 | 0.33** | 0.11 | -0.07 | 0.23* | 0.45*** |
| **EH** | | | | | 0.80*** | 0.11 | 0.04 | 0.24** | -0.01 | -0.15 | 0.26 | 0.48 |
| **EPO** | | | | | | 0.09 | 0.10 | 0.01 | -0.15 | -0.24** | 0.19 | 0.35** |
| **RL** | | | | | | | 0.45*** | 0.02 | -0.16 | -0.04 | -0.01 | 0.01 |
| **SL** | | | | | | | | -0.06 | -0.26 | -0.03 | -0.26** | 0.11 |
| **EPP** | | | | | | | | | 0.12 | 0.28*** | 0.10 | 0.15 |
| **HC** | | | | | | | | | | 0.20 | -0.03 | -0.23* |
| **ER** | | | | | | | | | | | 0.17 | -0.29** |
| **TEX** | | | | | | | | | | | | -0.12 |

*Note.* *, **, *** significant at 0.05, 0.01 and 0.001, respectively; grain yield (GYD), anthesis date (AD), ear height (EH), plant height (PH), stem lodging (SL), root lodging (RL), husk cover (HC), moisture content (MC), anthesis silking interval (ASI), ear position (EPO), texture (TEX), and ear per plant (EPP).

### *3.5 Path Coefficient Analysis*

We further partitioned correlations coefficients of secondary traits on grain yield into direct and indirect effects using the path coefficient analysis. We categorized the coefficient values according to Lenka and Mishra (1973) as: negligible 0.00-0.09; low 0.10-0.19; moderate 0.20-0.29 and high 0.30-0.99. Path analysis results from different management levels had similar trends (Data not shown). Correlations combined analysis was done and direct effects of anthesis date, root lodging, stem lodging, husk cover and texture were negligible (Table 5). Plant height, ear position, ear per plant and moisture content had positive direct effects on grain yield. Anthesis silking interval, ear height and ear rots had negative direct effects on grain yield. However, plant height and ear height had indirect positive effects on anthesis silking interval. Anthesis date, ear height, ear position, ears per plant, texture and moisture content had positive indirect effects on plant height whilst anthesis silking interval had negative indirect effects on plant height. Anthesis date, plant height, ear height and moisture content had positive indirect effects on ear position. Plant height and ear position had negative indirect effects on ear height and anthesis silking interval had positive indirect effect on ear height.

Table 5. Path coefficient analysis indicating direct and indirect effects of component characters on grain yield for 105 maize genotypes

| Trait | AD | ASI | PH | EH | EPO | RL | SL | EPP | HC | ER | TEX | MC | Total effects |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AD** | **0.05**** | 0.08 | 0.25 | -0.09 | 0.13 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.05 | 0.50*** |
| **ASI** | -0.02 | **-0.25**** | -0.35 | 0.10 | -0.08 | 0.00 | 0.00 | -0.03 | 0.00 | -0.01 | -0.01 | -0.03 | -0.69*** |
| **PH** | 0.02 | 0.16 | **0.54**** | -0.14 | 0.12 | 0.00 | 0.00 | 0.04 | 0.00 | 0.01 | 0.01 | 0.06 | 0.82*** |
| **EH** | 0.03 | 0.15 | 0.48 | **-0.16**** | 0.23 | 0.00 | 0.00 | 0.03 | 0.00 | 0.02 | 0.01 | 0.06 | 0.84* |
| **EPO** | 0.02 | 0.07 | 0.23 | -0.13 | **0.29**** | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.01 | 0.04 | 0.57*** |
| **RL** | 0.01 | 0.03 | 0.04 | -0.02 | 0.02 | **0.04**** | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.13*** |
| **SL** | 0.01 | 0.00 | -0.03 | -0.01 | 0.03 | 0.02 | **0.01**** | -0.01 | 0.01 | 0.00 | -0.01 | 0.01 | 0.04 |
| **EPP** | 0.00 | 0.06 | 0.18 | -0.04 | 0.00 | 0.00 | 0.00 | **0.12**** | 0.00 | -0.04 | 0.00 | 0.02 | 0.31*** |
| **HC** | -0.01 | 0.03 | 0.06 | 0.00 | -0.04 | -0.01 | 0.00 | 0.01 | **-0.02*** | -0.03 | 0.00 | -0.03 | -0.03* |
| **ER** | 0.00 | -0.02 | -0.04 | 0.02 | -0.07 | 0.00 | 0.00 | 0.03 | 0.00 | **-0.13*** | 0.01 | -0.04 | -0.24*** |
| **TEX** | 0.01 | 0.05 | 0.12 | -0.04 | 0.05 | 0.00 | 0.00 | 0.01 | 0.00 | -0.02 | **0.04*** | -0.01 | 0.21*** |
| **MC** | 0.02 | 0.07 | 0.24 | -0.08 | 0.10 | 0.00 | 0.00 | 0.02 | 0.00 | 0.04 | -0.01 | **0.12*** | 0.54*** |
| **RE** | 0.18 | | | | | | | | | | | | |

*Note*. *, **, *** significant at 0.05, 0.01 and 0.001, respectively; grain yield (GYD), anthesis date (AD), ear height (EH), plant height (PH), stem lodging (SL), root lodging (RL), husk cover (HC), moisture content (MC), anthesis silking interval (ASI), ear position (EPO), texture (TEX) and ear per plant (EPP).

## 4. Discussion

The analysis of variance for 105 genotypes exhibited significant variability among most of the characters under study except for ear rots which was not significant. The variability can be attributed to diverse source of germplasm coming from new lines and the CIMMYT elite lines crossed to form the single cross hybrids. Phenotypic and genotypic coefficients of variation estimates are very crucial in predicting selection efficiency. The difference between GCV and PCV indicates the environmental influence. In this study the PCVs were slightly higher than GCVs suggesting least environmental influence on characters. Similar results had been reported by Meena et al. (2016) and Alhussein et al. (2017). In contrast Ogunniyan and Olakojo (2015) reported higher GCVs compared to PCVs for traits such as plant height, ear height, number of leaf per plant, ear weight, grain weight, number of ears and leaf area and zero environmental influence for traits such as days to silking, days to anthesis and anthesis silking interval. Ayodele et al. (2020) also reported higher GCVs compared to PCVs suggesting low environmental influence on the expression of characters studied. Higher percentage of GCV provides ample scope for selection and in the current study traits such as grain yield, anthesis silking interval, root lodging, stem lodging, husk cover, ear rots and texture had higher GCV % estimates.

Heritability is the percentage of genotypic variance over phenotypic variance and is a predictor of the reliability of phenotypic value of quantitative characters. Higher heritability estimates were observed for the following traits: plant height, anthesis date, ear height, grain yield, ear position and texture. Therefore, these characters can be used for selection because variation for traits was under genetic control and traits were less influenced by environment. High heritability values for these traits also imply that traits can be easily passed on to the next generation enhancing selection efficiency in maize breeding programs. Jilo et al. (2018) also recorded high heritability estimates for grain yield and plant height. Bartaula et al. (2019) recorded high estimates for plant height at harvest (0.99) and ear height (0.99). In contrast, Alhussein and Idris (2017) recorded very low heritability estimates for grain yield, ear height and plant height. The traits that exhibited moderate heritability were anthesis silking interval, root logging, stem logging, moisture, ears per plant and husk cover may respond positively to phenotypic selection and progress can be made when improving them. Similar results have been recorded for the trait ears per plant by Atta (2016).

Genetic advance as a percentage of mean indicates mode of gene action in the expression of a trait (Meena, 2016) and reveals the degree of gain of a particular trait under selection pressure. The expected genetic advance was low for anthesis date, ear position, ears per plant moisture content and texture. In this study, traits with both high heritability and genetic advance estimates were plant height, ear height and grain yield. These traits can be used for selection because high estimates of heritability coupled with high genetic advance offers the most suitable condition for selection as it indicates the presence of additive genes and are usually more helpful than heritability alone in predicting the resultant effect for selecting the best genotypes (Johnson et al. 1955).

Significant genotypic and phenotypic environmental correlations between a few sites were observed using grain yield data. Strong environmental correlations were recorded between CZ and CIMMYT2 (0.85), CZ2 and RARS2 (0.81), however these environments are known to be different in terms of annual temperature, rainfall and altitude. CIMMYT2 and RARS2 (0.86) also had a very strong environmental correlation, suggesting that only one of the sites could be used for trial evaluation since they have the same discriminating effect.

The complexity of inheritance of traits in maize demands simultaneous selection of grain yield along with other closely related traits to effectively identify best genotypes (Atta, 2016). In this study Pearson's correlation coefficients and path coefficients values were used to establish relationships among grain yield and its components. Grain yield was strongly correlated to plant height and ear height. Plant height was strongly correlated to ear height while ear positon was strongly associated with ear height. Atta (2016) reported high positive and significant genetic correlations among grain yield and its several component traits under low nitrogen conditions. Similar results have been reported by Alhussein (2017). Secondary traits with positive correlations can be indirectly selected for each other and this ensures parallel improvement of these traits. Significant and positive correlation between two characters suggests that these characters can be improved simultaneously in a selection program. Negative correlation observed between traits indicates inverse relationships e.g. grain yield and anthesis silking interval (-0.69): as you increase ASI grain yield decreases.

Direct and indirect association depicts the importance of characters to the final product and in this case grain yield was used as a dependent character to calculate path coefficients at phenotypic level. The independent characters were plant height, ear height, anthesis date, ear position, anthesis silking interval, husk cover, root logging, stem logging, moisture at harvest, ear per plant, ear rots and texture. Path coefficients enable the breeder to concentrate on the variable with high direct effect on grain yield (Kumar & Babu, 2015). The study revealed that plant height had the maximum positive direct effect on grain yield (0.54), whilst ear position and ears per plant had high and moderate direct effects on grain yield, therefore may be considered together with plant height as selection criteria for grain yield in maize breeding programs. This means that these traits should be seriously considered when selecting high yielding genotypes. Plant height also revealed positive indirect effects via anthesis date, ear height, ear position, grain moisture content at harvest and texture. These traits have direct effects on grain yield and can be used to select for increased grain yield. Wuhaib et al. (2018) reported that ear height, plant height, number of ears and 50% days to silking had positive direct effect on grain yield. Thus by selecting plant height these traits will also be selected for. Ear height and anthesis silking interval had negative direct effect on grain yield and this means these traits need to be improved before using them as selection criterion.

There was environmental influence on character expression as shown by higher PCV than GCV. High genetic and phenotypic variance, heritability and genetic advance estimates were recorded for plant height, ear height and grain yield. Implication for breeding is that progress can be made through selection for these traits in this set of germplasm. The study was able to reveal trait association of this particular set of hybrids under diverse growing conditions. Grain yield had strong positive correlations with plant height and ear height under diverse environments. Plant height, ears per plant and ear position had positive direct effects on grain, whilst other characters such as anthesis date, ear height, ear position, grain moisture content at harvest and texture indirectly influenced grain yield. These characters' contribution to grain yield is important and the strong association with grain yield implied that these can be used as secondary traits to indirectly select for grain yield performance in this set of germplasm across all the environments.

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**References**

Alhussein, M. B., & Idris, A. E. (2017). Correlation and path analysis of grain yield components in some Maize (*Zea mays* L.) genotypes. *International Journal of Advanced Research and Publications, 1*, 79-82.

Atta, M. M. M. (2016). Genetic correlations and heritability in maize under Low-N and heat stress conditions. *Egyptian Journal of Plant Breeding, 20*, 241-260. https://doi.org/10.12816/0031701

Ayodele, I. S., Omolaran, B., Aremu, C., & Abolusoro, S. (2020). Estimation of genetic variation, heritability and genetic advance for yield and agronomic traits correlation of some low nitrogen tolerance maize (*Zea mays*) varieties in the tropics. *Research on Crops, 21*, 595-603. https://doi.org/10.31830/2348-7542.2020.093

Badu-Apraku, B., Fakorede, M. A. B., Menkir, A., & Sanago, D. (2012). *Conduct and Management of field trials* (p. 59). IITA, Ibadan, Nigeria.

Bartaula, S., Panthi, U., Timilsena, K., Acharya, S. S., & Shrestha, J. (2019). Variability, heritability and genetic advance of maize (*Zea mays* L.) genotypes. *Research in Agriculture Livestock and Fisheries, 6*, 163-169. https://doi.org/10.3329/ralf.v6i2.42962

Bello, O. B., Ige, S. A., Azeez, M. A., Afolabi, M. S., Abdulmaliq, S. Y., & Mahamood, J. (2012). Heritability and genetic advance for grain yield and its component characters in maize (*Zea mays* L.). *International Journal of Plant Research, 2*(5), 138-145, https://doi.org/10.5923/j.plant.20120205.01

Beyene, Y., Mugo, S., Oikeh, S. O., Juma, C., Olsen, M., & Prasanna, B. M. (2016). Hybrids performance of doubled haploid lines derived from 10 tropical bi-parental maize populations evaluated in contrasting environments in Kenya*. African Journal of Biotechnology, 16*(8), 371-379. https://doi.org/10.5897/AJB 2016.15697

CIMMYT. (1985). *Managing trials and reporting data for CIMMYT's maize testing program*. Mexico, D.F.

Das, B., Atlin, G. N., Olsen, M., Burgueno, J., Tarekegne, A., Babu, R., … Cairns, J. E. (2019). Identification of donors for low-nitrogen stress with maize lethal necrosis (MLN) tolerance for maize breeding in sub-Saharan Africa. *Euphytica, 215*, 80. https://doi.org/10.1007/s10681-019-2406-5

Davis, J., Amézquita, M., & Muñoz, J. (1981). Border Effects and Optimum Plot Sizes for Climbing Beans (*Phaseolus vulgaris*) and Maize in Association and Monoculture. *Experimental Agriculture, 17*, 127-35. https://doi.org/10.1017/S0014479700011364

De Mendiburu, F. (2009). *Una herramienta de analisis estadistico para la investigacion agricola* (Thesis, Departamento Academio de Estadistica e Informatica, Facultad de Economia y Planificacion, Universidad Nacional La Molina, Lima, Peru).

Dewey, D. R., & Lu, K. H. (1959). A correlation and path coefficient analysis of components of crested wheat grass and seed production. *Agronomy Journal, 51*, 515-517. https://doi.org/10.2134/agronj1959.0002196 2005100090002x

Elrod, S., & Stanfield, W. (2002). *Genetics* (4th ed.). Tata McGraw Hill Co. New Delhi.

FAOSTAT. (2017). *FAOSTAT database*. FAO, Rome. Retrieved August 7, 2017, from http://faostat.fao.org

Jahufer, M. Z. Z., & Luo, D. (2018). "DeltaGen" a comprehensive decision support tool for plant breeders. *Crop Science, 58*, 1-14. https://doi.org/10.2135/cropsci2017.07.0456

Jakhar, D. S., Singh, R., & Kumar, A. (2017). Studies on path coefficient analysis in maize (*Zea mays* L.) for grain yield and its attributes. *International Journal of Current Microbiology and Applied Science, 6*, 2851-2856. https://doi.org/10.20546/ijcmas.2017.604.327

Jilo, T., Tulu, L., Birhan, T., & Beksisa, L. (2018). Genetic variability, heritability and genetic advance of maize (*Zea mays* L.) inbred lines for yield and yield related traits in southwestern Ethiopia. *Journal of Plant Breeding and Crop Science, 10*, 281-289. https://doi.org/10.5897/JPBCS2018.0742

Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955). Estimation of genetic and environmental variability in soybean. *Agronomy Journal, 47*, 314-318. https://doi.org/10.2134/agronj1955.00021962004700070009x

Kassie, G. T., Abdulai, A., Greene, W. H., Shiferaw, B., Abate, T., Tarekegne, A., & Sutcliffe, C. (2017). Modelling preference and willingness to pay for drought tolerance (DT) in Maize in rural Zimbabwe. *World Development, 94*, 465-477. https://doi.org/10.1016/j.worlddev.2017.02.008

Kumar, S. V. V., & Babu, D. R. (2015). Character association and path analysis of grain yield and yield components in Maize (*Zea mays* L.). *Journal of Plant Breeding, 6*, 550-554.

Kwon, S. H., & Torrie, J. H. (1964). Heritability and interrelationship among traits of two soybean populations. *Crop Science, 4*, 196-198. https://doi.org/10.2135/cropsci1964.0011183X000400020023x

Lenka, D. B., & Mishra, Y. P. (1973). Path coefficient analysis of yield in rice varieties. *Indian Journal of Agricultural Science, 43*, 376-369.

Makumbi, D., Assanga, S., Diallo, A., Magorokosho, C., Asea, G., Worku, M., & Banziger, M. (2018). Genetic analysis of tropical Midaltitude-adapted maize populations under stress and non-stress conditions. *Crop Science, 58*, 1492-1507. https://doi.org/10.2135/cropsci2017.09.0531

Malik, H. N., Malik, S. I., Hussain, M., Chughtai, S. U. R., & Javed, H. I. (2005). Genetic correlation among various quantitative characters in Maize (*Zea mays* L.) hybrids. *Journal of Agriculture and Social Science, 1*, 262-265.

Meena, M. K, Singh, R., & Meena, H. P. (2016). Genetic variability, heritability and genetic advance studies in newly developed maize genotypes (*Zea mays* L.). *The Bioscan, 11*, 1787-1791.

Mhike, X., Okori, P., Magorokosho, C., & Gibson, P. (2012). *Genetic analysis of tropical maize inbreds and hybrids for grain yield and traits associated with drought tolerance*. Third Rofurom Biennial Meeting, September 24-28, 2012, Entebbe, Uganda.

Musundire, L., Derera, J., Dari, S., & Tongoona, P. (2019). Genetic variation and path analysis of introgressed maize inbred lines for economic traits. *Journal of Agricultural Science, 11*, 135-149. https://doi.org/10.5539/jas.v11n17p135

Ogunniyan, D. J., & Olakojo, S. A. (2014). Genetic variation, heritability, genetic advance and agronomic character association of yellow elite inbred lines of maize (*Zea mays* L). *Nigerian Journal of Genetics, 28*, 24-28. https://doi.org/10.1016/j.nigjg.201506.005

Patterson, H. D., Williams, E. R., & Hunter, E. A. (1978). Block Designs for Variety Trials. *Journal of Agricultural Science (Cambridge), 90*, 395-400. https://doi.org/10.1017/S0021859600055507

R Core Team. (2020). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing Vienna, Austria.

Raghu, B., Suresh, J., Kumar, S. S., & Saidaish, P. (2011). Character association and path analysis in maize (*Zea mays* L.). *Madras Agricultural Journal, 98*, 7-9.

Seshu, G., Jabeen, F., & Rani, K. J. (2013). Heritability, trait association and path analysis for grain yield and yield contributing characters in maize. *The Andhra Agricultural Journal, 60*, 563-567.

Shukla, S., Bhargava, A., Chatterjee, A., Sirivastava, J., Singh, N., & Singh, S. P. (2006). Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). *Plant Foods for Human Nutrition, 61*, 23-28. https://doi.org/10.1007/s11130-006-0004-x

Singh, R. K., & Chaudhary, B. D. (1985). *Biometrical methods in quantitative analysis* (p. 318). Kaylani Publishers, New Delhi.

Siva-Subranamanian, S., & Menon, M. (1973). Heterosis and inbreeding depression in rice. *Madras Agricultural Journal, 60*, 1139-1140.

Wright, S. (1921). Correlation and causation. *Journal of Agricultural Research, 20*, 557-585.

Wuhaib, K. M., Hadi, B. H., & Hassan, W. A. (2018). Genotypic and phenotypic correlation in maize and path coefficient I-Agronomic traits. *Iraqi Journal of Agricultural Sciences, 49*, 179-187.

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# Genetic Diversity in *Mimosa tenuiflora* (Willd.) Poir.: A Multipurpose Plant Genetic Resource of Semiarid Brazil

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**Abstract**

Caatinga is the third largest biome in Brazil but little is known about the species diversity from this biotic community, despite of its social, economic and environmental importance for the semiarid region. Among the several typical plant species from Caatinga, *Mimosa tenuiflora* (Willd.) Poir. (black jurema) stands out because it plays a major role in the maintenance of this ecosystem, besides being widely used to recover degraded areas. Therefore, the goal of this study was to evaluate the genetic diversity and structural analysis from 10 populations of *M. tenuiflora* from the state of Bahia, northeastern Brazil, using 10 ISSR (Inter Simple Sequence Repeat) markers. A total of 117 fragments were obtained from 218 individuals with a mean number of 11.8 bands per primer. The mean population polymorphism was 85.0%, while the values of genetic diversity (He) and the Shannon index (I) were equal to 0.295 and 0.442, respectively. Most of genetic variation was observed (87.0%) but high $F_{ST}$ values were observed (0.132), indicating the populations are genetically differentiated. Bayesian inference using Structure divided the populations into two groups while Geneland indicated five clusters that could be related to the fragmentation of Caatinga and to constraints in the dispersal of pollen and seeds. In conclusion, *M. tenuiflora* presents high levels of genetic diversity and natural populations might serve as potential sources for management and reforestation of degraded areas in Caatinga.

**Keywords:** Caatinga, genetic diversity, ISSR, reforestation

## 1. Introduction

Caatinga is a poorly known and highly threatened biome, restricted to Brazilian semiarid areas that have suffered from intensive deforestation for cattle and charcoal production as well as inappropriate irrigation systems, thus leading to desertification, sedimentation of rivers and soil erosion (Leal et al., 2005). However, the lack of detailed information about the regional biodiversity restrains effective conservation efforts in Caatinga. *Mimosa* L. is a species-rich genus in Leguminosae, comprising about 540 taxa (Simon et al., 2011) widespread throughout distinct phytophysiognomies over tropical and subtropical regions in Americas. Brazil is a major center of origin for this genus (Barneby, 1991), with a high number of species (350), being 38 of them found in Caatinga (Queiroz, 2009; Dutra & Morim, 2015a).

*Mimosa tenuiflora* (popularly known as black jurema) represents an important natural resource in Caatinga along semiarid regions of northeastern Brazil, being found in the states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte and Sergipe (Dutra & Morim, 2015b). Throughout its range, black jurema is widely used as timber for furniture, bridges, fences and studs, as charcoal in energy production (Maia, 2004; Riegelhaupt & Pareyn, 2010) and to feed cattle (Braga, 1989). On the other hand, the tree bark powder is popular in traditional medicine to treat burns, acne, and wounds, since it presents antibiotic, analgesic and astringent properties (Albuquerque, 2007) and antifungal activity in the control of *Alternaria alternata* in citrus (Pinto et al., 2018). Furthermore, it is considered a sacred plant for native tribes from semiarid Brazil who prepare a wine from the bark to be used as a ceremonial drink, a tradition that was later expanded to some Afro-Brazilian religious activities (Maia, 2004). As a matter of fact, psychoactive effects are recognized in jurema wine because it is rich in the alkaloid N, N-dimethyltryptamine or DMT (Souza, 2008). This specie has been regarded as "high conservation priority" after presenting local risk of extinction in the southern mesoregion of the state of Ceará because of overexploitation for medical purposes and animal feeding. The small number of individuals can be also related to losses of genetic variability contributing to lower population sizes (Santos et al., 2017).

Besides the social, cultural and economic relevance, black jurema also plays a major role in the maintenance of forest cover and ecological processes (*e.g.*, pollinator attraction) in Caatinga favored by its fast growth and propagation, being particularly recommended to recover degraded areas (Queiroz, 2009; Maia-Silva et al., 2012). Adaptative morphological features to arid and semiarid ecosystems, such as higher size and stem diameter, make *M. tenuiflora* suitable for recovering degraded areas in Caatinga (Lima et al., 2018). This plant is visited by moths, butterflies, flies and beetles, but bees represent the main visitors since black jurema produces pollen throughout most of the year, being particularly helpful to the survival of colonies raised by bee keepers during dry seasons (Maia-Silva et al., 2012; Silva et al., 2015).

The knowledge about population structure is essential to conservation, genetic improvement, sustainable management, and selection of seed trees to recover deforested areas (Kageyama et al., 2003). The genetic diversity of *M. tenuiflora* was analyzed from 15 natural population located in state of Rio Grande Norte, revealing higher genetic variation within populations and the formation of four genetic groups. This study suggested that conservation efforts should focus on six populations, such as those with the highest and the lowest levels of genetic variation. In particular, the population of Espírito Santo (Rio Grande do Norte) was selected as a priority for conservation, as it presents the highest genetic distinctiveness in relation to the other population (Chagas, 2018). Hence, identification of genetically rich and divergent populations helps defining operational units that should be prioritized in ex situ or in situ conservation programs (Diniz-Filho & Telles, 2002).

Forest fragmentation might eventually lead to decreased population effective size and high inbreeding levels, thus depleting the original genetic variation of natural populations that hence become more susceptible to environmental, demographic and genetic changes (Rajora & Mosseller, 2001) even in widespread species such as *M. tenuiflora.* Several methods of molecular analyses have been used to assess the genetic diversity in natural populations of plants, including ISSR (Inter Simple Sequence Repeats) markers (Almeida et al., 2009). In spite of their dominant behavior, ISSR markers are advantageous to assess the genetic diversity in wild plants because they disregard previous information about genomes, are easily obtained, indicate variation within unique regions of the genome at several loci at the same time and provide reproducible results with high levels of polymorphism (Reddy et al., 2002; Kumar & Yadav, 2018).

Therefore, the goal of this study was to evaluate the genetic diversity and population structure in *M. tenuiflora* along the state of Bahia, northeastern Brazil, based on ISSR markers. The present information is useful to the management and conservation of this species that represents a major plant resource in Caatinga.

## 2. Material and Methods

### *2.1 Sampling*

Leaf samples from 218 individuals of *M. tenuiflora* were collected in 10 sites from the state of Bahia, which comprises the largest area within the natural range of this species, comprising about 564 thousand $km^2$ (Table 1, Figure.1). To avoid the sampling of closely related individuals, a minimum distance of 10 m was applied among individual samples. A transect was defined across the state based on two major roads (BR-116 and BR-242) that cross the region and, thus, the sampling efforts comprised about 1,200 km from east to west and from north to south. Each sampled individual of *M. tenuiflora* was labeled *in situ* with a number code for identification and each sampled location was georeferenced using Garmin 12 GPS (Global Position System) receiver. The leaf samples were stored at -20 °C in the Laboratory of Molecular Genetics at UESB and voucher samples from each population were stored in the Herbarium from Universidade Estadual do Sudoeste da Bahia (HUESB) to confirm the species identification. The geographic distance among sampled populations was measured (Table 2) using the software DIVA-GIS (Hijmans et al., 2001) based on the coordinates of each collection site (Table 1). The coordinates were transformed into UTM (Universal Tranverse Mercator) values to calculate the distance among localities.

Table 1. Localities, coordinates, altitude (A) and number of samples (N) per population of *Mimosa tenuiflora* (Willd.) Poir. in the state of Bahia

| Localities | Abbreviation | Coordinates | A (m) | N |
|---|---|---|---|---|
| Vitória da Conquista | VC | 14°51′48″ S; 40°50'48″ W | 874 | 24 |
| Poções | PO | 14°40'17″ S; 40°18'42″ W | 911 | 20 |
| Jequié | JE | 13°49'18″ S; 40°05'51″ W | 218 | 15 |
| Itaberaba | IT | 12°44'25″ S; 40°19'53″ W | 272 | 21 |
| Seabra | SE | 12°24'58″ S; 41°46'09″ W | 812 | 21 |
| Ibotirama | IB | 11°59'40″ S; 43°12'50″ W | 115 | 25 |
| Barreiras | BA | 12°8'50″ S; 44°59'40″ W | 454 | 21 |
| Santo Amaro | SA | 12°32'26″ S; 38°42'39″ W | 8 | 20 |
| Serrinha | SR | 11°39'43″ S; 39°00'32″ W | 379 | 25 |
| Euclides da Cunha | EC | 10°30'29″ S; 39°00'47″ W | 472 | 26 |
| Total | - | - - | - | 218 |

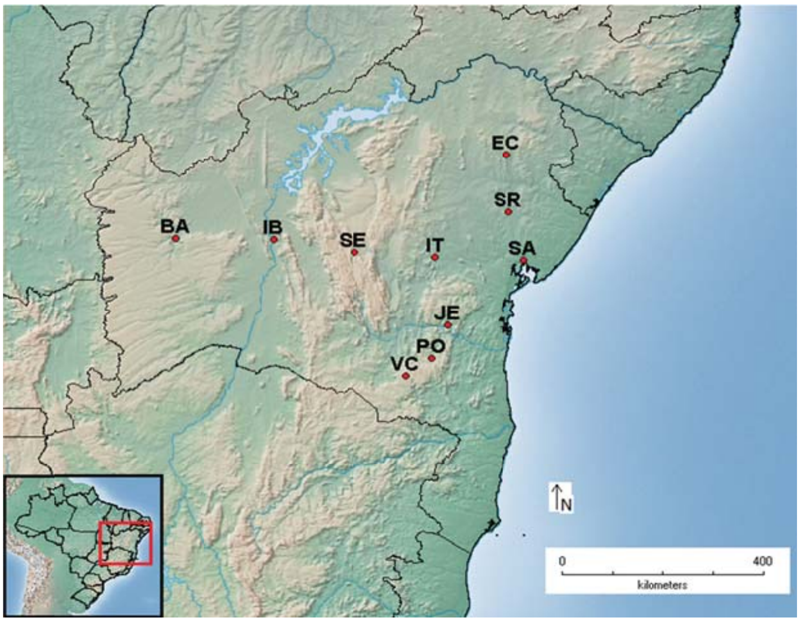


Figure 1. Mapping of the populations. Geographic distribution of the 10 sampled populations of *Mimosa tenuiflora* from the state of Bahia, Brazil (Table 1)

Table 2. Geographic distance among the populations of *Mimosa tenuiflora* collected in the state of Bahia, Brazil

| | VC | PO | JE | IT | SE | IB | BA | SA | SR | EC |
|---|---|---|---|---|---|---|---|---|---|---|
| VC | 0 | | | | | | | | | |
| PO | 63.0 | 0 | | | | | | | | |
| JE | 143.0 | 97.0 | 0 | | | | | | | |
| IT | 235.0 | 238.0 | 187.0 | 0 | | | | | | |
| SE | 288.0 | 297.0 | 244.0 | 160.76 | 0 | | | | | |
| IB | 412.0 | 433.0 | 401.0 | 322.01 | 166.0 | 0 | | | | |
| BA | 542.0 | 582.0 | 568.0 | 510.2 | 353.0 | 196.0 | 0 | | | |
| SA | 349.0 | 294.0 | 210.0 | 180.83 | 336.0 | 496.0 | 685.0 | 0 | | |
| SR | 408.0 | 366.0 | 274.0 | 188.31 | 313.0 | 456.0 | 656.0 | 107.0 | 0 | |
| EC | 523.0 | 484.0 | 391.0 | 285.74 | 371.0 | 487.0 | 680.0 | 232.0 | 130.0 | 0 |

### *2.2 DNA Extraction and Amplification*

The total DNA was isolated from leaves of *M. tenuiflora* according to the optimized protocol by Arruda et al. (2017). After isolation, the DNA samples were quantified in 0.8% agarose gel using L-Quant spectrophotometer

(Loccus). Forty ISSR primers provided by UBC (University of British Columbia) were tested and 10 of them were selected based on their repeatability and high-quality of band profiles (Table 3).

Table 3. ISSR primers and their respective sequences, annealing temperature (Tm), total number of loci, and number of polymorphic loci

| Primer | Sequence (5′-3′) | Tm (°C) | Number of loci | Number of polymorphic loci |
|---|---|---|---|---|
| UBC-807 | AGAGAGAGAGAGAGAGT | 53 | 10 | 8 |
| UBC-811 | GAGAGAGAGAGAGAGAC | 53 | 11 | 9 |
| UBC-812 | GAGAGAGAGAGAGAGAA | 52 | 10 | 9 |
| UBC-815 | CTCTCTCTCTCTCTCTG | 55 | 14 | 12 |
| UBC-827 | ACACACACACACACACG | 53 | 16 | 14 |
| UBC-836 | AGAGAGAGAGAGAGAGYA | 52 | 10 | 5 |
| UBC-845 | CTCTCTCTCTCTCTCTRG | 53 | 11 | 10 |
| UBC-854 | CTCTCTCTCTCTCTCRG | 54 | 16 | 15 |
| UBC-856 | ACACACACACACACACYA | 53 | 12 | 10 |
| UBC-864 | ATGATGATGATGATGATG | 53 | 7 | 7 |
| Total | - | - | 117 | 99 |
| Mean | - | - | 11.8 | 9.9 |

The DNA amplification via PCR (Polymerase Chain Reaction) was performed using 10 mM Tris-HCl pH 8.3, 2.5 mM of $MgCl_2$, 1 mM of dNTPs, 0.2 M of each primer, 5 U of Taq DNA polymerase (Biotools) and 40 ng of template DNA and ultrapure water to a final volume of 25 µL in a MG108+ thermocycler. The PCR conditions were: first denaturation step at 94 °C for 3 min, followed by 40 cycles of 1 min at 92 °C, 2 min at optimum annealing temperature (Table 3), 2 min at 72 °C, plus a final extension at 72 °C for 7 min. The amplified products were run in 1.2% agarose gel for 2h and 30 min at 100 volts and photodocumented under UV light using L-Pix system (Loccus). A 1000-bp ladder was used to estimate the fragment size while poorly define or weakly stained bands were disregarded from the present analyses.

*2.3 Data Analysis*

A binary matrix from the band profile for each ISSR primer was built in which "0" and "1" indicated the absence and the presence of a particular band, respectively. Based on these data, we estimated the Shannon's (I) and Nei's (He) genetic diversity (Nei, 1973), Nei's genetic distance (Nei, 1978), gene differentiation between populations ($F_{ST}$) and gene flow (*Nm*) levels using the software TFPGA 1.3 (Miller, 1997). The pairwise Nei's genetic distances was estimated using the software GenAlEx 6.5. In order to verify whether the genetic and geographic distances were correlated or not, we performed the Mantel's test (Manly, 1997) with 10,000 permutations, using AIS 1.0 (Miller, 2005). In the software Arlequin 3.0 (Excoffier & Schneider, 2005), we carried out the analysis of molecular variance (AMOVA) to estimate how the genetic variation was partitioned using 10,000 bootstrap permutations to test its significance.

To estimate the degree of admixture among sampled populations, a Bayesian inference of population structure was obtained in the software STRUCTURE 2.2.3 (Falush et al., 2007). The number of tested populations (K) ranged from 1 to 11, with 10 iterations and 1 million MCMC (Markov Chain Monte Carlo) generations with a burn-in of 100,000 generations. After adjustments in the software for dominant markers, we determined the best admixture model and assumed that the allele frequencies were uncorrelated among populations (Wang et al., 2012). The most likely K value was calculated using the platform Structure Harvester Web v.0.6.9 following the parameters established by Evanno et al. (2005). A second Bayesian model was tested using the GENELAND R package in order to analyze the geographic distribution of the genetic variation (Guillot, 2012). The number of clusters (K) was defined within an interval from 1 to 10 to infer the actual K value comprising the maximum number of populations following 1 million MCMC generations and a burn-in of 1,000 generations. A Principal Component Analysis (PCA) was also performed using the software PAST (Hammer et al., 2001) to visualize the degree of relatedness between populations based on the matrix of genetic distance for the 117 ISSR alleles.

## 3. Results

*3.1 Genetic Diversity*

A total of 117 bands were obtained from the 218 individuals of *M. tenuiflora*. The number of amplicons per ISSR primer ranged from 7 to 16 (UBC-864 and UBC-827, respectively), with a mean number of 11.8 bands/primer

(Table 3) and fragment sizes between 250 and 2000 base pairs (pb). At a population level, the mean percentage of polymorphic bands per locality was 85.0%, being the lowest and the highest values observed in Jequié (65.0%) and Serrinha (94.9%), respectively (Table 4). The mean values of genetic diversity for the 10 populations were estimated in 0.295 (He) and 0.442 (I). On the other hand, these values were highly differentiated among populations, ranging from He = 0.239 and I = 0.354 in Jequié (JE) to He = 0.369 and I = 0.538 in Serrinha (SR) (Table 4).

Table 4. Nei's genetic diversity (He), Shannon's index (I) and percentage of polymorphic loci (PLP) in populations of *Mimosa tenuiflora* from the state of Bahia, Brazil

| Population | He | *I* | PLP (%) |
|---|---|---|---|
| VC | 0.299 | 0.443 | 82.0 |
| PO | 0.315 | 0.466 | 84.6 |
| JE | 0.239 | 0.354 | 65.0 |
| IT | 0.339 | 0.496 | 88.0 |
| SE | 0.347 | 0.509 | 91.4 |
| IB | 0.348 | 0.510 | 90.6 |
| BA | 0.294 | 0.434 | 79.5 |
| SA | 0.305 | 0.450 | 81.2 |
| SR | 0.369 | 0.538 | 94.9 |
| EC | 0.368 | 0.536 | 94.0 |
| Mean | 0.322 | 0.474 | 85.0 |

### *3.2 Genetic Structure in Natural Populations of Mimosa tenuiflora*

The AMOVA revealed that most of genetic variation was found within populations (87.0%) of *M. tenuiflora* (Table 5). Nonetheless, high and significant levels of interpopulation genetic differentiation were detected ($F_{ST}$ = 0.132; p = 0.001) along with estimates of less than one migrant individual per generation (Nm = 0.87; p < 0.001). In fact, Mantel's test revealed significant correlation between genetic and geographic distances in the analyzed populations (r = 0.19, p = 0.001). The lowest values in pairwise genetic distance were observed between the samples from VC × PO (0.074) and PO × JE (0.087), while the populations from VC and EC showed the highest genetic divergence (0.225) (Table 6).

Table 5. Population structure and genetic divergence in populations of *Mimosa tenuiflora* from the state of Bahia based on 10 ISSR markers

| Source of variation | AMOVA[a] (%) | *p*-value[*] | $F_{ST}$[b] | *p*-value* | Nm[c] |
|---|---|---|---|---|---|
| Among populations | 13.0 | <0.000 | 0.132 | <0.001 | 0.870 |
| Within populations | 87.0 | <0.000 | | | |
| Total | 100 | | | | |

*Note*. [a] = analyses of molecular variance; [b] = index of genetic fixation; [c] = gene flow; [*] significance level.

Table 6. Pairwise Nei's genetic distance in populations of *Mimosa tenuiflora* from the state of Bahia, Brazil

| | VC | PO | JE | IT | SE | IB | BA | SA | SR | EC |
|---|---|---|---|---|---|---|---|---|---|---|
| VC | 0.000 | | | | | | | | | |
| PO | 0.074 | 0.000 | | | | | | | | |
| JE | 0.192 | 0.087 | 0.000 | | | | | | | |
| IT | 0.182 | 0.134 | 0.098 | 0.000 | | | | | | |
| SE | 0.178 | 0.135 | 0.140 | 0.134 | 0.000 | | | | | |
| IB | 0.148 | 0.122 | 0.104 | 0.088 | 0.123 | 0.000 | | | | |
| BA | 0.200 | 0.165 | 0.147 | 0.149 | 0.175 | 0.151 | 0.000 | | | |
| SA | 0.207 | 0.150 | 0.167 | 0.166 | 0.161 | 0.164 | 0.138 | 0.000 | | |
| SR | 0.216 | 0.171 | 0.154 | 0.119 | 0.171 | 0.139 | 0.220 | 0.158 | 0.000 | |
| EC | 0.225 | 0.218 | 0.215 | 0.180 | 0.167 | 0.210 | 0.199 | 0.193 | 0.165 | 0.000 |

Accordingly, the Bayesian inference of population structure confirmed that the K = 2 is the most suitable number of genetic groups in *M. tenuiflora* (Figure 2) (see Appendix A1 for details). The first group (green) comprised the populations VC, BA, SA and EC and the second (red) grouped the populations PO, JE, IT, SE, IB and SR. The analysis based on GENELAND separated the 10 populations into five clusters (Figure 3). The first cluster (dark green) was formed by samples from BA; the second group (light green) comprised the VC and PO populations; the third group (yellow) encompassed the SA and SR samples; the fourth group (beige) was formed by EC; and the fifth group (grey) grouped the samples from IB, SE, IT and JE. Moreover, the PCA discriminated only the population from BA in relation to the others, which composed a large group (Figure 4).

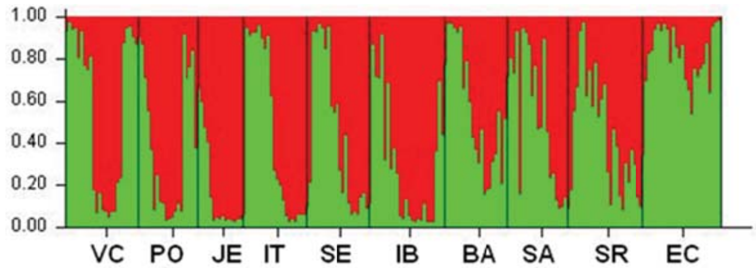


Figure 2. Result of population analysis of *Mimosa tenuiflora* showing the genetic relatedness of 10 populations of *Mimosa tenuiflora* (Table 1) estimated by STRUCTURE based on ISSR markers (K = 2; Mean (LnProb) = -14143.590). The vertical lines indicate the specimens and the colors represent the allele frequencies

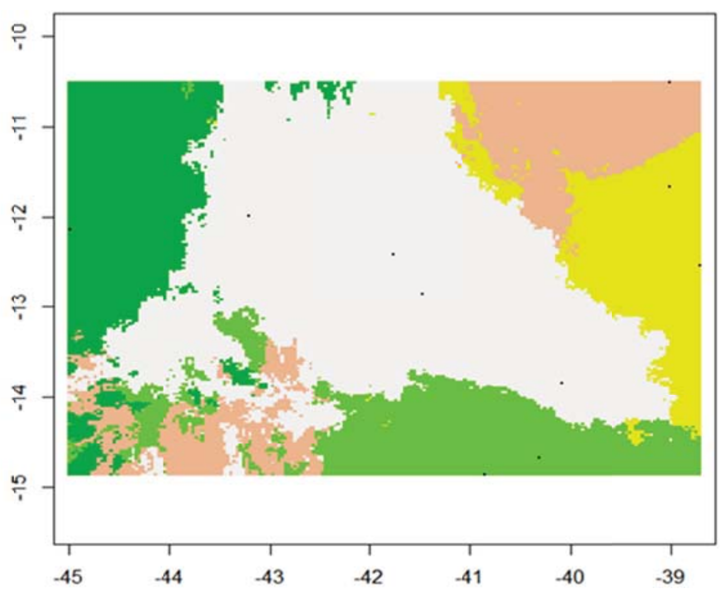


Figure 3. Mosaic of the spatial distribution of genetic groups in *Mimosa tenuiflora* based on the algorithm implemented in GENELAND (K = 5). The dots indicated the geographic location of populations and each color represents the genetic clusters, as follows: 1 (light green) comprising the BA samples, 2 (dark green) encompassing VC and PO, 3 (yellow) including SA and SR, 4 (beige) composed of EC and 5 (grey) comprising JE, IT, SE and IB

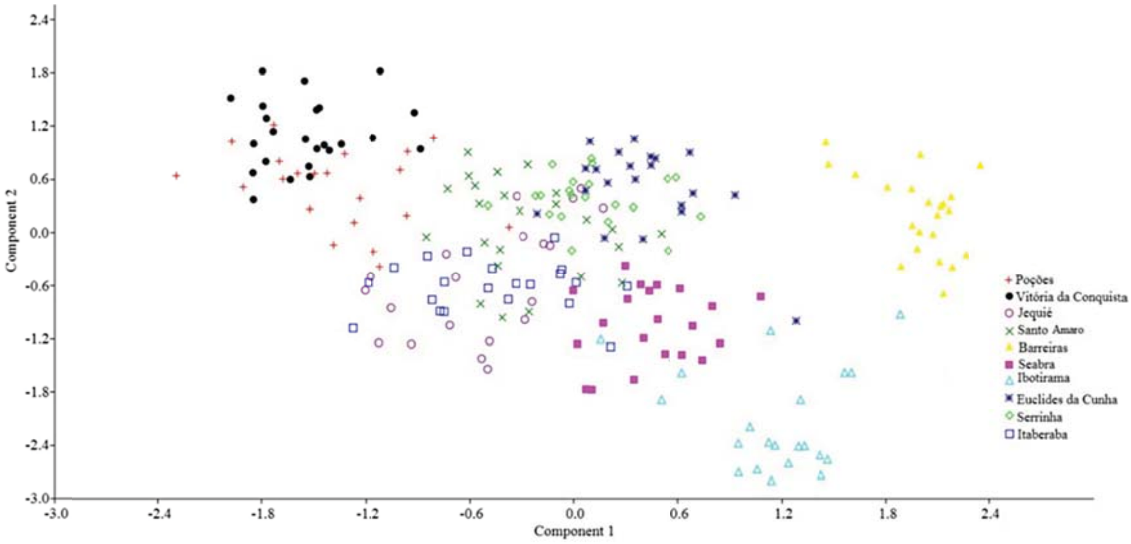


Figure 4. Graph of the PCA showing the position of each population along the axis. Principal Component Analysis (PCA) in populations *Mimosa tenuiflora* sampled in the state of Bahia, Brazil, based on the matrix of genetic distance of 117 ISSR alleles

## 4. Discussion

### *4.1 Genetic Diversity*

A high percentage of polymorphic loci (mean value around 85.0%) was detected in the present study, being superior to that reported in *M. tenuiflora* (65.3%) (Chagas, 2018) and *Mimosa caesalpiniaefolia* Benth (52.7%) (Araújo et al., 2016). The present values are typical of cross-fertilized plants as commonly observed in Fabaceae species, like *Enterolobium contortisiliquum* (Moreira et al., 2015) and *Erythrina velutina* Willd (Gonçalves et al., 2014). Increased levels of polymorphism have also been described in plant species from heterogeneous desert habitats, such as *Medicago ruthenica* (L.) Trautv. (Fabaceae) (Li et al., 2013), *Haloxylon ammodendron* (Sheng et al., 2004) and *H. salicornicum* moq (Amaranthaceae) (Salameen et al., 2018). Similarly, Caatinga is a semiarid biome characterized by habitats with differences in sunlight incidence, mean temperatures, rates of evapotranspiration, and rainfall, usually restricted to short seasonal periods (Leal et al., 2003). As a result, species living in differentiated habitats under stressful conditions usually present high values of genetic variation across their range related to local adaptation processes (Martínez-Palacios et al., 1999; Shrestha et al., 2002). The lowest polymorphism observed in the populations from JE (65.0%) is likely to reflect the high degree of deforestation for agriculture and timber exploitation in this area, determining population decline and high levels of inbreeding eventually reducing local genetic variation.

The Nei's genetic distance and Shannon index values in *M. tenuiflora* were similar to those observed in other leguminous plants (Fabaceae), such as *M. ruthenica* (Li et al., 2013) and *Adesmia bijuga* (Guerra et al., 2018). According to Cole (2003), non-rare plant species usually show increased genetic diversity, since genetic drift is affected by small populations, resulting in decreased heterozygosity levels. This statement corroborates the present study since *M. tenuiflora* is an abundant species with high levels of genetic polymorphism. It should be pointed out that the genetic diversity is a key feature to the evolutionary potential of species inasmuch as it allows selecting adaptive genotypic combinations to specific environmental conditions (Sebbenn et al., 2000; Freeland et al., 2011).

### *4.2 Genetic Structure and Gene Flow*

The present data revealed low levels of gene flow among populations of *M. tenuiflora* (Nm = 0.87) and moderate population genetic structure ($F_{ST}$~0.13; p = 0.001). In plants, $F_{ST}$ values above 0.15 are regarded as evidence of high population structure (Frankham et al., 2002), being inversely proportional to gene flow levels (Salameen et al., 2018). Most of genetic variation in *M. tenuiflora* was found within populations, as similarly observed in a previous report with this species in state of Rio Grande do Norte (Chagas, 2018) and other cross-fertilized plants such as *M. ruthenica* (Li et al., 2013), *Caryocar brasiliense* camb. (Melo Jr. et al., 2012), *H. ammodendron* (Sheng et al., 2004), and *H. salicornicum* (Salameen et al., 2018). Nonetheless, the reproductive biology of *M.*

*tenuiflora*, an essential aspect to infer the population genetic structure (Hamrick, 1989; Salameen et al., 2018), remains largely unknown.

As observed in other biomes, Caatinga has been impacted by human activities (*e.g.*, uncontrolled deforestation) thus leading to habitat fragmentation which restrains the gene flow among the populations of *M. tenuiflora*. Previous reports suggest that values of gene flow equal or higher than 1.0 are able to prevent the genetic differentiation among populations (Slatkin & Barton, 1989). According to the present cluster analyses and Nm values, the gene flow among the samples of *M. tenuiflora* are insufficient to avoid the population structure, putatively favored by the effects of low connectivity among individuals from fragmented habitats. Nonetheless, other features such as genetic isolation by geographic distance and genetic drift should also be taken into account (Khierallah et al., 2014). *M. tenuiflora* usually occur in aggregates with high population density, besides presenting reduced life cycles (~20 years) and short-distance seed dispersal, being thus more susceptible to limited gene flow and high genetic population structure (Wright, 1943).

The groups formed evidenced by the cluster analysis (Figure 2 and Figure 4) may result from the increased gene flow among nearby individuals along the distribution of populations, following the isolation-by-distance model by Wright (1943). Considering the high fragmentation of the Caatinga biome, gene flow can also be limited by the pollination by insects that usually fly over short distances. To date, there are no studies about the pollen dispersal in *M. tenuiflora*, but bees, wasps and flies have been regarded as their main pollinators (Maia-Silva et al., 2012). For instance, bees (*Apis mellifera* and *Bombus morio*) have been observed as effective pollinators of *M. bimucronata* (Silva et al., 2011) while domestic goats (*Capra hircus*) have been reported as occasional dispersers of *M. luisana*, because the infestation of bruchids in seeds can reduce the amount of seeds in the excrement from goats (Giordani, 2008). The Mantel's test corroborated the isolation model since a low but significant correlation between genetic and geographic distance was observed ($r = 0.19$, $p < 0.001$).

The number of clusters differed between both Bayesian approaches, what could be related to the distinct parameters from each analysis. It should be pointed out that Geneland takes into consideration the geographic and genetic values while Strucutre considers only the genetic estimates. According to the results based on Geneland, the cluster 1 included the first record of *M. tenuiflora* in cerrado (a Brazilian savannah biome), represented by the samples from BA. The discrimination of BA individuals in two out of the three cluster analyses should be related to the fact that this is the only population of this species found in cerrado and separated by the São Francisco River (Figure 1), thus placing this locality apart from all the other populations and putatively acting as a barrier to gene flow. The group 2 (VC and PO) comprised the populations separated by short geographic distances, high altitude and similar vegetation, favoring gene flow within this cluster. Similarly, the populations in group 3 (SA and SR) represented nearby localities in the northern portion of Bahia. The group 4 (EC) was separated from the others, representing the population located in the northern range of this species. The populations from the group 5 (IT, SE, IB, and JE) are geographically close to each other and inhabit areas that share similar altitudes, landscape and rainfall indexes (Tables 1 and 2). A similar separation of clusters was observed in the analysis using Structure, inasmuch four (IT, SE, IB and JE) out of six populations in group 2 (IT, SE, IB, JE, PO and SR) were clustered in both Bayesian approaches.

Once the genetic diversity is directly related to the survival of species in face of environmental changes (Freeland et al., 2011) and little is known about the genetic structure in most organisms from dry regions, the present data in populations of *M. tenuiflora* from semiarid regions are essential to the proper restoration of forest cover in degraded biomes, such as Caatinga. The use of pioneer plants such as *M. tenuiflora* enables the subsequent establishment of other species, the stabilization and increase of the biological activity of the soil (Chaves et al., 2006), thus being a key species to maintaining biodiversity and ecosystem functionality. In general, the populations presented high levels of genetic variation, being relevant to species conservation. Moderate population structure in black jurema is possibly related to the remarkable fragmentation of Caatinga and biological features of the analyzed species that favor the genetic differentiation among populations of *M. tenuiflora* across their range. Therefore, the local conservation of genetically divergent populations is strongly recommended. In particular, special attention should be drawn to those populations with the highest levels of polymorphism to be used in reforestation programs in Caatinga or as sources for ex situ and in situ conservation of *M. tenuiflora*.

Then, we indicate the SR, EC and IB populations was the best options to management and conservation plans inasmuch as they present high rates of genetic diversity, therefore, being the most genetically represented populations. We also point out the importance of conserving the populations from JE, BA and SA since they share low levels of genetic variation in order to avoid furthers genetic losses.

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**References**

Albuquerque, U. P., Medeiros, P. M., Almeida, A. L. S., Monteiro, J. M., Lins-Neto, E. M. F., Melo, J. G., & Santos, J. P. (2007). Medicinal plants of the Caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. *J. Ethnopharmacol, 114*(3), 325-54. https://doi.org/10.1016/j.jep.2007.08.017

Almeida, C. M. A., Lima, S. E. N., Lima, G. S. A., Brito, J. Z., Sabino, V. M. T., & Silva, M. V. (2009). Caracterização Molecular de Cultivares de Cana-de-açúcar Utilizando Marcadores ISSR. *Ciência e Agrotec., 33*(SP), 1771-1776. https://doi.org/10.1590/S1413-70542009000700012

Araújo, F. S., Pacheco, M. V., Vieira, F. A., Ferrari, C. S., Félix, F. C., & Chagas, K. P. T. (2016). ISSR molecular markers for the study of the genetic diversity of *Mimosa caesalpiniaefolia* Benth. *Idesia, 34*(3), 45-50. https://doi.org/10.4067/S0718-34292016000300007

Arruda, S. R., Pereira, D. G., Silva-Castro, M. M., Brito, M. G., & Waldschmidt, A. M. (2017). An optimized protocol for DNA extraction in plants with a high content of secondary metabolites, based on leaves of *Mimosa tenuiflora* (Willd.) Poir. (Leguminosae). *Genet Mol Res, 16*(3). https://doi.org/10.4238/gmr16039063

Barneby, C. R. (1991). Sensitivae Censitae. A description of the genus *Mimosa L.* (Mimosaceae) in the New World. *Mem N Y Bot Gard., 65*, 835.

Braga, R. (1989). *Plantas do Nordeste, especialmente no Ceará*. Editora Universitária da UFRN, Natal, Rio Grande do Norte.

Chagas, K. P. T. (2018). *Diversidade genética e modelagem preditiva de distribuição de Mimosa tenuiflora (Willd) Poiret* (Master's thesis, Universidade Federal do Rio Grande do Norte, Macaíba, Brazil). Retrieved from https://repositorio.ufrn.br/bitstream/123456789/26582/1/Diversidadegen%C3%A9ticamodelagem_Chagas_2018.pdf

Chaves, L. L. B. (2006). Crescimento de mudas de angico-vermelho em substrato fertilizado e inoculado com rizóbio. *Revista Árvore, 30*, 911-919. https://doi.org/10.1590/S0100-67622006000600006

Cole, C. T. (2003). Genetic variation in rare and common plants. *Annu Rev Ecol Evol Syst, 34*(1), 213-237. https://doi.org/10.1146/annurev.ecolsys.34.030102.151717

Diniz-Filho, J. A. F., & Telles, M. P. C. (2002). Spacial autocorrelation analysis and the identification of operational units for conservation in continuous populations. *Conserv Biol, 16*(4), 924-935. https://doi.org/10.1046/j.1523-1739.2002.00295.x

Dutra, V. F., & Morim, M. P. (2015a). *Mimosa in Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. Retrieved from http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB18831

Dutra, V. F., & Morim, M. P. (2015b). *Mimosa in Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. Retrieved from http://floradobrasil.jbrj.gov.br/jabot/fichapublicataxonuc/fichapublicataxonuc.do?id=fb18874

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol Ecol, 14*(8), 2611-20. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L. G., & Schneider, S. (2005). Arlequin, version 3.1 for Windows: An integrated software package for population genetics data analysis. *Evol Bioinform Online, 1*, 47-50. https://doi.org/10.1177/117693430500100003

Falush, D., Stephens, M., & Prichard, J. K. (2007). Inference of population structure using multilocus genotype data: Dominant markes and null alleles. *Mol Ecol, 7*(4), 574-578. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Frankham, R., Briscoe, D. A., & Ballou, J. D. (2002). *Introduction to conservation genetics*. Cambridge University Press. https://doi.org/10.1017/CBO9780511808999

Freeland, J. R., Kirk, H., & Petersen, S. (2011). *Molecular ecology*. Chichester, West Sussex. https://doi.org/10.1002/9780470979365

Giordani, L. (2008). *The role of goats in germination and dispersal of Mimosa luisana Brandegee (Leguminosae-Mimosoideae) seeds in Tehuacán-Cuicatlán Valley, Puebla State, Mexico* (Master's thesis, Norwegian University of life Sciences, Oslo, Norway). Retrieved from https://www.researchgate.net/publication/242144285

Gonçalves, L. O., Pinheiro, J. B., Zucchi, M. I., & Silva, R. (2014). Caracterização genética de mulungu (*Erythrina velutina* Willd.) em áreas de baixa ocorrência. *Ciênc. Agron., 45*, 290-298. https://doi.org/10.1590/S1806-66902014000200009

Guerra, F. P., Gomez, P., Gutierrez, A., & Hahn, S. (2018). Genetic diversity of *Adesmia bijuga* Phil., an endangered Fabaceae species from Central Chile. *Braz. J. Bot., 41*, 247-251. https://doi.org/10.1007/s40415-018-0440-5

Guillot, G., Mortier, F., & Estoup, A. (2005). GENELAND: A computer package for landscape genetics. *Molecular Ecology Notes, 5*, 712-715. https://doi.org/10.1111/j.1471-8286.2005.01031.x

Hammer, O., Harper, D. A. T., & Ryan, P. D. (2001). PAST, version 2.0 for Windows: Palaeontological Statistics software package for education and data analysis. *Palaeontol. Electron., 4*, 1-9.

Hamrick, J. L. (1989). Isozymes and the analysis of genetic structure in plant. In J. L. Hamrick (Ed.), *Isozymes in plant biology* (pp. 87-105). Springer, Dordrecht. https://doi.org/10.1007/978-94-009-1840-5_5

Hijmans, R. J., Guarino, L., Cruz, M., & Rojas, E. (2001). Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Plant Genetic Resources Newsletter, 127*, 15-19.

Kageyama, P. Y., Gandara, F. B., & Oliveira, R. E. (2003). Biodiversidade e restauração da floresta tropical. In P. Y. Kageyama, F. B. Gandara, R. E. Oliveira, L. F. D. Moraes, V. L. Engel, & F. B. Gandara (Eds.), *Restauração ecológica de ecossistemas naturais* (pp. 27-48). Botucatu, São Paulo.

Khierallah, H. S. M., Al-Sammarraie, S. K. I., & Mohammed, H. I. (2014). Molecular characterization of some Iraqi date palm cultivars using RAPD and ISSR markers. *J of Asian Sci Res, 4*, 490-503.

Kumar, V., & Yadav, H. K. (2018). Assessment of genetic diversity in *Lepidium sativum* L. using inter simple sequence repeat (ISSR) marker. *Physiol Mol Biol Plants, 25*(2), 399-406. https://doi.org/10.1007/s12298-018-0622-4

Leal, I. R., Silva, J. M. C., Tabarelli, M., Thomas, E., & Lacher, J. R. (2005). Mudando o curso da conservação da biodiversidade na Caatinga do Nordeste do Brasil. *Megadiversidade, 1*, 139-146.

Leal, I. R., Tabarelli, M., & Silva, J. M. C. (2003). *Ecologia e conservação da Caatinga.* Ed Universitária da UFPE, Recife.

Li, H., Li, Z., Cai, L., Shi, W., Mi, F., & Shi, F. (2013). Analysis of genetic diversity of Ruthenia Medic (*Medicago ruthenica* (L.) Trautv.) in Inner Mongolia using ISSR and SSR markers. *Genet Resour Crop Evol, 60*, 1687-1694. https://doi.org/10.1007/s10722-012-9950-3

Lima, A. T., & Meiado, M. V. (2018). Effect of hydration and dehydration cycles on *Mimosa tenuiflora* seeds during germination and initial development. *South African Journal of Botany, 116*, 164-167. https://doi.org/10.1016/j.sajb.2018.03.017

Maia, G. N. (2004). *Caatinga: Árvores e arbustos e suas utilidades.* D&Z Computação Gráfica e Editora, São Paulo.

Maia-Silva, C., Silva, C. I., Hrncir, M., Queiroz, R. T., & Imperatriz-Fonseca, V. L (2012). *Guia de plantas visitadas por abelhas na Caatinga*. Fundação Brasil Cidadão, Fortaleza.

Manly, B. J. F. (1997). *Randomization, Bootstrap and Monte Carlo Methods in Biology*. Chapman & Hall, London.

Martínez-Palacios, M., Eguiarte, L. E., & Furnier, G. R. (1999). Genetic Diversity of the Endangered Endemic Agave *Victoriae reginae* (Gavaceae) in the Chihuahuan Desert. *Am J Bot, 86*(8), 1093-8. https://doi.org/10.2307/2656971

Melo Jr., A. F., Carvalho, D., Vieira. F. A., & Oliveira. D. A. (2012). Spatial genetic structure in natural populations of *Caryocar brasiliense* Camb. (Caryocaraceae) in the North of Minas Gerais, Brazil. *Biochem Syst Ecol, 43*, 205-209. https://doi.org/10.1016/j.bse.2012.02.005

Miller, P. M. (1997). *TFPGA: Tools for populations genetics analyses*. IOP Publishing Physics. Retrieved from http://www.ccg.unam.mx/~vinuesa/tlem09/docs/TFPGADOC.PDF

Miller, P. M. (2005). Alleles In Space (AIS): Computer Software for the Joint Analysis of Interindividual Spatial and Genetic Information. *The Journal of Heredity, 96*(6), 722-4. https://doi.org/10.1093/jhered/esi119

Moreira, P. de A., Brandão, M. M., de Araujo, N. H., de Oliveira, D. A., & Fernandes, G. W. (2015). Genetic diversity and structure of the tree *Enterolobium contortisiliquum* (Fabaceae) associated with remnants of a seasonally dry tropical forest. *Flora—Morphology, Distribution, Functional Ecology of Plants, 210*, 40-46. https://doi.org/10.1016/j.flora.2014.10.005

Nei, M. (1973). Analysis of gene diversity in subdivided population. *Proc Natl Acad Sci USA, 70*(12), 3321-3. https://doi.org/10.1073/pnas.70.12.3321

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics, 89*, 583-1590.

Pinto, K. M. S., Melo, P. A. F. R., Nascimento, L. C., Cortez, M. I. G. M., Aires, A. A. C., Mondego, J. M., … Lemos, R. N. S. (2018). Biological potential of extracts of Caatinga plants in the control of *Alternaria alternata* f. sp. citri in citrus. *Journal of Agricultural Science, 10*(12), 116. https://doi.org/10.5539/jas.v10n12p116

Queiroz, L. P. (2009). *Leguminosas da Caatinga*. Universidade Estadual de Feira de Santana/Royal Botanic Gardens, Kew/Associação Plantas do Nordeste, Feira de Santana.

Rajora, O. M., & Mosseler, A. (2001). Challenges and opportunities for conservation of forest genetic resources. *Euphytica, 118*, 197-212. https://doi.org/10.1023/A:1004150525384

Reddy, M. P., Sarla, N., & Siddiq, E. A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica, 128*, 9-17. https://doi.org/10.1023/A:1020691618797

Riegelhaupt, E. M., & Pareyn, F. G. C. (2010). A questão energética e o manejo florestal da Caatinga. In M. A. Gariglio, E. V. S. B. Sampaio, L. A Cestaro, & P. Y. Kageyama (Eds.), *Uso sustentável e conservação dos recursos florestais da Caatinga* (pp. 65-75). Serviço Florestal Brasileiro, Brasília.

Salameen, F. A., Habibi, N., Kumar, V., Amad, S. A., Dashti, J., Talebi, L., & Bashayer, A. D. (2018). Genetic diversity and population structure of *Haloxylon salicornicum* moq. in Kuwait by ISSR markers. *PLoS ONE, 13*(11), e0207369. https://doi.org/10.1371/journal.pone.0207369

Santos, M. O., Almeida, B. V., Ribeiro, D. A., Macêdo, D. G., Macêdo, M. J. F., ... Souza, M. M. A. (2017). The conservation of native priority medicinal plants in a Caatinga area in Ceará, northeastern Brazil. *Annals of the Brazilian Academy of Sciences, 89*, 2675-2685. https://doi.org/10.1590/0001-3765201720160633

Sebbenn, A. M., Kageyama, P. Y., Siqueira, A. C. M. F., & Zanatto, A. C. S. (2000). Sistemas de cruzamento em populações naturais de *Cariniana legalis* Mart. O. ktze.: Implicações para a conservação e o melhoramento genético. *Sci For, 58*, 25-40.

Sheng, Y., Zheng, W. H., Pei, K. Q., & Ma, K. P. (2004). Population Genetic Structure of a Dominant Desert Tree, *Haloxylon ammodendron* (Chenopodiaceae), in the Southeast Gurbantunggut Desert Detected by RAPD and ISSR Markers. *Acta Bot Sin, 46*, 675-681.

Shrestha, M. K., Golan-Goldhirsh, A., & Ward, D. (2002). Population genetic structure and the conservation of isolated population of *Acacia raddiana* in the Negev Desert. *Biol Conserv, 108*(1), 119-127. https://doi.org/10.1016/S0006-3207(02)001003

Silva, A. S., Fernandes, N. S., Cavalcante, A. M., Lima, A. O. N., & Freitas, B. M. (2015). Florescimento induzido da jurema preta para fornecer pólen à abelha melífera na estiagem da Caatinga. *Rev Caatinga, 28*, 197-206. https://doi.org/10.1590/1983-21252015v28n302rc

Silva, L. A., Guimarães, E., Rossi, M. N., & Maimoni-Rodella, R. D. C. S. (2011). Biologia da reprodução de Mimosa bimucronata: Uma espécie ruderal. *Planta Daninha, 29*(SP), 1011-1021. https://doi.org/10.1590/S0100-83582011000500007

Simon, M. F., Grether, R., Queiroz, L. P., Sarkinen, T. E., Dutra, V. F., & Hughes, C. E. (2011). The evolutionary history of *Mimosa* (Leguminosae): Toward a phylogeny of the sensitive plants. *Am J Bot, 98*(7), 1201-21. https://doi.org/10.3732/ajb.1000520

Slatkin, S. M., & Barton, N. H. (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evolution, 43*(7), 1349-1368. https://doi.org/10.1111/j.1558-5646.1989.tb02587.x

Souza, R. S. O., Albuquerque, U. P., Monteiro, J. M., & Amorim, E. L. C. (2008). Jurema-Preta (*Mimosa tenuiflora* [Willd.] Poir.): A review of its traditional use, phytochemistry and pharmacology. *Braz. Arch. Biol. Technol., 51*(5), 937-947. https://doi.org/10.1590/S1516-89132008000500010

Wang, X., Yang, R., Feng, S., Hou, X., Zhang, Y., Li, Y., & Ren, Y. (2012). Genetic Variation in *Rheum palmatum* and *Rheum tanguticum* (Polygonaceae), Two Medicinally and Endemic Species in China Using ISSR Markers. *PloS ONE, 7*(12), e51667. https://doi.org/10.1371/journal.pone.0051667

Wright, S. (1943). Isolation by distance. *Genetics, 28*, 114-138.

## Appendix A

**Graphs identifying the optimal number of populations following Evanno's methods**

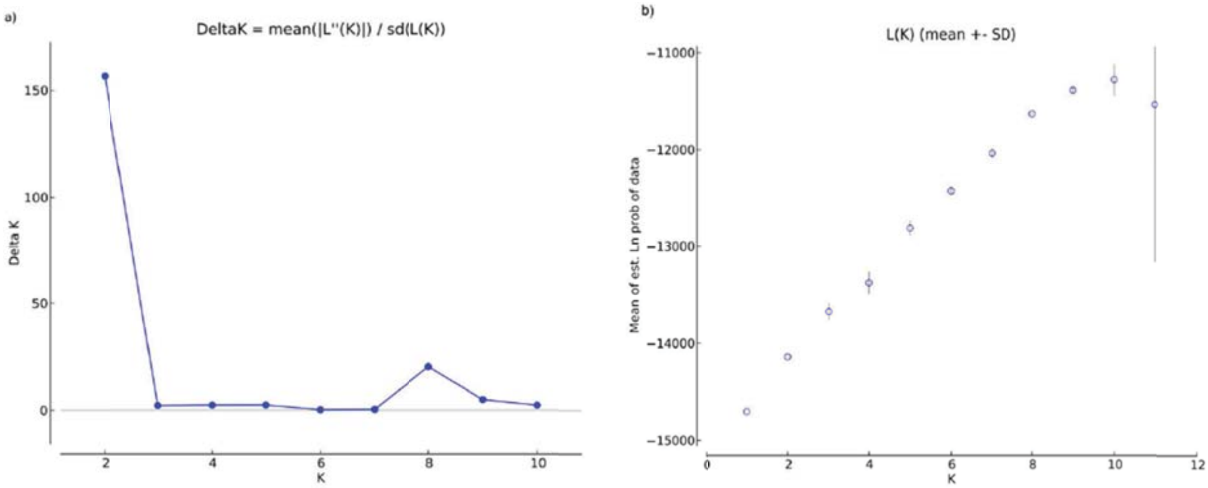


Figura A1. Graph used to demonstrate the DeltaK value (a) and the L(K) value (b), a single K value out of a range of K values, which captures the uppermost level of structure (Evanno et al., 2015)

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# *Basmati* Rice Quality Enhancement by Zinc Fertilization and Green Manuring on a Sub-tropical Inceptisol in Indo-Gangetic Plains of India

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**Abstract**

*Basmati* (aromatic) rice is premier rice grown in north-western India and Pakistan. This rice is preferred for their long and slender kernels which expand 3-4 times in length and remain fluffy and are well known all over the world, especially in the Middle East and South Asia for their long fluffy grains on cooking. Paddy soils are usually deficient in organic matter because of high temperature and moisture, which causes rapid decomposition of organic matter. The importance of leguminous green manure crops in improving soil fertility, and soil physical properties received increasing attention. Also, the zinc (Zn) deficiency in soils is prevalent worldwide, especially in high pH calcareous soils. No reports were available on combining green manuring crops and Zn fertilization on productivity, Zn content and kernel quality of *Basmati* rice. Therefore, the current investigation was undertaken to quantify the combined effects of summer green manuring crops and zinc fertilization on productivity, Zn content and kernel quality of *Basmati* rice in summer green manuring-*Basmati* rice cropping system. A field study was therefore conducted for two years (2009 and 2010) on a sandy clay-loam soil (*typic Ustochrept*) at the research farm of the ICAR-Indian Agricultural Research Institute, New Delhi, India. The experiments were conducted in split plot design, keeping three green manuring crops viz. *Sesbania aculeata* (Dhaincha), *Crotalaria juncea* (Sunhemp), and *Vigna unguiculata* (Cowpea) and one summer fallow treatment as main-plot treatments and six Zn sources viz. control (no Zn application), $ZnSO_4 \cdot 7H_2O$ (21% Zn), $ZnSO_4 \cdot H_2O$ (33% Zn), ZnO (82% Zn), $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) and EDTA-chelated Zn (12% Zn) in sub-plots and was replicated thrice. The experiments in both the years were conducted with a fixed lay-out plan on the same site. The results showed that incorporation of green manures along with zinc (Zn) fertilization increased grain and straw yield, enhanced Zn concentrations and improved the kernel quality before and after cooking in *Basmati* rice 'Pusa *Basmati* 1'. The application of EDTA-chelated Zn (12% Zn) was the best in terms of grain and straw yield and Zn concentrations in grain and straw and kernel quality before and after cooking *Basmati* rice. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was the second-best treatment followed by $ZnSO_4 \cdot H_2O$ (33% Zn) and $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%). Application of ZnO (82% Zn) had least effect in increasing the studied parameters. The lowest values were observed with control (no Zn application). Among the summer green manuring crops, incorporation of *Sesbania aculeata* (Dhaincha) was found to be the best over *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow in terms of grain and straw yield, Zn concentrations in grain and straw and kernel quality before and after cooking in *Basmati* rice. Zn fertilization with EDTA-chelated Zn (12% Zn) lead to 25.91 and 21.26% higher grain yield; 60.66 and 82.14% Zn-denser grains; with 13.33 and 10.92% increase in head rice recovery in *Basmati* rice over control (no Zn application) during 2009 and 2010, respectively.

**Keywords:** *Basmati* rice, India, kernel quality parameters, South Asia, sub-tropical region, summer green manuring crops, yields, zinc concentration, zinc sources

## 1. Introduction

*Basmati* (aromatic) rice is the premier rice grown in north-western India and Pakistan. This rice is preferred for their long and slender kernels which expand 3-4 times in length and remain fluffy. The varieties of *Basmati* rice differ greatly in kind and intensity of aroma. The aroma in rice is due to chemical diacetyl 1-pyrolline (V. P. Singh & A. K. Singh, 2009). These type of rice are well known all over the world, especially in the Middle East and South Asia for their long fluffy grains on cooking, a desirable characteristic for the dish *Biryani* or *Pulao* made by cooking rice with vegetables, mutton or chicken and flavoured with special oriental spices (Shivay et al., 2010). Traditional *Basmati* rice in India's Doon valley is tall *indicas* yielding 1.5 to 2.0 tonnes per hectare and lodge on heavy fertilization. Therefore, attempts were made at the ICAR-Indian Agricultural Research Institute, New Delhi to develop *Basmati* rice varieties having high yielding qualities of semi-dwarf *indicas* rice and having better quality aromas than the traditional one. Considering the great demand of *Basmati* rice globally, the researchers made concerted research efforts at the ICAR-Indian Agricultural Research Institute, New Delhi, India to develop rice varieties having both *Basmati* traits and high yield potential. This involved convergent breeding and simultaneous selection at field and laboratory for the complex inherited key characteristics of *Basmati* quality (extra-long slender grain, excessive elongation on cooking, aroma and ideal physico-chemical properties of starch) and high yield potential of new semi-dwarf rice varieties (Shivay et al., 2010). Pusa *Basmati*-1 resulted from this research, which took about 25 years and now occupies the largest area in north-western India and fetches about the US $ 500 million in the international market (Siddiq, 2006). 'Pusa *Basmati*-1' is high yielding *Basmati* rice with long and slender kernels as per classification by Jennings et al. (1979). *Basmati* rice varieties developed from IARI now covered about 60% of the total area under rice in north-western India, which is estimated at 5 million hectares.

Paddy soils are usually deficient in organic matter because of high temperature and moisture, which causes rapid decomposition of organic matter (Mohammed et al., 2005). The application of green manures to the soil is considered a good management practice in any agricultural production system because it can increase cropping system sustainability by reducing soil erosion and ameliorating soil physical properties, by increasing soil organic matter and fertility levels (Mandal et al., 2003), by increasing nutrient retention and by reducing global warming potential (Robertson et al., 2000). Leguminous and non-leguminous plants are used in the production of green manures. Leguminous plants form symbiotic associations with *Rhizobium* bacteria to fix $N_2$. This fact causes the green manures, which their principal component are leguminous plant debris, supply to the important soil amounts of N in relation to the green manures obtained from non-leguminous plants.

The importance of leguminous green manure crops in improving soil fertility, and soil physical properties received increasing attention (Whitbread et al., 2000; Ray & Gupta, 2001). The improvement in physical soil conditions as a result of build-up of organic matter by incorporation of green manure or crop residue is associated with a decrease in bulk density, increase in total pore space, water-stable aggregates and hydraulic conductivity of the soil (Tejada et al., 2008a, 2008b). Fast-growing leguminous green manures with their adaptability to fit in rice-based cropping patterns and their ability to fix atmospheric nitrogen may offer opportunities to increase and sustain productivity and income in the rice-based cropping systems (Yadvinder-Singh et al., 1991). Green manures enhance organic matter which is the most important benefit credited to green manures. The positive effect of green manures on paddy yield has been reported by Sharma and Prasad (1999). Hemalatha et al. (1999) observed that *in-situ* incorporation of cowpea before transplanting of rice increased the grain yield by 18% and straw yield by 16% and the quality of rice.

Similarly, Bhatti et al. (1983) reported that *Sesbania* green manuring substantially improved grain yield to 72%. Incorporation of green manures before transplanting rice can ameliorate the Fe and Zn deficiency by promoting reduced condition and improving other physico-chemical properties of soil. *Sesbania aculeata* (Dhaincha), *Crotalaria juncea* (Sunhemp) and *Vigna unguiculata* (Cowpea) are some of the important leguminous green manuring crops for north-western Indian region. It is expected that regular incorporation of green manuring crops before transplanting of rice may improve not only the physico-chemical properties of the earth but also the availability of macro and micronutrients in soil and zinc fertilization in *Basmati* rice may help in improving its grain quality.

Zinc deficiency in soils is prevalent worldwide, especially in high pH calcareous soils (Adriano, 2001; Fageria et al., 2003; Norman et al., 2003; Prasad, 2006; Alloway, 2008; Cakmak, 2002, 2008a, 2008b). A recent analysis of two hundred forty-one thousand soil samples in India showed that 49% soils are deficient in Zn (Behera et al., 2009) and Zn deficiency is widespread in north Indian rice-wheat cropping system belt (Prasad, 2005). In rice, zinc deficiency is characterized by brown spots, which appear first in the younger leaves and later in the lower leaves. In severe Zn deficiency, burnt dark brown patches of plants in rice fields (Dobermann & Fairhurst, 2000). Zinc deficiency in rice was first reported by Nene (1966).

The response of rice to Zn has been reported by several workers in India (Srivastava et al., 2006; Shivay et al., 2007, 2010; Pooniya et al., 2012; Ghasal et al., 2018; Yadav et al., 2019), China (Shihua & Wenqiang, 2000) and the USA (Slaton et al., 2005). Zinc is now recognized as the fifth leading health risk factor in developing Asian countries, where rice is the staple food (Anonymous, 2007) and Zn nutrition of humans and animals has recently received considerable attention (WHO, 2002). Hotz and Brown (2004) estimated that 1.2 billion (20% of the world population) are at risk of inadequate Zn uptake. There is a HarvestPlus Global Challenge Programme of the Consultative Group on International Agricultural Research (CGIAR) focusing on breeding Zn efficient cultivars (HarvestPlus, 2009) to evolve rice and other cereals varieties with the denser-Zn grain. Nevertheless, there are problems in developing rice varieties with high grain yields and denser-Zn grains. However, as pointed out by Prasad (2009) this can be achieved at a faster rate through application of Zn fertilizers and that too without any compromise on grain yield under Zn stress conditions. Even when Zn-efficient cultivars are developed, adequate Zn fertilizers would be required to make up the Zn depletion of soils (Shivay et al., 2010; Prasad & Shivay, 2020).

Zn fertilizers (oxides, sulphates and other Zn salts) since, as with most crops, the normal way of correcting Zn deficiencies in soils is to apply these fertilizers (Brennan & Bolland, 2006; Alvarez et al., 2009). However, In India, Zn deficiency is usually corrected through the application of inorganic salt, mainly $ZnSO_4·7H_2O$. Soil application of 5 kg Zn ha$^{-1}$ (25 kg $ZnSO_4·7H_2O$ ha$^{-1}$) is recommended for rice in India. However, the product is quite costly and generally not available to farmers', resulting in reduced rice yield. Other sources are the chelated forms of Zn such as Zn-EDTA, which supplies a substantial amount of Zn to the plants without interacting with soil components (Karak et al., 2005) because the central metal ion $Zn^{2+}$ is surrounded by chelate ligands (Mortvedt, 1979), needs to be investigated.

However, no reports are available on combining green manuring crops and Zn fertilization on productivity, Zn content and kernel quality of *Basmati* rice. Therefore, the current investigation was undertaken to quantify the combined effects of summer green manuring crops and zinc fertilization on productivity, Zn content and kernel quality of *Basmati* rice in summer green manuring-*Basmati* rice cropping system.

## 2. Materials and Methods

### *2.1 Description of the Study Area*

The field experiments were conducted for two consecutive years at the ICAR-Indian Agricultural Research Institute, New Delhi, India during summer-*Kharif*/rainy seasons (April-October) of 2009 and 2010 on a sandy clay-loam soil (*typic Ustochrept*). The experiments in both the years were conducted with a fixed layout plan on the same site. The institute farm is located at a latitude of 28$^o$38′ N, longitude of 77$^o$10′ E and altitude of 228.6 m above the mean sea level. The mean annual rainfall of New Delhi is 650 mm, and more than 80% generally occurs during the south-west monsoon season (July-September) with mean yearly evaporation of 850 mm.

The soils of the experimental field had 135.75 kg ha$^{-1}$ alkaline permanganate oxidizable nitrogen (N) (Subbiah & Asija, 1956), 16.04 kg ha$^{-1}$ available phosphorus (P) (Olsen et al., 1954), 292.10 kg ha$^{-1}$ 1 *N* ammonium acetate exchangeable potassium (K) (Hanway & Heidel, 1952) and 0.53% organic carbon (C) (Walkley & Black, 1934). The pH of the soil was 7.5 (1:2.5 soil and water ratio) (Prasad et al., 2006) and diethylene triamine penta acetic acid (DTPA)-extractable Zn (Lindsay & Norvell, 1978) in soil was 0.67 mg kg$^{-1}$ of soil. The critical level of DTPA-extractable Zn for rice grown on alluvial soils in the rice-wheat belt of North India varies from 0.38-0.90 mg kg$^{-1}$ soil (Takkar et al., 1997).

### *2.2 Experimental Treatments and Design*

The experiment was conducted in a split-plot design, keeping three green manuring crops viz. *Sesbania aculeata* (Dhaincha), *Crotalaria juncea* (Sunhemp), and *Vigna unguiculata* (Cowpea) and one summer fallow treatment as main-plot treatments and five Zn sources viz. $ZnSO_4·7H_2O$ (21% Zn), $ZnSO_4·H_2O$ (33% Zn), ZnO (82% Zn), $ZnSO_4·7H_2O$ + ZnO (50% + 50%), EDTA-chelated Zn (12% Zn) and a control (no Zn application), in sub-plots and was replicated thrice.

### *2.3 Application of Treatments and Fertilizers*

During summer seasons three summer green manuring crops viz. *Sesbania aculeata* (Dhaincha), *Crotalaria juncea* (Sunhemp), and *Vigna unguiculata* (Cowpea) were planted as main plot treatments. A summer fallow treatment was also considered as the control. After 40 days the summer green manuring crops were incorporated into the soil before transplanting of rice. After incorporating green manuring crops, each main plot (with green manure incorporation or otherwise) was divided into six sub-plots, which received the Zn-fertilization treatments. The experimental field was disk-ploughed twice, puddled three times with a puddler in standing water and levelled. At final puddling 26 kg, P $ha^{-1}$ as single superphosphate and 33 kg K $ha^{-1}$ as muriate of potash was broadcasted. Addition of 26 kg P as single super phosphate also supplied 45 kg S $ha^{-1}$ and took care of S deficiency (if any). It also takes care of the S advantage of $ZnSO_4{\cdot}7H_2O$ and permits a fair comparison of $ZnSO_4{\cdot}7H_2O$ and ZnO or EDTA-chelated Zn as a source of Zn. Nitrogen at 150 kg $ha^{-1}$ as prilled urea was applied into two equal splits, half at the time of transplanting and remaining half at panicle initiation stage (40 DAT). In all the Zn treatments, uniformly 5 kg Zn $ha^{-1}$ was applied at the time of transplanting.

### *2.4 Rice Transplanting*

Three 25-day-old seedlings of *Basmati* rice (*Oryza sativa* L.) variety 'Pusa *Basmati* 1' was transplanted per hill at 20 cm × 10 cm in the first fortnight of July in both the years of study. It is a *Basmati* (aromatic) variety released from ICAR-Indian Agricultural Research Institute, New Delhi, India during 1989 for its commercial cultivation. It is a cross between 'Pusa 150' and 'Karnal Lokal'. It produces long slender grains with good aroma and excellent cooking qualities (Rani et al., 2009; V. P. Singh & A. K. Singh, 2009; Siddiq et al., 2012). Irrigation channels measuring 1 m wide were placed between the replications to ensure an easy and uninterrupted irrigation water flow. An individual plot was independently irrigated from the irrigation channels. Rice crop was grown as per recommended package of practices and was harvested in the second fortnight of October in both the years of experimentation.

### *2.5 Measurements of Yields of Rice*

Harvesting of the *Basmati* rice was undertaken as soon as it attained the harvest maturity. The harvesting was done with the help of sickles after leaving the border area. Net plots were demarcated at first from the portion of the plot kept for recording grain yield. Plants from the demarcated net plot area were harvested, tied in bundles and taken to the threshing floor for drying and threshing. The harvested plants were dried for three days to bring down the moisture content to around 14%. After threshing, the seeds were cleaned, sun-dried and their weight was recorded. The yields in kg $plot^{-1}$ were converted to tonnes $ha^{-1}$. The weight of the harvested plants after sun drying and before threshing was recorded. The straw yield was obtained by deducting the seed weight from the total weight. The grain and straw yields were expressed in tonnes $ha^{-1}$. The 1000-filled grains, taken at harvest, were first counted by a seed counter and then weighed to compute the 1000-grain weight.

### *2.6 Chemical Analysis of Zn Concentration in Rice Grain and Straw*

At harvest, the dried plants were separated into grain and straw, ground in a milling machine, sieved through 0.7 mm sieve and analysed for Zn content separately. Dried plant samples were digested with di-acid [perchloric acid ($HClO_4$) + nitric acid ($HNO_3$) in 3:10 ratio] as per procedures described by Prasad et al. (2006). After digestion and extraction of samples, total Zn was estimated with the atomic absorption spectrophotometer (Perkin Elmer; Model-A. Analyst 100).

### *2.7 Kernel Quality Parameters*

#### 2.7.1 Milling Quality Parameter

*Hulling (%)*: Well sun-dried paddy (rough or unhulled rice) samples of each treatment weighing 100 g from each replication were hulled in a mini "Satake Rice Mill" (Satake, 1990), the weight of brown rice was recorded, and the hulling percentage was calculated as:

$$\text{Hulling (\%)} = [\text{Weight of brown rice (g)}/\text{Weight of rough rice (g)}] \times 100 \qquad (1)$$

*Milling (%)*: To obtain uniformly polished grains, the hulled brown rice was passed through a 'Satake Rice Polishing Machine' (Satake, 1990) for 2 minutes. The polished rice was weighed, and milling percentage was worked out as under:

$$\text{Milling (\%)} = [\text{Weight of milled rice (g)}/\text{Weight of rough rice (g)}] \times 100 \qquad (2)$$

*Head Rice Recovery:* The milled rice was passed through an appropriate sieve to separate whole kernels from the broken ones. Head rice recovery (%) was computed as:

$$\text{Head rice recovery (\%)} = [\text{Weight of whole milled rice (g)}/\text{Weight of rough rice (g)}] \times 100 \qquad (3)$$

2.7.2 Kernel Cooking Quality Parameters

(1) Kernel Length and Breadth Before Cooking

Ten milled kernels from each plot were taken at random and placed separately on a graph paper, and their length and breadth were measured using a 'Photo Enlarger' with a magnification of 3X. The actual mean kernel length and breadth was expressed in mm.

(2) Kernel Length and Breadth After Cooking

Rice cooking technique was a simple modification of the technique used by Juliano and Perez (1984). A sample of ten kernels was taken in 15 cm long and 2.5 cm wide test tubes and pre-soaked in 5 ml of tap water for 30 minutes. The tubes were then placed in a water bath maintained at boiling temperature (using a Thermotech temperature controller TH-013; Thermotech, Gujarat, India) for 6-7 minutes. After cooking, the tubes were taken out and cooled under running water for 2 minutes. Cooked kernels were taken out of the tubes, and excess water was removed with a blotting paper. Length and breadth of cooked kernels were measured, as mentioned above.

(3) Kernel Elongation Ratio

*Kernel Length Expansion Ratio:* The kernel length expansion ratio was calculated by dividing the cooked kernel's length by its original length.

$$\text{Length expansion ratio} = L_2/L_1 \tag{4}$$

where, $L_1$ and $L_2$ are kernel length before and after cooking, respectively.

*Kernel Breadth Expansion Ratio:* The kernel breadth expansion ratio was calculated by dividing the cooked kernel's breadth by its original breadth.

$$\text{Breadth expansion ratio} = B_2/B_1 \tag{5}$$

where, $B_1$ and $B_2$ are grain length before and after cooking, respectively.

*2.8 Protein Content*

Protein content in *Basmati* rice grain was obtained by multiplying N concentration with a coefficient factor 5.95 (Juliano, 1979; Juliano, 1985). This factor is based on the nitrogen content (16.8%) of the major rice protein (Glutelin). The protein content was expressed in percentage.

*2.9 Amylose Content*

A sample of 1 g milled rice grains was gently crushed and made into fine powder in a vitreous pestle and mortar. The flour samples were stored to uniform moisture of 12%. One hundred mg sample was weighed carefully on an electric meter balance and transferred to a 100 milliliter (ml) volumetric flask. One ml of distilled ethanol was added and mixed well. Ten ml of freshly prepared 1 *N* NaOH solution was added to it. After gelatinization, the sample suspension was heated for 10 minutes in a boiling water bath. The volume was made up to 100 ml with distilled water. After thoroughly shaking the content, an aliquot of 2.5 ml was pipetted out into a 50 ml volumetric flask and added about 20 ml of water. Three drops of phenolphthalein indicator were added mixed well. The content was acidified by adding 0.1 *N* HCl drop by drop until the pink colour disappears. Then one ml of iodine reagent was added to develop blue colour and volume was made up to 50 ml. The absorbance at 590 nm was recorded with the help of a Spectrophotometer.

A standard curve was prepared based on the absorbance values of known quantities of pure amylose (rice amylose). The amount of amylose in the sample using the standard curve was prepared from pure amylose (range 0.2-1.0 mg) against a blank for which diluted 1 ml of iodine reagent to 50 ml with distilled water was used (Sadasivam & Manickam, 1992; Thanyumanavan & Sadasivam, 1984; Thimmiah, 1999).

$$\text{Absorbance corresponds to 2.5 ml of test solution} = \text{'}\times\text{' mg amylose in a test solution} \tag{6}$$

$$\text{100 ml contains} = [X/2.5] \times 100\% \text{ amylose} \tag{7}$$

*2.10 Statistical Analysis*

All the data obtained from rice crop for consecutive two years of study were statistically analyzed using the F-test as per the procedure given by K. A. Gomez and A. A. Gomez (1984). Least significant difference (LSD) values at $P = 0.05$ were used to determine the significance of differences between treatment means.

## 3. Results

### *3.1 Grain and Straw Yields of Basmati Rice*

In general, the grain and straw yields were higher during the second year of experimentation (Table 1). *Basmati* rice yields were significantly influenced by incorporating summer green manuring crop residues and Zn sources. The significantly higher grain and straw yields of *Basmati* rice were recorded when it was grown after *Sesbania aculeata* (Dhaincha) incorporation compared with *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow treatments.

Table 1. Effect of summer green manuring crops and Zn fertilizer sources on the grain and straw yields and Zn concentration in grain and straw of *Basmati* rice

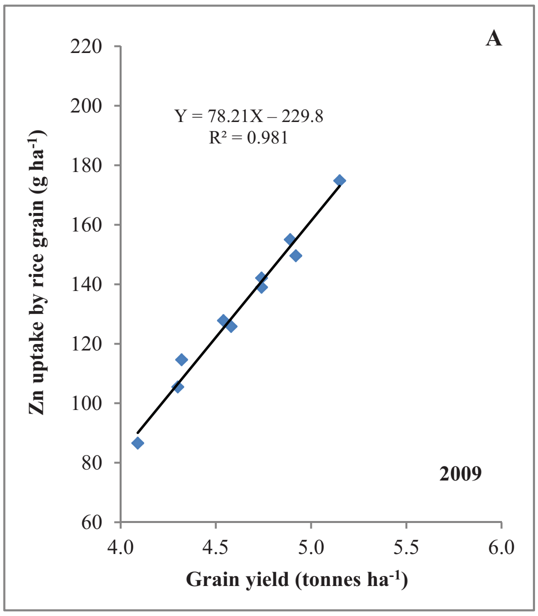
| Treatment | Grain yield (tonnes $ha^{-1}$) | | Straw yield (tonnes $ha^{-1}$) | | Zn concentration in grain (mg $kg^{-1}$ grain) | | Zn concentration in straw (mg $kg^{-1}$ straw) | |
|---|---|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 |
| *Summer-green manuring crops* | | | | | | | | |
| *Sesbania aculeata* (Dhaincha) | 4.89 | 5.56 | 9.04 | 10.21 | 31.5 | 32.4 | 167.5 | 172.1 |
| *Crotalaria juncea* (Sunhemp) | 4.74 | 5.34 | 8.83 | 10.02 | 29.8 | 29.6 | 162.1 | 162.4 |
| *Vigna unguiculata* (Cowpea) | 4.58 | 5.12 | 8.64 | 9.82 | 27.2 | 26.7 | 155.6 | 156.2 |
| Summer fallow | 4.30 | 4.86 | 8.36 | 9.63 | 24.2 | 23.7 | 150.2 | 151.0 |
| SEm± | 0.041 | 0.026 | 0.039 | 0.031 | 0.47 | 0.55 | 2.05 | 2.37 |
| LSD (P = 0.05) | 0.141 | 0.091 | 0.135 | 0.105 | 1.61 | 1.90 | 7.08 | 8.16 |
| *Zn sources* | | | | | | | | |
| Control | 4.09 | 4.75 | 8.13 | 9.39 | 21.1 | 19.6 | 146.4 | 146.8 |
| $ZnSO_4 \cdot 7H_2O$ (21% Zn) | 4.92 | 5.41 | 9.04 | 10.18 | 30.3 | 30.3 | 164.4 | 165.9 |
| $ZnSO_4 \cdot H_2O$ (33% Zn) | 4.74 | 5.27 | 8.81 | 10.02 | 29.3 | 29.1 | 159.6 | 161.3 |
| ZnO (82% Zn) | 4.32 | 4.98 | 8.40 | 9.61 | 26.5 | 25.6 | 152.5 | 153.2 |
| $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) | 4.54 | 5.15 | 8.61 | 9.85 | 28.1 | 28.1 | 156.7 | 158.1 |
| EDTA–chelated Zn (12% Zn) | 5.15 | 5.76 | 9.30 | 10.48 | 33.9 | 35.7 | 173.5 | 177.4 |
| SEm± | 0.043 | 0.027 | 0.046 | 0.032 | 0.29 | 0.35 | 0.68 | 0.94 |
| LSD (P = 0.05) | 0.123 | 0.077 | 0.131 | 0.090 | 0.84 | 0.99 | 1.96 | 2.70 |

Zn fertilizer sources also significantly influenced grain and straw yields of *Basmati* rice during 2009 and 2010. Among the Zn fertilization treatments, application of EDTA-chelated Zn (12% Zn) resulted into statistically higher values of grain (5.15 and 5.76 tonnes $ha^{-1}$) and straw yields (9.30 and 10.48 tonnes $ha^{-1}$) compared with all other Zn fertilizer sources and control (no Zn application), respectively during 2009 and 2010. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was second-best treatment with respect to grain (4.92 and 5.41 tonnes $ha^{-1}$) and straw yields (9.04 and 10.18 tonnes $ha^{-1}$). The lowest values of grain and straw were recorded with control (no Zn application). The performance of Zn sources in terms of yields of *Basmati* rice was in the order; EDTA-chelated Zn (12% Zn) > $ZnSO_4 \cdot 7H_2O$ (21% Zn) > $ZnSO_4 \cdot H_2O$ (33% Zn) > $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) > ZnO (82% Zn). The per cent increase in grain and straw yields with EDTA-chelated Zn (12% Zn) application over control (no Zn application) was 25.91, 21.26% and 14.39, 11.60%, respectively during 2009 and 2010.

### *3.2 Zn Concentration in Grain and Straw of Basmati Rice*

Different green manures and Zn sources had significantly influenced on the Zn concentrations in grain and straw of *Basmati* rice (Table 1). In our studies, the Zn concentration in rice straw was higher compared with rice grains. The significantly higher Zn concentration in grain and straw of rice was recorded with *Sesbania aculeata* (Dhaincha) incorporation and application of EDTA-chelated Zn (12% Zn) during both the years. Zn concentration in grain and straw were significantly higher with *Sesbania aculeata* (Dhaincha) incorporation compared with *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow treatments.

In general, application of Zn fertilizers, irrespective of its source, significantly increased the Zn concentration in grain and straw compared with control (no Zn application). Among the Zn fertilization treatments, the application of EDTA-chelated Zn (12% Zn) resulted in statistically higher values of Zn content in grain and straw than all other Zn sources and control (no Zn application) during 2009 and 2010, respectively. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was the second best treatment with respect to Zn concentration in grain and straw. The lowest values were recorded with control (no Zn application). A significantly higher correlation was recorded between grain yield and Zn uptake by grain (Figures 1A and 1B). This might be because zinc affects carbohydrate metabolism through its effects on photosynthesis, sugar transformations and seed development. Thus, increased Zn content and its uptake in grains which helped to produce bolder grains, thus increasing the grain yield (Alloway, 2008).



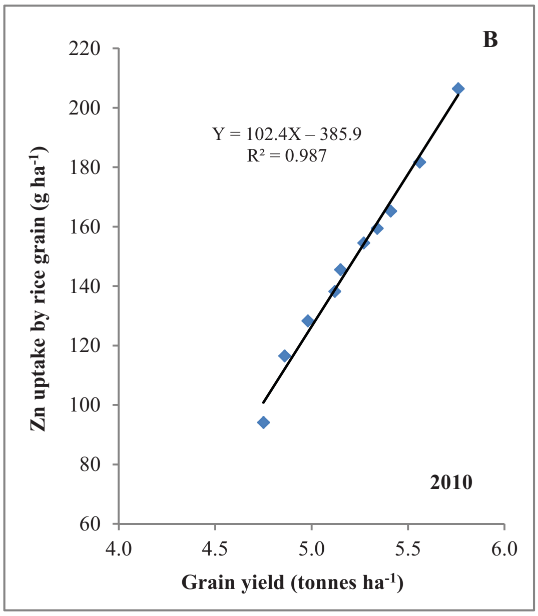




Figure 1. Rice grain yield correlation and regression with Zn uptake by *Basmati* rice grain

Percent increase in Zn concentration in grain and straw of rice with EDTA-chelated Zn (12% Zn) application over control (no Zn application) was 60.66, 82.14% and 18.51, 20.84%, respectively during 2009 and 2010. The per cent Zn concentration in grain and straw of rice was higher during 2010 as compared to 2009. It may be due to the higher residual effect (applied during 2009) of EDTA-chelated Zn in the experimental field as the same site with fixed lay out plan was used during both the years of experimentation. The performance of Zn sources in terms of Zn concentration in grain and straw was in the order; EDTA-chelated Zn (12% Zn) > $ZnSO_4 \cdot 7H_2O$ (21% Zn) > $ZnSO_4 \cdot H_2O$ (33% Zn) > $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) > ZnO (82% Zn).

### *3.3 Milling Quality Parameters of Basmati Rice*

The data on hulling, milling and head rice recovery are presented in Table 2. Milling quality parameters of *Basmati* rice got significantly influenced by incorporating summer green manuring crop residue and Zn sources. In general, hulling, milling and head rice recovery percentages significantly improved with green manuring over summer fallows, irrespective of the type of green manure. The significantly higher values of milling quality parameters were recorded when it was grown after *Sesbania aculeata* (Dhaincha) incorporation compared with *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow treatments.

Table 2. Effect of summer green manuring crops and Zn fertilizer sources on the hulling, milling and head rice recovery of *Basmati* rice

| Treatment | Hulling (%) | | Milling (%) | | Head rice recovery (%) | |
|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 |
| *Summer-green manuring crops* | | | | | | |
| *Sesbania aculeata* (Dhaincha) | 78.7 | 79.0 | 68.7 | 69.2 | 58.8 | 59.0 |
| *Crotalaria juncea* (Sunhemp) | 77.6 | 77.7 | 67.3 | 68.5 | 57.3 | 57.8 |
| *Vigna unguiculata* (Cowpea) | 76.9 | 77.1 | 66.6 | 67.0 | 56.0 | 56.5 |
| Summer fallow | 75.9 | 76.0 | 65.2 | 66.0 | 54.4 | 55.2 |
| SEm± | 0.23 | 0.30 | 0.33 | 0.25 | 0.44 | 0.31 |
| LSD (P = 0.05) | 0.80 | 1.04 | 1.12 | 0.88 | 1.51 | 1.08 |
| *Zn sources* | | | | | | |
| Control | 75.7 | 76.0 | 64.6 | 65.7 | 53.3 | 54.0 |
| $ZnSO_4·7H_2O$ (21% Zn) | 78.1 | 78.3 | 68.4 | 68.9 | 58.6 | 59.0 |
| $ZnSO_4·H_2O$ (33% Zn) | 77.2 | 77.6 | 67.0 | 68.0 | 57.5 | 57.7 |
| ZnO (82% Zn) | 76.2 | 76.5 | 65.7 | 66.6 | 54.6 | 55.5 |
| $ZnSO_4·7H_2O$ + ZnO (50% + 50%) | 76.7 | 77.0 | 66.4 | 67.1 | 55.6 | 56.7 |
| EDTA-chelated Zn (12% Zn) | 79.7 | 79.4 | 69.7 | 69.8 | 60.2 | 59.9 |
| SEm± | 0.23 | 0.18 | 0.22 | 0.16 | 0.37 | 0.24 |
| LSD (P = 0.05) | 0.64 | 0.51 | 0.63 | 0.44 | 1.04 | 0.69 |

Zn fertilization to *Basmati* rice also significantly influenced the hulling, milling and head rice recovery percentages. Among the Zn fertilization treatments, application of EDTA-chelated Zn (12% Zn) resulted in statistically higher values of milling quality parameters than all other Zn fertilizer sources including control (no Zn application), respectively during 2009 and 2010. Application of $ZnSO_4·7H_2O$ (21% Zn) was the second best treatment with respect to milling quality parameters, but was statistically inferior to it. The lowest hulling values, milling and head rice recovery, were recorded with control (no Zn application). The performance of Zn sources in terms of milling quality parameters of *Basmati* rice was in the order; EDTA-chelated Zn (12% Zn) > $ZnSO_4·7H_2O$ (21% Zn) > $ZnSO_4·H_2O$ (33% Zn) > $ZnSO_4·7H_2O$ + ZnO (50% + 50%) > ZnO (82% Zn). There was 13.33 and 10.92% increase in head rice recovery with EDTA-chelated Zn (12% Zn) application over control (no Zn application) during 2009 and 2010.

### *3.4 Kernel Length Before and After Cooking and Length Expansion Ratio*

Incorporation of green manures and Zn sources had influenced significantly the kernel length before and after cooking (Table 3). Our results indicate that the rice kernels were significantly lengthier, both before and after cooking with *Sesbania aculeata* (Dhaincha) incorporation and application of EDTA-chelated Zn (12% Zn) during both the years. Kernel length before and after cooking and length expansion ratio were significantly higher with *Sesbania aculeata* (Dhaincha) incorporation compared with *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow treatments.

Table 3. Effect of summer green manuring crops and Zn fertilizer sources on the rice kernel length before and after cooking and kernel length expansion ratio and 1,000-grain weight of *Basmati* rice

| Treatment | Rice kernel length before cooking (mm) ($L_1$) | | Rice kernel length after cooking (mm) ($L_2$) | | Rice kernel length expansion ratio ($L_2/L_1$) | | 1,000-grain weight (g) | |
|---|---|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 |
| *Summer-green manuring crops* | | | | | | | | |
| *Sesbania aculeata* (Dhaincha) | 6.84 | 6.90 | 14.26 | 14.41 | 2.08 | 2.09 | 25.37 | 25.88 |
| *Crotalaria juncea* (Sunhemp) | 6.74 | 6.80 | 14.05 | 14.17 | 2.07 | 2.08 | 24.95 | 25.13 |
| *Vigna unguiculata* (Cowpea) | 6.66 | 6.69 | 13.69 | 13.83 | 2.05 | 2.08 | 24.25 | 24.63 |
| Summer fallow | 6.56 | 6.57 | 13.42 | 13.55 | 2.04 | 2.06 | 23.47 | 24.01 |
| SEm± | 0.009 | 0.022 | 0.023 | 0.030 | 0.002 | 0.002 | 0.132 | 0.162 |
| LSD (P = 0.05) | 0.032 | 0.076 | 0.081 | 0.103 | 0.009 | 0.008 | 0.456 | 0.558 |
| *Zinc sources* | | | | | | | | |
| Control | 6.52 | 6.52 | 13.38 | 13.38 | 2.03 | 2.06 | 22.54 | 22.69 |
| $ZnSO_4·7H_2O$ (21% Zn) | 6.83 | 6.88 | 14.22 | 14.42 | 2.08 | 2.09 | 25.71 | 26.14 |
| $ZnSO_4·H_2O$ (33% Zn) | 6.73 | 6.78 | 13.94 | 14.10 | 2.07 | 2.08 | 24.82 | 25.21 |
| ZnO (82% Zn) | 6.59 | 6.60 | 13.54 | 13.67 | 2.05 | 2.07 | 23.33 | 23.96 |
| $ZnSO_4·7H_2O$ + ZnO (50% + 50%) | 6.64 | 6.71 | 13.68 | 13.84 | 2.06 | 2.06 | 24.07 | 24.69 |
| EDTA-chelated Zn (12% Zn) | 6.89 | 6.94 | 14.38 | 14.53 | 2.09 | 2.10 | 26.59 | 26.77 |
| SEm± | 0.015 | 0.028 | 0.032 | 0.052 | 0.003 | 0.069 | 0.153 | 0.148 |
| LSD (P = 0.05) | 0.043 | 0.080 | 0.090 | 0.148 | 0.008 | N.S. | 0.438 | 0.422 |

Application of Zn fertilizers, in general, irrespective of its source, appreciably increased the kernel length before and after cooking compared with control (no Zn application). Among the Zn fertilization treatments, application of EDTA-chelated Zn (12% Zn) resulted in statistically higher kernel length values before and after cooking compared with all Zn fertilizer sources and control (no Zn application), respectively during 2009 and 2010. Application of $ZnSO_4·7H_2O$ (21% Zn) was second best treatment with respect to kernel length after EDTA-chelated Zn (12% Zn) however; it was statistically inferior to it. The lowest values were recorded with control (no Zn application). The kernel length expansion ratio was affected significantly with Zn sources during 2009 only. Length expansion ratio got increased with Zn application during 2010 also, but up to non-significant levels. However, it was highest with EDTA-chelated Zn (12% Zn) application.

### 3.5 Kernel Breadth Before and After Cooking and Breadth Expansion Ratio

Application of Zn and green manure incorporation significantly influenced the kernel breadth of *Basmati* rice both before and after cooking (Table 4). Kernel breadth increased significantly, both before and after cooking with *Sesbania aculeata* (Dhaincha) incorporation and application of EDTA-chelated Zn (12% Zn) during both the years. Kernel breadth before and after cooking was significantly higher with *Sesbania aculeata* (Dhaincha) incorporation compared with *Vigna unguiculata* (Cowpea) and summer fallow treatments.

Table 4. Effect of summer green manuring crops and Zn fertilizer sources on the rice kernel breadth before and after cooking and kernel breadth expansion ration of *Basmati* rice

| Treatment | Rice kernel breadth before cooking (mm) ($B_1$) | | Rice kernel breadth after cooking (mm) ($B_2$) | | Rice kernel breadth expansion ratio ($B_2/B_1$) | |
|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 |
| *Summer-green manuring crops* | | | | | | |
| *Sesbania aculeata* (Dhaincha) | 1.72 | 1.74 | 2.46 | 2.47 | 1.43 | 1.42 |
| *Crotalaria juncea* (Sunhemp) | 1.70 | 1.72 | 2.42 | 2.43 | 1.42 | 1.41 |
| *Vigna unguiculata* (Cowpea) | 1.68 | 1.71 | 2.39 | 2.40 | 1.41 | 1.41 |
| Summer fallow | 1.66 | 1.68 | 2.34 | 2.36 | 1.40 | 1.41 |
| SEm± | 0.006 | 0.006 | 0.009 | 0.005 | 0.008 | 0.003 |
| LSD (P = 0.05) | 0.022 | 0.019 | 0.033 | 0.018 | NS | NS |
| *Zinc sources* | | | | | | |
| Control | 1.64 | 1.67 | 2.32 | 2.35 | 1.40 | 1.41 |
| $ZnSO_4 \cdot 7H_2O$ (21% Zn) | 1.72 | 1.74 | 2.45 | 2.46 | 1.42 | 1.42 |
| $ZnSO_4 \cdot H_2O$ (33% Zn) | 1.70 | 1.72 | 2.41 | 2.42 | 1.42 | 1.41 |
| ZnO (82% Zn) | 1.66 | 1.69 | 2.35 | 2.37 | 1.41 | 1.41 |
| $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) | 1.68 | 1.70 | 2.38 | 2.40 | 1.41 | 1.41 |
| EDTA-chelated Zn (12% Zn) | 1.75 | 1.76 | 2.50 | 2.49 | 1.43 | 1.42 |
| SEm± | 0.006 | 0.005 | 0.008 | 0.005 | 0.002 | 0.023 |
| LSD (P = 0.05) | 0.016 | 0.014 | 0.022 | 0.014 | NS | N.S. |

Kernel breadth before and after cooking was significantly higher with Zn application, irrespective of its source, compared with control (no Zn application). Among the Zn fertilization treatments, application of EDTA-chelated Zn (12% Zn) resulted in statistically higher kernel breadth values before and after cooking compared with all other Zn fertilizer sources and control (no Zn application), respectively during 2009 and 2010. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was the second best treatment with respect to kernel breadth increase. Application of ZnO (82% Zn) was least effective in affecting the kernel breadth of *Basmati* rice. The lowest values were recorded with control (no Zn application). However, incorporating green manures or Zn application could not significantly influence the kernel breadth expansion ratio during both the years.

### 3.6 Protein Content

Protein content in *Basmati* rice grain got significantly influenced by Zn application and green manure incorporation (Table 5). Significantly higher protein content in *Basmati* rice grain was observed when grown after *Sesbania aculeata* (Dhaincha) incorporation and application of EDTA-chelated Zn (12% Zn) during both the years. Protein content was significantly higher with *Sesbania aculeata* (Dhaincha) incorporation compared with *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow treatments.

Table 5. Effect of summer green manuring crops and Zn fertilizer sources on the quality parameters of *Basmati* rice

| Treatment | Protein (%) | | Amylose (%) | |
|---|---|---|---|---|
| | 2009 | 2010 | 2009 | 2010 |
| *Summer-green manuring crops* | | | | |
| *Sesbania aculeata* (Dhaincha) | 8.1 | 8.3 | 24.6 | 24.9 |
| *Crotalaria juncea* (Sunhemp) | 8.0 | 8.2 | 24.3 | 24.6 |
| *Vigna unguiculata* (Cowpea) | 7.9 | 8.0 | 23.8 | 24.1 |
| Summer fallow | 7.7 | 7.8 | 23.4 | 23.7 |
| SEm± | 0.03 | 0.03 | 0.06 | 0.07 |
| LSD (P = 0.05) | 0.09 | 0.10 | 0.20 | 0.23 |
| *Zn sources* | | | | |
| Control | 7.6 | 7.7 | 22.9 | 23.5 |
| $ZnSO_4 \cdot 7H_2O$ (21% Zn) | 8.1 | 8.3 | 24.6 | 24.7 |
| $ZnSO_4 \cdot H_2O$ (33% Zn) | 8.0 | 8.2 | 24.3 | 24.4 |
| ZnO (82% Zn) | 7.7 | 7.9 | 23.6 | 23.9 |
| $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) | 7.8 | 8.0 | 23.9 | 24.1 |
| EDTA-chelated Zn (12% Zn) | 8.3 | 8.4 | 24.9 | 25.3 |
| SEm± | 0.04 | 0.02 | 0.09 | 0.06 |
| LSD (P = 0.05) | 0.11 | 0.06 | 0.25 | 0.18 |

Zn application, irrespective of its source, appreciably increased the grain protein compared with control (no Zn application). Among the Zn fertilization treatments, application of EDTA-chelated Zn (12% Zn) resulted in statistically higher grain protein values compared with all other Zn fertilizer sources and control (no Zn application), respectively during 2009 and 2010. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was the second best treatment. The lowest values were recorded with control (no Zn application). Grain protein was 9.21 and 9.09% higher with EDTA-chelated Zn (12% Zn) application over control during 2009 and 2010, respectively. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) increased the grain protein content to the tune of 6.57 and 7.79% over control during 2009 and 2010, respectively. The performance of Zn sources in improvement in grain protein content was in the order; EDTA-chelated Zn (12% Zn) > $ZnSO_4 \cdot 7H_2O$ (21% Zn) > $ZnSO_4 \cdot H_2O$ (33% Zn) > $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) > ZnO (82% Zn).

### *3.7 Amylose Content*

Amylose content in *Basmati* rice grain got significantly influenced by Zn application and green manure incorporation (Table 5). The significantly higher amylose content values in *Basmati* rice grain were observed when it was grown after *Sesbania aculeata* (Dhaincha) incorporation and application of EDTA-chelated Zn (12% Zn) during both the years. It was significantly higher with *Sesbania aculeata* (Dhaincha) incorporation compared with *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow treatments.

Zn application, irrespective of its source, significantly improved the amylose content compared with control (no Zn application). Among the Zn fertilization treatments, application of EDTA-chelated Zn (12% Zn) resulted in statistically higher amylose content values than all other Zn fertilizer sources and control (no Zn application), respectively during 2009 and 2010. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was the second best treatment. The lowest values were recorded with control (no Zn application) during both the years.

### *3.8 1,000-Grain Weight*

Zn application and green manure incorporation significantly improved the 1,000-grain weight of *Basmati* rice (Table 5). Significantly higher values of 1,000-grain weight were recorded with *Sesbania aculeata* (Dhaincha) incorporation and EDTA-chelated Zn (12% Zn) application during both the years. It was significantly higher with *Sesbania aculeata* (Dhaincha) incorporation compared with *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow treatments.

Significantly higher values of 1,000-grain weight were observed with Zn application, irrespective of its source, compared with control (no Zn application). Among the Zn fertilization treatments, the application of EDTA-chelated Zn (12% Zn) resulted in a statistically higher 1,000-grain weight than all other Zn fertilizer sources and control (no Zn application), respectively during 2009 and 2010. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was the second best treatment. However, application of ZnO (82% Zn) was least effective in affecting the

1,000-grain weight during both the years. The lowest values were recorded with control (no Zn application). The performance of Zn sources in improvement in 1,000-grain weight was in the order of; EDTA-chelated Zn (12% Zn) > $ZnSO_4 \cdot 7H_2O$ (21% Zn) > $ZnSO_4 \cdot H_2O$ (33% Zn) > $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) > ZnO (82% Zn).

## 4. Discussion

Zinc deficiency is a well-documented problem in food crops, causing decreased crop yields and nutritional quality. Generally, the regions in the world with Zn-deficient soils are also characterized by low crop productivity. Cereal crops, especially rice play an important role in satisfying daily calorie intake in the developing world. They are still inherently very low in Zn concentrations in grain, particularly when grown on Zn-deficient soils or under intensive cropping systems. Soil Zn deficiency is also a well-documented problem that reduces crop production. It brings about a significant decrease in plant performance, as shown in various countries such as in India, Pakistan, Australia, Turkey, USA and China (Alloway, 2008; Prasad, 2009; Prasad & Shivay, 2020). Earlier studies have shown that the inclusion of a green manure crop like *Sesbania aculeata* and other legumes in the rotation improves soil organic matter status and nutrient content and increases crop productivity (Yadvinder-Singh et al., 1991). However, the role of different types of Zn fertilizers, combined with the incorporation of green manure crops on grain quality parameters had not been undertaken so far.

This present study showed that there was a significant improvement in grain and straw yields, grain and straw Zn concentrations and kernel quality parameters of *Basmati* rice with summer green manure incorporation and Zn fertilization. This could be attributed to the higher supply of N and other micronutrient cations by incorporating leguminous green manures into the soil (Bisht et al., 2006; Pooniya & Shivay, 2011; Yadav et al., 2019). The increase in studied parameters of *Basmati* rice might also be due to higher nutrient availability and better physico-chemical soil properties under green manure incorporated plots. The increased availability of Fe and other micronutrients in soil with regular summer green manuring every year before transplanting rice in the rice-wheat system was responsible for higher yields in the green manuring plot than the non-green manuring plot (Nayyar & Chhibba, 2000). *Sesbania aculeata* (Dhaincha) supplied a significantly higher amount of readily decomposable organic materials (data not shown in this manuscript), which improved soil organic matter and nutrient status. It leads to recycling of nutrients into the soil and increased availability of nutrients and thus improved the yields and quality parameters of *Basmati* rice. Increase in the Zn concentrations in rice grains through soil Zn applications were positively stimulated by increasing soil N availability (Kutman et al., 2010). The positive effect of increasing N supply through leguminous green manure crops' residue incorporation on the Zn concentration of rice grain and straw was reflected in our studies. Nitrogen nutritional status of plants may also exert positive effects on Zn's root uptake (Cakmak et al., 2010). There are several steps during uptake and transport of Zn in plants which might be affected by N nutrition (Cakmak et al., 2010). By affecting root growth and stimulating root exudation of organic compounds (Marschner, 1995; Paterson et al., 2006), N may influence Zn's mobility and root uptake from soils (Cakmak et al., 2010). Nitrogen status in soil tended to affect the endosperm Zn concentrations to a greater extent than the whole grain Zn concentrations. The positive effects of high N on the endosperm Zn concentration have important implications for human nutrition. This part of the grain is the most commonly eaten part in many countries (Kutman et al., 2011). Enhanced nitrogen and other nutrients' supply through green manure residue incorporation resulted in significantly larger/bolder grains, improving the 1,000-grain weight (Table 5), and larger grains most probably have greater endosperm-to-whole grain ratios.

The positive effect of increasing N and other nutrients' supply through leguminous green manure crops' residue incorporation on rice grain protein content was reflected in our studies. This could be attributed to the higher supply of N and other micronutrient cations by incorporating legumes into the soil (Bisht et al., 2006; Pooniya & Shivay, 2011; Yadav et al., 2019). Grain protein concentration is an important quality parameter for rice, as it is the major staple food for the people in Asian and other developing countries.

The performance of Zn sources was in the order of; EDTA-chelated Zn (12% Zn) > $ZnSO_4 \cdot 7H_2O$ (21% Zn) > $ZnSO_4 \cdot H_2O$ (33% Zn) > $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%) > ZnO (82% Zn). Application of ZnO (82% Zn) was least effective in affecting the studied parameters during both the years. The increase in *Basmati* rice yields with the application of EDTA-chelated Zn (12% Zn) might be due to the relatively greater amount of Zn uptake compared with other Zn sources. The relatively higher maintenance of available Zn in soil due to applied EDTA-chelated Zn (12% Zn) may be attributed from the very little or no interaction between soil components preventing various harmful reactions occurring in the soil as compared to soil treated with other Zn sources, which enhances greater fixation, adsorption etc., resulting from the greater interaction between soil components. Ortiz and Garcia (1998) reported that the chelated-Zn (Cosmo-Quel-Zn) is fixed lesser in soil than the sulphate source. Srivastava et al. (1999) also studied the comparative efficiency of different Zn sources for low land rice production. They reported that out of various sources, the chelated-Zn (Zn-EDTA) was the most efficient Zn for low land rice production. These results are in the agreement with the findings of Karak et al. (2005) who reported that chelated Zn was the most efficient source of Zn for lowland rice production. Further, incorporating green manuring crops before transplanting of *Basmati* rice improves the organic matter content in soil. The applied Zn might have been complexed with the humic substances present in soil due to organic matter addition and there might have been lesser Zn fixation by the formation of insoluble Zn complexes. Thus, resulting into increase in the availability of soil applied Zn to rice plants. Improvement in the applied fertilisers' nutrient use efficiency by transplanted rice after green manure incorporation was also reported by Yadvinder-Singh et al. (1991). These results also confirmed those reported by Maftoun and Karimian (1989). Further, they concluded that Zn-EDTA's has greater influence over other sources of Zn in terms of growth and its utilisation by plants might be due to lesser retention and greater transport and movement of chelated Zn to plant roots. This could be attributed to lesser fixation in soil of Zn applied as Zn-EDTA than $ZnSO_4$ (Ortiz & Garcia, 1998). The higher increase in the Zn content in rice with EDTA-chelated Zn (12% Zn) might be due to increased amounts of Zn in soil solution that facilitates greater absorption of Zn as compared to other Zn sources. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was second best treatment with respect to Zn concentration in grain and straw and its uptake after EDTA-chelated Zn (12% Zn). Singh et al. (1999) reported that applying the different sources of Zn up to 10 mg $kg^{-1}$ increased the Zn concentration of rice leaves, being a higher uptake with Zn-EDTA than $ZnSO_4$. Zinc content of rice grain and straw during the present investigation confirmed the results reported by Ugurluoglu and Kacar (1996) who studied the efficiency of ZnO, $ZnSO_4 \cdot 7H_2O$ and Zn-EDTA on rice and reported that the application of Zn at the rate of 8 mg $kg^{-1}$ as Zn-EDTA was found most effective in the enhancement of Zn content in rice plants. Rattan and Shukla (1991) studied the efficiency of Zn sources on rice cv. Pusa-33 in *Typic Ustipsament* and reported that the Zn content and uptake by rice were in the order of Zn-EDTA > Zn-DTPA > $ZnSO_4$. Application of ZnO (82% Zn) was found to be least effective among all the Zn sources. This might be due to the fact that the EDTA-chelated Zn (12% Zn), $ZnSO_4 \cdot 7H_2O$ (21% Zn) and $ZnSO_4 \cdot H_2O$ (33% Zn) are more water-soluble and therefore readily available, making its effects visible in the plants. In comparison, ZnO (82% Zn) is sparingly soluble and is not readily available. The water solubility of zinc sources is considered as an important criterion for Zn availability (Slaton et al., 2005a, 2005b). Mikkleson and Brandon (1975); Nayyar et al. (1990) also showed that ZnO was inferior to $ZnSO_4$, both in grain yield and Zn uptake. Our results indicated that Zn applied through EDTA-chelated Zn (12% Zn) remained available to crops for a longer period of time than that with the rest of the Zn sources. This might be due to lesser transformation of applied Zn through EDTA-chelated Zn (12% Zn) into unavailable forms (Naik & Das, 2008).

The increase in rice's studied parameters with Zn fertilization may also be due to higher Zn availability to plants in treated plots than control (no Zn application). This might have lead to higher Zn uptake with Zn fertilization, resulting into higher biomass production (Shivay et al., 2008; Shivay & Prasad, 2012; Pooniya et al., 2012; Prasad & Shivay, 2018) and photosynthates translocation to reproductive parts (Ozkutlu et al., 2006; Alloway, 2008). A significantly high correlation was recorded between grain yield and Zn uptake by grain (Figures 1A and 1B). This might be because zinc exerts an effect on carbohydrate metabolism through its effects on photosynthesis, sugar transformations and seed development. Thus, increased Zn content and its uptake in grains help in production of bolder grains, hence increasing the grain yield (Alloway, 2008). Our result showed that there was a significant improvement in grain and straw Zn concentrations with Zn application. High grain Zn concentration is considered a desirable quality factor that could increase the grains nutritional value for humans (Cakmak et al., 1998; Prasad, 2006; Shivay & Prasad, 2012; Prasad & Shivay, 2020). Also, the increased Zn concentration in rice straw is of immense importance from the viewpoint of cattle nutrition since in developing countries of Asia, rice straw is the major feed for farm cattle (Shivay et al., 2008). The positive correlations between Zn and protein content in grains of various cereal crops were observed by Cakmak et al. (2010). Strongly positive correlations between grain protein and Zn indicate that grain proteins represent a sink for Zn. Increase in protein content with Zn application in our results are in good agreement with the hypothesis that protein represents a sink for Zn in the grain (Morgounov et al., 2007; Cakmak et al., 2010; Kutman et al., 2010). The strength of the correlation between Zn and protein depends on sufficiently high Zn availability to the plants (Kutman et al., 2011). Application of Zn fertilizers in soil increased the Zn availability to the plants. Given that Zn plays a particular role in protein synthesis (Cakmak et al., 1989; Marschner, 1995; Kutman et al., 2011), enhancement in protein biosynthesis results from the increased N supply through leguminous green manures may also increase the sink strength for Zn. Ozturk et al. (2006) concluded that the highest accumulation of Zn in cereal grains occurs in the early stage of seed formation, the same stage during which the highest protein synthesis occurs (Kutman et al., 2011). In biological systems, proteins are highly dependent on Zn ions to maintain their activities. Zinc is needed for numerous proteins, having both a catalytic and a structural role

(Anzellotti & Farrell, 2008). Further, the higher N supply by incorporating large amounts of plant biomass as leguminous green manures resulted in higher protein concentrations in rice grains, accompanied by higher Zn concentrations. In addition to N contribution, these summer legumes' biomass also recycled considerable quantities of P, K and other nutrients; and thus improving the rice grain quality parameters. Our results show the beneficial effect of summer green manure crops and Zn fertilizer application on grain protein content. The presence of protein, and likely of starch-protein interactions, affect the packing arrangement of starch polymers within the granule resulted in better kernel quality parameters. Also, zinc affects carbohydrate metabolism through its effects on photosynthesis, sugar transformations and seed/grain development (Alloway, 2008). Shivay et al. (2007) and Shivay and Prasad (2012) also reported the improvement in kernel quality parameters of rice with Zn fertilization. Thus, increased Zn content and its uptake in grains help in production of bolder grains, and hence improving the quality characters of *Basmati* rice. The present study also demonstrates that summer green manure incorporation and Zn fertilization improve the milling and cooking quality of *Basmati* rice. The increase in kernel length varied from 0.27 to 0.86 mm with green manuring and from 0.29 to 1.15 mm after cooking due to Zn fertilization, a quality for which *Basmati* rices are preferred and sold on a premium price all over the world for making rice *Biryani* (a dish made by cooking rice with mutton/chicken/peas and spices etc.).

## 5. Conclusions

Zinc fertilization and summer green manure incorporation increased the grain and straw yield, enhanced Zn concentrations and improved kernel quality in *Basmati* rice. The application of EDTA-chelated Zn (12% Zn) was the best in terms of grain and straw yield and Zn concentrations in grain and straw and kernel quality before and after cooking of *Basmati* rice. Application of $ZnSO_4 \cdot 7H_2O$ (21% Zn) was second-best treatment followed by $ZnSO_4 \cdot H_2O$ (33% Zn) and $ZnSO_4 \cdot 7H_2O$ + ZnO (50% + 50%). Application of ZnO (82% Zn) was least effective in affecting the studied parameters. The lowest values were observed with control (no Zn application). Among the summer green manuring crops, incorporation of *Sesbania aculeata* (Dhaincha) was found to be the best over *Crotalaria juncea* (Sunhemp), *Vigna unguiculata* (Cowpea) and summer fallow in terms of grain and straw yield, Zn concentrations in grain and straw and kernel quality before and after cooking in *Basmati* rice. Zn fertilization with EDTA-chelated Zn (12% Zn) lead to 25.91 and 21.26% higher grain yield; 60.66 and 82.14% Zn-denser grains; with 13.33 and 10.92% increase in head rice recovery in *Basmati* rice over control (no Zn application) during 2009 and 2010, respectively. The best results were obtained with *Sesbania aculeata* (Dhaincha) green manure among different green manures and EDTA-chelated Zn (12% Zn) among various Zn sources. Incorporation of summer green manures and adequate Zn fertilizer application in *Basmati* rice can thus lead to higher grain yield and Zn-denser grains with improved cooking quality in *Basmati* rice. Such positive effects of green manuring and Zn application can help sustaining good crop yields over time without deteriorating grain quality and nutritional value, especially in intensive cropping systems.

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## References

Adriano, D. C. (2001). *Trace Elements in Terrestrial Environments-Biogeochemistry, Bioavailability and Risks of Metals* (2nd ed.). Springer Verlag, New York, USA.

Alloway, B. J. (2008). *Zinc in Soils and Crop Nutrition*. International Zinc Association Publication. International Zinc Association, Brussels, Belgium.

Alvarez, J. M., Almendros, P., & Gonzalez, D. (2009). Residual effects of natural Znchelates on navy bean response, Zn leaching and soil Zn status. *Plant Soil, 317*, 277-291. https://doi.org/10.1007/s11104-008-9808-9

Anonymous. (2007). *Help eliminate the fifth leading disease risk factor in developing countries* (p. 6). Fertilizer & Agriculture, International Fertilizer Agency, Paris, France.

Anzellotti, A. I., & Farrell, N. P. (2008). Zinc metalloproteins as medicinal targets. *Chemical Society Reviews, 37*, 1629-1651. https://doi.org/10.1039/b617121b

Behera, S. K., Singh, M. V., & Lakaria, B. L. (2009). Micronutrient deficiencies in India and their amelioration through fertilization. *Indian Farming, 59*, 28-31.

Bhatti, H. M., Yasin, M., & Rashid, M. (1983). Evaluation of Sesbania green manuring in rice-wheat rotation. In *Proceedings of International Symposium on nitrogen and the environment* (pp. 275-284). Lahore, Pakistan.

Bisht, P. S., Pandey, P. C., & Singh, D. K. (2006). Effect of different sources of nutrients on rice (Oryza sativa) yield and soil nutrient status in rice-wheat cropping. In: *National Symposium on Conservation Agriculture and Environment*. October 26-28, Banaras Hindu University, Varanasi, India.

Brennan, R. F., & Bolland, D. A. (2006). Residual values of soil-applied zinc fertilizer forearly vegetative growth of six crop species. *Australian Journal of Experimental Agriculture, 46*, 1341-1347. https://doi.org/10.1071/EA05154

Cakmak, I. (2002). Plant nutrition research: Priorities to meet human needs for food insustainable ways. *Plant and Soil, 247*, 3-24. https://doi.org/10.1023/A:1021194511492

Cakmak, I. (2008a). Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant and Soil, 302*, 1-17. https://doi.org/10.1007/s11104-007-9466-3

Cakmak, I. (2008b). Zinc deficiency in wheat in Turkey. In B. J. Alloway (Ed.), *Micronutrient deficiencies in global crop production* (pp. 181-200). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6860-7_7

Cakmak, I., Marschner, H., & Bangerth, F. (1989). Effect of zinc nutritional status on growth, protein metabolism and levels of indole-3-cetic acid and other phytohormones in bean (*Phaseolus vulgaris* L.). *Journal of Experimental Botany, 40*, 405-412. https://doi.org/10.1093/jxb/40.3.405

Cakmak, I., Pfeiffer, W. H., & McClafferty, B. (2010). Biofortification of durum wheatwith zinc and iron. *Cereal Chemistry, 87*, 10-20. https://doi.org/10.1094/CCHEM-87-1-0010

Cakmak, I., Torun, B., Erenoglu, B., Öztur-Marschner, H., Kalayci, M., Ekiz, H., & Yilmaz, A. (1998). Morphological and physiological differences in the response of cereals to zinc deficiency. *Euphytica, 100*, 349-357. https://doi.org/10.1023/A:1018318005103

Dobermann, A., & Fairhurst, T. (2000). *Rice: Nutrient disorders and nutrient management* (p. 192). Handbook series. Potash & Phosphate Institute (PPI), Potash & Phosphate Institute of Canada (PPIC) and International Rice Research Institute.

Fageria, N. K., Slaton, N. A., & Baligar, V. C. (2003). Nutrient management for improving lowland rice productivity and sustainability. *Advances in Agronomy, 80*, 63-152. https://doi.org/10.1016/S0065-2113(03)80003-2

Ghasal, P. C., Shivay, Y. S., Pooniya, V., Choudhary, M., & Verma, R. K. (2018). Zinc partitioning in aromatic rice varieties as influenced by Zn fertilization. *The Crop Journal, 6*, 136-147. https://doi.org/10.1016/j.cj.2017.09.001

Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research* (2nd ed.). John Wiley & Sons, New York, USA.

Hanway, J. J., & Heidel, H. (1952). *Soil analysis methods as used in Iowa State College Soil Testing Laboratory* (Bulletin 57, p. 131). Iowa State College of Agriculture, Iowa, USA.

HarvestPlus. (2009). Target countries and crops. Retrieved March 13, 2012, from http://www.harvestplus.org/content/crops

Hemalatha, M., Thirumurugan, V., & Balasubramanian, R. (1999). Influence of organic, biofertilizer and inorganic forms of nitrogen on rice quality. *International Rice Research Notes, 24*, 33.

Hotz, C., & Brown, K. H. (2004). Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin, 25*, 91S-204S.

Jennings, P. R., Coffman, W. R., & Kauffman, H. E. (1979). Grain quality. *Rice Improvement* (pp. 101-120). International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines.

Juliano, B. O. (1979). Chemical basis of rice grain quality. *Proceedings of the Workshop on Chemical Aspects of Rice Grain Quality* (pp. 69-70). International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines.

Juliano, B. O. (1985). Polysaccharides, proteins and lipids of rice. *Rice-Chemistry and Technology* (p. 98). International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines.

Juliano, B. O., & Perez, C. M. (1984). Results of collaborative test on the measurement ofgrain elongation of milled rice during cooking. *Journal of Cereal Science, 2*, 281-292. https://doi.org/10.1016/S0733-5210(84)80016-8

Karak, T., Singh, U. K., Das, S., Das, D. K., & Kuzyakov, Y. (2005). Comparative efficacy of $ZnSO_4$ and Zn-EDTA application for fertilization of rice (*Oryza sativa* L.). *Archives of Agronomy and Soil Science, 51*, 253-264. https://doi.org/10.1080/03650340400026701

Kutman, U. B., Yildiz, B., & Cakmak, I. (2011). Improved nitrogen status enhances zinc andiron concentrations both in the whole grain and the endosperm fraction of wheat. *Journal of Cereal Science, 53*, 118-125. https://doi.org/10.1016/j.jcs.2010.10.006

Kutman, U. B., Yildiz, B., Ozturk, L., & Cakmak, I. (2010). Biofortification of durum wheatwith zinc through soil and foliar applications of nitrogen. *Cereal Chemistry, 87*, 1-9. https://doi.org/10.1094/CCHEM-87-1-0001

Lindsay, W. L., & Norvell, W. A. (1978). Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal, 42*, 421-428. https://doi.org/10.2136/sssaj1978.03615995004200030009x

Maftoun, M., & Karimian, N. (1989). Relative efficiency of two zinc sources for maize in two calcareous soils from an arid area of Iran. *Agronomie, 9*, 771-775. https://doi.org/10.1051/agro:19890804

Mandal, U. K., Singh, G., Victor, U. S., & Sharma, K. L. (2003). Green manuring: Its effect on soil properties and crop growth under rice-wheat cropping system. *European Journal of Agronomy, 19*, 225-237. https://doi.org/10.1016/S1161-0301(02)00037-0

Marschner, H. (1995). *Mineral Nutrition of Higher Plants* (2nd ed.) Academic Press: London.

Mikkelson, D. S., & Brandon, D. M. (1975). Zinc deficiency in California rice. *California Agriculture, 29*, 8-9.

Mohammad, A., Hamad, R. J., Khan, E. A., & Mohammad, R. (2005). Comparative response of diverse rice varieties to green manuring (*Sesbania aculeata*). *Journal of Research (Science), 16*, 39-43.

Morgonuov, A., Gómez-Becerra, H. F., Abugalieva, A., Dzhunusova, M., Yessimbekova, M., Muminjanov, H., … Cakmak, I. (2007). Iron and zinc graindensity in common wheat grown in Central Asia. *Euphytica, 155*, 193-203. https://doi.org/10.1007/s10681-006-9321-2

Mortvedt, J. J. (1979). Crop response to zinc sources applied alone or with suspensions. *Fertilizer Solutions, 23*, 64-79.

Naik, S. K., & Das, D. K. (2008). Relative performance of chelated zinc and zinc sulphate for lowland rice (*Oryza sativa* L.). *Nutrient Cycling in Agroecosystems, 81*, 219-227. https://doi.org/10.1007/s10705-007-9158-7

Nayyar, V. K., & Chhibba, I. M. (2000). Effect of green manuring on micronutrientavailability inrice-wheat cropping system of northwest India. In I. P. Abrol & K. F. Brown (Eds.), *Long-term SoilFertility Experiments in Rice-Wheat Cropping Systems* (Rice-Wheat Consortium Paper Series 6). New Delhi, India.

Nayyar, V. K., Takkar, P. N., Bansal, R. L., Singh, S. P., Kaur, N. P., & Sadana, U. S. (1990). *Micronutrients in soils and crops of Punjab*. Research Bulletin, Department of Soils, Punjab Agricultural University (PAU), Ludhiana, Punjab, India.

Nene, Y. L. (1966). Symptoms, causes and control of *khaira* disease of paddy. *Bulletin of the Indian Phytopathological Society, 3*, 97-101.

Norman, R. J., Wilson, C. E. Jr., & Salton, N. A. (2003). Soil fertilization and mineral nutrition in US mechanized rice culture. In C. W. Smith & R. H. Hilday (Eds.), *Rice origin, history, technology and production* (pp. 331-412). John Wiley, New Jersey, USA.

Olsen, R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. Circular-939 United States Department of Agriculture, Washington DC, USA.

Ortiz, E. M. E., & Garcia, O. A. (1998). Process of sorption of zinc in three soils of Colombiacultivated with rice (Oryza sativa) of the dry land variety Llano-5. Proceedings ofthe VIII Congress of Soil Sciences, Santa Marta, Colombia. *Suelos-Ecuatoriales, 28*, 112-117.

Ozkutlu, F., Torun, B., & Cakmak, I. (2006). Effect of zinc humate on growth of soybean andwheatin zinc-deficient calcareous soils. *Communications in Soil Science and Plant Analysis, 37*, 2769-2778. https://doi.org/10.1080/00103620600832167

Paterson, E., Sim, A., Standing, D., Dorward, M., & McDonald, A. J. S. (2006). Rootexudation from *Hordeum vulgare* in response to localized nitrate supply. *Journal of Experimental Botany, 57*, 2413-2420. https://doi.org/10.1093/jxb/erj214

Pooniya, V., & Shivay, Y. S. (2011). Effect of green manuring and zinc fertilization onproductivity and nutrient uptake in Basmati rice (*Oryza sativa*)-wheat (*Triticum aestivum*) cropping system. *Indian Journal of Agronomy, 56*, 28-34.

Pooniya, V., Shivay, Y. S., Rana, A., Nain, L., & Prasanna, R. (2012). Enhancing soilnutrient dynamics and productivity of Basmati rice through residue incorporation and zinc fertilization. *European Journal of Agronomy, 41*, 28-37. https://doi.org/10.1016/j.eja.2012.03.004

Prasad, R. (2005). Rice-wheat cropping systems. *Advances in Agronomy, 86*, 255-339. https://doi.org/10.1016/S0065-2113(05)86006-7

Prasad, R. (2006). Zinc in soils and in plants, human & animal nutrition. *Indian Journal of Fertilizers, 2*, 103-119.

Prasad, R. (2009). Zinc malnutrition and its alleviation through zinc fortified cereal grains. *Proceedings of the Indian National Science Academy, 75*, 89-91.

Prasad, R., & Shivay, Y. S. (2018). Relative grain zinc loading ability of cereals (rice, wheat, maize) and a grain legume (chickpea). *The Indian Journal of Agricultural Sciences, 88*(2), 307-313.

Prasad, R., & Shivay, Y. S. (2020). Agronomic biofortification of plant foods with minerals, vitamins and metabolites with chemical fertilizers and liming. *Journal of Plant Nutrition, 43*(10), 1534-1554. https://doi.org/10.1080/01904167.2020.1738464

Prasad, R., Shivay, Y. S., Kumar, D., & Sharma, S. N. (2006). *Learning by Doing Exercises in Soil Fertility (A practical manual for soil fertility)* (p. 68). Division of Agronomy, Indian Agricultural Research Institute, New Delhi, India.

Rani, N. S., Prasad, G. S. V., & Viraktamath, B. C. (2009). National system for evaluation of *Basmati* rices for yield and quality traits. *Indian Farming, 59*, 7-11.

Rattan, R. K., & Shukla, L. M. (1991). Influence of different Zn carriers on the utilization ofmicronutrients by rice. *Journal of the Indian Society of Soil Science, 39*, 808-810. https://doi.org/10.1038/ki.1991.101

Ray, S. S., & Gupta, R. P. (2001). Effect of green manuring and tillage practices on physical properties of puddled loam soil under rice-wheat cropping system. *Journal of theIndian Society of Soil Science, 49*, 670-678.

Robertson, G. P., Paul, E. A., & Harwood, R. R. (2000). Greenhouse gases in intensiveagriculture: Contributions of individual gases to the radioactive forcing of the atmosphere. *Science, 289*, 1922-1925. https://doi.org/10.1126/science.289.5486.1922

Sadasivam, S., & Manickam, A. (1992). *Biochemical Methods for Agricultural Sciences* (pp. 12-13). Wiley Eastern Limited, New Delhi, India.

Satake, T. (1990). *Modern Rice Milling Technology*. University of Tokyo Press, Tokyo, Japan.

Sharma, S. N., & Prasad, R. (1999). Effect of *Sesbania* green-manuring and mung bean residue incorporation on productivity and nitrogen uptake of a rice-wheat cropping system. *Bioresource Technology, 87*, 171-175. https://doi.org/10.1016/S0960-8524(98)00101-1

Shihua, T., & Wenqiang, F. (2000). Nutrient management in the rice-wheat cropping systemin the Yangtze river flood plain. In P. R. Hobbs & R. K. Gupta (Eds.), *Soil and crop management practices for enhancedproductivity of the rice-wheat cropping system in the Sichuan province of China* (pp. 24-34). Rice-Wheat Consortium for the Indo-Gangetic Plains, New Delhi, India.

Shivay, Y. S., & Prasad, R. (2012). Zinc-coated urea improves productivity and quality of Basmati rice (*Oryza sativa* L.) under zinc stress condition. *Journal of Plant Nutrition, 35*, 928-951. https://doi.org/10.1080/01904167.2012.663444

Shivay, Y. S., Kumar, D., & Prasad, R. (2007). Effect of zinc fertilization on physical grain quality of basmati rice. *International Rice Research Notes, 32*(1), 41-42. https://doi.org/10.3860/irrn.v32i1.1092

Shivay, Y. S., Kumar, D., Ahlawat, I. P. S., & Prasad, R. (2007). Relative efficiency of zinc oxide and zinc sulphate coated urea for rice. *Indian Journal of Fertilizers, 3*, 51-56.

Shivay, Y. S., Kumar, D., Prasad, R., & Ahlawat, I. P. S. (2008). Relative yield and zinc uptake by rice from zinc sulphate and zinc oxide coatings onto urea. *Nutrient Cycling in Agroecosystems, 80*, 181-188. https://doi.org/10.1007/s10705-007-9131-5

Shivay, Y. S., Prasad, R., & Rahal, A. (2010). Genotypic variation for productivity, zincutilization efficiencies, and kernel quality in aromatic rices under low available zinc conditions. *Journal of Plant Nutrition, 33*, 1835-1848. https://doi.org/10.1080/01904167.2010.503832

Siddiq, E. A. (2006). Genetic improvement of rice in perspective. *Indian Farming, 56*, 10-16.

Siddiq, E. A., Vemireddy, L. R., & Nagraju, J. (2012). Basmatirices: Genetics, breeding and trade. *Agricultural Research, 1*, 25-36. https://doi.org/10.1007/s40003-011-0011-5

Singh, A. K., Khan, S. K., & Nongkynrih, P. (1999). Transformation of zinc in wetland rice soils in relation to nutrition of crop. *Journal of the Indian Society of Soil Science, 47*, 248-253.

Singh, V. P., & Singh, A. K. (2009). History of Basmati rice research and development inIndia. *Indian Farming, 59*, 4-6.

Singh, Y., Khind, C. S., & Singh, B. (1991). Efficient management of leguminous green manures in wetland rice. *Advances in Agronomy, 45*, 135-189. https://doi.org/10.1016/S0065-2113(08)60040-1

Slaton, N. A., Gbur, E. E., Wilson, C. E., & Norman, R. J. (2005a). Rice response to granular zinc sources varying in water-soluble zinc. *Soil Science Society of America Journal, 69*, 443-452. https://doi.org/10.2136/sssaj2005.0443

Slaton, N. A., Norman, R. J., & Wilson, C. E. (2005b). Effect of zinc source and application time on zinc uptake and grain yield of flooded-irrigated rice. *Agronomy Journal, 97*, 272-278. https://doi.org/10.2134/agronj2005.0272

Srivastava, P. C., Ghosh, D., & Singh, V. P. (1999). Evaluation of different zinc sources forlow land rice production. *Biology and Fertility of Soils, 30*, 168-172. https://doi.org/10.1007/s003740050604

Srivastava, P. C., Singh, S. K., & Mishra, B. (2006). Crop response and profitability to applied secondary and micronutrients. *Indian Journal of Fertilizers, 2*, 45-51.

Subbiah, B. V., & Asija, G. L. (1956). A rapid procedure for the estimation of available nitrogen in soils. *Current Science, 25*, 259-260.

Takkar, P. N., Singh, M. V., & Ganeshmurthy, A. N. (1997). A critical review of plantnutrient supply needs, efficiency and policy issues for Indian agriculture for the year2000: Micronutrients and Trace elements. In J. S. Kanwar & J. C. Katyal (Eds.), *Plant Nutrient, Supply Efficiency and Policy Issues: 2000-2025* (pp. 238-264). National Academy of Agricultural Sciences New Delhi, India.

Tejada, M., Gonzalez, J. L., Martinez, A. M. G., & Parrado, J. (2008a). Application of agreen manure and green manure composted with beet vinasse on soil restoration: Effects on soil properties. *Bioresource Technology, 99*, 4949-4957. https://doi.org/10.1016/j.biortech.2007.09.026

Tejada, M., Gonzalez, J. L., Martinez, A. M. G., & Parrado, J. (2008b). Effects of different green manures on soil biological properties and maize yield. *Bioresource Technology, 99*, 1758-1767. https://doi.org/10.1016/j.biortech.2007.03.052

Thanyumanavan, B., & Sadasivam, S. (1984). Physico-chemical basis for the preferential uses of certain rice varieties. *Plant Foods for Human Nutrition, 34*, 253-259. https://doi.org/10.1007/BF01126554

Thimmiah, S. K. (1999). Determination of amylose. *Standard Method of Biochemical Analysis* (pp. 60-61). Kalyani Publishers.

Ugurluoglu, H., & Kacar, B. (1996). Effect of different zinc sources on the growth of rice (*Oryza sativa* L.) plants. *Turkian Journal of Agriculture and Forestry, 20*, 473-478.

Walkley, A. J., & Black, I. A. (1934). An examination of the Degtjareff method for determination of soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science, 37*, 29-38. https://doi.org/10.1097/00010694-193401000-00003

Whitbread, A. M., Blair, G. J., & Lefroy, R. D. B. (2000). Managing legume leys, residuesand fertilisers to enhance the sustainability of wheat cropping systems in Australia 1. The effects on wheat yields and nutrient balances. *Soil and Tillage Research, 54*, 63-75. https://doi.org/10.1016/S0167-1987(99)00112-9

WHO. (2002). *The World Health Report 2002* (pp. 1-14). Geneva: World Health Organization.

Yadav, D., Shivay, Y. S., Singh, Y. V., Sharma, V. K., & Bhatia, A. (2019). Water use and soil fertility under rice-wheat cropping system in response to green manuring and zinc nutrition. *Communications in Soil Science and Plant Analysis,* 50(22), 2836-2847. https://doi.org/10.1080/00103624.2019.1686516

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# Evaluation of Different Starches as Gelling Agents for Micropropagation of Potato

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**Abstract**

Unavailability of clean planting material is a major constraint of potato production in Eritrea. *In vitro* multiplication is proved to be a reliable solution; however, due to high media cost and initial investment, its use has been limited. The current study was carried out to evaluate the effectiveness of three starches (corn, potato and barley) of both laboratory and commercial grades as agar substitute, for micropropagation of potato. Single nodes of potato were sub-cultured into a fresh modified Murashige and Skoog (MS) medium supplemented with sucrose (3% w/v) and gelled with 50 g/l of six types of starches in addition to 7 g/l of agar as a control. Gelling agents used showed a highly significant difference in all measured parameters. The pH of all starch based media increased by 0.23-1.3 units during the culture time except the control media which decreased by 0.4 units. Similarly EC showed a decreasing trend in all gelling agents. In most of the measured physical parameters such as plant height, root length, fresh weight and dry weight three starch based media namely laboratory grade potato, commercial corn and laboratory corn showed better results compared to the control media. Survival of *in vitro* plantlets ranged between 85 and 90% after acclimatization. Both laboratory and commercial grade starch based media resulted in cost reduction of 15-22% and 61-66%, respectively. This implies that using both corn and potato starches can be reliable and cost effective gelling agents for micropropagation of potato.

**Keywords:** Eritrea, micropropagation, starches, single node explant, MS medium

## 1. Introduction

Potato is the most important root and tuber crop in the world and is used as stable food in households, in food processing industries, alcohol production and as animal feed (Danci et al., 2012). Total world production in 2014 was approximately 370 million tons (FAO, 2015). Developing countries are now the world's biggest producers and importers of potatoes and its products, where its cultivation is expanded intensely because of its ease of cultivation and nutritional value (Lutaladio et al., 2009).

In Eritrea, potato is an integral part of the commonly grown vegetable crops. Its production is mainly in the highland areas of the country, particularly in Maekel and Debub administrative regions and limited areas in Anseba and Semenawi Keyh Bahri regions (Biniam et al., 2014b). Potato production system in Eritrea is still traditional and undeveloped (Biniam & Tadesse, 2008). As a result, the average yield is very low (6.4 tons $ha^{-1}$), compared to the averages for Africa (10.8 tons $ha^{-1}$) and the world (16.8 tons $ha^{-1}$) (FAO, 2007).

Lack of efficient seed potato supply system, unavailability of improved varieties and good quality seed tuber, among others, are characteristic problems of potato production in Eritrea (Biniam et al., 2014b; Biniam & Tadesse, 2008). Therefore, farmers use unimproved seed tuber saved from the previous season or purchased from non-reliable sources (Biniam et al., 2014a). This type of seed potato production system is characterized by low rates of multiplication and carry high risk of disease infection (Tadesse, 2000). Biniam and Tadesse (2008) reported detection of five of the six most important potato viruses (PVX, PVY, PLRV, PVS and PVA), in single and multiple infections. As a consequence the unavailability of standard seed supply systems coupled with disease and virus infection causes the overall potato yield losses in Eritrea.

One of the promising solutions to potato production constraints in Eritrea is to provide high yielding, improved quality and disease free seeds through the application of tissue culture technology. However, high production cost has been an impediment to tissue culture adoption. One of the factors contributing to the high cost of production is the cost of culture nutrient media which requires several chemicals that are often very expensive. Gelling agents such as agar constitute about 70% of the total cost (Purohit et al., 2011). Therefore, a quest for an alternative to agar as gelling agent will result in significant reduction of cost of tissue culture propagated seed potato material.

Agar has been the choice in the majority of tissue culture systems as it assures adequate support and translocation of water and mineral nutrients to explants (Ivanova & Van Staden, 2011). Thus it is very crucial that alternative gelling agents need not only be economical; but also of satisfactory qualities.

Several alternative gelling agents, such as corn starch, barley starch, white flour, laundry starch, semolina, potato starch, rice powder, sago, sweet potato, maize, rice, wheat, sorghum and cassava have been proposed since the 1980s (Sorvari, 1986a, 1986b, 1986c, Henderson & Kinnersley, 1988, Ullah et al., 2015; Teixeira da Silva, 2015). The current study was, thus, designed to assess the effectiveness of some starches (potato, barley and corn of commercial and laboratory grades) as substitute gelling agents for agar in the micro-propagation of potato.

## 2. Materials and Methods

### *2.1 Planting Material and Experimental Design*

This study was conducted in the Tissue Culture Laboratory of the National Agricultural Research Institute (NARI) at Halhale, Eritrea. Single-node explants from previously maintained disease and virus free plantlets of variety Kufri Chipsona-3 cultured in Gelrite gelatinized media was used as planting material for carrying out the experiment.

Six different starch sources, in addition to agar as a control, were accommodated in a complete randomized design (CRD). Each treatment had 10 replicate test-tubes, each containing a single-node ex-plant.

### *2.2 Media Preparation*

The MS medium including modified Vitamins (4.41 g/l) supplemented with sucrose 30 g/l and 2 mg/l D-calcium pantothenate was used. Then MS medium was supplemented with agar 7 g/l as a control and the six starch sources barley, potato and corn (commercial and laboratory grades each) were added at a rate of 50 g/l. The rates of potato and corn starches were decided based on Mohamed et al. (2010), while for barley starch Sorvari (1986b) was followed. Prior to autoclaving the pH of the medium was adjusted to 5.7.

Gelatinization of the nutrient medium was made by mixing the starch with cold distilled water and pouring the mixture slowly into hot (> 95 °C) nutrient medium and mixing continuously. Then 10 ml of the gelatinized medium was poured in each test tube. Due to the soft texture of barley commercial and barley laboratory media, polyester nets were used to prevent sinking of the cultures. Following that, the nutrient media were covered and autoclaved at 121 °C for 21min under 1.5 KPa pressure.

### *2.3 Inoculation and Multiplication*

The single node explants were excised essentially from middle nodes of the microplants for maintaining explants' homogeneity. The test tubes were sealed with Para-film tagged with the corresponding line number and date of subculture. They were then incubated at 21±1 °C under a 16 h photoperiod with a photosynthetic photon flux density of 40 µmol $m^{-2}$ $s^{-1}$ provided by overhead cool fluorescent lamps (Philips, India 30 Watts) and humidity range of 50-60%, for 28 days in a culture room.

Multiplication was carried out through sub-culturing the explants at 4 weeks interval, and each treatment was sub-cultured into its respective media type. This was done to confirm the starch capabilities of supporting the plant and to evaluate the effect of the starch in subsequent sub-cultures (generations).

### *2.4 Acclimatization of Plantlets*

Seven plantlets of each treatment with well-developed root and shoot systems were washed with tap water and transplanted into acclimatization trays filled with a soil mix of sand, clay and compost in a ratio of 2:1:1. The trays with plantlets were then kept in an acclimatization chamber for 21 days. Afterwards the plants were transferred into pots and evaluated by counting the number of live plants.

### *2.5 Data Collection and Analysis*

Twenty-eight days after culturing, three culture tubes each with one microplant from each treatment were randomly picked for data collection. Then observations on plantlet height, root length, number of leaves, plantlet fresh and dry weights were recorded and averaged.

The three media were homogenized individually after removing the plantlets then, the pH and EC were measured with Systronics 362 digital µ pH meter and Jenway Conductivity meter 4150 (UK), respectively.

GENSTAT Discovery edition 4, statistical software (VSN international) was used for data analysis. The data was subject to the analysis of variance and least significant difference at $P \leq 0.05$ was used to separate the difference between treatment means.

*2.6 Cost Comparison of Gelling Agents*

The cost of media with different gelling agents was computed based on the prices of media that were prevailing at the time of their use during the period of experimentation (2015/2016). The percentage cost reduction was calculated using the following formulae:

$$\text{Cost reduction (\%)} = \frac{\text{Cost of Agargelled Media} - \text{Cost of Alternative Media}}{\text{Cost of Agargelled Media}} \times 100 \quad (1)$$

## 3. Results and Discussion

*3.1 Gel State and Clarity*

The control treatment, agar, formed a solid and transparent media. While the alternative gelling agents showed either a semi-solid or semi-liquid gel state and were either semi-opaque or opaque in clarity. The media state is an important aspect of a gelling agent in supporting a plantlet from sinking, while gel clarity is vital in detecting microbial contamination.

Table 1. Media state and clarity of the different gelling agents

| Treatments | Gel state | Gel clarity |
|---|---|---|
| Agar | Solid | Transparent |
| Corn laboratory | Semi-Solid | Opaque |
| Potato laboratory | Semi-Solid | Transparent |
| Barley laboratory | Semi-Liquid | Semi-Opaque |
| Corn commercial | Semi-Solid | Opaque |
| Potato commercial | Semi-Solid | Opaque |
| Barley commercial | Semi-Liquid | Opaque |

*3.2 Effect on EC and pH*

After autoclaving the media, the Electrical Conductivity (EC) and pH of the different gelling agents was variable (Table 2). The EC ranged between 4.51 Millisiemns/cm (corn LG) and 6.17 (barley LG).While media pH showed a decrease from the adjusted 5.7 pH in all treatments except potato LG.

The results in Table 2 show that the difference on media EC and pH measured four weeks after culturing was significantly different ($p < 0.01$) among the seven gelling agents. The highest EC was recorded in barley LG gelled media (5.77 Millisiemns/cm) and corn LG showed the lowest (4.13Millisiemns/cm). While the highest pH was recorded in corn LG gelled media (6.12) compared to agar which showed the lowest result (4.92). The variation in pH and EC could be mainly due to ionic composition of the different gelling agents. Dougall (1980) attributed changes in pH to the ammonium ($NH_4$) and nitrate ($NO_3$) uptake by *in vitro* grown tissue which is affected by the initial pH of the medium. In agreement with Skirvin et al. (1986) agar gelled media of the current study showed a reduction in pH, while the starch based alternative gelling agents showed increased pH levels. This can be attributed to the presence of different chemical composition in the various media which creates variable amounts of $H^+$ and $OH^-$.

Media pH may affect the auxin and gibberellic acid stability, precipitate phosphate and iron salts and vitamin B1 and pantothenic acid to become less (Skirvin et al., 1986). Since in the current study the alternative media showed increase in pH, those negative effects might have been reduced.

Table 2. EC and pH of the different gelling agents before culturing and four weeks after culture

| Treatment | EC Before culturing | EC 4 weeks After | pH Before culturing | pH 4 weeks After |
|---|---|---|---|---|
| Agar | 5.52 | 4.808 | 5.34 | 4.916 |
| Corn LG | 4.52 | 4.133 | 4.85 | 6.117 |
| Potato LG | 4.80 | 4.333 | 5.69 | 5.953 |
| Barley LG | 6.12 | 5.773 | 4.71 | 5.897 |
| Corn CG | 4.58 | 4.387 | 4.76 | 5.687 |
| Potato CG | 4.64 | 4.517 | 4.82 | 5.580 |
| Barley CG | 5.54 | 5.481 | 4.69 | 5.537 |
| LSD (5%) | | 0.3718 | | 0.3545 |
| CV% | | 8.2 | | 6.6 |

*Note*. LG = Laboratory grade; CG = Commercial grade.

The chemical parameters of the culture medium could lead to undesirable effects on plant growth and nutrient element availability. The highest EC recorded in both commercial and laboratory grade barley might have caused low nutrient element availability, poor plant growth, and nutrient element imbalance (Shibli et al., 1999). This is in agreement with the poor physical performance of both barley starch grades of the current study. This result is also in agreement with that of Shatnawi et al. (2010) where the number of shoots, number of new leaves, fresh weight and dry weight decreased with increasing salt content in the medium.

### *3.3 Growth Parameters*

The results in Table 3 shows the difference among treatments in all growth parameters (plant height, number of leaves/plant, root length and fresh and dry weight) was significant ($P < 0.05$). Similarly the difference among the three generations and the interaction between the treatments and generations was significant ($P < 0.05$).

#### 3.3.1 Effect of Gelling Agenton Plant Height

The highest plant height was recorded in media gelled with potato LG (11.56 cm), while the lowest was recorded in media gelled with barley LG (4.91 cm). Potato LG, corn LG and corn CG, gelled media showed better results as compared to the control. These results are in agreement with those of Mohamed et al. (2010), where corn starch and potato starch gelled media showed higher plant height values as compared to the control (Agar). The reason could possibly lie in the nature, consistency and rigidity of the different media which are dependent on the gelling agent used. Moreover, the better response in starch gelled media could also be due to the absence of inhibitors which have been reported to be present in agar (Puchooa & Purseramen, 1999).

Barley LG generally showed lower plant height levels in comparison with the other gelling agents and the possible reason for this could be explained by earlier findings of Dobraski et al. (2011) where the rheological and diffusion properties of gelling agents affected shoot development in apple and black locus. In addition to that the saline conditions in the media gelled with barley can also be another possible reason. Shibli et al. (1999) found that when salinity increases significantly with time, low nutrient element availability, poor plant growth, and nutrient element imbalance are expected. This might be due to the greater osmotic stress and ionic contamination which would have decreased the optimum growth as reported in banana under *in vitro* conditions (Gebre & Sathyanarayana, 2001).

Table 3. Effect of gelling agent on plant physical parameters

| Treatment | Plant Height (cm) | | | | Leaf Number | | | | Root Length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | Mean | G1 | G2 | G3 | Mean | G1 | G2 | G3 | Mean |
| Agar | 10.20 | 10.00 | 8.73 | 9.64 | 14.00 | 11.00 | 12 | 12.33 | 7.40 | 4.83 | 6.40 | 6.21 |
| Corn LG | 10.20 | 14.37 | 8.67 | 11.08 | 10.33 | 12.00 | 10.33 | 10.89 | 4.93 | 5.18 | 5.00 | 5.04 |
| Potato LG | 13.67 | 10.83 | 10.17 | 11.56 | 9.67 | 11.00 | 10.67 | 10.45 | 6.67 | 6.90 | 5.8 | 6.46 |
| Barley LG | 4.07 | 4.83 | 5.83 | 4.91 | 10.67 | 7.67 | 6.33 | 8.22 | 2.77 | 5.6 | 1.6 | 3.32 |
| Corn CG | 10.17 | 11.77 | 8.77 | 10.24 | 12.67 | 10.00 | 11.67 | 11.45 | 7.77 | 5.07 | 9.33 | 7.39 |
| Potato CG | 7.27 | 10.80 | 6.90 | 8.32 | 10.00 | 11.67 | 10.33 | 10.67 | 5.57 | 8.50 | 8.40 | 7.49 |
| Barley CG | 9.50 | 6.30 | 7.20 | 7.67 | 10.00 | 9.67 | 9.00 | 9.56 | 3.67 | 5.8 | 3.93 | 4.47 |
| Mean | 9.30* | 9.84* | 8.04* | 9.06* | 11.05* | 10.43* | 10.05* | 10.51* | 5.54* | 5.98* | 5.78* | 5.98* |
| LSD (5%) | 2.9 | 2.96 | 2.20 | 1.5 | 2.51 | 1.62 | 1.83 | 1.122 | 0.65 | 0.65 | 0.73 | 0.37 |
| CV% | 17.8 | 17.1 | 15.6 | 17.4 | 13 | 8.9 | 10.4 | 10.77 | 6.7 | 6.2 | 7.2 | 6.7 |

*Note*. LG = Laboratory grade; CG = Commercial grade.

#### 3.3.2 Effect of Gelling Agent on Number of Leaves

The highest number of leaves per explant was 12.33 recorded in agar media, while barley CG performed the poorest of all the treatments with average leaf numbers of 8.22 (Table 3). These results are contrary to those of Mohamed et al. (2010) who found highest number of shoots/explant in medium gelled with potato starch. The lowest value was recorded in barley LG. The possible reason for this low performance of barley in the current study might be in the contents of the barley starch used in the current study which seems to contain compounds that might have interacted with the plant nutrient uptake activity negatively and further inhibited the differentiation of plantlets, but it is unclear if those compounds are similar to the impurities found in agar. Another possible reason might be the high salinity in the media (Table 2) recorded before inoculation of the plant that might have severely affected nutrient uptake of the plant from the media and hence caused ill effects on the plant's physiological development. Another study reported by Batty and Dunwell (1989), showed that potato anthers cultured on a medium containing maltose produced significantly fewer embryos than those cultured on sucrose. Therefore, the presence of maltose in barley LG may have inhibitory effects.

#### 3.3.3 Effect of Gelling Agent on Root Length

The performance of agar was lower compared to starches of commercial grade of potato (7.49 cm) and corn (7.39 cm) as well as potato LG (6.46 cm), while barley LG (3.32 cm) and barley CG (4.47 cm) had shorter root length compared to all gelling agents including the control agar (Table 3). Scholten and Pierik (1998) inferred that agar could have a number of drawbacks that could negatively affect morphogenesis and culture growth in some plants. In addition to that, toxic exudates from the cultured explants may take longer time to diffuse (Powell & Uhrig, 1987) since agar is more solid. The increase in number of roots and root length in the starches might be due to the low viscosity of the media, better availability of water and nutrients and closer contact between explants and the medium than in the media solidified with agar.

Mellor and Stace-Smith (1969) found the rooting of excised potato buds to be best at pH 5.7, root formation being inhibited at pH levels of 4.8 and 6.2 or above. However, in the current study, the effect of pH on rooting might have not been clearly influenced by pH levels where the pH of barley starch that recorded the lowest root length was similar to the pH of potato and corn starch gelled media that recorded the two highest root lengths (Tables 2 and 3). On the other hand Shibli et al. (1999) related high media EC with low nutrient availability, poor plant growth, and nutrient element imbalance. This is in agreement with the results of the current study where barley gelled media had the highest EC but lowest root length (Tables 2 and 3) compared to the rest of the gelling agents.

#### 3.3.4 Effectof Gelling Agent on Fresh and Dry Weight

The highest average fresh weight was recorded in potato LG gelled media (0.28 g) followed by corn LG (0.264 g), while the lowest average was obtained from barley LG (0.13 g) followed by barley CG which recorded an average fresh weight of 0.15 g (Table 4). This result is in agreement with the findings of Mohamed et al. (2010) where plantlets developed on media with corn starch or potato starch had higher shoot fresh weight.The possible reason for the poor performance of the plants in barley could be due to the presence of impurities in their content that could have triggered negative interaction with the plants.

Compared to agar which recorded a dry weight of 0.019 g, the highest average dry weight was recorded by media gelled with corn CG (0.021 g), whereas the lowest average (0.012 g) was recorded by media gelled with barley LG (Table 4). The result of corn starch is in agreement with the findings of Mohamed et al. (2010) who found corn starch to have significantly higher dry weight than agar.

In another study, Henderson and Kinnersely (1988) found similar results in using corn starch as agar alternative for wild carrot and tobacco cell cultures, in which cell dry weight increased more than three times with respect to cells grown in a medium gelled with agar. Similar results were also obtained by Sharma et al. (2011) who observed different potato varieties including Kufri-Chipsona-3, developed on media with 7 g/l of agar had less dry weight compared to those grown in medium with agar-alternative. The possible reasons could be due to the contribution of nutritional contents present in the corn starch used such as vitamin B-complex including B1 (thiamine), B2 (niacin), B3 (riboflavin), B5 (pantothenic acid) and B6, carbohydrates, protein in small amount and can easily be taken by the plant (Kumar & Jhariya, 2013). Similar to the fresh weight values, barley LG and barley CG showed the lowest dry weight values. This might be caused by the salinity of the media under which low nutrient elements availability, poor plant growth, and nutrient element imbalance are expected (Shibli et al., 1999).

Table 4. Effect of gelling agent on wet and dry weight

| Treatment | Fresh weight (mg) | | | | Dry weight (mg) | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | Mean | G1 | G2 | G3 | Mean |
| Agar | 0.333 | 0.249 | 0.203 | 0.2617 | 0.0210 | 0.0210 | 0.0140 | 0.01867 |
| Corn LG | 0.22 | 0.4 | 0.171 | 0.2637 | 0.0170 | 0.0270 | 0.0143 | 0.01944 |
| Potato LG | 0.315 | 0.318 | 0.203 | 0.2787 | 0.0180 | 0.0260 | 0.0133 | 0.01911 |
| Barley LG | 0.107 | 0.191 | 0.1 | 0.1327 | 0.0130 | 0.0170 | 0.0083 | 0.01278 |
| Corn CG | 0.268 | 0.281 | 0.237 | 0.262 | 0.0250 | 0.0200 | 0.0183 | 0.02111 |
| Potato CG | 0.191 | 0.317 | 0.138 | 0.2153 | 0.0100 | 0.0250 | 0.0133 | 0.01611 |
| Barley CG | 0.159 | 0.153 | 0.143 | 0.1517 | 0.0120 | 0.0130 | 0.0103 | 0.01178 |
| Mean | 0.2276 | 0.2727 | 0.1707 | 0.2237 | 0.0166 | 0.0213 | .01314 | 0.01700 |
| LSD (5%) | 0.03065 | 0.02648 | 0.03502 | 0.02909 | 0.003034 | 0.005694 | 0.7305 | 0.00430 |
| CV% | 7.7 | 5.5 | 11.7 | 7.9 | 10.5 | 15.3 | 7.2 | 15.4 |

*Note*. LG = Laboratory grade; CG = Commercial grade.

3.3.5 Generation Effect

In most of the results obtained from plant physical parameters (Tables 3 and 4), the highest performance was recorded in the second generation except for leaf number. First and third generations showed relatively lower performance in comparison with the second generation. The possible reason for this could be the fact that in the first generation the plantlets were transferred from a gelrite gelled media to the different gelling agents that were used in the current study. Therefore, the plantlets might have faced a possible shock and might have used that stage as an adjustment stage. In case of the third generation the results showed reduction and this is a usual phenomenon in tissue culture since the plant is manipulated with every subculture and as a result reduction in performance of the plant is expected in subsequent generations. This is in agreement with the findings of Madege et al. (2015) who found significant difference between different gelling agents and subsequent generations.

### *3.4 Survival Percentage*

When plantlets were transferred from *in vitro* to external conditions for the purpose of acclimatization; the survival rate was generally high with all gelling agents. The percentage ranged between 85 and 90%. The highest results were obtained in plantlets that were grown in media gelled with corn CG and potato LG. While the survival percentage of plantlets from all the media gelled with the rest of starches and agar recorded a survival percentage of 85%. Since plantlets were grown previously *in vitro* under controlled environment of temperature, light and humidity some anatomical and morphological changes could be induced in plantlets. Consequently, transferring to harsh conditions may result in death of some of these plantlets.

### *3.5 Cost Analysis*

Cost analysis has been done to observe the cost of each starch media compared to the control. According to the cost analysis conducted, agar was very high compared to all types of starches used. Similarly the cost of the laboratory grade starches was more expensive compared to the commercial grades of starches bought from super markets.

The cost of agar per litre of media is 38.5 Nakfa (local currency). However, substituting agar with alternative starches showed a significant reduction in the cost of tissue culture media. The highest cost reduction was observed in media gelled with barley and potato commercial starches (66%), while the lowest cost reduction was recorded in laboratory starches of both corn and potato (15%) (Table 4).

Table 5. Cost reduction (in Nakfa) for the different gelling agents compared to agar

| Gelling Agent | Price/Kg | Conc. (g/l) | Cost/l | | Cost of media/l | Cost reduction (%) |
|---|---|---|---|---|---|---|
| | | | Gelling agent | Other ingrid | | |
| Agar | 5500.00 | 7 | 38.50 | 16.50 | 55.00 | - |
| Corn laboratory | 607.50 | 50 | 30.40 | 16.50 | 46.90 | 15 |
| Potato laboratory | 607.50 | 50 | 30.40 | 16.50 | 46.90 | 15 |
| Barley laboratory | 526.50 | 50 | 26.30 | 16.50 | 42.80 | 22 |
| Corn commercial | 100.00 | 50 | 5.00 | 16.50 | 21.50 | 61 |
| Potato commercial | 45.90 | 50 | 2.30 | 16.50 | 18.80 | 66 |
| Barley commercial | 42.50 | 50 | 2.10 | 16.50 | 18.60 | 66 |

*Note*. 1 USD = 15 Nakfa.

## 4. Conclusion

The results of this study suggest that using corn and potato starches at 50 g/l as solidifying agent instead of agar can be efficient and cost effective for single node potato micropropagation. While both commercial and laboratory grades of barley were not satisfactory for micropropagation of potato.

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**References**

Batty, N., & Dunwell, J. (1989). Effect of maltose on the response of potato anthers in culture. *Plant Cell Tissue Organ Culture, 18*, 221-226. https://doi.org/10.1007/BF00047748

Biniam, M. G., Githiri, S. M., Tadesse, M., & Remmy, W. K. (2014a). Potato Seed Supply, Marketing and Production Constraints in Eritrea. *American Journal of Plant Sciences, 5*, 3684-3693. https://doi.org/10.4236/ajps.2014.524384

Biniam, M. G., Githiri, S. M., Tadesse, M., & Remmy, W. K. (2014b). Diagnostic survey on potato production practices in Eritrea. *ARPN (Asian Research Publishing Network) Journal of Agricultural and Biological Science, 9*(12).

Biniam, T., & Tadesse, M. (2008). A survey of viral status on potatoes grown in Eritrea and *in vitro* virus elimination of a local variety '*Tsaeda embaba*'. *African Journal of Biotechnology, 7*(4), 397-403.

Danci, M., Danci, O., Mike, L., Baciu, A., Olaru, D., Petolescu, C., … David, I. (2012). Production of virus free potato plantlets. *Journal of Horticulture, Forestry and Biotechnology, 16*(1), 232-238.

Dougall, D. K. (1980). Nutrition and metabolism. In E. J. Staba (Ed.), *Plant Tissue Cultures and Source of Biochemicals* (pp. 21-58). Chemical Huibber Company Press, Boca Raton, FL.

FAO (Food and Agriculture Organization of the United Nations). (2015). *FAOSTAT*. FAO, Rome.

Gebre, E., & Sathyanarayana, B. N. (2001). Tapioca . A new and cheaper alternative to agar for direct microtuber production from nodal culture of potato. *African Crop Science Journal, 9*, 1-8. https://doi.org/10.4314/acsj.v9i1.27618

Henderson, W. E., & Kinnersley, A. M. (1988). Corn starch as an alternative gelling agent for plant tissue culture. *Plant Cell and Tissue Organs Culture, 15*, 17-22. https://doi.org/10.1007/BF00039885

Ivanova, M., &Van, S. J. (2011). Influence of gelling agent and cytokinins on the control of hyperhydricity in *Aloe polyphylla*. *Plant Cell Tissue and Organ Culture, 104*, 13-21. https://doi.org/10.1007/s11240-010-9794-5

Kumar, D., & Jhariya, A. N. (2013). Nutritional, Medicinal and Economical importance of Corn: A Mini Review. *Research Journal of Pharmaceutical Sciences, 2*(7), 7-8.

Kuria, P., Demo, P., Nyende, A., & Kahangi, E. (2008). Cassava starch as an alternative cheap gelling agent for the *in vitro* micro-propagation of potato (*Solanum tuberosum L.*). *African Journal of Biotechnology, 7*(3), 301-307.

Lutaladio, N., Ortiz, O., Haverkort, A., & Caldiz, D. (2009). *Sustainable Potato Production: Guidelines for Developing Countries*. FAO, Rome.

Madege, R., Mneney, E., Misangu, R., & Maerere, A. (2015). Characterization of botanical starches as a potential substitutes of agar in tissue culture media. *African Journal of Biotechnology, 14*(8), 702-713. https://doi.org/10.5897/AJB2014.14289

Mellor, F. C., & Stace-Smith, R. (1969). Development of excised potato buds in nutrient medium. *Can. J. Bot., 47*, 1617-1621. https://doi.org/10.1139/b69-232

Mohamed, M. A. H., Alsadon, A. A., & Al Mohaidib, M. S. (2010). Corn and potato starch as an alternative for *Solanum tuberosum* micropropagation. *African Journal of Biotechnology, 9*(1), 012-016.

Powell, W., & Uhrig, H. (1987). Anther culture of Solanum genotypes. *Plant Cell Tissue and Organ Culture, 11*, 13-24. https://doi.org/10.1007/BF00036572

Puchooa, D., & Purseramen, R. (1999). Effects of medium support and gelling agent in tissue culture of tobacco (*Nicotiana tabacum*). *Sci. Technol. Res. J., 3*, 129-144.

Purohit, S. D., Teixeira da Silva, J. A., & Habibi, N. (2011). Current Approaches for Cheaper and Better Micropropagation Technologies. *International Journal of Plant Development Biology, 5*(1), 1-36.

Scholten, H. J., & Pierik, R. L. M. (1998). Agar as a gelling agent: chemical and physical analysis. *Plant Cell Rep., 17*, 230-235. https://doi.org/10.1007/s002990050384

Shatnawi, M., Al-Fauri, A., Megdadib, H., Al-Shatnawic, M. K., Saead, R. S., Rommana, A., & Al-Ghzawi, A. (2010). *In vitro* multiplication of *Chrysanthemum morifolium* Ramat and it is Responses to NaCl Induced Salinity. *Jordan Journal of Biological Sciences, 3*(3), 101-110.

Shibli, R. A., Mohammad, M. J., Ajlouni, M. M., Shatnawi, M. A., & Obeidat, A. F. (1999). Stability of chemical parameters of tissue culture medium (pH, osmolarity, electrical conductivity) as a function of time of growth. *Journal of Plant Nutrition, 22*(3), 501-510. https://doi.org/10.1080/01904169909365647

Skirvin, R. M., Chu, M. C., Mann, M. L., Young, H., Sullivan, J., & Fermanian, T. (1986). Stability of tissue culture medium pH as a function of autoclaving time and cultured plant material. *Plant Cell Rep., 5*, 292-297. https://doi.org/10.1007/BF00269825

Sorvari, S. (1986a). The effect of starch gelatinized nutrient media in barley anther cultures. *Annales Agriculturae Fenniae, 25*, 127-133.

Sorvari, S. (1986b). Differentiation of potato tuber discs in barley starch gelatinized nutrient media. *Annales Agriculturae Fenniae, 25*, 135-138.

Sorvari, S. (1986c). Comparison of anther cultures of barley cultivars in barley-starch and agar gelatinized media. *Annales Agriculturae Fenniae, 25*, 249-254.

Tadesse, M. (2000). *Manipulating the physiological quality of in vitro plantlets and transplants of potato* (PhD Thesis, Wageningen University, The Netherlands).

Teixeira da Silva, J. A. (2015). Response of *Syngonium podophyllum* L. 'White Butterfly' shoot cultures to alternative media additives and gelling agents, and flow cytometric analysis of regenerants. *Nusantara Bioscience, 7*(1), 26-32. https://doi.org/10.13057/nusbiosci/n070105

Ullah, M. A., Uddin, M. I., Puteh, A. B., Haque, M. S., & Islam, M. S. (2015). Alternative Gelling Agents for *in vitro* propagation of orchid (*Dendrobiumsonia*). *Journal of Animal and Plant Sciences, 25*(3), 792-797.

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# Cultivation and Propagation of *Iris laevigata* Fisch., an Endangered Ethno-medicinal Plant of Imphal Valley Manipur, India

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**Abstract**

*Iris laevigata* Fisch., is restricted geographically in Manipur, north eastern India. An experiment was planned with Factorial Randomized Block Design during 2018 to 2020 (3 seasons) in the study plots of ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat. The study encompasses on the growth and development of *I. laevigata* seedlings collected from Ipa Thoukok Complex: influenced by spacing, trimming and correlations amongst the growth parameters, so as to validate the most favourable conservation method for this endangered plant. Among the treatments, $T_6 = S_2T_3$ (45 cm × trimming 40 DAT) was found the best treatment. $T_9 = S_3T_3$ (60 cm × trimming 40 DAT) was second, $T_3 = S_1T_3$ (30 cm × trimming 40 DAT) was the third and lowest was found in case of $T_1 = S_1T_1$ (30 cm × 0 trimming, *i.e.*, without trimming). Strong positive correlation between leaf surface area and plant height on the growth and development was found to be effective among other correlations.

**Keywords:** *Iris laevigata* Fisch., endangered, growth and development, correlation, soil nutrient, water quality and depth

## 1. Introduction

Water Iris Plant: *Iris laevigata* Fisch., belongs to the family Iridaceae, grows in moist habitats like marshy grassy slopes, meadowlands, bogs and lakes. The plant is commonly known as Water Iris, *Kombirei* in Manipuri, Japanese iris, rabbit-ear iris, or shallow-flowered iris, is native to Japan. Water Iris is distributed from Assam to Myanmar. In Manipur the plant grows naturally in Lamphel pat, Yaral pat, Loktak Lake, etc., as an endemic water plant presently in the endangered status under the RET (Rare Endangered and Threatened) category. The genus Iris comprises more than 300 herbaceous species, growing in the temperate regions of the northern hemisphere (Rodionenko, 1987).

Regarding *Iris* species, Kostrakiewicz (2000) studied the analysis of spatial horizontal structure of *I. sibirica* L. population on the station in Stanisławice near Bochnia. The studied species growing in wet habitats belongs to rare, legal protected plants in Poland. The distribution of the individuals within all phytocenosis as well as on 100 $m^2$ selected area was presented.

Earlier in Manipur, *I. laevigata* was wrongly identified by some researchers as *Iris bakeri* Wall. (Deb, 1961a, 1961b; Sinha, 1996). However, one scientist H. B. Singh identified *Kombirei* after confirmation from RBG, Kew (Sobhapati, 2017). Four species of *Iris* were reported in Manipur. Unfortunately this beautiful flower, which has very close connections with the traditions of Manipuris since time immemorial, is facing a great threat due to lack of attention. Height is the major distinct differences between *I. laevigata* (106.68 cm) and *I. singuinea* (60.96 cm) besides their leaves. *Singuinea* has no straight leaves unlike *Laevigata*. Even though other species such as *I. wattii*, *I. singuinea*, *I. kumaon* grow wild in the State, this flower grows only in the wetlands of Lamphelpat and Loktak Lake. Interestingly, *I. singuinea* which is used as real *Kombirei* (*I. laevigata* Fisch.) during annual *Cheiraoba* festival in April is being cultivated in private nurseries in view of the huge public demand (Sobhapati, 2017).

Lee et al. (2018) reported the effect of water levels and soil nutrients on the growth of endangered *I. laevigata* seedlings in Korea. The study was conducted to examine the effects of environmental conditions such as water levels and soil nutrient conditions on the growth and survival of *I. laevigata* seedlings. Complete submergence lowered the total number of leaves, biomass, and survival rates. A rise in soil nutrients increased overall seedling growth and increased tiller numbers via the promotion of asexual reproduction. The water level must be lower than the seedling height for effective growth and management of *I. laevigata*.

Earlier, water Iris grows naturally, when the monsoon comes in Manipur during March-April, however, without people's care these plants could complete its mature stage and blooms flower. One can understand that *Kombirei* will be available in the marshy areas, when we need the flower. But nowadays, because of urbanization, drying up of lakes, expansion of agricultural lands, etc., it is high time to take care and artificial human protection and conservation of the plant.

In this regard, an understanding of the life cycle of a plant, such as the method of reproduction, seed dispersion, germination, and survival conditions of seedlings is essential for the stable establishment of the species (Mahoney & Rood, 1998). Here it can be mentioned that, water level is the most important factor affecting seedling survival and the establishment of submerged plants (Nicol & Ganf, 2000; Fraser & Karnezis, 2005; Kwon et al., 2007; Casanova & Brock, 2000).

From China, Wang et al. (2017) observed the plant growth parameters between reproductive ramets and non-reproductive ramets and reported that leaves of reproductive ramets stopped growing around the time that flowering began (in late May), while the leaves of non-reproductive ramets would continue growing until late August, dramatically increasing the leaf area available for photosynthesis.

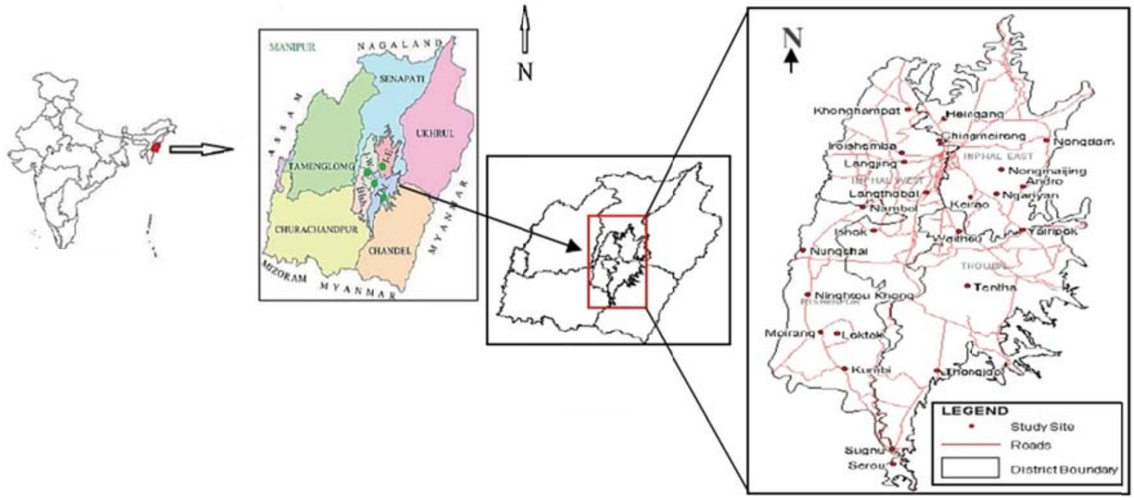
In Manipur, there is continuous exploitation, habitat-degradation, unsustainable harvesting and over-exploitation bringing substantial loss of the habitat of *I. laevigata*, is still going on. Most problematic one is during the annual *Cheiraoba* festival, *i.e.*, Annual New Year, which falls during the month of April, two *Cheiraoba* festivals are being observed: one on the day of *Shajibu nongma panba* for Meiteis, and another one, as *Charak puja* observed by the *Meitei* following Hinduism religion. On these two days *Kombirei* flower is being offered to the God as one of the most important constituents. On this day people purchase from the local market at Rs. 10 to 50 per bunch of *Kombirei* flower. People use to over harvest from the marshy natural home of *Kombirei* or from the market.

Some researchers from Manipur State worked on the distribution and mapping of some endangered plants and their status under the RET category (Devi & Das, 2016; Singh et al., 2017, 2020; Devi et al., 2021). So far, no scientific work has been done regarding *I. laevigata*, which is endangered plant under the RET category. Here, we focused on the growth and development of *I. laevigata* seedlings with specific objectives of this paper as follows: (1) influenced by spacing, (2) trimming and (3) correlations amongst the growth parameters of the plant, so as to validate the most favourable conservation method for this endangered plant.

## 2. Materials and Methods

### *2.1 Study Site*

The present work has been taken up to cultivate Water Iris plant, which is under the RET category, so as to conserve the plant species by following modern tools of agricultural practices under ICAR, Lamphelpat, Imphal; field condition of Ipa Thoukok Complex (Figure 1).




**(A)** **(B)** **(C)** **(D)**

Figure 1. Study area: (A) Map of India showing the location of Manipur; (B) Map of Manipur showing districts; (C) Map of Manipur showing the location of Imphal valley; (D) Map of Imphal valley and study sites ICAR, Lamphelpat

### *2.2 Experimental Design*

Factorial Randomized Block Design was made with a Plot Size of 4.0 m × 5.0 m. The Layout Plan for the cultivation and propagation of *Iris laevigata* Fisch., was done during 2018 to 2020 (3 seasons) in the study plots of ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal. Treatments: Two factors: Factor 1: Spacing 3 levels ($S_1$ = 30 cm, $S_2$ = 45 cm, $S_3$ = 60 cm); Factor 2: Trimming 3 levels ($T_1$ = 0 trimming, $T_2$ = trimming 20 DAT (Days after Transplantation), $T_3$ = trimming 40 DAT) (Table 1).

Table 1. Layout Plan for the Cultivation and Propagation of *Iris laevigata* Fisch

**Plot Size:** 4.0 m × 5.0 m

**Design:** Factorial Randomized Block Design

**Treatment:**

*Factor 1*: Spacing 3 levels: ($S_1$ = 30 cm, $S_2$ = 45 cm, $S_3$ = 60 cm);

*Factor 2*: Trimming 3 levels: ($T_1$ = 0 trimming, $T_2$ = trimming 20 DAT, $T_3$ = trimming 40 DAT)

| **Rows (R)** | | | **Treatments** |
|---|---|---|---|
| **$R_1$** | **$R_2$** | **$R_3$** | |
| $T_5$ | $T_6$ | $T_3$ | $T_1 = S_1T_1$ (30 cm × 0 trimming) |
| $T_3$ | $T_4$ | $T_5$ | $T_2 = S_1T_2$ (30 cm × trimming 20 DAT) |
| $T_9$ | $T_8$ | $T_7$ | $T_3 = S_1T_3$ (30 cm × trimming 40 DAT) |
| $T_6$ | $T_9$ | $T_6$ | $T_4 = S_2T_1$ (45 cm × 0 trimming) |
| $T_1$ | $T_1$ | $T_1$ | $T_5 = S_2T_2$ (45 cm × trimming 20 DAT) |
| $T_4$ | $T_7$ | $T_4$ | $T_6 = S_2T_3$ (45 cm × trimming 40 DAT) |
| $T_8$ | $T_5$ | $T_2$ | $T_7 = S_3T_1$ (60 cm × 0 trimming) |
| $T_2$ | $T_3$ | $T_9$ | $T_8 = S_3T_2$ (60 cm × trimming 20 DAT) |
| $T_7$ | $T_2$ | $T_8$ | $T_9 = S_3T_3$ (60 cm × trimming 40 DAT) |

### *2.3 Physico-chemical Soil and Water Composition*

Soil and water samples of the experimental plots were analyzed at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal.

*2.4 Meteorological Data*

Meteorological Data of the experimental farm was recorded from ICAR Complex during the tenure of the research program. Soil and water analysis of the experimental farms were analysed at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal. In Manipur there are four marked seasons namely, spring (March-May), rainy (June-Aug.), summer (Sept.-Nov.) and winter (Dec.-Feb.); because of the fluctuation and changing environment, each and every seasons overlapping each other (Figure 2).

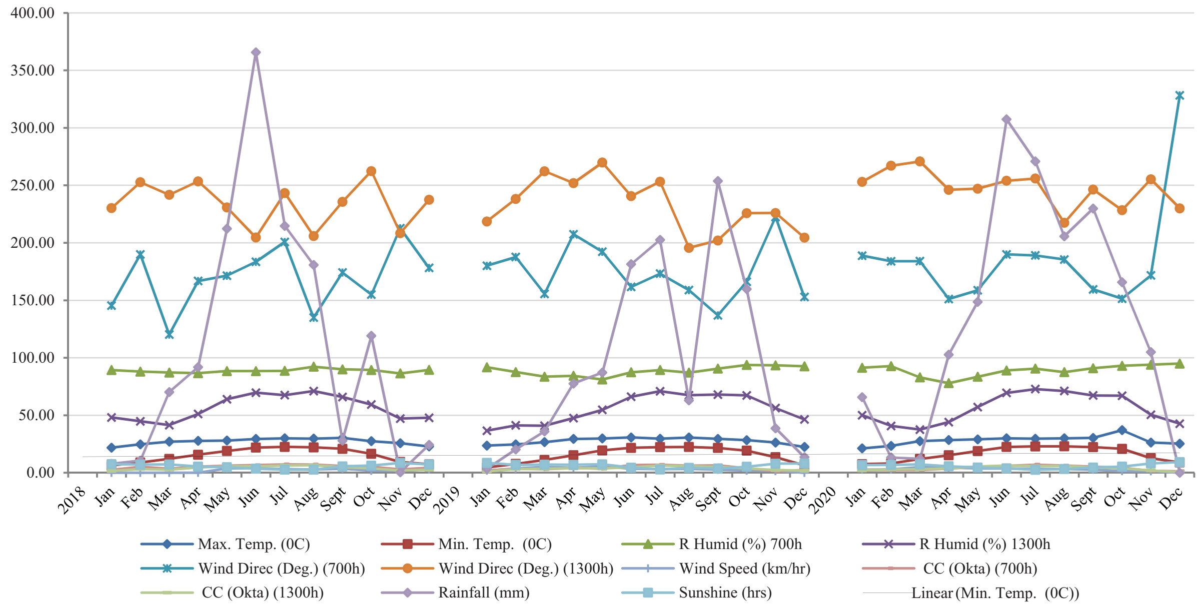



Figure 2. Weather data of *Iris laevigata* Fisch., experimental farm at ICAR Research Complex, Lamphelpat, Imphal from 2018 to 2020

*2.5 Plant Growth Parameters*

Plantation of water iris was done in the month of July, trimming started after 20 and 40 DAT, avoid cutting of leaves completely as the rhizomes will need some foliage to collect nutrients and recover from the transplant shock. The leaf will develop erectly after the transplantation in the field. The leaf surface area, plant height, number of leaves, number of plants per clump, number of flowers per pike, number of rhizomes per clump, etc., will be measured. The correlations between plant height and leaf surface area and other growth parameters will be calculated. Flowering will be during the month of March to May and harvesting follows.

*2.6 Statistical Anlysis*

All the analyses were carried out using SPSS 22.0 for Windows (SPSS, Inc., Chicago, Illinois, USA). The experiment was laid out under factorial randomized block design. The data were analyzed by Fisher's analysis of variance (ANOVA) technique and then results were interpreted.

**3. Results and Discussion**

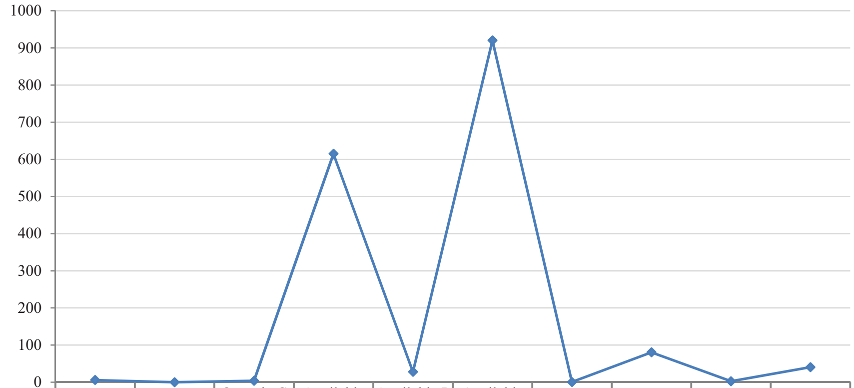
In Table 2, among the treatments, $T_6 = S_2T_3$ (45 cm × trimming 40 DAT) was found the best treatment. $T_9 = S_3T_3$ (60 cm × trimming 40 DAT) was second, $T_3 = S_1T_3$ (30 cm × trimming 40 DAT) was the third and lowest was found in case of $T_1 = S_1T_1$ (30 cm × 0 trimming, *i.e.*, without trimming). From the results it can be concluded that, in water iris cultivation spacing of 45 cm with trimming at 40 DAT treatment $T_6$ was the best and whereas, lowest value of treatment $T_1$ in which 30 cm spacing without trimming is unfavorable for the growth of water iris. The plant can grow luxuriantly in the ICAR Experimental Farm, Lamphelpat with the Treatment No. $T_6 = S_2T_3$ (45 cm × trimming 40 DAT) (Table 2).

Soil pH value was 5.8; E.C. was 0.048; organic carbon was 3.76%; available N, P and K were found to be 615, 28 and 920 kg/ha respectively. Some micronutrients *viz.*, Cu, Fe, Zn and Mn ranges from 0.4, 80.4, 2.5 and 40.50 mg/kg respectively. Water pH value was 7.26; E.C. was 0.48; P and K ranges from 0.014 and 14.7 ppm respectively.

Table 2. Influence of spacing and trimming on growth and development of *Iris laevigata* Fisch.

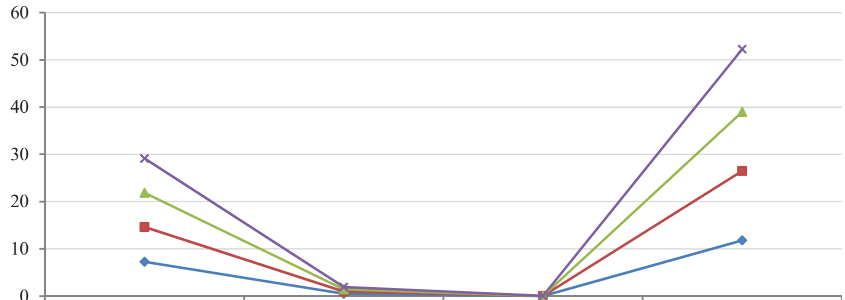
| **Treatment** | **Plant height (cm)** | **No. of leaves/ plant** | **No. of plants/ clump** | **Days to spike emergence** | **Days to flower initiation** | **No. of spikes/ plant** | **No. of flowers/ spike** | **Spike length (cm)** | **No. of rhizomes/ clump** | **Weight of rhizome (g)** |
|---|---|---|---|---|---|---|---|---|---|---|
| *Year (Y)* | | | | | | | | | | |
| $Y_1$-2018 | 56.207 | 15.488 | 6.363 | 247.934 | 9.635 | 3.302 | 3.180 | 48.474 | 6.363 | 94.074 |
| $Y_2$-2019 | 56.905 | 15.423 | 7.626 | 247.749 | 9.693 | 3.556 | 3.780 | 55.327 | 7.626 | 99.249 |
| $Y_3$-2020 | 58.329 | 17.643 | 8.437 | 249.737 | 9.869 | 4.129 | 3.375 | 54.564 | 8.437 | 100.881 |
| S.E(m)± | **1.510** | 0.364 | 0.201 | 1.427 | 0.286 | 0.133 | 0.114 | 0.867 | 0.201 | 1.873 |
| C.D. at 5% | **NS** | 1.036 | 0.573 | **NS** | **NS** | 0.379 | 0.325 | 2.464 | 0.573 | **NS** |
| *Spacing (S)* | | | | | | | | | | |
| $S_1$(30 cm) | 48.170 | 12.509 | 5.437 | 253.883 | 10.129 | 3.410 | 3.282 | 48.774 | 5.437 | 79.241 |
| $S_2$(45 cm) | 60.659 | 16.762 | 6.941 | 243.983 | 9.251 | 3.854 | 3.411 | 54.417 | 6.941 | 103.725 |
| $S_3$(60 cm) | 62.613 | 19.284 | 10.048 | 247.553 | 9.817 | 3.723 | 3.640 | 55.173 | 10.048 | 111.237 |
| S.E(m)± | 1.510 | 0.364 | 0.201 | 1.427 | 0.286 | 0.133 | 0.114 | 0.867 | 0.201 | 1.873 |
| C.D. at 5% | 4.294 | 1.036 | 0.573 | 4.059 | **NS** | **NS** | **NS** | 2.464 | 0.573 | 5.327 |
| *Trimming (T)* | | | | | | | | | | |
| $T_1$(No trimming) | 51.936 | 14.011 | 6.578 | 259.572 | 10.907 | 3.011 | 2.961 | 47.983 | 6.578 | 92.442 |
| $T_2$(20 DAT) | 56.864 | 15.961 | 7.400 | 246.933 | 9.503 | 3.634 | 3.492 | 51.902 | 7.400 | 97.926 |
| $T_3$(40 DAT) | 62.641 | 18.582 | 8.448 | 238.913 | 8.788 | 4.341 | 3.881 | 58.480 | 8.448 | 103.836 |
| S.E(m)± | 1.510 | 0.364 | 0.201 | 1.427 | 0.286 | 0.133 | 0.114 | 0.867 | 0.201 | 1.873 |
| C.D. at 5% | 4.294 | 1.036 | 0.573 | 4.059 | 0.813 | 0.379 | 0.325 | 2.464 | 0.573 | 5.327 |
| *Interaction (SXT)* | | | | | | | | | | |
| $S_1T_1$ | 43.179 | 11.411 | 4.956 | 266.103 | 11.803 | 2.800 | 2.609 | 42.799 | 4.956 | 70.970 |
| $S_1T_2$ | 47.813 | 12.467 | 5.511 | 250.677 | 9.807 | 3.460 | 3.429 | 48.851 | 5.511 | 79.476 |
| $S_1T_3$ | 53.517 | 13.650 | 5.844 | 244.869 | 8.778 | 3.971 | 3.809 | 54.673 | 5.844 | 87.279 |
| $S_2T_1$ | 53.839 | 13.258 | 5.822 | 259.699 | 10.574 | 3.058 | 2.886 | 48.799 | 5.822 | 99.169 |
| $S_2T_2$ | 59.770 | 16.163 | 6.644 | 240.660 | 8.757 | 3.709 | 3.382 | 53.071 | 6.644 | 103.940 |
| $S_2T_3$ | 68.368 | 20.864 | 8.356 | 231.591 | 8.422 | 4.794 | 3.967 | 61.382 | 8.356 | 108.066 |
| $S_3T_1$ | 58.791 | 17.365 | 8.956 | 252.914 | 10.343 | 3.177 | 3.388 | 52.350 | 8.956 | 107.187 |
| $S_3T_2$ | 63.009 | 19.253 | 10.044 | 249.463 | 9.944 | 3.733 | 3.666 | 53.783 | 10.044 | 110.361 |
| $S_3T_3$ | 66.039 | 21.233 | 11.144 | 240.280 | 9.163 | 4.258 | 3.868 | 59.386 | 11.144 | 116.164 |
| S.E(m)± | 2.616 | 0.631 | 0.349 | 2.472 | 0.495 | 0.231 | 0.198 | 1.501 | 0.349 | 3.245 |
| C.D. at 5% | **NS** | 1.794 | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |
| C.V | **13.731** | 11.693 | 14.002 | 2.985 | 15.262 | 18.889 | 17.221 | 8.531 | 14.002 | 9.926 |

Soil and Water samples for the cultivation of water iris should be maintained as follows: Soil pH value at 5.8; E.C. at 0.048; organic carbon 3.76%; available N, P and K should be maintained as 615, 28 and 920 kg/ha respectively. Some micronutrients *viz*., Cu, Fe, Zn and Mn required from 0.4, 80.4, 2.5 and 40.50 mg/kg respectively. Water pH value should be maintained at 7.26; E.C. at 0.48; P and K should be maintained from 0.014 and 14.7 ppm respectively. Some micronutrients like, Cu, Fe, Zn and Mn content in water samples were found to be in trace amounts. In some places where water iris could not grow were low water level, eutrophic condition, alkaline pH of water and soil; and low nutrients (Figures 3 and 4).




| | pH | E.C. | Organic C (%) | Available N (Kg/ha) | Available P (Kg/ha) | Available K (Kg/ha) | Cu (mg/kg) | Fe (mg/kg) | Zn (mg/kg) | Mn(mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Series 1 | 5.8 | 0.048 | 3.76 | 615 | 28 | 920 | 0.4 | 80.4 | 2.5 | 40.5 |

Figure 3. Soil analysis of the Experimental Fields of *Iris laevigata* Fisch. Lamphelpat




| | pH | E.C. | P (ppm) | K (ppm) |
|---|---|---|---|---|
| Mean | 7.26 | 0.48 | 0.01 | 13.3 |
| 3 | 7.25 | 0.47 | 0.01 | 12.5 |
| 2 | 7.36 | 0.48 | 0.014 | 14.7 |
| 1 | 7.26 | 0.49 | 0.008 | 11.8 |

Figure 4. Water analysis of the Experimental Fields of *Iris laevigata* Fisch., Lamphelpat

As water iris is an aquatic plant with almost all the aerial half is above water in amphibious manner, maintenance of water level is mandatory for seedling growth. Submerged and flooding of water levels with eutrophic conditions are badly affected to the plant. So, any marshy and waterlogging areas are not suitable for Iris cultivation. Lacoul and Freedman (2006) supported this view and opined that "rapid seedling growth is required to quickly escape the stressful environment of submergence and low nutrient seedlings". Lee et al. (2018) reported the effect of water levels and soil nutrients on the growth of endangered *Iris laevigata* seedlings in Korea. A rise in soil nutrients increased overall seedling growth and increased tiller numbers via the promotion of asexual reproduction. The water level must be lower than the seedling height for effective growth and management of *I. laevigata*.

Environmental factors of Manipur State favours for the natural growth of water iris. The plant is a perennial plant with rhizomatous stalk keep drying as a dormant phase during winter (Dec.-Feb.), when monsoon (March-May) comes during the end of February month starts sprouting and has reached the mature stage and flowering begins during the first week of April and continues till May end. As there were fluctuations of meteorological data during the three crop seasons (2018 to 2020) of water iris maximum rainfall reach maximum during the month of July, however, in the year 2019 rainfall was meagre and attain maximum in October. Maximum and minimum temperatures also fluctuate every year. If so fluctuations could be seen during three crop seasons. Even though, the normal temperatures did not cross an average of 32 $^{o}$C every year, the plant maintains its growth (Figure 2).

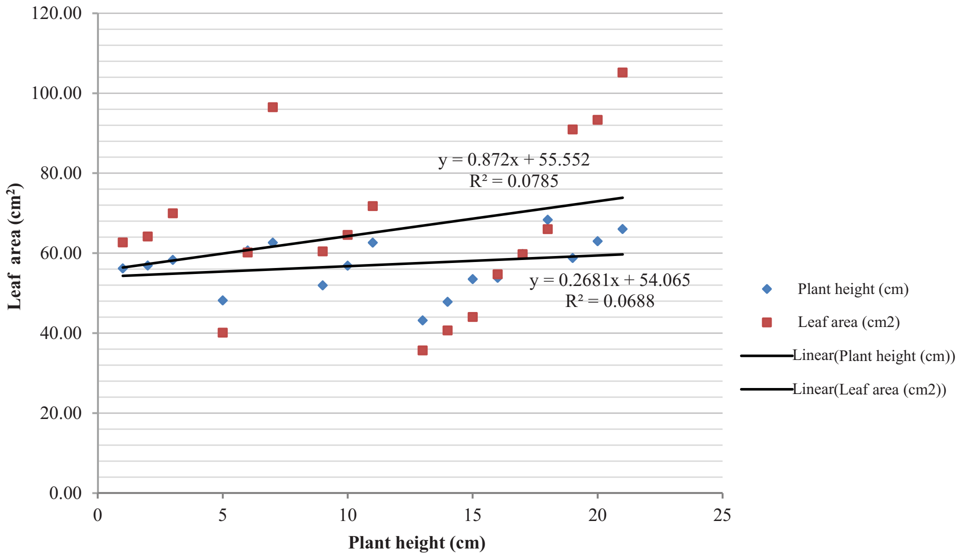



Figure 5. Correlation between leaf surface area and plant height on the growth and development of *Iris laevigata* Fisch.

Strong positive correlation between leaf surface area and plant height on the growth and development was found to be effective among other correlations (Figure 5). This work is in conformity with Wang et al. (2017), he reported that leaves of reproductive ramets stopped growing around the time that flowering began (in late May), while the leaves of non-reproductive ramets would continue growing until late August, dramatically increasing the leaf area available for photosynthesis.

## 4. Conclusion

From the above mentioned facts, scientific method of cultivation as an objective of conservation measures was done at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal. QPMs (Quality Planting Materials) were adopted in the present research program. Plant Growth Analysis and good harvesting techniques were also adopted to conserve the plant and the findings should be reached to the farmers. Water iris should be planted at a spacing of 45 cm during the month of July, trimming at 40 DAT (days after transplantation), flowering during March to May and harvesting of rhizomes followed.

## References

Casanova, M. T., & Brock, M. A. (2000). How do depth, duration, and frequency of flooding influence the establishment of wetland plant communities? *Plant Ecology, 147*(2), 237-250. https://doi.org/10.1023/A:1009875226637

Deb, D. B. (1961a). Dicotyledonous plants of Manipur territory. *Bulletin of Botanical Survey of India, 3*.

Deb, D. B. (1961b). Monocotyledonous plants of Manipur Territory. *Bulletin of Botanical Survey of India, 3*.

Devi, K. Y., Singh, S. S., Devi, M. H., Dhabe, A. S., & Singh, P. K. (2021). Ethno-medicinal Plants from Bishnupur District, Manipur, north eastern India. *Bioinfolet, 17*(4a), 602-608.

Devi, T. S., & Das, A. K. (2016). Occurrence of some Rare and Threatened vascular plants in Senapati district, Manipur, Northeast India. *NeBio, 7*(3), 111-114.

Fraser, L. H., & Karnezis, J. P. (2005). A comparative assessment of seedling survival and biomass accumulation for fourteen wetland plant species grown under minor water-depth differences. *Wetlands, 25*(3), 520-530. https://doi.org/10.1672/0277-5212(2005)025[0520:ACAOSS]2.0.CO;2

Kostrakiewicz, K. (2000). The analysis of special structure of *Iris sibirica* (Iridaceae) population on the station in Stanislawice near Bochniaouthern Poland. *Fragm. Flor. Geobot. Polonica, 7*, 209-214.

Kwon, G. J., Lee, B. A., Nam, J. M., & Kim, J. G. (2007). The relationship of vegetation to environmental factors in Wangsuk stream and Gwarim reservoir in Korea: II. Soil environments. *Ecological Research, 22*(1), 75-86. https://doi.org/10.1007/s11284-006-0188-4

Lacoul, P., & Freedman, B. (2006). Environmental influences on aquatic plants in freshwater ecosystems. *Environmental Reviews, 14*(2), 89-136. https://doi.org/10.1139/a06-001

Lee, E. H., Lee, B. E., & Kim, J. G. (2018). Effect of water levels and soil nutrients on the growth of Iris laevigata seedlings. *Journal of Ecology and Environment, 42*(5), 1-7. https://doi.org/10.1186/s41610-018-0065-4

Mahoney, J. M., & Rood, S. B. (1998). Streamflow requirements for cottonwood seedling recruitment—An integrative model. *Wetlands, 18*(4), 634-645. https://doi.org/10.1007/BF03161678

Nicol, J. M., & Ganf, G. G. (2000). Water regimes, seedling recruitment and establishment in three wetland plant species. *Marine and Freshwater Research, 51*(4), 305-309. https://doi.org/10.1071/MF99147

Rodionenko, G. I. (1987). *The genus Iris L.: Questions of morphology, biology, evolution and systematics*. London: British Iris Society.

Singh, H. D., Ningombam, D., Singh, P. K., & Borah, L. (2017). Status of Threatened Ethno-medicinal Plants Found in Sub-alpine areas of Ukhrul and Senapati Districts of Manipur. *International Journal of Applied Research, 3*(6), 544-548.

Singh, S. S., Devi, M. H., Dhabe, A. S., & Singh, P. K. (2020). Status of Threatened Ethno-medicinal Plants from Imphal Valley (Manipur) India. *Bioinfolet, 17*(3a), 386-394.

Sinha, S. C. (1996). *Medicinal plants of Manipur*. Manipur Association for Science & Society (MASS), Manasi Press, Calcutta.

Sobhapati, S. (2017). *The Assam Tribune* (26-04-2017). Guwahati, India.

Wang, L., Wang, H., He, C., Sheng, L., & Tang, Z. (2017). An irreversible division of labour through a sexually dependent system in the clonal plant *Iris laevigata* (Iridaceae). *Ecosphere, 8*(3), 1-11. https://doi.org/10.1002/ecs2.1757

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# Group Cohesion for Enhanced Operation of Agricultural Community-Based Projects in Gauteng Province, South Africa

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**Abstract**

Collective operation of smallholder farmers and cooperatives has been attributed to many mishaps and malfunctions. Such knowledge creates misperceptions regarding agricultural cooperatives and their usefulness in development. This study investigated member commitment, group cohesion and membership retention in agricultural production cooperatives. The main aim was to identify possible practical measures for enhanced performance and increased sustainability in farmer organizations. Data was collected from 92 participants that were currently operating as cooperative members. A combination of descriptive statistics, Perceived Cohesion (PC) and Binary Logistic Regression methods were employed for analysis. Results of the study indicate that group cohesion is influenced by trust among members, internal communication, financial performance of the cooperative, involvement of members in decision making, and role of the organization in the community. Strategies for increased group cohesion that were recommended in the study include information sharing and transparency at all levels of operation, and collective decision making and planning in organizations.

**Keywords:** cooperative organizations, smallholder farmers, commercialization, cohesion

## 1. Introduction

Commercialization of smallholder and emerging farmers is receiving increasing support from the South African government, especially through policy reforms and public investment. Commercialization is advocated because it plays a vital role in minimizing poverty and food insecurity, and in enhancing food production and income generation, which boost the overall development of the agricultural sector (DAFF, 2010; Khapayi & Celliers, 2016). In a rural economy, increased agricultural commercialization contributes substantially to labour absorption. Thus, several jobs are created from direct employment on the farms, as well as from other linked services created through multiplier effect in the input and out markets (Wiggins et al., 2011).

As much as commercialization is advocated, it has proven impractical for individual smallholder farmers in certain instances, due to an array of challenges that are faced by the farmers in both production and marketing (Khapayi & Celliers, 2016). For instance, the majority of smallholder farmers lack capital, infrastructure, mechanization, adequate labour and knowledge, collateral, bargaining power and access to lucrative markets. In some situations, they fail to access production land as individuals. In cases where commercialization of individual farmers is impractical, farmers are often encouraged to operate collectively in cooperative organizations, which are also known as Community-Based Projects (CBPs). Collective farming is generally supposed to benefit members (farmers engaging in collective farming) through shared responsibilities, increased access to resources, enhanced economies of scale, risk reduction, establishment of countervailing power, auxiliary services, and improved positioning in mainstream agriculture (Ito et al., 2012; Yang et al., 2013). On overall, effective collective farming provides a governance structure with implicit cost-savings and risk-sharing devices (Francesconi & Heerink, 2011).

It is indisputable that agricultural production cooperatives (hereafter CBPs) have far-reaching benefits, however, they are also known for their high failure risk (Garnevska et al., 2011). There is enough evidence in literature showcasing the inability of several CBPs to meet the intended objective of commercialization. CBPs often face challenges of high governance costs (resulting from collective ownership and decision-making), power struggles, opportunistic behaviour and pursuance of personal agenda by few influential members (Paulus, 2012; Bernard et al., 2013; Nkonki-Mandleni & Anim, 2014). Nonetheless, some CBPs successfully operate and manage to bring tangible benefits to their members. This suggests that there exist certain elements in each CBP which either permit or restrict the functioning and performance of an organization. According to Paulus (2012), when members are committed, they willingly work together to complete tasks and achieve group goals. In addition, when members value their group membership, they actively seek to remain part of the group by adhering to the objectives of the group. Based on this background, the current research investigated the factors that influence member commitment to their CBP and their willingness to remain part of the organization. The research is intended to identify possible practical measures for enhancing performance and sustainable operation of farmer organizations.

## 2. Conceptual Framework

### *2.1 Cooperative Relations in Agricultural Production*

A cooperative is defined by the International Cooperative Alliance as "an autonomous association of persons united voluntarily to meet their common economic, social, and cultural needs and aspirations through a jointly-owned and democratically-controlled enterprise" (ICA, 1995). Cooperatives have a long history in many parts of the world, including Africa. However, they have recently received increased attention in Africa as a tool for alleviating poverty and socio-economic development (Wanyama et al., 2009; Develtere et al., 2008). There are different types of cooperatives in different economic sectors; depending on the activities that they are engaged. Agricultural cooperatives can be categorised into production and marketing cooperatives; can either be legally registered or not; and can take any size and scope (FAO, 2012). For the purposes of this publication, the word cooperative refers to agricultural producer organizations. These organizations encompass joint production and income-generating activities, risk-sharing and profit-sharing.

In principle, agricultural producer organizations are established to help farmers reap greater benefits through increased yields and incomes; by pooling their resources together. As they operate jointly as an organization, their bargaining power is enhanced, as well as their access to market information, agricultural resources and external support services (Getnet & Anullo, 2012; FAO, 2012). These are all necessary conditions that allow for commercialization of smallholder farmers (Francesconi & Heerink, 2011). Whilst cooperatives are often formed with good intentions, several challenges can emerge which cause ineffectiveness, and eventually lead to the dissolution of the cooperative. These challenges include structural and managerial problems, dependence syndrome, opportunistic behaviour, limited participation and commitment of members, limited external support, limited capacity to perform effectively, unfulfilled expectations and conflicting agendas (Bernard et al., 2013; Getnet & Anullo, 2012).

Several authors indicated that the effectiveness, sustainability and success of any cooperative are highly dependent on the commitment of its members and their ability to patronize the cooperative. If members are not committed to their cooperative, they often lack the motivation to perform optimally towards the activities of that cooperative (Jussila et al., 2012; Paulus, 2012; Bijman & Verhees, 2011). Paulus (2012) decomposes member commitment into loyalty, identity and participation.

### *2.2 Group Cohesion*

Group cohesion refers to a sense of attraction or a bond that pulls people towards membership in a certain group and a feeling of morale associated with their membership in that group. It further measures the strength of members' desire to remain in a group (group pride), their sense of belonging and their commitment to it. Members who lack a sense of belonging to the group would not desire to continue associating with their cohorts (Forsyth, 2006; Paulus, 2012). One of the presumptions on the subject of group cohesion is that it influences group task performance. Thus, teams that have a strong bond and committed members, who take pride in their group, are often motivated to achieve organizational goals and objectives (Paulus, 2012; Bijman & Verhees, 2011). According to Hansen et al. (2002), group cohesion occurs when members have a positive feeling towards each other and the group at large. That way, they can easily relate to each other and work towards set targets. The ability of a group to be more or less cohesive is dependent on several factors, such as past group experiences, stage of the group, size of the group, time spent together, the level of trust among members and similarities of members (Gikunda & Lawver, 2019; Evans & Dion, 2012; Paulus, 2012). In principle, group cohesiveness can be easily developed when group members have similar values, aspirations and beliefs, and when they feel that they can trust each other. In addition, group sizes need to be monitored, in order to maintain a high level of cohesion (Gikunda & Lawver, 2019).

## 3. Methods

The study employed a survey methodology to collect primary data, where a semi-structured questionnaire was utilized for data collection, through face-to-face interviews. To collect relevant data, nine community-based projects (CBPs) were selected randomly from the list of projects obtained from the Gauteng Department of Agriculture and Rural Development (GDARD), and all members belonging to the 9 CBPs were interviewed, giving a total of 92 respondents. While carrying out interviews, research ethics were followed, where respondents were assured of confidentiality of the information they provided. Table 1 provides the details of 9 CBPs in the study. As part of confidentiality agreement, community-based projects were referred to as CBP 1, CBP 2 up to CBP 9.

Table 1. Details of community-based projects in the study

| CBP Name* | Municipality* | Year established | Land size (ha) | Number of respondents |
|---|---|---|---|---|
| **1** | Sedibeng DM | 2007 | 0.75 | 4 |
| **2** | City of Ekurhuleni MM | 2008 | 2.8 | 13 |
| **3** | City of Tshwane MM | 2000 | 7.0 | 7 |
| **4** | West Rand DM | 2014 | 2.5 | 5 |
| **5** | City of Johannesburg MM | 2012 | 0.4 | 5 |
| **6** | City of Tshwane MM | 2009 | 2.5 | 11 |
| **7** | City of Ekurhuleni MM | 2005 | 3.2 | 16 |
| **8** | City of Tshwane MM | 2014 | 4.0 | 22 |
| **9** | West Rand DM | 2011 | 2.0 | 9 |
| **Total** | | | | **92** |

*Note*. * There are abbreviations under these columns (CPB: Community-Based Project; DM: District Municipality; MM: Metropolitan Municipality).

Source: Field survey, 2019.

Data obtained from the survey was analysed using both qualitative and quantitative analysis tools. Qualitative data was analysed through interpretation and conceptual generalization and a mix of quantitative methods were employed to analyse quantitative data. These include descriptive statistics, Perceived Cohesion (PC) and Binary Logistic Regression analysis. Perceived Cohesion and Binary Logistic Regression analysis addressed in detail the focal point of the study. They were both used for analysing member commitment, willingness to belong to an organization and group cohesion in CBPs in the study. To analyse the data, Statistical Package for Social Science (SPSS version 24) software was used.

### *3.1 Perceived Cohesion*

The analysis of Perceived Cohesion originated with Bollen and Hoyle (1990). It measures an individual's sense of belonging to a particular group and his or her feelings of morale associated with membership in the group. It is understood that a sum of group members' individual perceptions characterise the cohesion of the entire group because the individual's perceptions inform their behaviour as well as that of the entire group. Bollen and Hoyle (1990) identified the importance of perceived cohesion at individual and group levels. At individual level, perceived cohesion reflects the role of the group in the lives of group members; and at group level, it reflects the role of individuals in the life of the group.

In order to measure perceived cohesion, Bollen and Hoyle (1990) developed a set of analysis measures known as Perceived Cohesion Scale (PCS). This is a six-item measure reflecting the two underlying dimensions of cohesion; a sense of belonging and feelings of morale. The perceived cohesion measure is attached on Appendix A. The contents of the PCS were captured in the questionnaire that was developed for this study. Responses from the PSC were rated using a five point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The individual responses were then used to construct mean scores of each group and draw conclusions on group cohesiveness.

### *3.2 Binary Logistic Regression Model*

A Binary Logistic Regression Model (BLRM) was employed to investigate the factors that influence group cohesion in CBPs. The model was used to predict the likelihood of developing strong inseparable bonds in community-based projects. A binary logistic regression model is used to predict the probability of occurrence of a certain event (dependent variable), based on continuous and/or categorical independent variables, where the dependent variable is dichotomous. The use of logistic regression model is valuable when dependent variables are qualitative in nature (Hilbe, 2015). In BLRM, a single outcome variable $Y_i$ follows a Bernoulli probability function that takes the value of 1 with probability $P_i$ and 0 with probability $1 - P_i$. The value of $P_i$ varies over the observations as an inverse logistic function of a vector $X_i$. In the equation, $P_i/(1 - P_i)$ refers to the odds of an event occurring (Hilbe, 2015).

A typical logistic regression model is of the form:

$$\text{Logit}\,(P_i) = \ln\left(\frac{P_i}{1 - P_i}\right) = \beta_0 + \beta_1 X_1 + \ldots + \beta_n X_n + U \quad (1)$$

Where, $P_i$ = probability of presence of the characteristic of interest; $1 - P_i$ = probability of absence of the characteristic of interest; $\beta = (\beta_1, \beta_2, \ldots \beta_n)$ = coefficients; $X = (X_1, X_2, \ldots X_n)$ = explanatory (independent) variables and $U$ = error term.

In the current study the dependent variable is represented by group cohesiveness, where the existence of strong group cohesion takes the value of 1, and the absence of strong group cohesion takes the value of 0. The dependent variable in the study captures the elements of group cohesiveness, *i.e.*, a feeling of morale, a sense of belonging and commitment to the group. The independent variables in the logistic regression were chosen based on wide literature reviewed on the subject of group cohesion. Seventeen variables were chosen and incorporated in the logistic regression model as summarised in Table 2, including their description. The variables were categorized into demographic, economic, organizational, psychological and social factors. Statistical Package for Social Sciences (SPSS version 24) was used to run the Binary logistic regression analysis.

Table 2. Description of independent variables used in the model

| Category | Variable | Description |
|---|---|---|
| **Demographic** | Gender | Gender of the member (dummy; female = 0; male = 1) |
| | Education | Level of education of the member |
| | Age | Age of the member (years) |
| **Economic** | Financial performance | Direct monetary benefits from the CBP |
| | Value addition practice | Involvement of the CBP in value adding practices |
| | Accessibility of CBP produce to members | The ability to access produce from CBP at an advantage |
| **Organizational** | Communication within the CBP | Competence in information dissemination within the CBP |
| | Leaders' capability | Competence and reliability of CBP leadership |
| | Involvement in decision making | Members' involvement in decision making in the CBP |
| | Training | Access to training through the CBP |
| | External influence | Level of external influence in CBP activities |
| **Psychological** | Trust | General level of trust among group members |
| | Group size | Total number of members |
| | Group stage | Number of years in operation |
| | Group composition | The level of homogeneity among members regarding their interests and goals |
| **Social** | Member social networking | Friendships among members of the same CBP |
| | Role in the community | Contribution of the CBP towards the betterment of the community |

## 4. Results and Discussion

Based on the data gathered from the survey, the nine CBPs in the study are highly diverse in terms of the number of group members, the structure of the CBP, years in operation, size of land, the choice of crops under production, involvement in marketing and the choice of markets, amongst others. Some of these differences in the CBPs have influenced the operations and the functionality of the CBPs as discussed in detail under descriptive and binary logistic regression results. Although the nine CBPs differ widely, they share a common characteristic that they are all located in urban or peri-urban areas. These areas are characterised by land scarcity, especially for agricultural purposes. In fact, the sites of five out of nine CBPs were former dumping sites that were converted to agricultural production sites. It is noteworthy that all CBPs in the study were allowed access to agricultural land because they were operating in a group.

In six out of nine CBPs, the projects became operational due to the existence of a visionary leader in the group. In these cases the visionary leader was actively involved in recruiting other group members. When all CBPs commenced, all members received a stipend from the government departments, for at least the first two years of operation, to allow them to stabilize. Thereafter, the projects were expected to generate enough money to fend for the members. As expected, the withdrawal of the stipend resulted in the withdrawal of some members from the CBPs. However, the rate at which group members withdraw from the group differs from one CBP to the other.

*4.1 Descriptive Results*

Descriptive results of the study include the results on demographic characteristics of members in community-based projects. According to Francesconi and Heerink (2011), there is normally a relationship between demographic characteristics of the members and their commitment to the cooperative. For instance, Trechter et al. (2002) posited that the members' commitment level declines as the level of formal education of a member increases. Hakelius (1999) established that young members often commit to their cooperatives for economic benefits, while older members view cooperatives as a means to show solidarity with peers. Demographic characteristics of members also influence their participation in decision making in the cooperatives (Francesconi & Heerink, 2011).

Table 3 summarizes the respondents' gender, age, educational level, social status in the community and access to training on cooperative relations. There were slightly more male respondents (54.3%) as compared to female respondents (45.7%). These results give an impression that the participation of males and females in agricultural cooperatives was almost balanced. However, by zooming in each of the CBP, the results show that there are some CBPs that are male-dominated and some female-dominated. Actually, one of the CBPs had only young male members.

Table 3. Demographic characteristics of respondents

| **Respondent characteristic** | **Frequency** | **Percent** |
|---|---|---|
| *1. Gender* | | |
| Male | 50 | 54.3% |
| Female | 42 | 45.7% |
| *2. Age (years)* | | |
| ≤ 35 | 38 | 41.3% |
| 36-59 | 46 | 50.0% |
| ≥ 60 | 8 | 8.7% |
| *3. Educational status* | | |
| No formal education | - | - |
| Primary | 19 | 20.7% |
| Secondary | 73 | 79.3% |
| Tertiary | - | - |
| *4. Social status in the community* | | |
| Community development recognition | 4 | 4.4% |
| Religious affiliation | 7 | 7.6% |
| Political affiliation | 5 | 5.4% |
| None | 76 | 82.6% |
| *5. Access to training on cooperative relations* | | |
| Yes | 36 | 39.1% |
| No | 56 | 60.9% |

Source: Field survey, 2019.

In terms of age, Table 3 shows that there was a dominance of middle-aged (36-59 years) members and the youth (≤ 35 years). All CBPs, except one had a mixture of different age categories. All respondents had some form of education, although none had acquired tertiary education. A minute number (17.4%) of the respondents held social responsibility status from community development participation (4.4%), religious affiliation (7.6%) and political affiliation (5.4%). As for access to training on cooperative relations, the majority (60.9%) did not have access. This is identified as an area of possible improvement because the members' knowledge generally impacts their confidence level and participation (Getnet & Anullo, 2012).

Table 4 shows the mean scores of the respondents when they were asked several questions related to group cohesion aspects of belonging (B) and morale (M). A five point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree) was adopted in the study, therefore, a mean score of above 3 indicated a certain level of agreement to the statement in question. Table 4 indicates that mean scores for all the questions were above 3 for CBP 8. These results suggest that on overall, the members in this project had a significant sense of belonging and a feeling of morale associated with their membership in the group. These results are in agreement with the results on project member retention, where the project experiences a small annual average member exit value of 0.06. In context, the results point out that the members have a strong attitude towards their project and are willing to continue participating as members. Even though CBP 8 is fairly new (established in 2014) as compared to the other projects in the study, regression results do not indicate a significant relationship between group cohesiveness and group stage.

Table 4. Descriptive statistics on group cohesion and member retention

| Group cohesion & member retention indicators | Community-Based Project (CBP) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| *Perceived cohesion (Group mean scores)* | | | | | | | | | |
| 1. I feel that I belong to this group. (B-1) | 2.00 | 2.15 | 2.14 | 2.60 | 1.20 | 3.09 | 3.06 | 3.95 | 1.33 |
| 2. I am happy to be part of this group. (M-1) | 3.00 | 4.38 | 3.46 | 4.40 | 2.40 | 4.18 | 4.25 | 4.32 | 2.56 |
| 3. I see myself as part of this group. (B-2) | 2.50 | 2.92 | 1.71 | 2.80 | 1.80 | 3.73 | 3.25 | 3.68 | 1.77 |
| 4. This group is one of the best. (M-2) | 1.75 | 3.07 | 2.29 | 3.00 | 1.60 | 3.63 | 3.19 | 3.86 | 1.89 |
| 5. I feel that I am a member of this group. (B-3) | 2.25 | 3.23 | 2.71 | 3.60 | 2.20 | 3.73 | 3.63 | 4.36 | 2.11 |
| 6. I am content to be part of this group. (M-3) | 3.25 | 4.46 | 3.86 | 4.20 | 3.20 | 3.82 | 4.00 | 4.55 | 3.22 |
| *Project member retention* | | | | | | | | | |
| Annual average member exit value | 0.43 | 0.16 | 0.38 | 0.19 | 0.67 | 0.12 | 0.13 | 0.06 | 0.51 |
| Member retention category | Bad | Fair | Bad | Fair | Bad | Fair | Fair | Good | Bad |

*Note*. N = 92; B: Belonging; M: Morale.

Member retention: number of people that exit the project on yearly basis (yearly average) [> 20%-Bad; 10 to 20%-Fair; < 10%-Good].

Source: Field survey, 2019.

Four community-based projects with the highest annual average member exit values [CBP 5(0.67); CBP 9(0.51); CBP 1(0.43) and CBP 3(0.38)] scored lower means on most of the group cohesion questions. These results support the argument that members who lack a sense of belonging to the group would not desire to continue associating with their cohorts (Paulus, 2012). However, it is interesting that all CBPs had mean scores of above 3 for the question related to contentedness in being part of their group. These results suggest that the members (including those in CBP 5, CBP 9, CBP 1 and CBP 3) are motivated to continue operating in a group because they receive some form of satisfaction from being part of a group. The only challenge is that if members receive minimal satisfaction from their group, they are bound to consider other attractive options. As such, projects that provide little satisfaction to its members are at risk of losing a significant number of members on a regular basis (Zeuli & Bentancor, 2005). Such projects will suffer from instability because they will continuously need to adjust to the loss of members.

### *4.2 Binary Logistic Regression Results*

Binary logistic regression results presented in Table 5 illustrate the factors that influence group cohesion in CBPs. The signs on the coefficients (β) of the factors show the direction of the relationship with group cohesion and the β-value shows the magnitude of influence. In the table, the goodness-of-fit test determines the level of acceptance of the model. A p-value for the goodness-of-fit test ($p = 0.084$) is higher than the 1% and 5% significance levels, which implies that the model is acceptable and the observed data is not statistically different from the expected values (Hilbe, 2015). The availability of many variables (17) in the model prompted the need to perform a correlation analysis before running the model, in order to eliminate the problem of multi-collinearity.

The correlation analysis results indicate that there was not any significant relationship between the independent variables.

Five factors, which are financial performance, communication within the CBP, involvement in decision making, trust and role in the community, were significantly related to group cohesion in the study at either 1% or 5% significance level. All significant factors had a positive relationship with group cohesion, indicating that an improvement in these factors result in an increase in group cohesiveness. Among the significant factors, 'trust' had the highest β-value of 0.642 and is highly significant at 1% level, highlighting its great influence on group cohesion. These results substantiate results from Barraud et al., (2012) which recognized trust as a key indicator of group cohesion in cooperatives. Further explanations reveal that group members' desire to remain in a cooperative and their commitment to the group is highly dependent on trust. When group members develop trust amongst themselves and collectively in the cooperative, they actively participate in the cooperative's different affairs in favour of its success (Barraud et al., 2012).

Table 5. Regression results of factors influencing group cohesion in CBP

| Independent variable | Unstandardized Coefficients | | Sig. (*p*) |
|---|---|---|---|
| | Coef. (β) | Std. Error | |
| (Constant) | .129 | .098 | .042 |
| *1. Demographic factors* | | | |
| Gender | .037 | .078 | .163 |
| Education | -.122 | .038 | .124 |
| Age | .088 | .071 | .398 |
| *2. Economic factors* | | | |
| Financial performance | .235 | .068 | .001*** |
| Value addition practice | .119 | .072 | .237 |
| Accessibility of CBP produce to members | .134 | .009 | .195 |
| *3. Organizational factors* | | | |
| Communication within the CBP | .174 | .011 | .048** |
| Leaders' capability | .289 | .045 | .276 |
| Involvement in decision making | .188 | .112 | .033** |
| Cooperative training | .311 | .013 | .192 |
| External influence | -.023 | .065 | .215 |
| *4. Psychological* | | | |
| Trust | .642 | .087 | .000*** |
| Group size | .136 | .087 | .109 |
| Group stage | .245 | .106 | .127 |
| Group composition | .167 | .038 | .321 |
| *5. Social factors* | | | |
| Member social networking | .198 | .091 | .283 |
| Role in the community | .203 | .036 | .042** |
| $R^2$ | .844 | | |
| Adjusted $R^2$ | .816 | | |
| **Goodness-of-fit test** | **Chi-square** | **df** | **Sig.(p)** |
| Pearson | 187.354 | 91 | .084 |
| Deviance | 86.109 | 91 | .812 |

*Note*. *N* = 92; ***, ** indicate significance at 1% and 5% level respectively.

Source: Field survey, 2019.

The variable 'financial performance' of the cooperative was highly significant (p = 0.001), which portrays the importance of financial gains in cooperative relations. These results support assertions from Österberg and Nilsson (2009) that cooperatives' good financial performance brings members commitment and satisfaction. In general, people engage in cooperative relations for financial gains. If they fail to see any financial benefits in cooperatives, they will not have an incentive to remain participating. Therefore, collective relations need to be entwined with financial incentives for the members to stay with their organization (Österberg & Nilsson, 2009; Zeuli & Bentancor, 2005). These results explain why a number of CBPs in the study lost members after government departments withdrew stipends; guaranteed financial gains were removed and members were not sure of the cooperatives' financial future.

'Role in the community' was statistically significant at the 5% level and had a β-value of 0.203. This variable represents the role that the cooperative takes in the community, particularly in improving the lives of community members. The results indicate that the higher the cooperative's activities are recognised in the community, the more the cooperative members are commitment to their cooperative. This variable is closely related to people's esteem—generally, humans seek to be associated with winning teams. Thus, a cooperative that performs distinguishable work in the community attracts many members (Tuna & Karantininis, 2017).

One of the organizational factors that was statistically significant at the 5% level is 'involvement in decision making.' The positive relationship between this variable and group cohesion denotes that members easily form strong ties in organizations where they are involved in making organizational decisions. When members perceive that their ideas are valuable in an organization, they become committed. According to Borgen (2001), it is easy for members to participate actively towards implementing decisions that they contributed. Involvement of members in decision making helps in developing a sense of member inclusiveness and acceptance in the cooperative, which ultimately strengthen their emotional attachment and commitment to the cooperative. Dakurah et al. (2005) emphasise the importance of members' involvement in decision making towards long term survival and growth of an organization.

'Communication within the CBP' is another organizational factor that was statistically significant at the 5% level and had a β-value of 0.174. Effective communication within a cooperative has always been advocated for increased participation among members (Taruvinga et al., 2017; Österberg & Nilsson, 2009). Effective communication embraces the channels, timeliness, language and quality of information, and the ability to raise concerns and receive feedback. An effective communication system is vital for disseminating information, coordinating tasks and keeping members informed of the direction of their cooperative. It also helps in creating social ties among cooperative members. The existence of an effective communication system influences the commitment of members and the success of an organization (Österberg & Nilsson, 2009).

## 5. Conclusion

The study investigated the factors that influence member commitment to their Community-based projects and their willingness to remain part of the organization, by utilizing a survey approach. Evidence from the study shows that there are several factors of consideration that guide people to continue participating or exit a cooperative. Five variables namely: financial performance, communication within the CBP, involvement in decision making, trust and role in the community, were statistically significant at either 1% or 5% level. The variables represented economic, social, psychological and organizational factors. Out of all the significant variables, 'trust' was identified as the most important indicator in influencing group cohesion. The explanations provided indicated that trust instils a sense of belonging and commitment among members to perform economic activities. Members that are driven by trust are willing to nature the functionality of the cooperative organization, and motivated to remain part of the organization for longer periods. Financial performance of a cooperative was also identified as an important indicator because people mostly engage in cooperative relations for financial gains. This is supported by tendency of people to exit an organization upon exposure to the availability of other options offering better financial gains. Communication within the CBP, involvement in decision-making and role in the community influence group cohesion and member commitment through creating a sense of recognition and importance.

Based on the results of the study, there are several recommendations that can be made in order to enhance group cohesion in agricultural cooperatives. Standards need to be set at cooperative formation stage. There is need for transparency and information sharing, from the commencement of the cooperative. In addition, members should agree on a clear code of conduct, communication channels and members' responsibilities. That way, members will have a clear vision of the cooperative and their roles and responsibilities in the organization. Moreover, transparency is essential in creating trust among members. The responsibility of planning, budgeting and evaluating performance of the cooperative should be done collectively, with the consultation of all members. This will assist towards setting measurable and practical goals, and in building knowledge among members regarding financial opportunities from the cooperative. The knowledge on internal financial opportunities will shift their focus from the external financial support (which is not sustainable) towards suggestions that improve internal finances.

**References**

Barraud, D. V., Henninger, M. C., & El Akremi, A. (2012). The relationship between members' trust and participation in the governance of cooperatives: The role of organizational commitment. *International Food and Agribusiness Management Review, 15*(1), 1-24.

Bernard, T., Abate, G. T., & Lemma, S. (2013). *Agricultural cooperatives in Ethiopia: Results of the 2012 ATA Baseline Survey*. The International Food Policy Research Institute (IFPRI). Washington, DC.

Bijman, J., & Verhees, F. (2011). Member or customer? Farmer commitment to supply cooperatives. International Conference on the Economics and Management of Networks.

Bollen, K. A., & Hoyle, R. H. (1990). Perceived Cohesion: A conceptual and empirical examination. *Social Forces, 69*(2), 497-504. https://doi.org/10.2307/2579670

Borgen, S. (2001). Identification as a Trust Generating Mechanism in Cooperatives. *Annals of Public and Cooperative Economics, 72*(2), 209-228. https://doi.org/10.1111/1467-8292.00165

DAFF (Department of Agriculture Forestry and Fisheries). (2010). *The Strategic Plan for South African Agriculture*. Department of Agriculture, Directorate of Agricultural Information Services, Pretoria.

Dakurah, H. A., Goddard, E., & Osuteye, N. (2005). *Attitudes towards and satisfaction with cooperatives in Alberta: A survey analysis* (pp. 24-27). American Agricultural Economics Association Annual Meeting, Providence, Rhode Island.

Develtere, P., Pollet, I., & Wanyama, F. (2008). *Cooperating out of poverty: The renaissance of the African cooperative movement*. ILO.

Evans, C. R., & Dion, K. L. (2012). Group cohesion and performance a meta-analysis. *Small Group Research, 43*(6), 690-701. https://doi.org/10.1177/1046496412468074

FAO (Food and Agriculture Organization of the United Nations). (2012). *Agricultural cooperatives key to feeding the world*. Retrieved on January 20, 2021, from http://www.fao.org/fileadmin/templates/getinvolved/images/WFD2012_leaflet_en_low.pdf

Forsyth, D. R. (2006). *Group Dynamics* (4th ed.). Thomson/Wadsworth, Belmont.

Francesconi, G. N., & Heerink. N. (2011). Ethiopian agricultural cooperatives in an era of global commodity exchange: Does organisational form matter? *Journal of African Economies, 20*(1), 153-177. https://doi.org/10.1093/jae/ejq036

Garnevska, E., Liub, G., & Shadbolt, N. M. (2011). Factors for Successful Development of Farmer cooperatives in Northwest. *International Food and Agribusiness Management Review, 14*(4), 69-84.

Getnet, K., & Anullo, T. (2012). Agricultural cooperatives and rural livelihoods: Evidence from Ethiopia. *Annals of Public and Cooperative Economics, 83*(2), 181-198. https://doi.org/10.1111/j.1467-8292.2012.00460.x

Gikunda, R. M., & Lawver, D. E. (2019). Group cohesion and application of best horticultural farming practices among farmer groups in Meru County. Kenya. *Journal of international Agricultural and Extension Education, 26*(1), 60-72. https://doi.org/10.5191/jiaee.2019.26106

Hakelius, K. (1999). Farmer cooperatives in the 21st century: young and old farmers in Sweden. *Journal of Rural Cooperation, 27*(1). 31-54.

Hansen, M. H., Morrow Jr., J. L., & Batista, J. C. (2002). The impact of trust on cooperative membership retention, performance, and satisfaction: An exploratory study. *The International Food and Agribusiness Management Review, 5*(1), 41-59. https://doi.org/10.1016/S1096-7508(02)00069-1

Hilbe, J. M. (2015). *Practical guide to logistic regression*. Chapman and Hall, Boca Raton. https://doi.org/10.1201/b18678

ICA (International Cooperative Alliance). (1995). Statement on the Cooperative Identity. *Review of International Cooperation, 88*(3), 12.

Ito, J., Bao, Z., & Su, Q. (2012). Distributional effects of agricultural cooperatives in China: exclusion of smallholders and potential gains on participation. *Food Policy, 37*, 700-709. https://doi.org/10.1016/j.foodpol.2012.07.009

Jussila, I., Goel, S., & Tuominen, H. (2012). Member commitment in co-operatives: The utilitarian approach. *Business and Management Research, 1*(3), 9-16. https://doi.org/10.5430/bmr.v1n3p9

Khapayi, M., & Celliers, P. R. (2016). Factors limiting and preventing emerging farmers to progress to commercial agricultural farming in the King Williams Town area of the Eastern Cape Province, South Africa. *South African Journal of Agricultural Extension, 44*(1), 25-42. https://doi.org/10.17159/2413-3221/2016/v44n1a374

Nkonki-Mandleni, B., & Anim, F. D. K. (2014). Determining factors of support for co-operatives in South Africa. *Journal of Human Ecology, 47*(2), 171-174. https://doi.org/10.1080/09709274.2014.11906750

Österberg, P., & Nilsson, J. (2009). Members' perception of their participation in the governance of cooperatives: The key to trust and commitment in agricultural cooperatives. *Agribusiness, 25*(2), 181-197. https://doi.org/10.1002/agr.20200

Paulus, W. (2012). *Strengthening member commitment in agricultural cooperatives: An overview on how Dutch agricultural cooperatives seek to strengthen the commitment of their members*. Wageningen, The Netherlands: Wageningen University, Management Studies.

Taruvinga, B., Ndou, P., Hlerema, I. N., Maraganedzha, T. L., du Plooy, C. P., & Venter, S. (2017). Fostering linking social capital for successful agricultural development projects in South Africa. *Agrekon, 56*(1), 28-39. https://doi.org/10.1080/03031853.2017.1283243

Trechter, D. D., King, R. P., & Walsh, L. (2002). Using communications to influence member commitment in cooperatives. *Journal of Cooperatives, 17*, 14-30.

Tuna, E., & Karantininis, K. (2017). *Cooperatives as agents of social capital: An evidence from a post-socialist country*. International Congress, August 28-September 1, 2017, European Association of Agricultural Economists, Parma, Italy.

Wanyama, F. O., Develtere, P., & Pollet, J. (2009). Reinventing the wheel? African cooperatives in a liberalized economic environment. *Annals of Public and Cooperative Economics, 80*(3), 361-392. https://doi.org/10.1111/j.1467-8292.2009.00390.x

Wiggins, S., Argwings-Kodhek, G., Leavy, J., & Poulton, C. (2011). *Small farm commercialisation in Africa: Reviewing the issues September 2011* (Research Paper 023). Retrieved July 20, 2020, from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.360.7442&rep=rep1 &type=pdf

Yang, H., Klerkx, X., & Leeuwis, C. (2013). Functions and limitations of farmer cooperatives as innovation intermediaries: Findings from China. *Agricultural Systems, 127*, 115-125. https://doi.org/10.1016/j.agsy.2014.02.005

Zeuli, K. A., & Bentancor, A. (2005). *The effects of cooperative competition on member loyalty*. 2005 Annual Meeting, November 8-9, NCERA-194 Research on Cooperatives.

**Appendix A**

| **Perceived Cohesion Measures** |
|---|
| 1. I feel that I belong to this group. (B-1) |
| 2. I am happy to be part of this group. (M-1) |
| 3. I see myself as part of this group. (B-2) |
| 4. This group is one of the best anywhere. (M-2) |
| 5. I feel that I am a member of this group. (B-3) |
| 6. I am content to be part of this group. (M-3) |

Source: Bollen and Hoyle (1990).

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# Tropical Viticulture Diagnosis in the North and Northwest Fluminense

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**Abstract**

Viticulture has proved to be an alternative for farmers in the northen and northwestern Rio de Janeiro State; however, the activity is still very recent and requires the development of agronomic and managerial techniques. Therefore, the objective of this work was to diagnose the production areas and the characteristics inherent to the inner and outer environment of this farming enterprise. It was observed that the grape-growing farms predominate in an average area of 1 hectare, with productivity between 20 and 25 t/ha, with offer in the harvest and in the off-season. The inner points are stronger than the weak ones, and can be adjusted with the joint execution of the viticulturists allied to the opportunities, such as agrotourism and the diversification of available cultivars that allow a greater offer of the product and minimize the inherent threats observed, such as climatic variations and the shortage of skilled labor. These identified points may indicate competitiveness strategies for the wine market in the studied regions.

**Keywords:** grape, productive chain, SOWT matrix, *Vitis labrusca*

## 1. Introduction

The wine sector is one of the most important for the Brazilian agricultural economy, as it provides livelihood for a number of small grape-growing farms. Although present in many States and regions, it is especially significant in Southern Brazil where a large part of the production is intended for agribusiness of juice and wine (Baldin et al., 2018). The southern region of Brazil stands out as the largest producer of grapes, for the production of wine, while in the Southeast and Northeast regions, the production of grapes for fresh consumption predominates (Mello, 2016).

Among the grape-producing states in the southeastern region, Rio de Janeiro has an area of 17 hectares (IBGE, 2018) ranking the 11th position among the grape-producing states in Brazil. Although it presents a very small production, it is observed the importance of this sector for the generation of income for small farmers in the north and northwestern Fluminense regions (Pommer et al., 2009).

The grape production in the State of Rio de Janeiro is much lower than that in areas in São Paulo State and with a smaller grape production area in the southeastern region. Nevertheless, it is likely that its grape is a factor that boosts local economies particulary because it is in expansion in the northern and northwestern Fluminense Region. It should be observed that the latter region is among the regions of Rio de Janeiro with the worst rates of economic development, with a poorly diversified agricultural sector.

The Fluminense viticulture is still an incipient activity. It has been disseminated through scientific research and by farmers who have been seeking for alternatives in this agricultural business. According to Pommer et al. (2009) the northern region of Rio de Janeiro has conditions to establish itself as a hub for table grapes in the State, with more than two harvests a year. It is possible to find information about the behavior of this crop in these regions in studies by Almeida et al. (2017), Silva et al. (2017), Deus et al. (2016), among others.

The viticulture chain is relevant due to the diversification of the local productive matrix, the added value, the generation of income and jobs as well as the increase in the Gross Domestic Product (GDP) of the municipalities involved. Because those crops are at their beginning, a diagnosis of the inner and outter points can serve as a reference regarding the development of the Fluminense viticulture and a guide to agricultural policies. In addition, management tools can be used within rural properties as a strategy to increase productivity (Parré, Bankutti, & Zanmaria, 2011).

According to Lopes et al. (2018) these tools can optimize the activity as a whole, both in the administration and in the management of the rural property, resulting in increased profitability and return. The adequate management can provide winegrowers with improvements in their socioeconomic conditions, strategic decisions and advances in the productive activity.

In the agricultural sector, SWOT analysis is used to assess the strategic position of projects. In India, the tool was used to analyze the agricultural sector in terms of capacity to meet further food security requirements (Parveen & Nain, 2013). In relation to the Fluminense viticulture, which is still in recent development, it can be a tool for the analysis of the inner and outter environment that can characterize the development of the activity.

The knowledge of the inner and outter factors linked to the development of this activity in a sustainable way is relevant to make the productive units more competitive. Thus, the objective of this work was to analyze viticulture in the North and Northwest Fluminense Regions, identifying the strengths and weaknesses, the potential and threats that can influence their development.

## 2. Method

### *2.1 Study Area*

The study area is located in the north and northwest Regions of the State of Rio de Janeiro (Figure 1). In the Northern Fluminense the following municipalities are found: Campos dos Goytacazes, Carapebus, Cardoso Moreira and São Fidelis. And the following municipalities are found in the northwest Region: Bom Jesus do Itabapoana, Cambuci, Italva, São José de Ubá and Varre Sai. These municipalities were selected taking into account the fact that they are the grape-producing municipalities in the regions, adding to the lack of economic information on production costs, the lack of data on the behavior of grape prices and analyzes of economic viability viticulture in development in these regions.

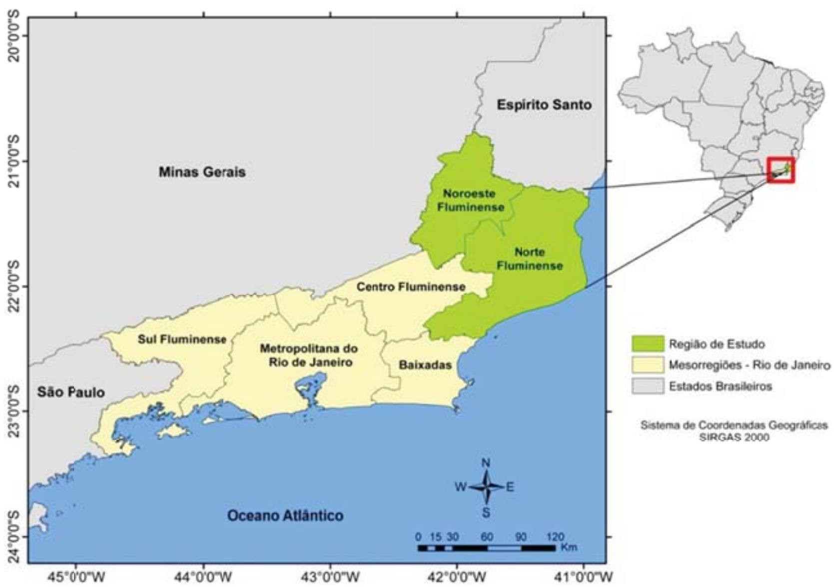


Figure 1. Characterization of grape production areas

### *2.2 Data Collection*

Data were collected by means of semi-structured interviews, through field research, from April 2017 to May 2018. The interviews were conducted directly with all winegrowers in the regions, considering the total number of 16 producers, and grape cultivation area already implemented in the regions in this period. Firstly, through these interviews, the following data were collected: location of grape production areas, size of production areas and average productivity.

### *2.3 Data Analysis*

Following data collection, an organizational assessment tool denominated SWOT Matrix was applied, in order to observe the following variables: Strengths, Weaknesses, Opportunities and Threats. This analysis was carried out on two levels: in the outter and inner environments. The inner environment examines the strengths and weaknesses that need to be monitored constantly and can be controlled. The external environment, in turn, comprises changes that occur outside the organization, but which can affect its performance, being characterized by threats and opportunities.

After analyzing the outter and inner environments, companies can decide which competitive strategies should be used to achieve their competitive objective. Competitive strategies could be used to "exploit opportunities in the company's environment with the company's strength and neutralize threats in the company's environment, avoiding the company's weaknesses". This analysis can be the starting point in the identification and implementation of the most appropriate strategy for the wine business in the areas surveyed. Thus, this work allows the identification of a strategy that can explore the strengths of viticulture in Rio de Janeiro and eliminate or minimize, at least, weaknesses and the outter threats.

As a result, this matrix allows analyzing the strengths and weaknesses as perspectives that depend only on the viticulturist and on his or her productive system and the opportunities and threats, which, in turn, do not depend on the action of the producer and on the response of his or her productive systems, but on outter factors.

The results obtained from the interviews were organized into tables with data in relative frequency, as well as the studies by Biassio and Crespo Silva (2015); Sarmento (2017); Nascimento Mendes, Dos Anjos, and Raphael (2018), and Schneider, Zilli, and Vieira (2017).

## 3. Results and Discussion

### *3.1 Characterization of the Grape Production Areas*

The diagnosis carried out in the field allowed to identify nine producing municipalities, among them, the municipality of Bom Jesus do Itabapoana as the largest in production area (7 ha) in the Northwest Region and Cardoso Moreira in the North Region (4 ha), while São José de Ubá had the largest number of producers per municipality (Table 1). Also, the producing areas were distributed in the regions. Small and medium farmers predominate in both regions, with a strong presence of family labor. Similar data for viticulture were found in other regions by Anzanello (2012) in Rio Grande do Sul and Cappello, Spósito, and Osaki (2017) in the Northwest of São Paulo State.

Table 1. Grape production units and number of winegrowers in northern and northwestern Rio de Janeiro State

| Municipalities | Area (ha) | (%) | *No. | (%) |
|---|---|---|---|---|
| Nothwest Region | 11.5 | 67.65 | 11 | 68.75 |
| Bom Jesus do Itabapoana | 7 | 41.18 | 1 | 6.25 |
| São José de Ubá | 2 | 11.76 | 4 | 25 |
| Varre Sai | 1 | 5.88 | 3 | 18.75 |
| Cambuci | 1 | 5.88 | 2 | 12.5 |
| Italva | 0.5 | 2.94 | 1 | 6.25 |
| North Region | 5.5 | 32,.35 | 5 | 31.25 |
| Cardoso Moreira | 4 | 23.53 | 1 | 6.25 |
| São Fidélis | 1 | 5.88 | 2 | 12.5 |
| Campos dos Goytacazes | 0.25 | 1.47 | 1 | 6.25 |
| Carapebus | 0.25 | 1.47 | 1 | 6.25 |
| Total | 17 | 100 | 16 | 100 |

*Note*. *No.: Number of grapegrowers/municipalities.

In 88% of the farms, the grape cultivation occupies an area of less than 1 ha. This reduced size is due to the amount that was subsidized by the Frutificar Program, and the other producers that invested on their own since they consider the activity as a novelty in the regions. Similar data were observed by Cappello et al. (2017) and Duarte (2013). It should be observed that, in order to expand the areas, more capital and greater availability of labor and guarantees for placing the product on the market are needed.

Productivity per hectare is between 20 and 25 t/ha/year, for 10 respondents (62.5%). Different productivity may be achieved depending on the variety, driving system and technological level and year of production. In Rio Grande do Sul, Anzanello (2012) found that the average yield was 9.6 t/ha, and Cappello et al. (2017) in the State of São Paulo observed 14 t/ha in the harvest, and 10 t/ha in the off-season in the region of Campinas. The comparison between the average of these leading traditional grape regions in the country showed that the local edaphoclimatic conditions, as well as, the appropriate management for cultivation has provided a very attractive productivity.

*3.2 Analysis of the SWOT (Strengths, Weaknesses, Opportunities, Threats) Matrix*

The strengths and weaknesses are related to the capacity and influence of the factors that can contribute or harm the grape production systems found in the regions. While, the opportunities were defined as aspects that are important to encourage the productive areas until the flow of the production, and the threats were listed as aspects that represent risks to the maintenance of the production systems (Table 2).

Table 2. Factors that influence the competitiveness of grape production and trade in the North and Northwest Fluminense Regions

| SWOT MATRIX | |
|---|---|
| INTERNAL ENVIRONMENT | |
| STRENGTHS: | WEAKNESSES: |
| 1. Favorable edaphoclimatic conditions | 1. Lack of producer's organization |
| 2. More than one harvest per year | 2. Little experience in viticulture |
| 3. Proximity to consumers | 3. Market shortages by producers |
| 4. Trade in the property | 4. Little administrative knowledge |
| 5. Strengthening of the family workforce | |
| 6. Agricultural diversification | |
| 7. Possibility of local trade | |
| EXTERNAL ENVIRONMENT | |
| OPPORTUNITIES: | THREATS: |
| 1. Fruiting Program | 1. High investment cost |
| 2. Agrotourism | 2. Low water availability |
| 3. Availability of variety diversity | 3. Absence of agricultural policies |
| 5. Research development | 4. Shortage of skilled labor |
| 6. Few intermediaries | 5. Few courses and field days |
| 7. Additional income source | |

3.2.1 Strengths

Among the strengths, the edaphoclimatic conditions of the regions promote the development of grape cultivation, such as solar radiation, temperature, relative humidity, precipitation and soil fertility. Associated with these conditions, management practices adapted to local conditions, such as scheduled pruning, use of irrigation and plant regulators, enable the grape producer to obtain more than one harvest throughout the year. In addition, the phenological cycle and thermal needs of the grapevine are similar to those found in the State of São Paulo.

The proximity of the farmers to the main consumer, in addition to providing a trade with other agricultural products within the properties, has strengthened viticulture in the regions. This trade on the property narrows the relationship between the producer and the consumer, and generally results in better prices for the producer, since the product does not pass through different agents in the chain up to retail. These data differ from the observations by Alves, Tonin, and Carrer (2013) in Paraná, where producers cannot classify the product and are unable to offer a large volume without the presence of an intermediary, with a trade that generally results in lower prices for the producers.

3.2.2 Weaknesses

One weaknesses in the chain is the little experience in viticulture. As it is still an incipient activity, many producers do not know the behavior of the grapevine over the year, and throughout the development of the crop they need to adapt the management combinied to the local edaphoclimatic conditions, and identify the physiological and nutritional demand of this fruit.

Technical agents also point out the lack of organization of the farmers as a major weakness in almost every rural sector, and it is not characteristic only of grape producers. In particular, it can be considered that at the stage of viticulture development, the existence of associations and cooperatives would strengthen the chain and the development of culture in the regions. Most municipalities in Rio de Janeiro have family farming with low levels of modernization, in particular, the North and Northwest regions show family farming with relatively low rates of use of the technologies, considered in the analysis carried out by De Souza, Souza, and Fereira Neto (2018). In view of this weakness, it is necessary to emphasize, the search for local organization can be an alternative for strengthening winegrowers, constituting an instrument for competitiveness. The formation of associations and cooperatives is an important tool for social, political and economic organization (Costa, Junior, Gomes, & Silva, 2015).

3.2.3 Opportunities

Agricultural policies can be arrangements of instruments that stimulate agricultural production. In this context, the Frutificar Program is an agricultural policy that developed the cultivation of grapes in the regions, since it encouraged and subsidized 62.5% of the interviewed wine growers. Costa, Tarsitano, and Conceição (2012) observed that the majority of winegrowers invested in the grape, through the National Program for Strengthening Family Agriculture (PRONAF) in the municipality of Jales (SP).

There is also a possibility of local vineyards to provide the “sale” of the tourist territory of the regions, also highlighting the sustainability of the regional grape. Agrotourism has been promoted by the consumption of grapes and by-products within the crop. Some producers report having received 30 people per month in the field in harvest months. Viticulture can even trigger a greater dissemination of tourism, not only by motivating wine tourism but also focusing on other attractions in the regions, as also reported by Sarmento (2017).

The good performance of winegrowing in different regions of the country is linked to the great variability in the genetic material used. There are more than 120 cultivars of Vitis vinifera and more than 40 cultivars of American grapes, including *Vitis labrusca* varieties (Camargo, Tonietto, & Hoffmann, 2011). The diversity of cultivars is another promising variable that favors viticulture in Rio de Janeiro, considering that the different characteristics of cultivars can aid ompetitiveness in this productive sector. In the distribution of cultivated varieties there are nine varieties, among which stands out the cultivation of ‘Niagara Rosada’, followed by cultivars, BRS Vitória, BRS Isabel Precoce, BRS Isis, Bordô, and others in smaller areas. This diversity allows a greater variety in the offer of the grape, giving the producer and consumer more options in the wine market, such as seedless grapes, and grapes that can be used for the production of wines, juices and jams, increasing the farm’s gross revenue, corroborating with Mello (2017).

The insertion of grape cultivation not only diversifies agricultural production in the regions, as observed in some properties with only an economic activity as a source of income, such as dairy farming, tomato and coffee farming but also allows to balance the income when a product has a drop in its value. This diversification is of great importance in terms of social reproduction strategy, as it guarantees yields in seasonal periods of production, minimizing the risks of having a single activity.

The expansion of viticulture to vitiviniculture mainly in northwestern Rio de Janeiro is a factor that boosts the local economies, since it is a region that has the lowest economic development rates in the State. In addition to the opportunities, the agents reported the maintenance of rural people in the region since throughout the crop, there is a considerable demand for labor, similar to what is reported by Pommer et al. (2009), being an important ameliorator of social problems in these regions.

Among the nine institutions that support local agriculture, six had the knowledge of grape production, but they only assist in commercialization through the National School Food Program (PNAE). Although, for some producers, the price paid for this program has already been advantageous, they stressed that it has suffered a reduction and delay in the payment, corroborating with Agapto et al. (2012). It seems that some farmers have the potential to participate in this process; however, these factors may discourage them.

3.2.4 Threats

In contrast, it is known that agriculture is vulnerable to the effects that the climate changes has on hydrological balances and natural resources. The interference of water shortage in the pruning and harvest seasons, observed by some producers, affected the possibility of more than one harvest per year. Regardless of being natural or anthropogenic, it is necessary to seek strategies mainly for small farmers as because of their dependende on agricultural production, will suffer the greatest impacts (Mertz et al., 2009; Deressa, Hassan, & Ringler, 2011).

The high investment value for the implementation is also a threat to the expansion of new vineyards. With the incentive of Frutificar (Agricultural development program of the State of Rio de Janeiro that aims to finance fruit-growing projects in the State), many producers had subsidies to start, the production; however, it is currently active only for maintenance. In addition to this failure in the operation of Frutificar, the lack of agricultural policies aimed at the development of viticulture in the regions is highlighted.

There is a lack of technical assistance for the management, the investment and the risk analysis in these regions. Thus, knowledge about administrative processes may provide advances in the production of the activity and added-value to the final product. There is also little skilled labor to guide them in cultivation techniques, such as pruning, phytosanitary management and harvesting, as found by Costa et al. (2012) in Jales, SP. Due to the high cost and the increasing difficulty in the availability of labor, winegrowers need to adjust themselves by intensifying the search for more productive cultivars and training systems for vines, as observed by Pedro Junior, Hernandes, and Moura (2018).

## 4. Conclusion

The edaphoclimatic conditions and the possibility of more than one harvest are points that contribute to the diversification of the productive matrix, job creation and sustainable development in the regions. The Frutificar Program and the development of studies are the outer factors that promoted the performance of viticulture in Rio de Janeiro. This demonstrates that the strengths and opportunities of this activity indicate the great potential to establish competitive advantages for the regions.

There was a lack of specialized technical assistance, management training for local producers, and water issues, which need to be adjusted for the better performance of the grapevine and the continuity of the recent development of the activity in the regions.

There was a need for strategies for the production and organization of producers, in order to better explore the strengths and opportunities, and keep production the best as possible in the face of unfavorable conditions, both in terms of climate changes and the market.

Finally, the present study can support further works on the scenario of the development of viticulture in the regions of Rio de Janeiro, and to contribute to the exploration of the points of competitiveness presented in the study.

## References

Agapto, J. P., Borsatto, R. S., Esquerdo, V. D. S., & Bergamasco, S. M. P. (2012). Avaliação do Programa de Aquisição de Alimentos (PAA) em Campina do Monte Alegre, estado de São Paulo, a partir da percepção dos agricultores. *Informações Econômicas, 42*, 13-21.

Almeida, L. V. B., Figueiredo, F. A. M. M. A., Deus, B. C., Viana, L. H., Ferraz, T. M., Martins, A. O., ... Campostrini, E. (2017). Plastic covering film can reduce midday depression photosynthesis of field-grown tropical grapevine in high photosynthetic photon flux. *Acta Horticulturae, 1*, 255-262. https://doi.org/10.17660/actahortic.2017.1157.36

Alves, A. F., Tonin, J. M., & Carrer, M. J. (2013). Assimetria de transmissão de preço na comercialização da uva fina de mesa no Paraná: 1997 a 2011. *Revista de Economia e Sociologia Rural, 51*, 479-498. https://doi.org/10.1590/S0103-20032013000300004

Anzanello, R. (2012). Caracterização da viticultura no Rio Grande do Sul por meio da análise dos dados do Cadastro Vitícola. *Pesquisa Agropecuária Gaúcha, 18*, 67-73.

Baldin, T., da Silveira, A. G., Talgatti, M., Santini, E. J., & Cardoso Marchiori, J. N. (2018). Grape rachis in composites manufacturing. *Brazilian Journal of Agricultural Sciences, 13*, 1-6. https://doi.org/10.5039/agraria.v13i1a5503

Biassio, A., & Crespo Silva, I. (2015). Análise SWOT como ferramenta para avaliação da agrobiodiversidade em sistemas tradicionais de produção nos municípios de Antonina e Morretes/PR. *Scientia Agraria, 16*, 71-76. https://doi.org/10.5380/rsa.v16i2.41051

Camargo, U. A., Tonietto, J., & Hoffmann, A. (2011). Progressos na viticultura brasileira. *Revista Brasileira de Fruticultura, SP*, 144-149. https://doi.org/10.1590/S0100-29452011000500017

Cappello, F. P., Spósito, M. B., & Osaki, M. (2017). Production costs and profitability of Niagara Rosada Table grape grown in different regions of São Paulo State. *Revista Brasileira de Fruticultura, 39*, e-774. https://doi.org/10.1590/0100-29452017774

Costa, A. B., Junior, A., Gomes, P. C., & Silva, M. G. (2015). As cooperativas de agricultura familiar e o mercado de compras governamentais em Minas Gerais. *Revista de Economia e Sociologia Rural, 53*, 109-26. https://doi.org/10.1590/1234-56781806-9479005301006

Costa, V. T., Tarsitano, M. A. A., & Conceição, M. A. F. (2012). Caracterização social e tecnológica da produção de uvas para mesa em pequenas propriedades rurais da região de Jales-SP. *Revista Brasileira de Fruticultura, 34*, 766-773. https://doi.org/10.1590/S0100-29452012000300016

De Souza, H. M., De Souza, P. M., & Ferreira Neto, J. A. (2018). Heterogeneidade da agricultura familiar: uma análise dos municípios fluminenses a partir de aspectos da modernização. *Revista de Economia e Agronegócio, 16*(2), 202-225. https://doi.org/10.25070/rea.v16i2.549

Deressa, T. T., Hassan, R. M., & Ringler, C. (2011). Perception of and adaptation to climate change by farmers in the Nile basin of Ethiopia. *Journal of Agricultural Science, 149*, 23-31. https://doi.org/10.1017/S0021859610000687

Deus, B. C. D. S. D., Figueiredo, F. A. M. M. D., Almeida, L. V. B. D., Ferraz, T. M., Martins, A. O., Rodrigues, W. P. V. L. H., ... Campostrini, E. (2016). Photosynthetic capacity of 'Niagara Rosada' grapes grown under transparent plastic covering. *Ciência Rural, 46*, 950-956. https://doi.org/10.1590/0103-8478cr20150400

Duarte, V. N. (2013). Estudo da Cadeia Produtiva do Vinho em Santa Catarina: Características e Estágio Atual. *Evidência-Ciência e Biotecnologia, 13*, 41-56. Retrieved from https://portalperiodicos.unoesc.edu.br/evidencia/article/view/2472

IBGE (Instituto Brasileiro de Geografia e Estatística). (2018). *Levantamento sistemático da Produção Agrícola*. Retrieved November 20, 2018, from http://www.sidra.ibge.gov.br/bda/tabela/listabl.asp?z=t&c=1613

Lopes, M. A., Reis, E. M. B., Demeu, F. A., Mesquita, A. A., Rocha, A. G. F., Pelegrini, D. F., ... Teixeira Junior, F. E. P. (2018). Uso de ferramentas de gestão na atividade leiteira: um estudo multicascos em Uberlândia, MG. *Revista Agropecuária Técnica, 39*, 73-86. https://doi.org/10.25066/agrotec.v39i1.34302

Mello, L. M. R. (2016). *Desempenho da vitivinicultura brasileira em 2015* (Comunicado Técnico 191, p. 5). Bento Gonçalves, Embrapa Uva e Vinho. Retrieved from https://www.embrapa.br/uva-e-vinho/busca-de-noticias/-/noticia/9952204/artigo-desempenho-da-vitivinicultura-brasileira-em-2015

Mello, L. M. R.(2017). *Panorama da produção de uvas e vinhos no Brasil* (Comunicado Técnico 207, p. 11) Bento Gonçalves, Embrapa Uva e Vinho. Retrieved from https://ainfo.cnptia.embrapa.br/digital/bitstream/item/187913/1/Comu nicado-Tecnico-207.pdf

Mertz, O., Mbow, C., Reenberg, A., & Diouf, A. (2009). Farmers' Perceptions of Climate Change and Agricultural Adaptation Strategies in Rural Sahel. *Environmental Management, 43*, 804-816. https://doi.org/10.1007/s00267-008-9197-0

Nascimento Mendes, L. N., Dos Anjos, I. L. J., & Raphael, G. A. (2018). Agricultural marketing in the syb-middle São Francisco Valley: the importance of the Juazeiro-BA producers market. *Brazilian Journal of Development, 4*, 3451-3477.

Parré, J. L., Bankutti, S. M. C., & Zanmaria, N. A. (2011). Perfil socioeconômico de produtores de leite da região sudoeste do Paraná: um estudo a partir de diferentes níveis de produtividade. *Revista de Economia e Agronegócio, 9*, 275-300. https://doi.org/10.25070/rea.v9i2.187

Parveen, K., & Nain, M. S. (2013). Agricultura na Índia: Uma análise SWOT. *Indian Journal Applied Research, 3*, 4-6. https://doi.org/10.15373/2249555X/JULY2013/2

Pedro Júnior, M. J., Hernandes, J. L., & Moura, M. F. (2018). Performance of juice and wine grape cultivars in different training systems. *Revista Brasileira de Fruticultura, 40*, e-055. https://doi.org/10.1590/0100-29452018055

Pommer, C. V., Mendes, L. S., Hespanhol-Viana, L., & Bressan-Smith, R. (2009). Potencial climático para a produção de uvas em Campos dos Goytacazes, região norte fluminense. *Revista Brasileira de Fruticultura, 31*, 1076-1083. https://doi.org/10.1590/S0100-29452009000400022

Sarmento, M. B. (2017). Diagnóstico da Vitivinicultura na Campanha Gaúcha: Uma análise SWOT. *Agropampa: Revista de Gestão do Agronegócio, 1*, 65-85.

Schneider, M. D., Zilli, J. C., & Vieira, A. C. P. (2017). Diagnóstico mercadológico: um estudo em uma vinícola nos Vales da Uva Goethe-Santa Catarina. *Revista da Universidade Vale do Rio Verde, 15*, 759-768. https://doi.org/10.5892/ruvrd.v15i2.3132

Silva, J. R., Patterson, A. E., Rodrigues, W. P., Campostrini, E., & Griffin, K. L. (2017). Photosynthetic acclimation to elevated CO2 combined with partial rootzone drying results in improved water use efficiency, drought tolerance and leaf carbon balance of grapevines (*Vitis labrusca*). *Environmental and Experimental Botany, 134*, 82-95. https://doi.org/10.1016/j.envexpbot.2016.11.007

## Appendix

### Data collection

| | ha | N | % | t/ha |
|---|---|---|---|---|
| Grape production units | | | | |
| Number of winegrowers | | | | |
| Productivity | | | | |
| Points | S | W | O | T |
| Favorable edaphoclimatic conditions | | | | |
| More than one harvest per year | | | | |
| Proximity to consumers | | | | |
| Trade in the property | | | | |
| Strengthening of the family workforce | | | | |
| Agricultural diversification | | | | |
| Possibility of local trade | | | | |
| Lack of producer's organization | | | | |
| Little experience in viticulture | | | | |
| Market shortages by producers | | | | |
| Little administrative knowledge | | | | |
| Fruiting Program | | | | |
| Agrotourism | | | | |
| Availability of variety diversity | | | | |
| Research development | | | | |
| Few intermediaries | | | | |
| Additional income source | | | | |
| High investment cost | | | | |
| Low water availability | | | | |
| Absence of agricultural policies | | | | |
| Shortage of skilled labor | | | | |
| Few courses and field days | | | | |

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# Mapping and Environmental Diagnosis in Native Acai Areas in the Amazon

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**Abstract**

For several decades, the Acai orchards (*acaizais*) have directly influenced the survival of the families in the Amazonian floodplains. In this period, the production of the Acai fruit for local consumption was ceased and became an export item produced in intensive management, resulting in an increase in orchards in the floodplains and the emergence of dryland plantations, no longer representing a typical extractive activity in the Amazon. The objective of this study was to map the classes of use and coverage, and the occurrences of the Acai orchards massifs, as well as to analyze the physical and chemical parameters of five islands in the municipality of Igarapé-Miri, State of Pará, Brazil, where there is a great occurrence of productive Acai orchards. This work evaluated the following islands: Jarimbu, Mamangal, Itaboca, Mutirão, and Buçu, where geolocalized collections were carried out in the areas with the highest occurrence of Acai orchards, both to assist in the classification of images and for soil sampling. August 2019 Planet images were processed using the unsupervised method, where seven classes of cover use were obtained: hydrography, exposed soil, urban, alluvial, lowland, arboreal, and agriculture areas. Therefore, occurrences of productive orchards were identified and correlated to the good attributes of soil fertility in the floodplains under continuous flooding and sedimentation. The correlation confirmed the higher productivity of Acai in the Alluvial and Lowland classes, which predominate in the evaluated area, presenting soils considered fertile with a loam-clay-silty and loam -silty texture, high base saturation (greater than 50%), high organic matter content, and significant presence of potassium and phosphorus.

**Keywords:** Acai, Amazon floodplains, geotechnologies, soil parameters

## 1. Introduction

Among the native cultures of the Amazon region, Acai (*Euterpe oleracea* Mart.) stands out as the most economic, social, and culturally important crop. This is a species that is part of the native forest, predominantly found in lowland areas (Nogueira, 1997; Tregidgo et al., 2020). The State of Pará stands out nationally as the largest Acai producer, with an annual production of 1,274,056 tons of fruit (IBGE, 2019).

The rise in the demand for Acai fruit caused a great interest in the management of Acai palms in the floodplain areas (Homma et al., 2006) and the expansion of Acai forests managed in non-flooded areas, with the use of irrigation (Farias Neto et al., 2011). These changes in the management have already been recorded, but studies in the non-flooded forest are still rare (Jardim et al., 2004; Lôbo et al., 2011; Arroyo-Rodríguez et al., 2013).

The native Acai areas had an increase in production, on the other hand, it has modified the floodplain ecosystem, also causing significant erosion on the islands (Tagore et al., 2018), causing the weakening of the slopes and, consequently, the deposition of large quantities of sediment in the riverbed due to the uncontrolled deforestation (Gonçalves & Brasil, 2016). However, Brito et al. (2020) consider that studies of this nature, reinforce the importance of the production/conservation of native species.

However, the Acai fruit trade is an alternative with great economic potential and that essentially contributes to the income of extractive communities in the Amazon (Silva et al., 2019) promoting the sustainable exploitation of floodplain forests (Homma et al., 2006) and expanding the local and national economies (Tagore et al., 2018).

In this context, studies on the mapping of large areas of Acai orchards in the Legal Amazon are still scarce. Not even the local community has an estimate of the size of the areas explored there, as well as their transformations and impacts over the years. In the most recent studies by Lima et al. (2018) on environmental degradation identified by coverage analysis, the use of technologies was effective for assessing the behavior of vegetation, allowing to monitor and estimate the expansion of changes in the environment. The use of digital image classification techniques provides a synoptic view and information on the temporal dimension of spatial phenomena, allowing the generation of information on the dynamics and spatial patterns of the landscape in areas of large territorial extensions (De Souza et al., 2019).

The use of high-spatial-resolution sensors, as is the case of the Planet sensor with a spatial resolution of three meters, has proven to be real sources in the evaluation of the characteristics of the mapped areas, consisting of reliable, solid, and active information in the analysis of the dynamics of land use and cover.

Knowledge of the soil quality through its physical-chemical and biological attributes is essential to assess impacted and cropped areas (Colodel et al., 2018), as well as the lowland areas of the Amazon (Fajardo et al., 2009). Therefore, knowing the spatial variability of soil attributes may assist in the management of areas of native Acai in this ecosystem with the use of geo-technologies, which supports the high-productive potential of the areas with the rich-natural fertility of the soils and productivity in the islands.

Thus, Rodríguez-Echeverry et al. (2018) recommend that such strategies for changing land use obtained with the use of geotechnologies can serve as crucial information for planning conservation strategies and also for subsidizing programs and other planning and other tools of territorial and environmental management and planning.

In this context, the objective of this work was to analyze through the unsupervised classification of images the thematic maps of representativeness of the areas with the highest occurrence of Acai by the ISODATA algorithm the five islands of the municipality of Igarapé-Miri/PA and to correlate the generated maps with results of the analysis of physical and chemical attributes of the soils of the areas.

## 2. Material and Methods

### *2.1 Study Area*

The study was carried out in the municipality of Igarapé-Miri, located in the Low Tocantins region in the mesoregion in the northeastern Para State. The municipality is located 78 km from the capital of the state of Pará, Brazil, and has a territorial area of 199.679 ha (IBGE, 2019) (Figure 1). The climate in the region is of the tropical humid type corresponding to the megathermic type Ami according to Köppen's classification. The annual rainfall is greater than 2,000 mm with an average annual temperature of 27 °C and air relative humidity of 80% (Alvares et al., 2013).

Five islands participating in the Agroextractive Settlement Project (PAE) and that are also more representative for the Acai fruit were selected (Table 1).

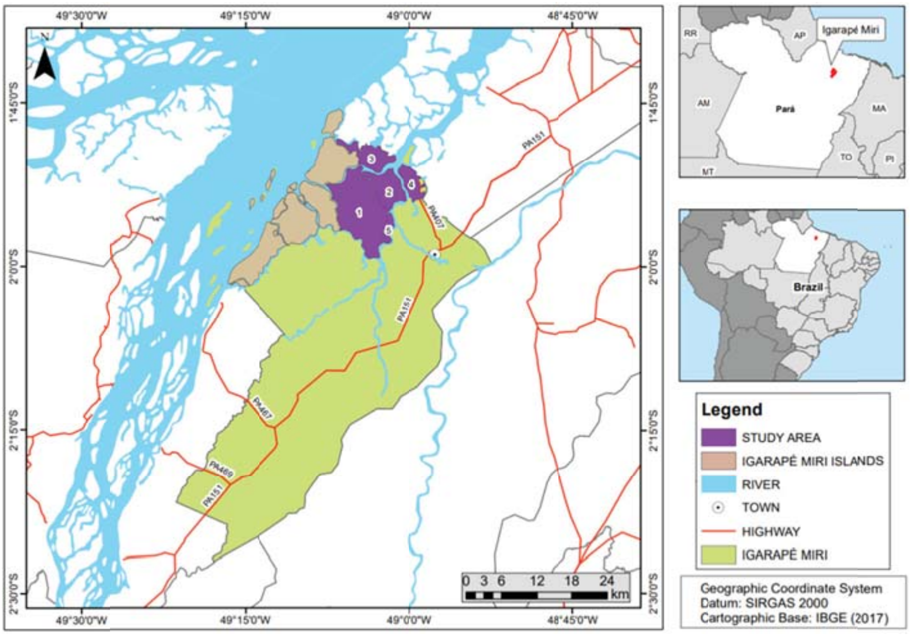



Figure 1. Study area location, islands region—Igarapé-Miri

Table 1. Characteristics of the islands in the study area

| Island | Coordinates | | Altitude (m) | Area (ha) |
|---|---|---|---|---|
| | Latitude | Longitude | | |
| JARIMBU | 1°54′27.18″S | 49° 4′17.25″W | 6 | 8,673.71 |
| MAMANGAL | 1°53′13.67″S | 49° 1′30.95″W | 8 | 2,590.76 |
| ITABOCA | 1°50′14.17″S | 49° 2′54.44″W | 16 | 2,806.13 |
| MUTIRÃO | 1°52′21.30″S | 49° 0′8.44″W | 12 | 1,708.52 |
| BUÇU | 1°56′3.01″S | 49° 1′48.35″W | 7 | 506.57 |

### *2.2 Collection and Pre-processing of Satellite Images*

Eight images of the Planet sensor obtained on August 9 and 10, 2019, were used, upon request to the State Secretariat of Environment and Sustainability of Pará (SEMAS), and then transferred to the Federal Rural University of the Amazon (UFRA) for use in research. The images have a spatial resolution of 3 meters composed of the following spectral bands: Band 1 (455-515 nm), Band 2 (500-590 nm), Band 3 (590-670 nm), and Band 4 (590-670 nm). The islands' shapefile was downloaded directly from the land collection of the National Institute for Colonization and Agrarian Reform (INCRA).

The images were georeferenced and submitted to atmospheric correction. All pre-processing of the satellite images used in the experiment was carried out individually for each island using the QGis software. The image was cut out with the delimitation of each island and, later, the mosaic was done.

### *2.3 Unsupervised Image Classification*

After the image pre-processing, the unsupervised classification was developed in the ENVI 5.3 software using the ISODATA algorithm (Iterative Selforganizing Data Analysis Technique) developed by (Geoffrey & Hall, 1965). The advantages of using this algorithm are related to not needing the knowledge of the evaluated area, the minimization of human errors as the fact that the pixels are spectrally separated and the control over the number of classes and interactions by the analyst (Morariu & Burescu, 2018).

For the formation of a cluster and grouping, based on the Euclidean distance, the minimum spectral distance formula was used as (Swain & Davis, 1978), according to (Equation 1):

$$SD_{xyc} = \sqrt{\sum_{i}^{n} = 1\left(\mu_{ci} - X_{xyi}\right)^2} \tag{1}$$

Where, n: number of bands; i: band number; c: private class; $X_{xyi}$: file value of the pixel data x, y in band i; $\mu_{ci}$: mean of the data file values (digital numbers) in *i* for the c class sample; $SD_{xyc}$: Spectral distance of pixels x, y the c class mean.

The definition standard was applied to a classification with a parameter of 30 classes, allowing the software to separate the different targets from the image and with interaction parameters specified in 15 for monitoring the process.

*2.4 Satellite Image Post-Processing*

The image analysis for post-processing of the results was performed through visual interpretation. This procedure consists of interpreting the image directly on the computer screen, using basic elements, such as color, texture, shape, shade, size, shadow, pattern, adjacencies, and geographic location (Loch, 1993; Temba, 2000; Gomes, 2001; Moreira, 2003; Barcellos et al., 2005). The reclassification was performed manually, pixel by pixel using the ClassEdit complement of the no Envi software. The complete processing from image collection to the classification analysis is represented in (Figure 2).

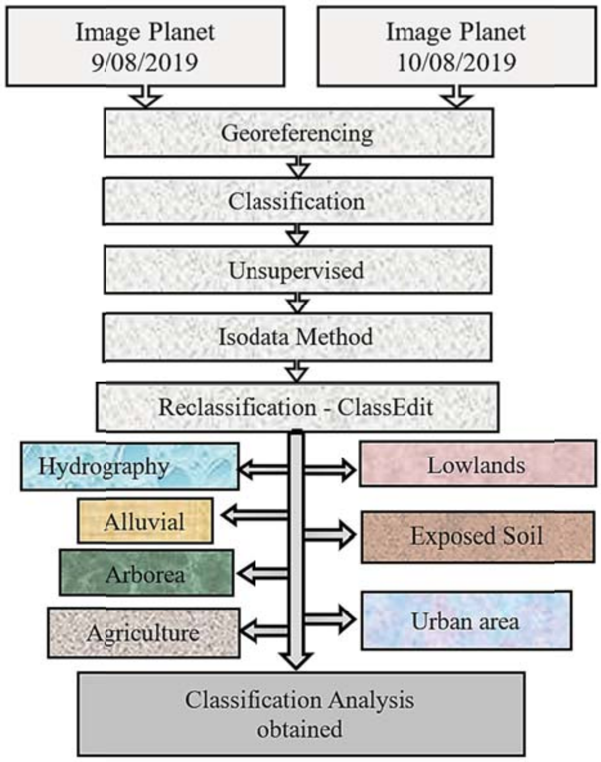



Figure 2. Flow chart of the developed methodology

The objective of the reclassification is to gather subclasses in their corresponding classes, which will promote the understanding of the classification (Matsukuma, 2002). This technique automatically separates the pixels into groups (clusters) of a similar spectral character (Verma et al., 2017), generating a highly successful analysis of the clusters (Meneses & Almeida, 2012). For a better understanding of the classification, descriptions were generated based on the type of class generated in the study (Table 2).

Table 2. Classification and description of land use and occupation

| Class type | Description |
|---|---|
| Hydrography | Rivers, canals, holes, lakes, igarapés (Amazon streams) |
| Exposed soil | Sandbank, roads, uncovered soil |
| Urban areas | Houses, village, built areas |
| Alluvial | Acai palm with the constant presence of water |
| Lowlands | Acai palms without the presence of water |
| Arborea | Tall and dense trees: Ucuuba (*Virola surinamensis*), Andiroba (*Carapa guianensis*), Ruber tree (*Hevea brasiliensis*), Mututi (*Pterocarpus santalinoides*), Munguba (*Pachira aquatica*), among others |
| | Small and fruit trees: mango (*Mangifera indica*), cacao (*Theobroma cacao*), Buriti (*Mauritia flexuosa*), Taperebá (*Sponidas nombin*) among others |
| Agriculture | Monocultivation and Agroforest systems |

For noise elimination and image smoothing, the Majority parameters 3 × 3 filter was applied (Vasconcelos et al., 2016). Then, the classification was edited through visual interpretation according to the spectral behavior of each pixel (Duarte & Silva, 2019). After, the file was transformed into a vector file (shapefile) and quantified, generating tabulated data.

The following methodological actions were used to measure the classification process of Planet images:

i) Evaluation of errors of omission and commission (statistics of the percentage of errors and successes of the pixel-by-pixel classification, attributed by the Confusion matrices); ii) Post-classification visual inspection checking the distribution and connection between the generated classes, the existence of isolated pixels, and the need for classification editing; iii) Validation and *in loco* confirmation through the georeferenced collection in the mapped classes at visiting the study area before and after the image classification process.

### *2.5 Soil Sampling*

In the areas with the highest occurrence of Acai, disturbed soil samples were collected in the 0-0.10 m layer, at a distance of 50 to 100 m from the river bank and 1 to 2 km from one collection point to another, totaling 19 samples for the five islands. Then, the samples were air-dried, ground, and passed through a 2-mm opening sieve to obtain the air-dried fine earth (ADFE). This sampling was carried out in August, a period of low rainfall in the region. All collection points were geo-referenced with the aid of GPS (Global Position System).

### *2.6 Soil Laboratory Analysis*

The chemical attributes of the soil were determined according to the methodology of Teixeira et al. (2017): pH in water and KCl in the soil:solution (1:2.5) rate. Available P and K, extracted by Mehlich-1 (0.0125 mol $L^{-1}$ of $H_2SO_4$ + 0.05 mol $L^{-1}$ of HCl), and P determined by colorimetry and K by flame photometry; Al, Ca and Mg extracted with KCl 1 mol $L^{-1}$; Al determined by titration (neutralization volume), while Ca and Mg were determined by complexometry with EDTA; H + Al extracted with 1 mol $L^{-1}$ calcium acetate solution at pH 7.0 and determined by titration. Organic carbon was determined by the modified method of (Walkley & Black, 1934), based on the principle of oxidation of organic matter, with potassium dichromate in the sulfuric medium.

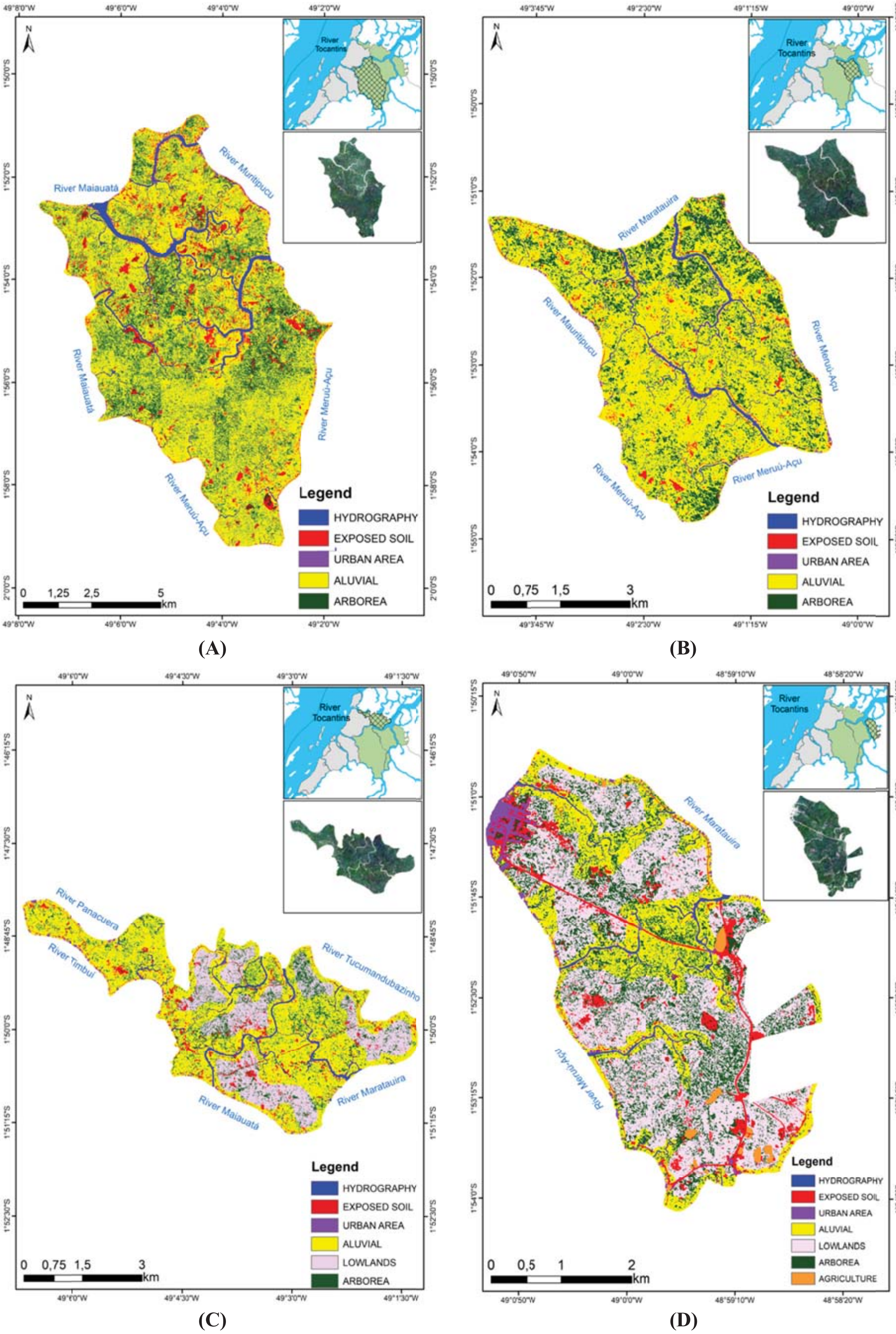
The particle size distribution was determined using the pipette method using 1M NaOH. Before dispersion, samples with an organic matter content > 5% were pre-treated to remove organic matter with $H_2O_2$ (Gee & Bauder, 1986). The sand fraction was separated through sieving, the clay by sedimentation, and the silt fraction calculated by the difference.

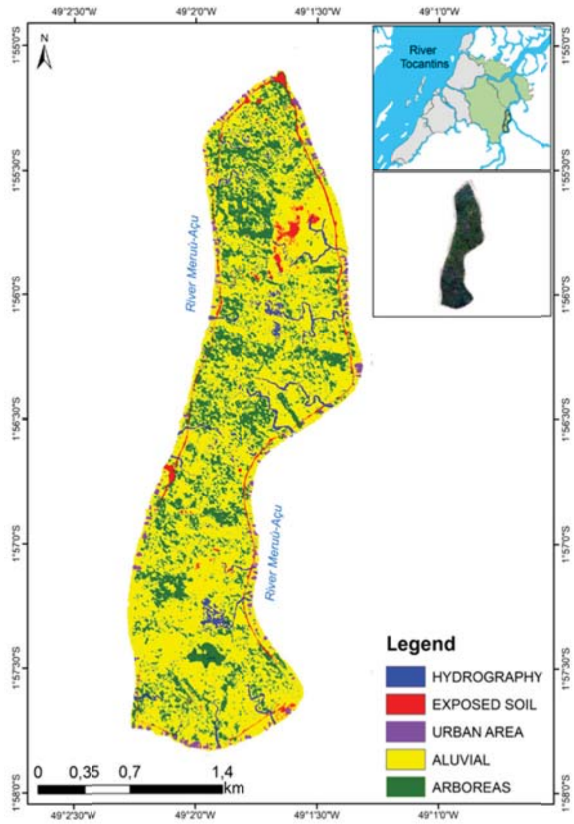
## 3. Results and Discussion

### *3.1 Land Use and Soil Cover Classification on the Islands*

The results obtained for the unsupervised reclassification of land use and the cover of the five islands using the IsoData method generated the identification of ash level patterns. These patterns are defined as samples grouped by clusters of space units (Olofsson et al., 2014).

It was observed the generation of a new classification by grouping applied in the Planet 2019 Images (Figure 3). The images were selected exactly during the Acai harvest period, which provided a good spatial resolution of the areas and, consequently, allowed the identification of the Acai massifs on each island and the most relevant classes.


River Tocantins
River Maiauatá
River Muritipucu
River Meruú-Açu
River Marataúira
River Mauritipucu
River Panacuera
River Timbuí
River Tucumandubazinho
Legend
HYDROGRAPHY
EXPOSED SOIL
URBAN AREA
ALUVIAL
LOWLANDS
ARBOREA
AGRICULTURE
km
(A)
(B)
(C)
(D)




**(E)**

Figure 3. Classification of the five areas using the unsupervised methods using Isodata algorithm (A: Pae Jarimbu; B: Pae Mamangal; C: Pae Itaboca; D: Pae Mutirão; E: Pae Buçu)

The Alluvial and Lowland classes were observed in all the islands and, together, they represented 67.72% of the total evaluated area, *i.e.*, 10 thousand hectares where the Acai is found (Figure 3). Homma (2014) estimated that roughly 80 thousand hectares of floodplain were transformed into homogeneous Acai areas. On the Jarimbu, Mamangal, and Buçu islands, five classes of interest were identified; Itaboca presented six classes and Mutirão seven classes, as shown in (Table 3). The characterization of the dense Ombrophilous Forest formations has two different formations: The Dense Ombrophilous Forest of the Lowlands and the Alluvial Dense Ombrophilous Forest (Veloso et al., 1991), the latter being a formation with many palms (IBGE, 2012), typical of these lowland areas in this region. For each island analyzed with its respective class raised, the values were obtained in the number of pixels and converted to the area, in hectares (Table 3).

Table 3. Representation of the classification of the areas per hectare of the five islands

| Classes | PAE-ISLANDS (ha) | | | | |
|---|---|---|---|---|---|
| | Jarimbu | Mamangal | Itaboca | Mutirão | Buçu |
| Hydrography | 364.48 | 105.30 | 96.37 | 27.26 | 13.22 |
| Exposed soil | 645.50 | 71.54 | 143.05 | 123.84 | 11.31 |
| Urban area | 80.14 | 33.18 | 29.48 | 39.75 | 7.94 |
| Alluvial | 5529.29 | 1823.05 | 1508.43 | 320.00 | 350.53 |
| Lowlands | - | - | 611.81 | 601.83 | - |
| Arborea | 2054.30 | 557.68 | 417.00 | 413.89 | 123.57 |
| Agriculture | - | - | - | 16.95 | - |
| Total area (ha) | 8,673.71 | 2,590.76 | 2,806.13 | 1,543.52 | 506.57 |

The percentage regarding the types of land use and occupation found for each island are shown in (Figure 4). The Lowland class was observed in only two islands, Itaboca and Mutirão, with 21.80% and 38.99% of the total area, respectively (Figure 4) due to the higher average altitudes of these areas (16 m and 12 m, respectively) compared to the others (Table 1). In the other evaluated islands, such as Jarimbu, Mamangal, Itaboca, and Buçu, the Alluvial class represents, more than 50% of all land occupation, which demonstrates the great presence of Acai in these areas, being directly linked to networks of rivers that involve these areas.

The Mutirão island showed only 20.73% for the Alluvial class (Figure 4), which may be associated with its distribution in other classes such as lowlands, farming, urban area, and the island with the smallest network of rivers has also been confirmed by the classification of hydrography in 1.77% and, consequently for being the island that suffers the most influence by human action in the use and cover of the soil. According to Assis (2011), it is very difficult to distinguish the alluvial and lowland formations due to the physiognomic similarity between them, with great difficulty in establishing the limits between alluvial sedimentation (which constitutes the alluvial plain) and the other types of sedimentation and geomorphological features.

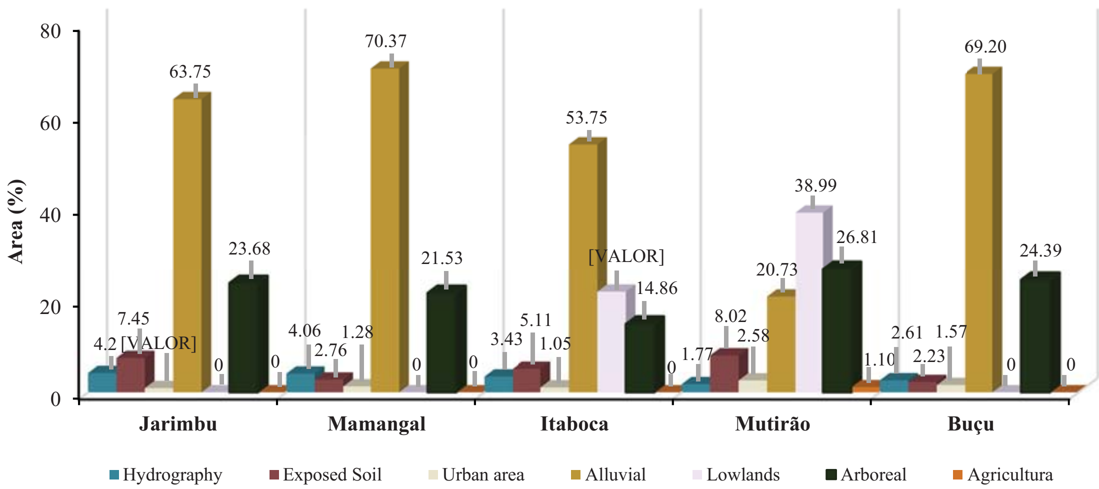



Figure 4. Percentage distribution of land use and occupation classes on Igarapé-Miri islands

The Arboreal class comprises an average value of 22% for all the evaluated islands, except for Itaboca island. Due to its distribution into classes such as Alluvial with 53.75% and Lowlands with 21.8% (Figure 4). According to these results, the arboreal class is the consequence of the anthropic activities carried out in the forest, such as intensive management of Acai. The rise in in the demand for the Acai fruit resulted in inadequate removal of the trees resulting in more than 50% loss of tree species diversity and a 63% reduction in the number of pioneer species (Freitas et al., 2015a).

The alteration in the landscape in the dense alluvial rain forest, resulting from the management of the Acai palms, was also reported by Braga and Jardim (2019). Low diversity is associated with restrictive environmental conditions, such as soil water saturation, which leads to the formation of a non-uniform community, with the dominance of a few species, known as oligo-species (few species with large numbers of individuals) (Almeida et al., 2004; Carvalho et al., 2009). However, this has been being accelerated by anthropic practices. Santos Júnior et al. (2015) reports that the reduction in the floodplain biodiversity has been disfavoring other species, corroborating with studies by Tregidgo et al. (2020) in the selective removal of other tree species.

Regarding the Exposed Soil class, the Mutirão, Jarimbu, and Itaboca islands stood out with values of 8.02%, 7.45%, and 5.11%, respectively. This proportion is attributed to areas of farming, roads, soil cleaning, and sandbanks. Braga and Jardim (2019), in their studies of Alluvial Dense Rainforest in Pará, also conclude that these areas are possibly cleared using thinning which promoted the development of the palm and inhibited the regeneration mechanisms, chasing away the dispersing fauna and opening clearings. Because it is a class that exposes the islands' soils, it becomes a negative point as native species were removed, leading to the erosion of these soils.

As for the Agriculture class, only one island displayed this characteristic, the Mutirão island with 16.95 (ha) in its extension, representing 1.1%, as this is located closer to the margin of the municipality of Igarapé-Miri and acts as Acai Road, an important highway which is the outlet for all fruit production from the adjacent islands.

Farming carried out by riverside dwellers occurs through clearing and thinning processes in their areas, as a way of implementing some crops, as reported by Silva et al. (2018) in their studies which show that of the 130 settlers on Island Mamangal, 28% have agroforestry systems (AFSs) in their areas, explained mostly by diversification (production), family food and shading of the Acai (*Euterpe oleracea* Mart.) crop. This reality coincides with what occurs on the other islands, as these are practices found by the riverside people to implement the AFSs as a form of income for these families.

The Urban Area class characterized in this study is represented by houses, villages, and built areas (Table 2). Mutirão Island is the most representative in this class, with 2.58% of the total area. On the Other hand, Jarimbu, Mamangal, Itaboca, and Buçu islands present, respectively, 0.92%, 1.28%, 1.05%, and 1.57% of the urban area (Figure 4). It was observed that the urban area in all islands is predominantly on the banks of rivers. In general, traditional populations in the Amazon maintain a strong connection with the river network, since they provide connectivity and mobility for their populations (Amaral et al., 2013).

The class characterized as Hydrography is found on all islands. Canto (2007) reports that the floodplains are large strips that border the rivers and are periodically covered by water. However, Tagore et al. (2018) point out that river courses have been being altered by the flow of the vessels, increasing the width of channels. It is worth mentioning that only the internal areas of each island were classified as Hydrography, therefore its outer edge in the image was not classified as in (Figure 3), which allowed to observe and determine the amount of water within each island.

*3.2 Classification of the Island Soil*

The results of soil chemical attributes are shown in (Table 4). The soils on the islands Mamangal, Itaboca, and Mutirão had the lowest mean pH values in water and KCl, in relation to the soil of the islands Jarimbu and Buçu. The more acidic pH in the soils of the islands of Mamangal, Itaboca, and Mutirão is the outcome of the ferrolysis process, which is common in soils subject to hydromorphism and rich in iron (Van Breemen & Buurman, 2002).

A work of soil genesis with flooding cycles has shown that high acidity has been observed in superficial horizons in lowland soils due to ferrolysis (Barbiero et al., 2010; Coringa et al., 2012; Da Silva et al., 2019). Studies in Gleisoil under native vegetation on the banks of the Guamá River attested acid pH in water and KCl associated with the alternation between flood and ebb tide (Lopes et al., 2006).

The nutrient content in the evaluated soils showed wide distribution and high variation within each area, with base saturation > 50% in the topsoil and also higher values of organic carbon (OC) and (pH) for the Jarimbu and Buçu islands. Freitas et al., (2015 b) in a floodplain study, reports that high values of base saturation and pH are widely used in the indication of soil fertility. While for the other three islands, Mamangal, Itaboca, and Mutirão, base saturation was < 50% and OC with lower values.

Table 4. Descriptive statistics of the soil chemical properties on native Acai palm plantation located in five municipalities in the state of Pará (n = 19). Depth: 0-0.10 m

| | pH | | OC | P | K | Ca | Mg | Al | H+Al | SB | CEC | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $H_2O$ | KCl | g kg$^{-1}$ | mg dm$^{-3}$ | -------------------------------- cmol$_c$ dm$^{-3}$ -------------------------------- | | | | | | | % |
| *$S_{JAR}$ (Jarimbu Island)* | | | | | | | | | | | | |
| Min. | 5.48 | 4.37 | 45.06 | 12.97 | 0.40 | 4.48 | 1.04 | 0.12 | 1.65 | 5.92 | 7.56 | 78.23 |
| Max. | 7.96 | 7.10 | 149.81 | 263.71 | 0.54 | 13.15 | 1.89 | 0.47 | 16.67 | 15.58 | 32.25 | 48.32 |
| Mean | 6.28 | 5.42 | 102.48 | 90.61 | 0.47 | 8.38 | 1.31 | 0.23 | 9.75 | 10.16 | 19.91 | 51.02 |
| SD | 1.13 | 1.18 | 43.16 | 118.74 | 0.08 | 3.57 | 0.39 | 0.16 | 6.18 | 4.05 | 10.23 | 39.56 |
| CV (%) | 18.03 | 21.73 | 42.12 | 131.04 | 16.92 | 42.64 | 30.22 | 72.93 | 63.42 | 89.79 | 153.21 | 58.61 |
| *$S_{MAM}$ (Mamangal Island)* | | | | | | | | | | | | |
| Min. | 5.58 | 4.76 | 24.65 | 5.16 | 0.32 | 5.09 | 1.47 | 0.12 | 8.31 | 6.88 | 15.19 | 45.28 |
| Max. | 5.92 | 5.00 | 38.54 | 13.67 | 0.47 | 7.68 | 3.49 | 0.27 | 11.52 | 11.64 | 23.16 | 50.26 |
| Mean | 5.74 | 4.88 | 31.93 | 9.03 | 0.39 | 5.77 | 2.21 | 0.20 | 9.90 | 8.37 | 18.27 | 45.82 |
| SD | 0.14 | 0.13 | 5.90 | 3.99 | 0.07 | 1.28 | 0.89 | 0.06 | 1.31 | 2.24 | 3.55 | 63.05 |
| CV (%) | 2.46 | 2.61 | 18.48 | 44.22 | 18.02 | 22.13 | 40.32 | 32.66 | 13.26 | 80.47 | 93.73 | 85.85 |
| *$S_{ITA}$ (Itaboca Island)* | | | | | | | | | | | | |
| Min. | 5.77 | 4.54 | 22.11 | 7.83 | 0.32 | 4.91 | 1.63 | 0.20 | 8.52 | 6.85 | 15.37 | 44.57 |
| Max. | 6.17 | 5.18 | 44.46 | 20.16 | 0.53 | 7.71 | 2.00 | 0.24 | 10.04 | 10.24 | 20.28 | 50.49 |
| Mean | 5.93 | 4.95 | 34.01 | 16.26 | 0.43 | 6.27 | 1.77 | 0.21 | 9.21 | 8.47 | 17.67 | 47.90 |
| SD | 0.19 | 0.28 | 9.18 | 5.67 | 0.09 | 1.16 | 0.16 | 0.02 | 0.71 | 1.42 | 2.13 | 66.59 |
| CV (%) | 3.16 | 5.62 | 26.99 | 34.90 | 22.26 | 18.55 | 9.06 | 9.52 | 7.73 | 49.87 | 57.60 | 86.58 |
| *$S_{MUT}$ (Mutirão Island)* | | | | | | | | | | | | |
| Min. | 5.49 | 4.72 | 28.88 | 6.79 | 0.39 | 5.92 | 1.65 | 0.12 | 11.60 | 7.97 | 19.57 | 40.71 |
| Max. | 5.97 | 5.24 | 86.50 | 240.87 | 0.50 | 11.12 | 2.85 | 0.24 | 13.99 | 14.47 | 28.46 | 50.85 |
| Mean | 5.81 | 5.04 | 51.74 | 69.20 | 0.44 | 7.89 | 2.38 | 0.19 | 12.73 | 10.71 | 23.43 | 45.69 |
| SD | 0.22 | 0.24 | 25.87 | 114.51 | 0.04 | 2.27 | 0.53 | 0.05 | 1.00 | 2.84 | 3.84 | 73.93 |
| CV (%) | 3.73 | 4.73 | 50.00 | 165.48 | 10.07 | 28.77 | 22.17 | 26.49 | 7.87 | 61.02 | 68.89 | 88.57 |
| *$S_{BU}$ (Buçu Island)* | | | | | | | | | | | | |
| Min. | 5.59 | 4.98 | 40.23 | 9.52 | 0.35 | 6.96 | 0.67 | 0.12 | 2.55 | 7.97 | 10.52 | 75.76 |
| Max. | 7.79 | 6.77 | 97.37 | 198.15 | 0.52 | 8.56 | 2.72 | 0.24 | 13.62 | 11.80 | 25.42 | 46.43 |
| Mean | 6.42 | 5.61 | 61.33 | 86.27 | 0.41 | 7.53 | 1.98 | 0.20 | 9.51 | 9.92 | 19.43 | 51.07 |
| SD | 1.20 | 1.00 | 31.36 | 99.10 | 0.10 | 0.89 | 1.14 | 0.07 | 6.06 | 2.14 | 8.19 | 26.07 |
| CV (%) | 18.64 | 17.80 | 51.14 | 114.86 | 24.18 | 11.88 | 57.62 | 34.64 | 63.71 | 93.68 | 157.39 | 59.52 |

*Note*. OC: organic carbon; H + Al: potential acidity; SB: sum of bases; CEC: cation exchange capacity; V%: base saturation; SD: standard deviation; CV: variation coefficient.

The sum of bases highly contributed to the five soils, which showed high concentrations of $Ca^{2+}$, where the highest mean value was shown in Jarimbu island., 8.38 cmol$_c$ dm$^{-3}$, which is the island with a high concentration of (OC), therefore corroborating with studies of Neto et al. (2018) indicating that in secondary forests, the natural dynamics promotes the incorporation and maintenance of organic matter in the soil (Delarmelinda et al., 2017) concentrating high fertilization in native areas.

The OC with a highly considerable value for Jarimbu island of 102.48 g kg$^{-1}$ is justified because on the margin of this there are many sawmills where all the tailings are discharged directly into the waters, and with the movement of flood and ebb from the river, these sediments are deposited, resulting in high values of organic matter in the soil, which was also found by Freitas et al. (2015b) reporting that large scale deposition of fresh organic material on the soil surface periodically without time for humification, explains the highest concentration of organic carbon in the first layer.

The highest average concentrations of $Mg^{2+}$ were for the soils on the islands Mamangal and Mutirão (2.21 and 2.38 cmol$_c$ dm$^{-3}$), respectively, and the lowest concentration value was found for the soils of the islands Jarimbu, Itaboca, and Buçu, 1.31 cmol$_c$ dm$^{-3}$; 1.77 cmol$_c$ dm$^{-3}$ and 1.98 cmol$_c$ dm$^{-3}$, respectively; the contents of $K^+$; $Al^+$ showed a small dispersion in the five soils in relation to the other chemical variables, showing a lower $K^+$ value (0.39 cmol$_c$ dm$^{-3}$) for Mamangal island and lower $Al^+$ value for Mutirão island (0.19 cmol$_c$ dm$^{-3}$) and higher mean values of $K^+$ and $Al^+$ (0.47 and 0.23 cmol$_c$ dm$^{-3}$) only on the Jarimbu island.

$K^+$ is found in large amounts in plant tissues with energetic functions, and water absorption by cells (Forster et al., 2019), where all the evaluated elements favored good spatialization of the Acai palm areas in these islands, presenting higher alluvial classes in this study.

The high P concentration in the soils on the Jarimbu islands, 90.61 mg $dm^{-3}$; Mutirão, 69.20 mg $dm^{-3}$, and Buçu 86.27 mg $dm^{-3}$, has a great contribution on organic matter, Where P is a fundamental element for the initial development of Acai plant, promoting a greater increase in the aerial part and biomass when in high availability (Araújo et al., 2018).

The cation exchange capacity and the potential acidity had a high contribution to the exchange complex in all five soils. Salviano et al. (1998) also report the similarities between CEC and potential acidity (H + Al) and how much they exert direct influence on the CEC calculation. This was also found by Freitas et al. (2015b), in which high values reflect the natural conditions of this soil and have a high capacity to retain cations.

The soils on Jarimbu, Mamangal, Itaboca, and Mutirão islands presented a silty clay-loam textural class. The soils of Buçu Island, on the other hand, presented a silt-loam textural class. However, in the soil of Jarimbu island., sand contributed more, 135.74 g $kg^{-1}$ in comparison to the other islands, while the soil of Itaboca island, presented a lower proportion of silt/clay, 1.91 g $kg^{-1}$ (Table 5).

Table 5. Texture classification of soils under native Acai palm in five islands in the municipality in Pará State (n = 19). Depth: 0-0.10 m

| Areas | Sand | Clay | Silt | Silt/Clay | Texture class |
|---|---|---|---|---|---|
| | ------------------------------ g $kg^{-1}$ | | | ----------------------------- | |
| $S_{JAR}$-Jarimbu Island | 135.74 | 238.44 | 625.83 | 2.62 | Silty clay-loam |
| $S_{MAM}$-Mamangal Island | 36.59 | 306.00 | 657.41 | 2.15 | Silty-clay-loam |
| $S_{ITA}$-Itaboca Island | 49.73 | 326.40 | 623.88 | 1.91 | Silty-clay-loam |
| $S_{MUT}$-Mutirão Island | 94.29 | 285.60 | 620.11 | 2.17 | Silty-clay-loam |
| $S_{BU}$-Buçu Island | 74.22 | 301.84 | 623.94 | 2.07 | Silty-loam |

Although studies for floodplain soils in the islands in the Low Tocantins region are scarce and incipient (Lopes et al., 2019), the results of this experiment are relevant, as all the five soils showed similar characteristics. According to Guedes et al. (2018), they are fertile soils with fragile peculiarities, in which the soil texture particles under flood are sedimented in the lowest part of the landscape, which induces changes in texture, culminating in siltier soils (Wang et al., 2017).

## 4. Conclusions

Based on the results of this experiment, we concluded that in the areas of the Igarapé-Miri islands, large Acai orchards massifs were consolidated, expanded through favorable soil fertility conditions and efficient management practices in the floodplain areas. The zoning and spatial distribution of Acai orchards in the five studied islands (Jarimbu, Mamangal, Itaboca, Mutirão, and Buçu) through the processing and use of image classification algorithms, allowed the correlation of the mappings carried out with the major physical-chemical attributes of the soils.

The mapping established seven classes of use and cover (Hydrography, Exposed Soil, Urban Area, Alluvial, Lowlands, Arboreal, and Agriculture) where it was identified occurrences of productive orchards, correlated to the soil fertility attributes naturally imposed under flooding and sedimentation in the lowest parts in the floodplains. This correlation confirmed the indications of higher productivity of the Acai fruit in the Alluvial and Lowland classes. In these classes of greater predominance in the studied area, the soils are considered fertile and presented similar physical-chemical characteristics with a loam-clay-silty and loam-silty texture, high base saturation (greater than 50%), high levels of organic matter, and significant presence of potassium and phosphorus.

The local community started to combine efficient management practices with environmental sustainability, with positive socio-environmental and cultural returns, resulting in promoting employment and income in the periods of Acai harvest (July to December) and off-season (January to July), supported by other local economic activities (fishing and other family farming activities).

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**References**

Almeida, S. S., Amaral, D. D., & Silva, A. S. L. (2004). Análise florística e estrutura de florestas de várzea no estuário amazônico. *Acta Amazônica, 34*(4), 513-524. https://doi.org/10.1590/S0044-59672004000400005

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Moraes, G., Leonardo, J., & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift, 22*(6), 711-728. https://doi.org/10.1127/0941-2948/2013/0507

Amaral, S., Dal'asta, A. P., Brigatti, N., Pinho, C. M. D. D., Medeiros, L. C. D. C., Andrade, P. R. D., … Monteiro, A. M. V. (2013). Comunidades ribeirinhas como forma socioespacial de expressão urbana na Amazônia: uma tipologia para a região do Baixo Tapajós (Pará-Brasil). *Revista Brasileira de Estudos de População, 30*(2), 367-399. https://doi.org/10.1590/S0102-30982013000200003

Araújo, C. S., Rufino, C. P. B., Bezerra, J. L. S., Andrade Neto, R. De C., & Lunz, A. M. P. (2018). Crescimento de mudas de açaizeiro (*Euterpe oleracea* Mart) Submetidas a diferentes doses de fósforo. *South American Journal of Basic Education, Technical and Technological, 5*(1).

Arroyo-Rodríguez, V., Rös, M., Escobar, F., Melo, F. P. L., Santos, B. A., Tabarelli, M., & Chazdon, R. (2013). Plant b-diversity in fragmented rain forests: Testing floristic homogenization and differentiation hypotheses. *J. Ecol., 101*, 1449-1458. https://doi.org/10.1111/1365-2745.12153

Assis, M. A., Prata, E. M. B., Pedroni, F., Eisenlohr, P. V., Martins, F. R., Santos, F. A. M. D., & Martins, S. C. (2011). Florestas de restinga e de terras baixas na planície costeira do sudeste do Brasil: Vegetação e heterogeneidade ambiental. *Biota Neotropica, 11*(2), 103-121. https://doi.org/10.1590/S1676-060320110 00200012

Barbiero, L., Kumar, M. M., Violette, A., Oliva, P., Braun, J. J., Kumar, C., & Valles, V. (2010). Ferrolysis induced soil transformation by natural drainage in Vertisols of sub-humid South India. *Geoderma, 156*(3-4), 173-188. https://doi.org/10.1016/j.geoderma.2010.02.014

Barcellos, C., Pustai, A. K., Weber, M. A., & Brito, M. R. V. (2005). Identificação de locais com potencial de transmissão de dengue em Porto Alegre através de técnicas de geoprocessamento. *Revista da Sociedade Brasileira de Medicina Tropical, 38*(3), 246-250. https://doi.org/10.1590/S0037-86822005000300008

Braga, E. O., & Jardim, M. A. G. (2019). Floristica, estrutura e formas de vida do estrato inferior de uma Floresta Ombrofila Densa Aluvial, Para, Brasil/Floristic, structure and life forms of lower stratum in a Alluvial Dense Rain Forest, Para state, Brazil. *Ciência Florestal, 29*(3), 1048-1060. https://doi.org/ 10.5902/1980509821834

Brito, A. D., Coelho, R. De F. R., & Rosal, F. L. (2020). Os extrativistas de andiroba em projetos de assentamentos agroextrativistas (paex) da várzea de Igarapé-Miri, Pará, Brasil. *Revista Agroecossistemas, 11*(2), 82-101. https://doi.org/10.18542/ragros.v11i2.7303

Canto, O. D. (2007). *Várzea e Varzeiro da Amazônia*. Belém: MPEG.

Carvalho, J., Marques, M. C. M., Roderjan, C. V., Barddal, M., & de Sousa, S. G. A. (2009). Relações entre a distribuição das espécies de diferentes estratos e as características do solo de uma floresta aluvial no Estado do Paraná, Brasil. *Acta Botanica Brasilica, 23*(1), 1-9. https://doi.org/10.1590/S0102-33062009000100002

Colodel, J. R., Pierangeli, M. A. P., Souza, M. F. P., Carvalho, M. A. C. D., & Dalchiavon, F. C. (2018). Atributos físicos e biológicos de Argissolo Vermelho-Amarelo Amazónico sob diferentes sistemas de uso e manejo. *Revista de Ciências Agrárias, 41*(2), 1-10. https://doi.org/10.19084/RCA17253

Coringa, E. D. A. O., Couto, E. G., Otero Perez, X. L., & Torrado, P. V. (2012). Atributos de solos hidromórficos no Pantanal Norte Matogrossense. *Acta Amazonica, 42*(1), 19-28. https://doi.org/10.1590/S0044-59672 012000100003

Da Silva, L. F., Fruett, T., Zinn, Y. L., Inda, A. V., & Do Nascimento, P. C. (2018). Genesis, morphology and mineralogy of Planosols developed from different parent materials in southern Brazil. *Geoderma, 341*, 46-58. https://doi.org/10.1016/j.geoderma..12.010

De Souza, A. R., Escada, M. I. S., Marujo, R. D. F. B., & Monteiro, A. M. V. (2019). Cartografia do Invisível: Revelando a Agricultura de Pequena Escala com Imagens Rapideye na Região do Baixo Tocantins, PA. *Revista Do Departamento De Geografia, 38*, 137-153. https://doi.org/10.11606/rdg.v38i1.151603

Delarmelinda, E. A., De Souza Júnior, V. S., Wadt, P. G. S., Deng, Y., Campos, M. C. C., & Câmara, E. R. G. (2017). Soil-landscape relationship in a chronosequence of the middle Madeira River in southwestern Amazon, Brazil. *Catena, 149*, 199-208. https://doi.org/10.1016/j.catena.2016.09.021

Duarte, M. L., & Da Silva, T. (2019). Avaliação do desempenho de três algoritmos na classificação de uso do solo a partir de geotecnologias gratuitas. *Revista de Estudos Ambientais, 21*(1), 6-16. https://doi.org/10.7867/1983-1501.2019v21n1p6-16

E Silva, J. I. D. S., Junior, F. K. R., De Lima, H. V., Dos Santos, M. A. S., Dos Santos, P. C., & Lopes, M. L. B. (2019). Socio-economics of Acai Production in Rural Communities in the Brazilian Amazon: A Case Study in the Municipality of Igarapé-Miri, State of Pará. *Journal of Agricultural Science, 11*(5), 215. https://doi.org/10.5539/jas.v11n5p215

Fajardo, J. D. V., Souza, L. A. G. De, & Alfaia, S. S. (2009). Características químicas de solos de várzeas sob diferentes sistemas de uso da terra, na calha dos rios Baixo Solimões e Médio Amazonas. *Acta Amazonica, 39*, 731-740. https://doi.org/10.1590/S0044-59672009000400001

Farias Neto, J. T., Resende, M. D. V., & Oliveira, M. S. P. (2011). Seleção simultânea em progênies de açaizeiro irrigado para produção e eso do fruto. *Revista Brasileira de Fruticultura, 33*(1), 532-539. https://doi.org/10.1590/S0100-29452011000200025

Forster, S., Lena, K. S., Eva, K., Erwin, G., Jörg, K., Dirk, B., … Dirk, B. (2019). Wounding-Induced Stomatal Closure Requires Jasmonate-Mediated Activation of GORK K+ Channels by a $Ca^{2+}$ Sensor-Kinase CBL1-CIPK5 Complex. *Developmental Cell, 48*, 87-99. https://doi.org/10.1016/j.devcel.2018.11.014

Freitas, L. Da J., Santos Dos, E. S., & Oliveira, De A. F. (2015). Avaliação de atributos químicos do solo em ambientes de terra firme e várzea no município de Santana, Amapá. *Biota Amazônia, 5*(4), 27-33. https://doi.org/10.18561/2179-5746/biotaamazonia.v5n4p27-33

Freitas, M. A. B., Vieira, I. C. G., Albernaz, A. L. K. M., Magalhães, J. L. L., & Lees, A. C. (2015). Floristic impoverishment of Amazonian floodplain forests managed for açaí fruit production. *Forest Ecology and Management, 351*, 20-27. https://doi.org/10.1016/j.foreco.2015.05.008

Gee, G. W., & Bauder, J. W. (1986). Particicle-size Analysis. In C. A. Klute (Ed.), *Methods of soil Analysis* (2nd ed., pp. 383-411). Medison, Wisconsin. https://doi.org/10.2136/sssabookser5.1.2ed.c15

Geoffrey, H. B., & Hall, D. J. (1965). *ISODATA, a novel method of data analysis and patern classification* (Technical Report April 1965). Stanford Research Institute Menlo Park, California.

Gomes, J. C. (2001). *Fotointerpretação I*. Guaratinguetá: Centro de Instrução e Adaptação da Aeronáutica.

Gonçalves De, L. D., & Brasil, D. D. S. B. (2016). Problemas ambientais e sustentabilidade nas várzeas da Amazônia Tocantina: Um estudo no Projeto de Assentamento Agroextrativista São João Batista II, Abaetetuba, Estado do Pará, Brasil. *Revista Pan-Amazônica de Saúde, 7*(4), 11-11. https://doi.org/10.5123/S2176-62232016000400011

Guedes, A. C. L., Ferreira, J. A., Júnio, N. J. M., & Guedes, M. C. (2018). *Coletores para quantificar a entrada de sedimentos em florestas de várzea Estuarina*. Embrapa Amapá-Boletim De Pesquisa E Desenvolvimento (INFOTECA-E).

Homma, A. K. (2014). Extrativismo vegetal ou plantio: Qual a opção para a Amazônia? In A. K. O. Homma (Ed.), *Extrativismo vegetal na Amazônia: História, ecologia, economia e domesticação*. Brasília, DF: Embrapa.

Homma, A. K. O., Nogueira, O. L., De Menezes, A. J. E. A., De Carvalho, J. E. U., Nicoli, C. M. L., & De Matos, G. B. (2006). Açaí: Novos desafios e tendências. *Amaz. Ciência Desenvol, 1*, 7-23.

IBGE (Instituto Brasileiro de Geografia e Estatística). (2012). *Manual técnico da vegetação brasileira*.

IBGE (Instituto Brasileiro de Geografia e Estatística). (2019). *Censo do município de garapé-mirim-PA*. Retrieved October 21, 2020, from https://cidades.ibge.gov.br/brasil/pa/igarape-miri/panorama

Jardim, M. A. G., Amaral, D. D., Santos, G. C. D., Medeiros, T. D. S., Silva, C. A. D., Francez, D. D. C., & Costa Neto, S. V. (2004). *Análise florística e estrutural para avaliação da fragmentação nas florestas de várzea do estuário amazônico*. Museu Paraense Emilio Goeldi.

Lima, A. M. T., Ataídes, A. G. De., Souza, E. P. B. De., Seibert, C. S., & Mucari, T. (2018). Área de (des) proteção ambiental Serra do Lajedo-TO: Degradação ambiental identificada por análise de cobertura vegetal e crimes registrados no período de 2001 a. *Gaia Scientia, 12*(1), 259-272. https://doi.org/10.22478/ufpb.1981-1268.2018v12n1.32877

Lôbo, D., Leão, T., Melo, F. P. L., Santos, A. M. M., & Tabarelli, M. (2011). Forest fragmentation drives Atlantic forest of northeastern Brazil to biotic homogenization. *Divers. Distrib., 17*, 287-296. https://doi.org/10.1111/j.1472-4642.2010.00739.x

Loch, C. (1993). *Noções básicas para interpretação de imagens aéreas, bem como algumas de suas aplicações nos campos profissionais* (3rd ed., p. 120). Florianópolis, UFSC.

Lopes, E. L. N., Fernandes, A. R., Grimaldi, C., Ruivo, M. D. L. P., Rodrigues, T. E., & Sarrazin, M. (2006). Características químicas de um Gleissolo sob diferentes sistemas de uso, nas margens do rio Guamá, Belém, Pará. *Bol. Mus. Para. Emílio Goeldi. Ciências Naturais*. https://doi.org/10.5123/S1981-81142006000100009

Lopes, M. R., Nogueira, A. V. M., Lopes, B. M., Xavier, B. H. M., Coelho, N. C. O., Mata, E. L. O., & Lopes, E. L. N. (2019). Caracterização físico-química de gleissolo de várzea da região do baixoTocantins, PA. *Revista Craibeiras de Agroecologia, 4*(SP), e8953.

Matsukuma, C. K. (2002). *Análise comparativa de algoritmos de classificação digital não-supervisionada, no mapeamento do uso e cobertura do solo* (Tese de Doutorado, Universidade de São Paulo, Brazil).

Meneses, P. R., & Almeida, De T. (2012). *Introdução ao processamento de imagens de sensoriamento remoto UNB, Brasília* (p. 276).

Morariu, O., Burescu, F., Herbei, P. E. M. V., & Chis, P. C. (2018). Agricultural land cover classification using Rapideye satellite imagery. *Journal of Young Scientist, 6*.

Moreira, M. A. (2003). Fundamentos do sensoriamento remoto e metodologias de aplicação. 2ed. Viçosa: UFV.

Nogueira, O. L. (1997). *Regeneração, manejo e exploração de açaizais nativos de várzea do estuário amazônico* (Tese Apresentada ao Curso de Pós-graduação em Ciências Biológicas do Centro de Ciências Biológicas da Universidade Federal do Pará, do Museu Paraense Emílio Goeldi e da Empresa Brasileira de Pesquisa Agropecuária).

Olofsson, P., Foody, G. M., Herold, M., Stehman, S. V., Woodcock, C. E., & Wulder, M. A. (2014). Good practices for estimating area and assessing accuracy of land change. *Remote Sensing of Environment, 148*, 42-57. https://doi.org/10.1016/j.rse.2014.02.015

Rodrigues, T. E., Silva, J. M. L. Da, Silva, B. N. R. Da, Valente, M. A., Gama, J. R. N. F., Santos, E. S. Dos, … Ponte, F. C. Da. (2005). *Caracterização e classificação dos solos do município de Tailândia, estado do Pará* (p. 55). Belém, PA: Embrapa Amazônia Oriental.

Rodríguez-Echeverry, J., Fuentes, R., Leiton, M., & Jaque, E. (2018). Changing Landscapes Forest: Implications for its Conservation. *Environment and Natural Resources Research, 8*(3), 44. https://doi.org/10.5539/enrr.v8n3p44

Salviano, A. A. C., Vieira, S. R., & Sparovek, G. (1998). Variabilidade espacial de atributos de solo e de Crotalaria juncea L. em área severamente erodida. *Revista Brasileira de Ciência do Solo, 22*(1), 115-122. https://doi.org/10.1590/s0100-06831998000100016

Santos Júnior, H. B., Batista Júnior, W. F., & Jardim, M. A. G. (2015). Formas tradicionais no manejo de palmeiras como alternativas de conservação ambiental. 9º Congresso Brasileiro de Agroecologia, Belém. *Cadernos de Agroecologia, 10*, 54-59.

Silva, A., Brito, A., Mendes, F., Chagas, H., Silva, T., & Coelho, R. (2018). Os ribeirinhos e os Sistemas agroflorestais agroecológicos na ilha Mamangal, Igarapé-Miri-Pará. *Cadernos de Agroecologia, 13*(1).

Swain, P. H., & Davis, S. M. (1978). *Remote Sensing: The Quantitative Approach* (p. 375). McGraw Hill Book Company, New York, NY.

Tagore, M. D. P. B., Do Canto, O., & Sobrinho, M. V. (2018). Políticas públicas e riscos ambientais em áreas de várzea na Amazônia: O caso do PRONAF para produção do açaí. *Desenvolvimento e Meio Ambiente, 45*. https://doi.org/10.5380/dma.v45i0.51585

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). *Manual de Métodos de Análise de Solos* (3rd ed., p. 573). Revista e Ampliada, Brasília: Embrapa.

Temba, P. (2000). *Fundamentos da fotogrametria*. Belo Horizonte: Departamento de Cartografia, Universidade Federal de Minas Gerais.

Tregidgo, D., Campbell, A. J., Rivero, S., Freitas, M. A. B., & Almeida, O. (2020). Vulnerability of the Açaí Palm to Climate Change. *Human Ecology, 48*(4), 505-514. https://doi.org/10.1007/s10745-020-00172-2

Van Breemen, N., & Buurman, P. (2002). Hydromorphic soils. *Soil Formation* (2nd ed.). New York: Kluwer Academic Publishers. https://doi.org/10.1007/0-306-48163-4

Vasconcelos, M. A. D., Oliveira, M. A. F. D., Guimarães, D. F. D. S., & Pereira, H. D. S. (2016). As transformações sazonais da paisagem na Ilha do Careiro e suas consequências socioambientais. *Revista Amazônica de Ensino de Ciências*.

Veloso, H. P., Rangel Filho, A. L. R., & Lima, J. C. A. (1991). *Classificação da vegetação brasileira, adaptada a um sistema universal*. IBGE, Rio de Janeiro.

Verma, A. K., Garg, P. K., & Prasad, K. H. (2017). Sugarcane crop identification from LISS IV data using ISODATA, MLC, and indices based decision tree approach. *Arabian Journal of Geosciences, 10*(1), 16. https://doi.org/10.1007/s12517-016-2815-x

Walkley, A., & Black, I. A. (1934). An examination of the degtyareff method for determining soil organic matter and a proposed modification of the cromic acid titration method. *Soil Science, 37*, 29-38. https://doi.org/10.1097/00010694-193401000-00003

Wang, Y., Chen, F., Zhang, M., Chen, S., Tan, X., Liu, M., & Hu, Z. (2018). The effects of the reverse seasonal flooding on soil texture within the hydro-fluctuation belt in the Three Gorges reservoir, China. *Journal of Soils and Sediments, 18*(1), 109-115. https://doi.org/10.1007/s11368-017-1725-1

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