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Ethnobotanical Studies of Medicinal Plants used to Treat Human and Livestock Ailments in Southern Nations, Nationalities and Peoples' Region, Ethiopia: A Systematic Review

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Abstract

Like many other parts of Ethiopia, people in the Southern Nations, Nationalities and Peoples' Region (SNNPR) do have indigenous knowledge on the preparation and use of traditional medicinal plants. Even though different studies have been conducted to document medicinal plants in different zones of SNNPR separately, there is no previous review work which summarizes the medicinal plants and the associated indigenous knowledge at the regional level (at SNNPR region as a whole or in large scale). Also, there is no previous review work that prioritizes the factors that affect medicinal plants at the regional level (including threatened medicinal plants). The purpose of this paper was to review habitat, growth forms, the method of remedy preparation and administration, marketability of medicinal plants, and to prioritize the factors that affect medicinal plants in SNNPR. Most of the medicinal plants in the majority of the reviewed areas are harvested from wild. Herbs are the most utilized life forms and leaves are the most utilized plant part in the preparation of remedies. Fresh plant materials are the most employed in the preparation of remedies. Majority of medicinal plants are not marketable. Agricultural land expansion is a major threat to medicinal plants which followed by deforestation. *Olea europaea* subsp. c*uspidata*, *Prunus africana*, *Echinops kebericho*, *Croton macrostachys*, *Cordia africana* and *Dodonaea angustifolia*, *Hagenia abyssinica*, *Withania somnifera* and *Ficus spp* are the highly affected medicinal plant species which require conservation and management priority in the region.

Keywords: conservation, growth form, habitat, indigenous knowledge, management medicinal plants, remedy, threat

1. Introduction

Starting from early times plants have been important sources of both defensive and curative traditional medicine preparations for both human beings and livestock. Native people around the world have exceptional knowledge of plant resources on which they depend for medicine and other uses (Martin, 1995). According to Farnsworth (1994), much of an indigenous knowledge system from the earliest times is found linked with the use of traditional medicine in different countries.

Ethiopia is a country characterized by a wide range of ecological, edaphic, and climatic conditions. As a result of its wide range of ecological, edaphic and climate diversity, is an important regional center for biological diversity (Kelbessa et al., 1992; Teketay, 2001; Friis et al., 2011) and possesses a wide range of potentially useful medicinal plants, more extensive indeed than available in many other parts of the world (Abebe, 1986; Pankhurst, 2001; Yirga, 2010). This wide potential has been made reachable by a rich biodiversity and an ancient indigenous knowledge on the use of plants in traditional medicine (Balemie et al., 2004), on which 80% of the rural communities in the country depend (Addis et al., 2001; Bekalo et al., 2009; Birhan et al., 2018). Even in cities where modern health services are more available and specialized; numerous people still continue to go to traditional healers for their primary healthcare requirements (Lambert, 2001; Mesfin et al., 2009). Incomplete coverage of the modern medical system, lack of pharmaceuticals, poor staffing and expensive prices of modern drugs (Zerabruk and Yirga, 2012; Hishe and Asfaw, 2015) can be reasons why the majority of Ethiopians still

depend on traditional medicine. Since the knowledge and use of plants for medicinal purposes is an essential part of many ethnic rural cultures in Ethiopia, cultural acceptability (Bekele, 2007; Bekalo et al., 2009) and efficiency of traditional medicines against certain types of disease as compared to modern medicine can be the other reasons(Omoruyi et al., 2012).

Popular knowledge of plants used by humans is based on thousands of years of experience, careful observations and trial and error experiments (Martin, 1995). By "trial and error", people learned how to recognize and use plants, including those with a magic-religious function (Camejo-Rodrigues et al., 2003). Almost all traditional medical preparations in Ethiopia (95%) are of plant origin (Wubetu et al., 2018). Medicinal plants are the basis for the development of new drugs and the survival of mankind as well as their livestock.

The knowledge and use of plants for medicinal purposes is an integral part of many ethnic rural cultures in Ethiopia (Bekalo et al. 2009). Due to the existence of diverse cultures, beliefs and languages, the expectation of a wide variety of traditional knowledge and uses of medicinal plant species in the country are high.

The ancient knowledge concerning the use of traditional medicinal plants is maintained by traditional healers. In the rural part of Ethiopia, which is much of the knowledge on traditional medicinal plants in most cases available, either the knowledge from herbalists is passed mysteriously from one generation to the next orally (Jansen, 1981) or their descendants inherit the medico-spiritual manuscripts (Tilahun and Giday, 2007).

1.1 Statement of the Problem

Ethiopia is believed to be home for about 6500 species of higher plants, with approximately 12% endemic (UNEP, 1995; Bekele, 2007; Mesfin et al., 2014). Medicinal plants comprise one of the important components of the vegetation. They play a key role in the development and advancement of modern studies by serving as a starting point for the development of innovations in drugs (Balick and Cox, 1996). According to Tora and Heliso (2017), there are 600 species of medicinal plants constituting a little over 10 percent of Ethiopia's vascular flora. As compared to the other areas, the greater concentration of medicinal plants is found in the south and southwestern parts of the country following the presence of more biological and cultural diversity (Belayneh et al. 2012). However, irrespective of their great concentration in the past these plants are currently facing a problem of continuity and sustainability. The current loss of medicinal plants can be due to lack of proper documentation of indigenous knowledge on medicinal plants (Lulekal et al., 2008; Giday et al., 2009; Regassa, 2013), increase of modern education, which has made the younger generation to undervalue the local indigenous knowledge on medicinal plants (potential acculturation) (Zerabruk and Yirga, 2012; Lulekal et al., 2008). According to Bekele (2007), the issue of medicinal plant conservation in Ethiopia calls for study and documentation before the accelerated ecological and cultural transformation distort the physical entities and the associated knowledge base. Like many other parts of Ethiopia, people in the Southern Nations, Nationalities and Peoples' Region (SNNPR) do have indigenous knowledge on the preparation and use of traditional medicinal plants. Even though different studies have been conducted to document medicinal plants in different areas of SNNPR separately, there is no previous review work which summarizes the medicinal plants and the associated indigenous knowledge at the regional level (at SNNPR region as a whole or in large scale). Also, there is no previous review work that prioritizes the factors that affect medicinal plants at the regional level (including threatened medicinal plants).

Prioritizing the factors that affect medicinal plants and identifying threatened medicinal plants could be useful in giving conservation and management priority for identified threatened medicinal plant species (Mesfin et al., 2014).

1.2 The Purpose of the Article

- ✓ To review growth forms, habitat, method of remedy preparation and administration and marketability of medicinal plants in SNNPR
- ✓ To prioritize the factors that affect medicinal plants in most areas (in most reviewed areas) of SNNPR
- ✓ To identify plant species those require special attention in future conservation and management efforts in the region.

2. Growth Forms

The majority of medicinal plants used in Amaro (Mesfin et al., 2014), Bench (Giday et al., 2009), Cheha (Bizuayehu and Assefa, 2017), Dawro (Andarge et al., 2015), Kembatta (Maryo et al., 2015), South Omo (Tolossa et al., 2013), Mirab-Badwacho (Temam and Dillo, 2016) and Lowlands of Konta (Bekalo et al., 2009) areas of SNNPR are herbs (see Figure 1A). The high use of herbs as medicinal plants as compared to the other growth forms in these areas can be due their better abundance (availability) (Giday et al., 2009; Andarge et al.,

2015). It may also due to the higher chance of obtaining pharmacologically active compounds (like alkaloids and flavonoids) in herbs as compared to woody plant forms (Thomas et al., 2009). In addition to availability and a chance of obtaining pharmacologically active compounds, socio-cultural beliefs and practices of the healers may contribute to the high use of herbs. The common use of herbs is also reported in studies carried out elsewhere in Ethiopia (Yineger et al., 2007; Teklehaymanot et al., 2007; Giday et al., 2003) and other parts of the world (Tabuti et al., 2003; Muthu et al., 2006).

The trend of using more of herbaceous plants in most areas SNNPR can be seen as a benefit because it is easier to cultivate herbs when they are in short supply and availability of more herbaceous plant species as compared to trees (Bekalo et al., 2009).

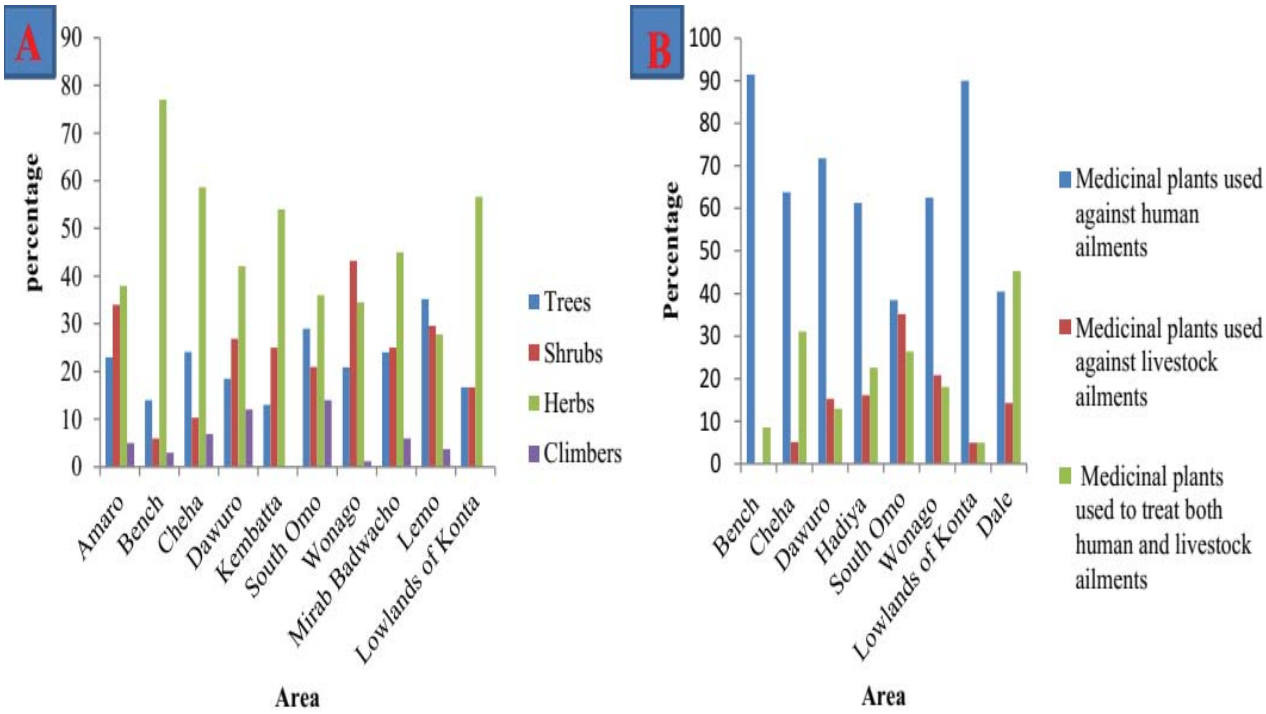

Figure 1. (A) Growth forms of medicinal plants (Mesfin et al., 2014; Giday et al., 2009; Bizuayehu and Assefa, 2017; Andarge et al., 2015; Maryo et al., 2015; Tolossa et al., 2013; Mesfin et al., 2009; Temam and Dillo, 2016; Kebebew and Mohamed, 2017; Bekalo et al., 2009), (B) Proportion of medicinal plants used to treat human, livestock and both human and livestock ailments in the reviewed areas of SNNPR (Giday et al., 2009; Bizuayehu and Assefa, 2017; Andarge et al., 2015; Agisho et al., 2014; Tolossa et al., 2013; Mesfin et al., 2009; Bekalo et al., 2009; Kewessa et al., 2015)

3. Habitat (Degree of Management)

The bulk of medicinal plants in most of the reviewed areas of SNNPR (Amaro, Bench, Dawro, Kembatta, Wonago, Mirab Badwacho and Lowlands of Konta) with the exceptions of Cheha and Lemo, are harvested from wild (see Figure 2). This implies that the majority of plants of medical importance are not yet cultivated by traditional healers. Thus, the high dependence on medicinal plants which are harvested from the wild habitats may have a considerable impact on the future availability of these resources and it will likely account for their vulnerability to being overexploited. Whereas, the exceptional case in the two areas (Cheha and Lemo) showed that the practitioners (traditional healers) have enhanced attitude on the importance of ex-situ conservation (since the society in these two areas depending on the home-garden source or cultivated rather than wild or natural environment to obtain the medicinal plants) in having long-lasting advantage on the sustainability of medicinal plants.

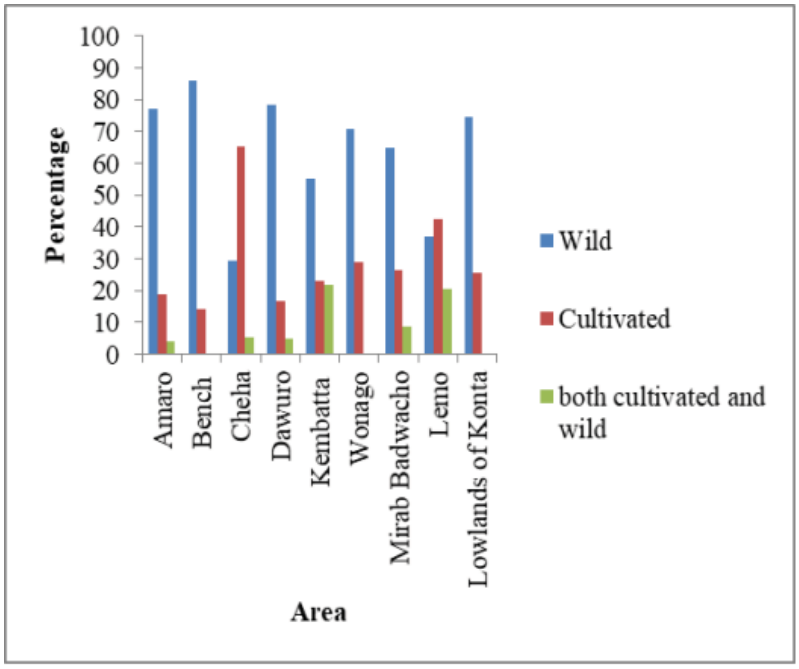

Figure 2. Habitat (degree of management) of medicinal plants (Andarge et al., 2015; Bekalo et al., 2009; Bizuayehu and Assefa, 2017; Giday et al., 2009; Kebebew and Mohamed, 2017; Maryo et al., 2015; Mesfin et al., 2009; Mesfin et al., 2014;Temam and Dillo, 2016)

4. Ailments Treated

In SNNPR, the reported medicinal plant species were used to treat ailments of human, livestock or both. However, a majority of the reported medicinal plants were used to treat human ailments only (see Figure 1B).

5. Remedy Preparation

5.1 Plant Parts Used in the Preparation of Remedies

Leaves, seeds, roots, flowers, stem bark, and fruits are the identified plant parts that are used in the preparation of remedies. However, leaves are the majorly used plant part in preparation of remedies in most of the reviewed areas (Dawuro (Andarge et al., 2015), Bench Maji (Giday et al., 2009), Cheha (Bizuayehu and Assefa, 2017), Kembatta (Maryo et al., 2015), Amaro (Mesfin et al., 2014), Lemo (Kebebew and Mohamed, 2017), Lowlands of Konta (Bekalo et al., 2009), Mirab-Badwacho (Temam and Dillo, 2016) and Wolaita (Tora and Heliso, 2017)) of SNNPR.

Relative easiness of finding because of better accessibility during field collection (Giday et al., 2009), easiness of preparation (Gazzaneo et al. 2005) as well as effectiveness due to bioactive components (Chekole, 2017; Mesfin et al., 2014) can be the reasons for a major utilization of leaves as compared to other plant parts, in the preparation of remedies in most of the areas of SNNPR.

Use of leaves (appreciable amount of leaves) in the preparation of remedies in most of the areas can be considered as an opportunity for the long life of the plant. Since it may reduce the chance of plant diversity declination (plants in which their leaves are harvested have a low level of wilting unlike that of root and stem harvest) (Andarge et al., 2015; Bizuayehu and Assefa, 2017). Thus, it can be said that areas in which plant roots were primarily used as a major plant part in the preparation of remedies are in question of sustainable use of medicinal plant species.

5.2 The Condition of Plant Materials Used in the Preparation of Remedies

Like in many other parts of Ethiopia, fresh and dry plant materials are used in the preparation of remedies in the SNNPR region. However, fresh (newly harvested) plant materials are the most utilized plant materials in the preparation of remedies in the region (Giday et al., 2009; Mesfin et al., 2014; Kewessa et al., 2015; Andarge et al., 2015; Maryo et al., 2015; Bekalo et al., 2009). Availability of plant materials in the people vicinity that can be picked at any time, an attempt not to lose volatile oils, the concentrations of which might deteriorate when dry (Mesfin et al., 2014), can be the reasons for fresh materials are majorly utilized in the remedy preparation. Also,

it can be due to believing that fresh preparations are effective in healing ailments (Maryo et al., 2015).

5.3 Method of Remedy Preparation and Mode of Administration

Decoction (Giday et al., 2009; Mesfin et al., 2014; Agisho et al., 2014; Maryo et al., 2015; Bekalo et al., 2009; Mesfin et al., 2009; Bizuayehu and Assefa, 2017),concoction (Mesfin et al., 2014; Bizuayehu and Assefa, 2017; Agisho et al., 2014; Bekalo et al., 2009; Tolossa et al., 2013; Mesfin et al., 2009), powder (Giday et al., 2009; Bizuayehu and Assefa, 2017; Mesfin et al., 2009; Maryo et al., 2015), infusion and juice (Giday et al., 2009; Mesfin et al., 2014),crushing (Mesfin et al., 2014; Bizuayehu and Assefa, 2017; Kewessa et al., 2015; Agisho et al., 2014; Maryo et al., 2015; Bekalo et al., 2009; Mesfin et al., 2009), paste (Giday et al., 2009; Mesfin et al., 2014; Tolossa et al., 2013), boiling (Kewessa et al., 2015), squeezing (Kewessa et al., 2015; Maryo et al., 2015), burning (Kewessa et al., 2015), filtrate (a liquid from which insoluble impurities have been removed)(Tolossa et al., 2013) and unprocessed are the identified methods of remedy preparation in SNNPR region.

In the preparation of remedies, medicinal plants can be used with or without the use of diluents (Giday et al., 2009). Local drinks (Tolossa et al., 2013), water, boiled coffee, milk**,** human saliva (Giday et al., 2009) and butter (Maryo et al., 2015) are the identified diluents that are used in the preparation of remedies in the region.

Different criteria can be used to select diluents that can be used in the preparation of remedy for a particular ailment. According to Giday et al. (2009), availability is one of the criteria that healers may use in the selection of diluents. Diluents may serve as a vehicle to administer the remedies (Etana, 2010) and may also enhance the efficacy and healing conditions.

Applications through mouth (orally), ear (auricular), nose, (nasally), eyes (trough eyes) and skin are the identified routes of administration of traditional medicines in SNNPR region. Selection of the route of administration of traditional medicines may be dependent on the ailment being treated.

According to Giday et al. (2009), the dominance of one route of administration on the others can be an indication for the high occurrence of certain ailments in an area (for example in an area if most remedies are applied topically on the skin it can be an indication to the high occurrence of skin-related ailments in that area).

6. Marketability

The majority of the medicinal plant species used in the traditional medicines were not available for sale at local markets in SNNPR (Giday et al., 2009; Maryo et al., 2015; Bekalo et al., 2009). The harvest of medicinal plants from the immediate environment in which medicinal plant species are abundantly found can be why most medicinal plants were not available for sale at the local markets (Giday et al., 2009; Mesfin et al., 2014). Among those which are available for sale in the local markets, some of them are marketed merely for their medicinal value and the rest for other use values (e.g. foods or spices).

Twelve medicinal plant species are identified and sold in the market for their medicinal value: *Acacia abyssinica* Hochst., *Artemisia afra* Jacq.ex willd*., Aframomum corrorima* (Braun) Jansen*.*, *Amaranthus caudatus seed* L*., Artemisia absinthium* L*., Brassica juncea, Echinops kebericho* Mesfin*., Hagenia abyssinica* (Bruce) J.F.Gmelin*, Ipomoea batatas* (L.) Lam*., Lepidium sativum* L*., Ocimum basilicum var. thyrsiflorum* (L.) Benth*., and Securidaca longipedunculata* Fresen.. On the other hand, *Allium cepa* L., *Allium sativum* L., *Carica papaya* L., *Citrus aurantiifolia* (Christm.) Swingle, *Cymbopogon citratus* (DC.) Stapf., *Nigella sativa* L*., Plectranthus ornatus* Codd, and *Ruta chalepensis* L. are the medicinal plant species that are marketed for other use-values (as spices or fruits).

7. Threats to Medicinal Plants

A threat is a status or the position of a species which determines whether it will survive or be extinct in the future (Bizuayehu and Assefa, 2017). Various human-induced and natural factors can threaten the survival of many medicinal plant species. However, the degree of threats varies from place to place and species to species (Agisho et al., 2014).

In the reviewed areas, numerous threats to medicinal plant species are identified by different researchers (Bekalo et al., 2009; Mesfin et al., 2009, Agisho et al., 2014; Mesfin et al., 2014; Kewessa et al., 2015; Maryo et al., 2015; Bizuayehu and Assefa, 2017). According to the result of this review, the major threat to medicinal plants in the reviewed areas of SNPPR is agricultural land expansion (it is mentioned as the first threat to medicinal plants in four areas out of eight) (see Table 1) followed by deforestation. Other threats claimed to medicinal plants in the areas include deforestation, fire, firewood, charcoal production, overgrazing, drought, trade, climate change, urbanization, construction, overutilization, timber production, and home use.

Olea europaea L. subsp*. cuspidata* (Mesfin et al., 2014)*, Prunus Africana* (Hook.f.) Kalkm. (Giday et al., 2009)*,*

Echinops kebericho Mesfin, *Croton macrostachys* Del., *Cordia Africana* Lam., *Dodonaea angustifolia* L.f., *Hagenia abyssinica* (Bruce) J.F.Gmelin, *Withania somnifera* (L.) Dunal (Bizuayehu and Assefa, 2017) *and Ficus spp* (Kebebew and Mohamed, 2017) are the identified medicinal plant species which are highly affected by the threats.

Table 1. Threats to medicinal plants in SNNPR

Area	Df	Ae	F	Fw	Cp	Fw&Cp	Og	D	Tr	Cc	Urb	Con	Ou	Tp	Hu	Source
Amaro	1	2	3			4	5	6								Mesfn et al., 2014
Cheha	2	1				2							4			Bizuayehu and Assefa, 2017
Hadiya		1		2	3		5	7	6						4	Agisho et al., 2014
Kembatta	1	2		6			3			5			4			Maryo et al., 2015
Wonago		1		2			3	4			5	6				Mesfin et al., 2009
Dale		1		5				4				2		3		Kewessa et al., 2015
Lowlands of Konta			2	1	4							3				Bekalo et al., 2009

Where, Df= Deforestation, Ae= Agricultural land expansion, F=Fire, Fw= Firewood, Cp= Charcoal production, Og= Overgrazing, D= Drought, Tr= Trade, Cc= Climate change, Urb=Urbanization, Con= Construction, Ou= Overutilization, Tp= Timber production, Hu= Home use

1= First threat, 2= second threat, 3= Third threat, 4= Fourth threat, 5 = Fifth threat, 6= Sixth threat, 7= Seventh threat

8. Medicinal Plant Knowledge Secrecy and Way of Transfer

The secrecy of traditional medical practice is a common phenomenon in the SNNPR. (Andarge et al., 2015; Giday et al., 2009; Agisho et al., 2014; Mesfin et al., 2014; Tora and Heliso, 2017; Mesfin et al., 2009). This secrecy is also found in other parts of the country and worldwide (Yineger and Yewhalaw, 2007). The presence of a strong belief that medicinal plants will lose their healing power or die if other people know them (Agisho et al., 2014) and the fear of losing societal recognition and reputation which traditional healers have earned due to their knowledge (Mesfin et al., 2014) can be the major reasons why traditional healers kept their knowledge secret on the use of medicinal plants.

Traditional healers in SNNPR transfer their indigenous knowledge orally to the family members (to selected family members to keep up the secrecy) usually to their sons (Giday et al., 2009; Mesfn et al., 2014; Maryo et al., 2015; Tora and Heliso, 2017).

9. Conclusions

This review shows that herbs are the most utilized life forms in the preparation of remedies in SNNPR. This trend of using more of herbaceous plants can be seen as a benefit due to ease of cultivating herbs when they are in short supply and due to their better availability as compared to the other growth forms. Utilization of leaves in most areas of the region as a major consumable medicinal plant part should be kept because it may reduce the chance of risk of depletion of medicinal plant in the region. Harvest of considerable amounts of plants from wild in most areas may introduce a risk of reduction in the wild. So people and other responsible stakeholders should work on a better achievement of ex situ conservation. This will help the society to develop the habit of cultivating plant resources which ensure medicinal plants and indigenous knowledge continuity for future generations. Majority of the reported medicinal plants were used to treat human ailments only. Thus, further studies that give emphasis on medicinal plants that used to treat livestock ailments should be conducted. People should also develop the habit of drying and storing medicines (for those medicines that can be utilized in the dry and fresh form; i.e. storage may also depend on the shelf-life of the material as well as the availability of proper storage facility) for future uses in order to reduce the risk of threat on medicinal plants. Agricultural land expansion and deforestation are the major threats to medicinal plants that require special attention in SNNPR. *Croton macrostachys, Cordia Africana, Dodonaea angustifolia* , *Echinops kebericho, Ficus spp, H. abyssinica, Olea europaea* subsp. *cuspidata*, *Prunus africana*, and, *Withania somnifera* are plant species which require conservation and management priority in the region.

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Moringa oleifera Lam.: A Biofunctional Edible Plant from India, Phytochemistry and Medicinal Properties

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Abstract

Moringa oleifera is a versatile horticulture tree with important medicinal, nutritional and industrial applications, widely distributed and used in India. The *Moringa* tree originated in India and was introduced to Africa from India and other countries as a health supplement. Almost all parts of the plant have shown nutritional value and are used in India for a variety of food preparations. In India, *M. oleifera* leaves are available in powder to treat mild malnourishment in children. About all parts like leaves, seeds and pods are used as vegetables. Phytochemicals such as flavonoids, tannins, triterpenoids, saponins, flavonoids, anthraquinones, alkaloids and others, are responsible for the medicinal value of this plant. This species is rich in protein, fatty acids, vitamins and minerals that form part of its quality as superfood. It has been reported to have strong antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, diuretic, anthelminthic and antiurolithiatic properties, among others. People in India use this species to treat common illnesses because of its availability and easy preparation. This review provides information on the significant potential of *Moringa* and its nutritional, medicinal, pharmaceutical and industrial values.

Keywords: *Moringa oleifera,* medicinal value, phytochemicals, underutilized nutritional plant

1. Introduction

The genus *Moringa* belongs to the monogeneric family Moringaceae and comprises thirteen species distributed from semi-arid Africa to Asia. Of these, *M. oleifera* Lam. (Figure 1) is the most commonly known species distributed in the northwest India (Mabberley, 2017; Nadkarni, 1976; Ramachandran *et al*., 1980; Jahn 1988; Somali *et al*., 1984; Mughal *et al*., 1999,). The tree ranges in height from 5 to 10m (Morton, 1991). This tree grows rapidly, with recording growth of 6-7 m in areas with rainfall of less than 400 mm per year (Odee, 1998). Although it is wild in NW India, it can be cultivated in different areas, growing at elevations of up to 1,000 m above sea level on pasturelands, river basins or hillsides.

Moringa oleifera is also known with different names including, horseradish tree, ben oil tree, drumstick tree, miracle tree, and “Mother’s Best Friend", Kelor tree (Anwar and Bhanger, 2003, Prabhu *et al*., 2011). This species was introduced to Africa at the beginning of the twentieth century as a health supplement (Muluvi *et al*., 1999). The ben oil seems to show promise for the manufacture of soap with high washing efficiency. This makes it suitable for poor areas where people cannot afford buying these products but they have the plant available for use.

This nutritional plant is little known in the western world despite of being considered one of the world’s most beneficial trees due to the potential use of each part of plant (Table 1) either as fodder, vegetable or medicine in South Asia (Odebiyi and Sofowora, 1999). The leaves, fruit, flower and immature pods (Figure 1) of this tree are commonly used as a nutritious vegetable in several countries, making it a great potential food source in dry season areas where food could be scarce. Countries such as India, Pakistan and Philippines (Gopalakrishnan et al., 2016; Anwar *et al*., 2005; Anwar and Bhanger, 2003; D’souza and Kulkarni.1993) use this species widely. *Moringa* is being used in diverse culinary ways and as a health supplement (McBurney *et al*., 2004; Fahey, 2005; DanMalam *et al*., 2001; Iqbal *et al*., 2006). A leaf powder from the plant can be used for children with malnutrition, pregnant and lactating woman (Price, 1985). For example, In the Philippines, this plant is commonly used to increase the production of milk in lactating women, hence its name of “mother’s best friend”; and it has also been prescribed to patients with anemia (Siddhuraju and Becker, 2003; Estrella *et al*., 2000).

Interestingly, this species is reported to have natural and organic sunscreens with an SPF value of 2 at low concentrations (2-4%), giving a 50% sun protection (Baldisserotto et al., 2018), a potential alternative for use in children in tropical countries.

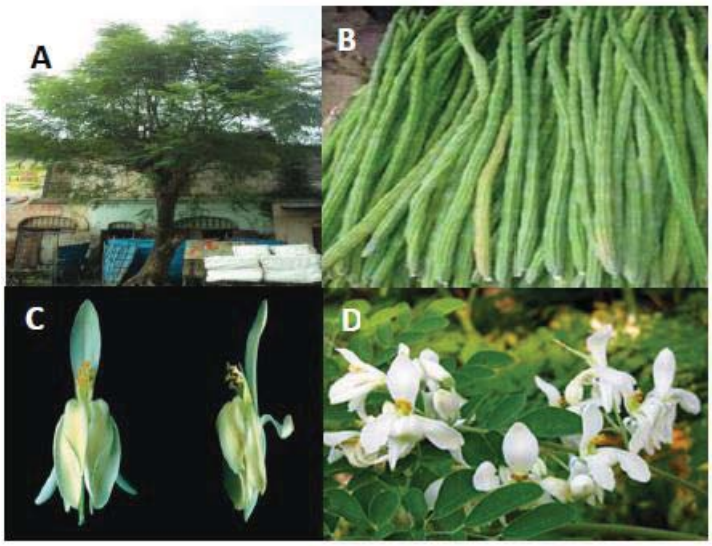


Figure 1. A *Moringa oleifera* tree planted along a street in Buxipur, Gorakhpur (A), green pods (B); frontal and side view of the flowers (C) and flowering branch (D)

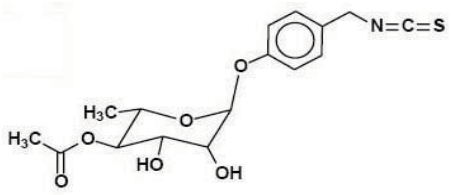
Table 1. Common uses of different parts of *Moringa oleifera* Lam

plant parts	Uses / applications	
	food	medicine
Leaves	Salads, vegetables curries	Powder for treating tumors; poultice for sores, piles, reduce glandular swelling, headaches, or body cleanser, to promote digestion; hypocholesterolemic, juice lowering glucose levels, eye and ear infection
Flower	extracts used for honey preparations	remedy for tumors, infections, muscle diseases, spleen enlargement and to lower cholesterol level
Pods / fruits	Salad, vegetable	treat malnutrition
Seeds	as a snack, oil for salads, cooking, cosmetics, lubricant; water purifying	To treat abdominal tumors and to remove harmful bacteria
Bark	For tanning	Promote digestion
Root	Used as a substitute for horseradish	To treat tumors, promote digestion, antilithic, antifertility and anti-inflammatory. Beneficial against rheumatism, constipation, kidney pain or back pain.

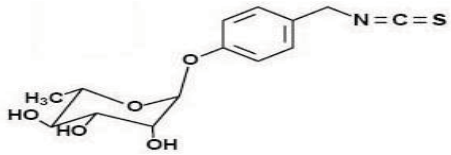
2. Phytochemistry and Chemical Constituents

The Phytochemicals (chemical compounds) responsible for the properties of this species have not been thoroughly studied (Liu, 2004, Brow and Arthur, 2001). Three structural types of phytochemicals of medicinal interest in this species includes, flavonoids, glucosinolates and phenolic compounds (Saleem, 1995; Manguro and Lemmen, 2007; Kasolo *et al*., 2010; Amaglo *et al*., 2010). The phytochemical of leaves of *Moringa* (Figure 2) can vary depending on the growing climate conditions where the plant grows, the method of collection and processing (Coppin, 2008; Mukunzi *et al*., 2011). These include tannins, saponins, alkaloids, flavonoids, anthraquinones, glycosides and steroids (Idris and Adamu, 2018). In addition, other compounds have been isolated from leaves, including different types of glycosides (Faizi *et al*., 1998, 1995, 1994, 1992; Murakami *et al*., 1998; Miean *et al*., 2001; Bennett *et al*., 2003; Wu *et al*., 2003). Leaves also contain carotenoids, tocopherols and vitamin C that can prevent free-radical damage linked to many diseases (Smolin

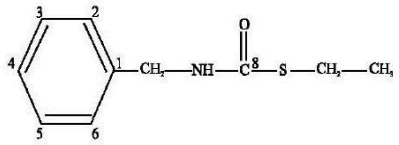
and Grosvenor, 2007). The root bark is rich in two alkaloids: moringine and moringinine.



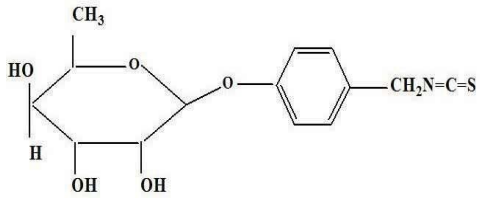
Benzyl isothiocyanate 4-(4'-O-acetyl-Lrhamnopyranosyloxy)



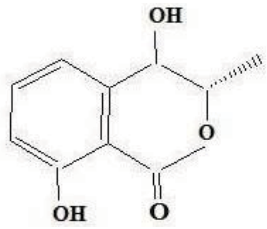
Benzyl isothiocyanate 4-(-L-rhamnopyranosyloxy)



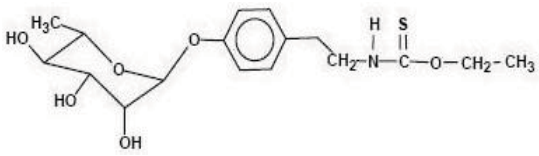
Aglycone of Deoxy- Niaziicine (N-benzyl, S- ethyl thioformate)



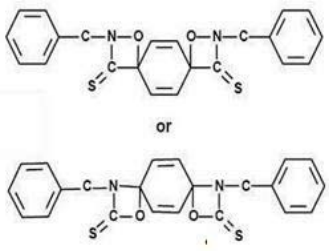
4- (α-L-rhamno Pyroanosyloxy) benzyl isothiacyanate



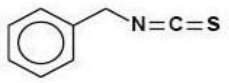
4-hydroxy mullein



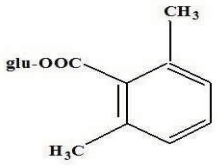
Niazimicin



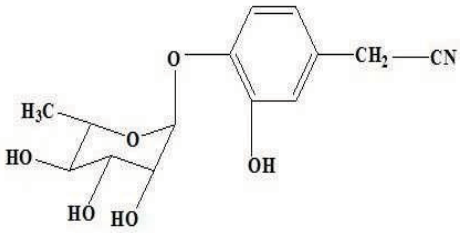
Pterygospermin



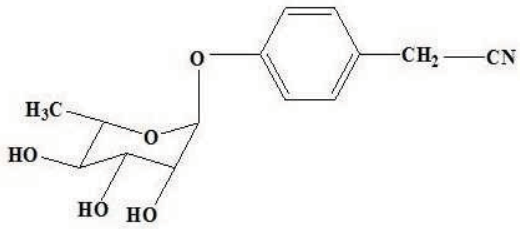
Benzyl isothiocyanate



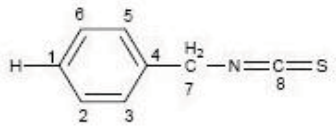
Moringyne



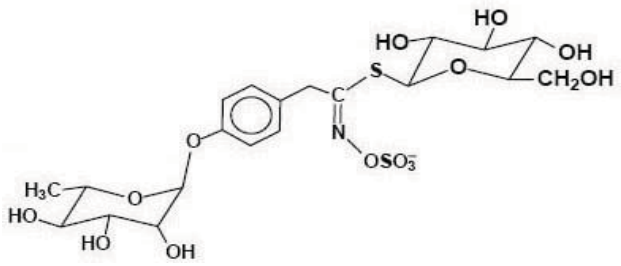
Niaziridin



Niazirin



Isothiocyanato methyl benzene



Benzyl glucosinolate

4(-α -L-rhamnopyranosyloxy)

Figure 2. Structures of some phytochemicals from *Moringa oleifera*

Moringa contains a substantial amount of palmiti, di-oleic, and stearic acids, proteins, saponins, proteins and a variety of vitamins, including Vitamin A, B1, B2, B3, C, nicotinic acid, pyridoxine, ascorbic acid and folic acid (Dahot and Memon 1985). In addition, it is rich in minerals such as iron, calcium, magnesium and phosphorus (Gopalakrishnan et al., 2016). A substantial amount of essential amino acids are found in the pods (Lako *et al*.,

2007) along with tocopherols (Gomez-Coronado *et al*., 2004; Sánchez-Machado *et al*., 2006). All these phytochemicals make *Moringa* a plant of great value.

3. Medicinal Value

Moringa is known in folk medicine in India (Fuglie, 1999.) because of its versatile medicinal properties and it has been used since ancient times by people. It is recognized for its anti-inflammatory, diuretic, abortifacient, antispasmodic, emmenagogue and ecbolic properties and it has been reported to be useful in the treatment of tumors, leukoderma and biliousness etc. (Das et al., 1957, Shaw and Jana, 1982). Interestingly, ethanolic extracts of Moringa leaves seem to have a radioprotection effect against damaged induced by high doses of ionizing radiation (Elwan et al., 2018) showing the versatility and potential uses of this plant.

3.1 Antimicrobial Properties

Virtually all parts of this species have shown antimicrobial properties (Caceres *et al*., 1991; Ali *et al*., 1999; Senthil Kumar and Reetha 2009); this includes leaves, bark, fruit, root and flowers (Khalil 1996; Tsaknis *et al*., 1999). This is important because there is a great need for antimicrobial compound in the medical community due to the development of antibiotic resistance. More studies need to be done to evaluate the use of this species and to isolate the proper compounds that can be use in allopathic medicine.

3.2 Antioxidant Properties

The antioxidant properties of this species is important to prevent of damage to important macromolecules such as DNA, proteins and lipids so cells can function properly (Ryter *et al*., 2007; Limon-Pacheco and Gonsebatt, 2009). The seeds and leaves of *Moringa* are a good source of antioxidants (Chumark *et al*., 2008) with 65.1 % in methanolic extracts and 66.8% in ethanolic extracts (Lalas and Tsaknis, 2002; Siddhuraju and Becker, 2003). Some studies have shown that quercetin and kaempferol seem to be responsible for this antioxidant activity (Bajpai *et al*., 2005; Siddhuraju and Becker, 2003). The qualitative properties of antioxidant in *Moringa* have been highlighted. Sreelatha and Padma (2009) indicated that the antioxidants in both mature and young leaves have a great potential to scavenge free radicals, along with nutritional antioxidants such as Vitamins A, C, and E (Limon-Pacheco and Gonsebatt, 2009). The highest therapeutic value of *M. oleifera* is further substantiated in terms of its high antioxidant level present in leaves, seeds, flowers and pods (Chumark *et al*., 2008; Verma *et al*., 2009; Atawodi *et al*., 2010). Kumar *et al*. (2007) demonstrated these antioxidant properties could be responsible for reduction of the chance of cancer development and other damage to the cell.

3.3 Anti-inflammatory Properties

The pathophysiology of many diseases, such as diabetes, obesity, hypertension and others can involve immunological responses (Rana *et al*., 2007). Leaf extracts from *Moringa* leaf extracts seem to modulate cellular immunity in rats and mice (Sudha *et al*., 2010; Gupta *et al*., 2010). Leaf preparations have shown anti-inflammatory properties in rodent models (Sulaiman *et al*., 2008; Mahajan and Mehta, 2009). Studies suggest that the anti- inflammatory properties are stronger in fruit and seed extracts (Mahajan and Mehta, 2010; Cheenpracha *et al*., 2010; Muangnoi *et al*., 2011).

3.4 Hypocholesterolemic Activity

Moringa leaves contain phytosterols (Jain *et al*., 2010); that seem to be implicated in the reduction of cholesterol uptake by the intestines (Lin *et al*., 2010). It is possible that this could result in a decrease in cholesterol levels and an increase in excreted cholesterol seen in experimental rodents (Mehta *et al*., 2003; Jain *et al*., 2010). The high-fiber content (12% w/w) may also play a role in the hypocholesterolemic effect due to enhanced excretion/gastric emptying (Bortolotti *et al*., 2008; Joshi and Mehta, 2010).

3.5 Anti-asthmatic Activity

The *Moringa* alkaloid moringine closely resembles ephedrine, inducing relaxation of the bronchioles (Kirtikar and Basu, 1975). The seeds and kernels of *Moringa* have been used successfully for the treatment of bronchial asthma, by decreasing the severity of the symptoms, resulting in the improvement of respiratory problems in patients (Agrawal and Mehta, 2008).

3.6 Analgesic Activity

Ethanolic extracts of *Moringa* fruits have shown good analgesic properties when tested in Male Sprague-Dawley rats (Rao *et al*., 2008). The authors used the Ugo Basile 37215 analgesia-meter to evaluate pain and adjuvant-induced arthritis to evaluate anti-inflammatory properties. They found strong analgesic activity and anti-inflammatory properties in this plant. Sutar et al. (2008) used Wister male albino rats and tested pain threshold using the licking or paw/jumping response of the animal and the tail immersion method. The authors

found that *Moringa* seems to have a strong analgesic activity. There is a great potential for this plant to be used by people that may not have access to more expensive analgesics but have the plant available for their use. In addition, extracts of this plant could eventually be commercialized for medicinal uses.

3.7 Antidiabetic Activity

Experiments in Wistar and Goto-Kakizaki (GK) rats with type 2 diabetes show a positive effect of *Moringa* leaves, with a significant decrease in blood glucose levels (Ndong *et al*., 2007). In addition, in high-fat-diet mouse models, the extracts of this plant have shown improved glucose tolerance (Jaja-Chimedza et al., 2018). The lowering blood sugar levels within 3 h after ingestion has been observed (Mittal *et al*., 2007) and is likely to be due to extracts' inhibitory activity against α-Glucosidase enzyme (Natsir et al., 2018). It is possible that polyphenols such as 3-glycoside, kaempferol glycosides and others (Ndong *et al*., 2007) could have an effect in this antidiabetic activity. Studies have shown the antioxidant activity of Moringa can rescue apoptosis of beta cells, therefore preventing damage to these cells, leading to the beneficial properties against diabetes (Al-Malki et al., 2015; Mbikay, 2012; Kaneto et al., 1999). Additional studies may lead to the commercialization of *Moringa* extracts for conventional treatment of diabetes.

4. Conclusion

This review shows that different parts of *Moringa* (leaves, flowers, fruits and seeds) possess a great variety of compounds with high nutritional and medicinal content that could make it an ideal complementary and alternative medicine and/or nutritional supplement. The presence of antimicrobial compounds could lead to the development of alternative treatments against infectious diseases. Research has shown that every part of the plant has some medicinal application that could be further develop to produce more affordable medicines. *M. oleifera* is nutritional and medicinally very active against free radicals formed during oxidative stress due to the strong phytochemical constituents present in virtually all parts of the plant. There is a great potential for the widespread use of this plant.

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Primary and Secondary Igapó Forests in the Peruvian Amazon: Floristics, Physical Structure and the Predictive Value of Soil Bulk Density

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Abstract

Igapó forests are a key part of the Amazon. And so, it is important to know their floristics and physical structure, and how they may be influenced by their soil. The floristics and physical structure of 16 primary [1^o] and secondary [2^o] igapó forest plots in Loreto Province, Peru was described and linear regressions were computed to explore whether soil bulk density could predict structural parameters. In the 1^o forest, Fabaceae, Malvaceae and Rubiaceae were the most common families and *Calycophyllum spruceanum*, *Ceiba samauma*, *Inga* spp., *Cedrela odorata*, *Copaifera reticulata*, *Phytelephas macrocarpa, Guazuma rosea*, and *Piptadenia pteroclada* were the most common species. And as flooding increased, bulk density, stem density, stem size, species richness, Fishers α, basal area and above-ground biomass all decreased. In the 2^o forest, Urticaceae, Rubiaceae and Euphorbiaceae were the most common families and *Cecropia membranacea*, *Sapium glandulosum*, *Pourouma guianensis* and *Byrsonima arthropoda* were the most common species. The number of stems was greatest in the island 2^o forest and lowest in the 1^o forest under water for more than four months, and mean stem size, species richness, Fishers α, basal area and above-ground biomass was lowest in the sandy beach 2^o forest and highest in the 1^o forest under water one to two months. Soil bulk density predicted mean stem size, species richness and Fishers α well, where all three decreased as soils became more sandy. I conclude that as soil becomes less sandy with more clay content there is an increase in forest structural complexity, unpredictable flooding in 2^o forests reduces structure more than the predictable flood pulse 1^o forests receive, and soil bulk density may have a causal role for diversity in igapó forests.

Keywords: island, oxbow lake, river margin, sandy beach, side creek

1. Introduction

The Amazon contains several of the ecosystems most important to life on earth and the relationship between their vegetation and soils are key to understanding them (Baudoin, et al., 2003; Haichar et al., 2008; Lauber et al., 2008). Indeed, many Amazon forests have their own unique soils (Pitman et al., 2001; Tuomisto et al., 2003), for example non-flooded *terra firme* forest is found on clay/loam soils, non-flooded white sand forest is found on soils with a large amount of quartz, and non-flooded palm forest is found on water-logged clay soils (Tuomisto et al., 2003; Myster, 2017). Flooded forests also cover much of the Amazon (Junk, 1989), and include flooding that is due to "black" water defined as nutrient poor forest runoff. Igapó forests occur in those flooded areas (Junk, 1989; Parolin et al., 2004; Honorio, 2006) and an important distinction is between 1^o igapó forests – subject to regular, predictable flood pulses – and 2^o igapó forests – subject to more irregular, unpredictable flooding events (Ferreira, 1997; Ferreria, 2000; Wittman et al., 2010). Both 1^o and 2^o igapó have low diversity and reduced physical structure compared to other Amazon forests (Ruokolainen et al., 2007; Myster, 2009; Myster, 2018a; Myster, 2018b;Myster, 2018c).

Studies have shown that among igapó soil parameters, soil bulk density may be particularly important in determining igapó floristics and physical structure (Haugaasen & Peres, 2006; Wittman et al., 2010). Soil grain sizes in igapó are of utmost importance, because there are floristic differences depending on whether the substrate is sandy or clay (F. Wittmann, pers. comm.), that is soil bulk density being inversely correlated with the ability of the soil to retain water and nutrients (Parker, 2010). Thus, floristic and physical structural patterns that

correspond to gradients of soil bulk density – from sandy soils (with their relatively large particles and higher bulk density) to loam soils (with medium size particles and medium bulk density) to clay soils (with small particles and low bulk density) – could be due to variation in water and nutrient retention capacity.

And so, in order to better understand the floristics and physical structure of both 1° and 2° igapó, and to explore which aspects of that structure (if any) can be predicted by soil bulk density, I expanded on my own past plot samplings of Amazon flooded forests (Myster, 2007; Myster, 2010; Myster, 2015; Myster, 2016; Myster, 2018a; Myster, 2018b) by sampling 16 1° and 2° igapó forest plots in the Peruvian Amazon. I first use that sampling data to compile floristics (species, genera, families) and compute physical structure parameters (stem density, mean stem diameter at breast height [dbh], species richness, Fishers α, stem dispersion pattern, canopy closure, basal area and above-ground biomass) for each of the igapó forests, and then explore if soil bulk density can predict any of these structural parameters.

2. Materials and Methods

2.1 Study Areas

The first study area, Sabalillo Forest Reserve (SFR: 3° 20' 3"S, 72° 18' 6" W: Frederickson et al., 2005; Moreau, 2008: www.projectamazonas.org) was on both sides of the upper Apayacu River, 172 km east of Iquitos, Peru. SFR is within 25,000 preserved acres and is made up of upper Amazon low, seasonally flooded river basins. Alluvial and fluvial Holocene sediments from the eastern slopes of the Andes dominate the soils. The average temperature is 25.2° C and precipitation is 3.3 m per year (Choo et al., 2007). Both 1° and 2° igapó and *terra firme* forest, palm forest and white sand forest are commonly found at SFR. The rainy season is from November and April.

The second study area is the Area de Conservacion Regional Comunal de Tamshiyacu-Tahuayo (ACRCTT: www.perujungle.com: Myster, 2007; Myster, 2010; Myster, 2015b) located in Loreto Province, 80 miles southeast of Iquitos, Peru (~2° S, 75° W) with an elevation of ~100 m above sea level (a.s.l.). The reserve is included in a large Amazon protected area, which has wet lowland tropical rainforest (Holdridge, 1967) of large diversity (Daly & Prance, 1989). The study site has low, seasonally inundated river basins of the upper Amazon, and is named for the white-water rivers (the Tahuayo and the Tamshiyacu) which form large fringing floodplains (Junk, 1989). The substrate is composed alluvial and fluvial Holocene sediments. The average temperature is 26.5° C and rainfall ranges between 2.4 – 3.0 m per year. 1° igapó forests are common and the rainy season occurs from November to April. Common tree species include *Calycophyllum spruceanum*, *Ceiba samauma*, *Inga* spp., *Cedrela odorata*, *Copaifera reticulata*, *Phytelephas macrocarpa, Guazuma rosea*, and *Piptadenia pteroclada* (Puhakka et al., 1992; Myster 2007).

2.2 Field Sampling

In June 2013, my field assistants and I sampled five 2° igapó forests named for their location – island, oxbow lake, river margin, sandy beach and side creek – close to a black-water river at SFR. We set up two plots of 250 m^2 each in each of the five forests, and measured diameter at breast height (dbh) of each tree at least 1 cm dbh within each plot. We took the dbh measurement at the nearest low point where the stem was cylindrical and above the buttresses when present. For each tree, we identified its species or genus using the taxonomic sources Romoleroux et al. (1997) and Gentry (1993). We also used the Universidad Nacional de la Amazonia Peruana herbarium and the Missouri Botanical Garden web site <www.mobot.org>. We collected three soil samples at random locations in each plot by driving a 3-inch diameter ring into a depth of 10 cm and extracting the soil. Back in Iquitos, each soil sample was dried for three 4-minute cycles in a microwave and then weighted. Soil bulk density was then computed for each sample as the weighted soil sample divided by the volume of container, and expressed as g/cm^3.

In June 2001 we sampled two plots of the same size (250 m^2), using the same protocol as above (for both plants and soil), in three 1° igapó which are subject to predictable, annual flooding at the ACRCTT. This design sufficiently samples floristics and physical structure of these forests (data published in Myster, 2007; Myster, 2010; Myster, 2015) and so I reuse that data here to address other questions. One 1° igapó forest was flooded 1-2 month per year (dry), one 1° igapó forest was flooded 3-4 months every year (wet) and one 1° igapó forest was flooded over 4 months per year (very wet). Each tree at least 1 cm dbh was measured and then identified to species or genus using, again, Romoleroux et al. (1997) and Gentry (1993) and the Universidad Nacional de la Amazonia Peruana herbarium and the Missouri Botanical Garden web site <www.mobot.org>. Comparison of a 1° 1 ha plot at ACRCTT with the smaller 1° ACRCTT mentioned earlier has shown that the smaller plots are large enough to sample floristics and physical structure well (Myster, 2016). Three soil samples were taken in each plot of the three 1° forests, using the same protocol as the 2° forests.

2.3 Data Analysis

Floristic tables of family, genus, and species were compiled giving the total number of stems in each family and the stem size structure for each of the secondary forest plots taken together by forest-type, and a complete species list with the number of stems in each of the five 2° forests. These parameters were then computed for each of the eight forest-types (1° igapó under water 1-2 months per year, 1° igapó under water 3-4 months per year, 1° igapó under water more than 4 months per year, 2° igapó island, 2° igapó oxbow lake, 2° igapó river margin, 2° igapó sandy beach, 2° igapó **s**ide creek) sampled from 500 m^2 combined plot area (1) total number of stems and the average dbh, (2) species richness and Fisher's α diversity index (procedure in Rosenzweig, 1995), (3) stem dispersion pattern (random, uniform, clumped) computed by comparing plot data to Poisson and negative binomial distributions using Chi-square analysis and, if clumped, using Green's index to find degree of clumping (Ludwig & Reynolds, 1988), (4) canopy closure using the formula in Buchholz et al. (2004) for tropical trees, (5) total basal area (sum of the basal areas of all individual stems where area = πr^2 and r = the dbh of the individual stem / 2) and (6) above-ground biomass (AGB) using the formula in Nascimento & Laurance (2010) suggested for Amazon tropical trees of these stem sizes.

Finally, linear regression analysis (SAS, 1985) was performed using soil bulk density as the independent variable and six different structural parameters as the dependent variables. Structural parameters from this study and from previously published data collected at ACRCTT were used (Myster, 2007; Myster, 2010; Myster, 2015). Each regression was graphed and expressed as: (1) the Y-intercept of the best-fit regression line, (2) the slope of that line, (3) the amount of variation explained by that line (R^2), and (4) the p-value of the best-fit line.

3. Results

There were a total of 24 plant families found in the five successional plots (Table 1). Urticaceae was the most common family, but the Rubiaceae and Euphorbiaceae were also common. Cannabaceae, Malvaceae, Meliaceae, Picramniaceae and Quiinaceae had only one stem. Every family but Malpighiaceae, Siparunaceae, Rutaceae and Meliaceae showed a monotonic decline, or a reverse J pattern, in stem number as stems get thicker. There were no stems with a dbh greater than 29 cm (Table 1).

Table 1. The family and total stems for the 10 secondary forest plots pooled together. Total stems are then divided into dbh size clases truncated to whole cm.

Family	total	1 < 9 cm	10 < 19 cm	20 < 29 cm	≥30 cm
Urticaceae	42	39	3	0	0
Rubiaceae	26	23	2	1	0
Euphorbiaceae	24	23	1	0	0
Lauraceae	14	14	0	0	0
Fabaceae	13	12	1	0	0
Malpighiaceae	11	9	1	1	0
Apocynaceae	10	10	0	0	0
Violaceae	9	8	1	0	0
Melastomataceae	5	5	0	0	0
Polygonaceae	5	5	0	0	0
Siparunaceae	5	3	1	1	0
Clusiaceae	4	4	0	0	0
Moraceae	4	4	0	0	0
Eeythropalaceae	3	2	1	0	0
Lamiaceae	3	2	1	0	0
Myrsinaceae	3	3	0	0	0
Nyctaginaceae	3	3	0	0	0
Rutaceae	3	0	2	1	0
Ebenaceae	2	2	0	0	0
Cannabaceae	1	1	0	0	0
Malvaceae	1	1	0	0	0
Meliaceae	1	0	1	0	0
Picramniaceae	1	1	0	0	0
Quinaceae	1	1	0		0

The most common species found in the five successional plots were *Cecropia membranacea*, *Sapium glandulosum*, *Pourouma guianensis* and *Byrsonima arthropoda* (Table 2).

Table 2. All species sampled in the 10 secondary forest plots and their number of stems in each forest-type

Species	sandy beach	oxbow lake	side creek	island	river margin
Aegiphila integrifolia	0	0	1	0	2
Alibertia stenantha	0	2	0	3	1
Amphirrhox longifolia	2	0	3	4	0
Aspidosperma excelsum	0	2	0	0	2
Bellucia pentamera	0	0	0	0	1
Byrsonima arthropoda	4	0	2	5	0
Calycophyllum spruceanum	0	2	0	5	0
Cecropia ficifolia	0	0	3	0	2
Cecropia membranacea	9	0	3	8	3
Cedrela fissilis	0	0	0	0	1
Coussarea ampla	0	0	0	1	0
Croton cuneatus	4	0	0	3	0
Diospyros guianensis	0	0	1	0	1
Duroia saccifera	5	0	0	0	2
Endicheria verticillata	0	4	2	0	0
Faramea sessilifolia	0	0	0	2	0
Heisteria spruceana	0	3	0	0	0
Himatanthus bracteatus	3	0	0	0	3
Macrolobium acacifolium	0	5	2	0	0
Miconia spichigeri	0	0	0	4	0
Mollia lepidota	0	0	0	0	1
Neea floribunda	0	3	0	0	0
Perebea longipedunculata	0	0	2	2	0
Picramnia sellowii	0	0	0	1	0
Pleurothyrium parviflorum	2	4	0	0	2
Pourouma guianensis	0	6	4	0	4
Pterocarpus amazonum	0	4	0	0	2
Quiina amazonica	0	0	1	0	0
Randia armata	0	0	1	0	1
Sapium glandulosum	5	0	4	5	3
Siparuna guianensis	0	2	0	0	3
Stachyarrhena spicata	0	0	0	1	0
Stylogyne longifolia	0	0	0	3	0
Trema micrantha	0	0	1	0	0
Triplaris americana	0	0	3	0	2
Vismia macrophylla	4	0	0	0	0
Zanthoxylum spruce	0	3	0	0	0

Sandy beach soil bulk density was greatest and soil bulk density in the forest under water at least four months was the lowest (Table 3). The island plot had the greatest number of stems (47) and the plot in primary forest under water the smallest (18). Sandy beach mean stem size was lowest but the highest in the forest under water only one month. This was also seen for species richness, Fishers α, basal area and AGB (Table 3).

Table 3. Mean soil bulk density and six structural parameters for all eight forests in their 500 m^2 plot. For each primary forest, the number of months under water is also given

	Secondary forests					Primary forests		
	Sandy beach	Oxbow lake	Side creek	Island	River margin	1-2	3-4	>4
Bulk density (g/cm^3)	1.31	1.03	0.91	0.90	0.70	0.68	0.63	0.59
Number of stems	39	40	33	47	36	31	29	18
mean stem size (cm)	2.1	3.0	2.8	3.1	3.2	4.8	3.6	3.5
species richness	9	12	15	14	18	21	18	14
Fishers α	25	32	46	40	55	72	70	60
basal area (m^2)	0.14	0.28	0.21	0.35	0.29	0.54	0.31	0.18
above-ground biomass (Mg)	8.21	8.35	10.12	11.55	12.36	27.14	16.21	13.52

Linear regression analysis was not significant when stem number was the dependent variable (Y-intercept = 18, slope = 0.9, $R^2 = 0.65$, p = 0.08: Figure 1a) but was significant when mean stem size was the dependent variable (Y-intercept = 5.8, slope = -3.1, $R^2 = 0.82$, p = 0.03: Figure 1b).

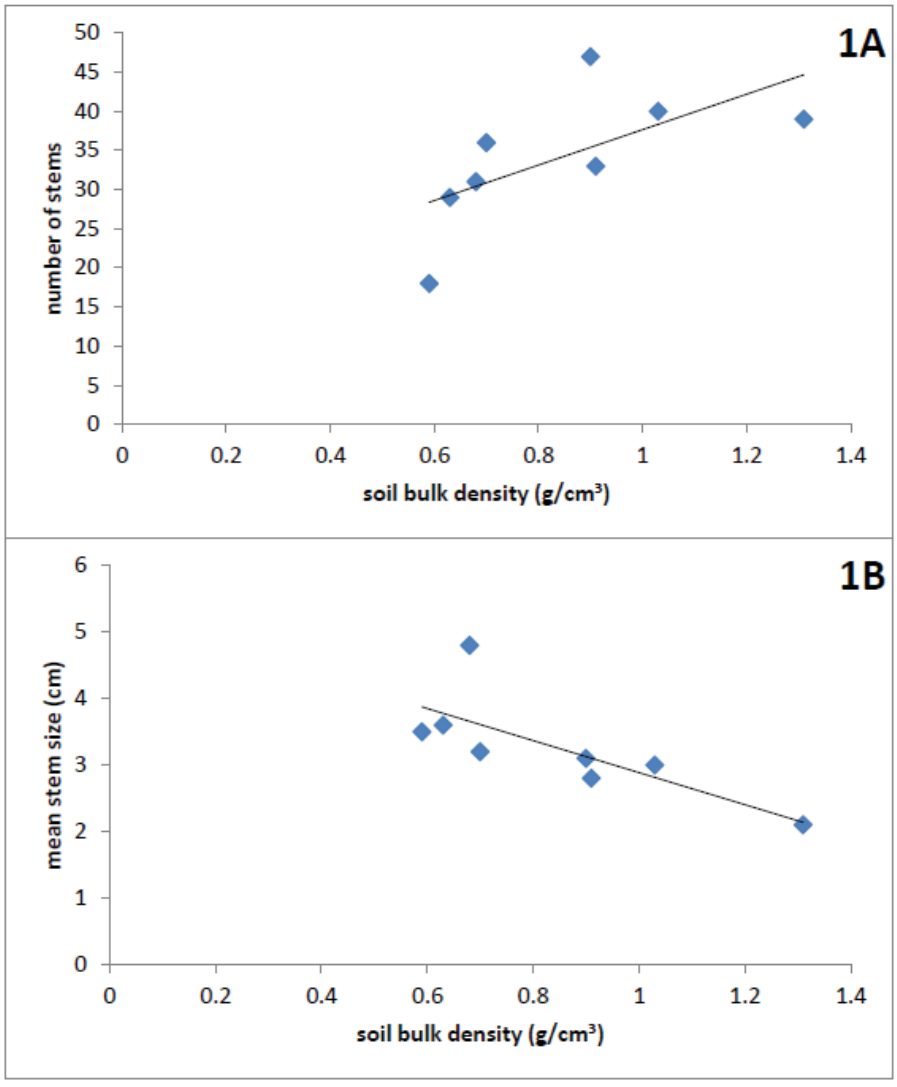

Figure 1. Regression line where (A) number of stems is the dependent variable and soil bulk density is the independent variable, and (B) mean stem size is the dependent variable and soil bulk density is the independent variable

Linear regression analysis was significant when species richness was the dependent variable (Y-intercept = 26.4, slope = -12.2, R^2 = 0.78, p = 0.04: Figure 2a) and significant when Fishers α was the dependent variable (Y-intercept = 91.1, slope = -25.4, R^2 = 0.95, p = 0.01: Figure 2b).

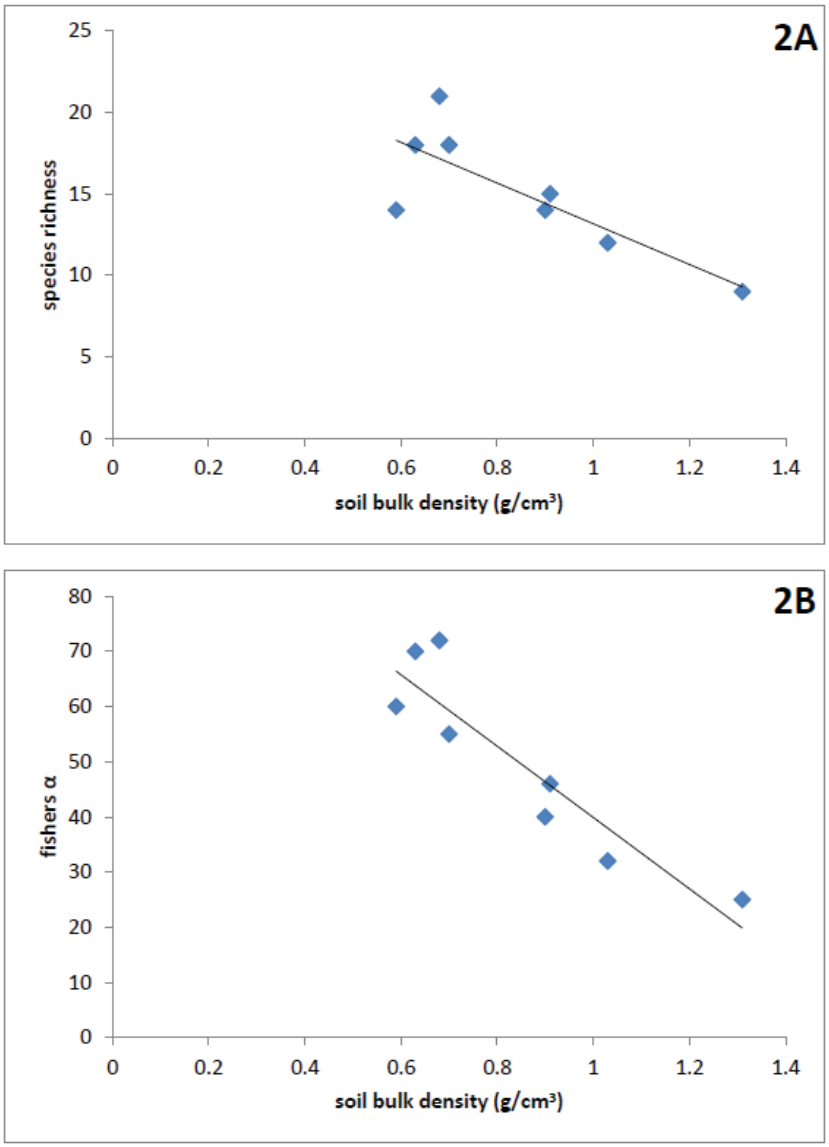

Figure 2. Regression line where (A) species richness is the dependent variable and soil bulk density is the independent variable, and (B) fishers α is the dependent variable and soil bulk density is the independent variable

Linear regression analysis was not significant when basal area was the dependent variable (Y-intercept = 0.49, slope = -0.5, R^2 = 0.42, p = 0.19: Figure 3a) and not significant AGB was the dependent variable (Y-intercept = 28.7, slope = -14.1, R^2 = 0.55, p = 0.12: Figure 3b).

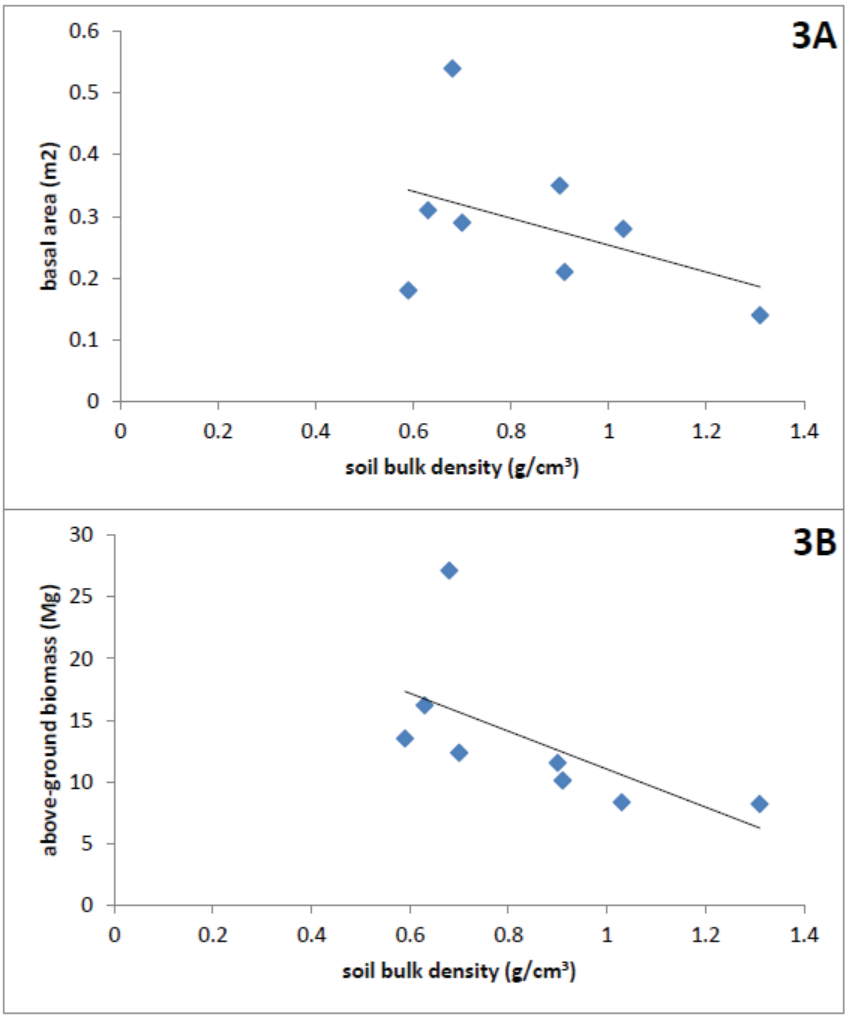

Figure 3. Regression line where (A) basal area is the dependent variable and soil bulk density is the independent variable, and (B) above-ground biomass is the dependent variable and soil bulk density is the independent variable

We see soil bulk density predicted mean stem size, species richness and Fishers α well, and all three decreased as soils became more sandy.

4. Discussion

In general, (1) as soil becomes less sandy with more clay content – i.e. increases in water retention capacity and nutrients – there is an increase in forest community complexity, (2) soil bulk density predicted both diversity indexes well from among the forest structural parameters, (3) the unpredictable flooding seen in 2^{o} forests reduces structure more than the predictable, annual flood pulse that 1^{o} forests receive, and (4) patterns between soil bulk density and igapó diversity – igapó tree diversity decreases as soils became more sandy – suggest that the ability of these soils to retain water and nutrients is important in determining plant diversity in igapó**.**

The 1^{o} forest plots had no species in common with the 2^{o} forest plots but did have three genera: Niconia, Pourouma and Siparuna. The most common families that the 1^{o} forest plots had in common with the 2^{o} forest plots were Fabacae, Rubiacaae and Myriticeae. In the study plots family, genus and species show great similarity with other samplings of similar igapó areas (Ferreira, 1997; Ferreira, 2000) although relative abundances do vary. Gaps within black water forests (Ferreira & Almeida, 2005; Myster, 2007) however, are quite different from the study plots in 2^{o} igapó. Sandy beaches, next to black-water rivers, suffer severe drought stress because of low water retention capacity, and their herbaceous vegetation is not well developed, common in black water river systems (Junk, 2011).

In addition to the structural parameters reported here, I also found in the ACRCTT 1^{o} forests tree stems and

canopy coverage declined as flooding increased, more so than reductions due to tree-fall, trees were clumped only in the gaps for wet forest, and there were smaller stems in gaps compared to all adjacent forests (Myster, 2007; Myster, 2010; Myster, 2015). Flooding was a greater stressor than the tree-fall, but flooding and tree-fall may present plants with similar cues. There were species in common between wet forests and their gaps and between wet and very wet gaps, and tree richness was highest in dry forest and lowest in very wet gaps. There was also a significant effect of tree-fall gap formation on canopy average height, canopy maximum height (height as measured using a tangent height gauge: Myster, 2010; Myster, 2015), basal area, density, aboveground biomass, turnover, and alpha diversity, and a flooding effect on species richness, genera richness, density, turnover, and alpha diversity. There were fewer but larger trees and more production in the forest plots compared with the gap plots, and the very wet plots had fewer trees, species, and genera compared with the other plots. The most turnover was in the very wet forest, and the wet forest had the greatest richness and alpha diversity. There was also support for a "mass" effect because species from both the non-flooded and most flooded forests and their gaps had overlapping ranges in the less flooded forest and gaps, increasing diversity (Myster, 2007; Myster, 2010; Myster, 2015**)**.

These 2^{o} forests gain stems (although smaller in size) but lose richness and diversity compared to the floodplain forests which receive regular, annual flooding from black-water rivers. Flooded forests in the Amazon have a greater number of larger trees, and their stem distribution is more normally distributed (not a monotonic decline in numbers with increasing size) leading to fewer trees, genera, and species as flooding increased (Myster, 2010). Flooding eliminates both vertical and horizontal heterogeneity reducing availability of commonly logged tree species and animal populations. The 1^{o} and 2^{o} igapó forests are between várzea and palm forest in most of the structural parameters (Myster, 2016). Várzea forests, for example, had more stems, larger stems and more basal area compared to igapó forests with the same duration of flooding. The structure in the 1^{o} and 2^{o} igapó forests is less than *terra firme* forests which have similar clay soils, but are not flooded. Finally results show support for the hypothesis that soil bulk density can predict physical structure in several parameters of the secondary black-water forests. While Tuomisto et al. (2002) reported a negative relationship between soil extractable bases and both plant density and species richness, and Ruokolainen et al. (2007) showed that soil differences explained 50% of floristic differences among *terra firme* plots, and geographic distances explained 16%**,** patterns between soil bulk density and igapó diversity suggest that the ability of the soils of both 1^{o} and 2^{o} igapó forests to retain water and nutrients is important in determining biodiversity of these forests.

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Screening Common Bean (*Phaseolus vulgaris* L.) Germplasm for Resistance against Angular Leaf Spot (*Pseudocercospora griseola*) Disease under Field Condition

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Abstract

Angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* is one of the most destructive disease in Latin America and eastern Africa countries. The fungus, *P. griseola* is highly variable and a diverse sources of resistance genes is required to manage this economically important disease. The use of genetic resistance is the most practical and economic way to manage angular leaf spot of the common bean. Common bean (*Phaseolus vulgarise* L.) germplasm were screened for resistance against Angular leaf spot (ALS) under field conditions at Wonodogenet and Areka Research farms. Out of 300 common bean accessions evaluated only 14 (4.6%) common bean accessions were resistant to naturally epidemics of angular leaf spot disease under field condition. Therefore, all common bean germplasm that showed resistance reaction can be involved in breeding program for the improvement of the common bean.

Keywords: screening, angular leaf spot disease, disease reactions

1. Introduction

Common bean (*Phaseolus vagaries* L.) one of the most important pulse crop in terms of nutrition, food security and economy, is cultivated in different regions of Ethiopia (Lemessa and Tessfaye, 2005). Among the most cause of poor yield in common bean production includes fungal, bacterial and viral diseases (Ferreira et al., 2003; Rezene et al., 2018). Angular leaf spot caused by *Pseudocercospora griseola*, may induce up to 40 to 80% yield loss when a susceptible variety is grown in a region with cool to moderate temperatures (13-26 ^{0}C) and abundant moisture (Lemessa et al., 2011).

Several strategies can be used to manage angular leaf spot but the use of genetically resistant common bean cultivars is the most effective controlling measures farmers to adopt (Pastor-Corrales et al., 1998; Schwartz et al., 1999; Wagara et al., 2003). The main drawback to resistant cultivars is the possible breakdown of the resistance caused by adaptation of the pathogen to host resistance (Guzman et al., 1979; McDermott et al., 1993). A number of exotic sources of ALS resistance do exist and have been utilized in breeding programs targeting ALS and the cultivars with sources of resistant includes Mexico 54, MAR1, MAR2, AND277, G5686, G10909 and G10474 (Mahuku et al., 2003; Caixeta et al., 2005). Besides, majority of resistance sources are with a climbing growth pattern; such attributes are not readily accepted by farmers in Africa at large (Beebe et al., 1981) and specifically in Ethiopia. But landraces and introductions with bush type growth habit adapted and maintained by farmers have for a long time been known to have useful agronomic traits. Indeed, most existing resistant sources developed elsewhere, have been derived from landraces (Busogoro et al., 1999). For instance, G5686, which is a good source of ALS resistance and a member of the ALS differential set, is a landrace that originated from Ecuador (Mahuku et al., 2009). Though resistance may exist in bean collections, the high degree of genetic variability of *P. griseola* often compromises the use of ALS resistance derived from landraces (Nietsche et al., 2001). This is due to continuous emergency of new races, which break down disease resistance (Young et al., 1998). Hence, the need for continuous screening of germplasm to identify new sources of resistance that can regularly be introgressed into commercial cultivars (Young & Kelly, 1996). This will counteract the new emerging races and reinforce resistance in existing ALS resistant sources.

Therefore, this screening study was conducted to asses and identify resistant common bean accessions to be used

in the breeding program to develop resistant cultivar.

2. Materials and Methods

2.1 Experimental Site and Design

The experiment was carried out at the Wondogenet and Areka research stations located in southern Ethiopia, respectively at N07"03.968' E037"41.124' and 1803 m.a.s.l and 7° 4′ 0″ N, 37° 42′ 0″ E and 1774 m.a.s.l with an average precipitation 1290mm. These two locations are known for high infestation of the angular leaf spot disease. Both experimental sites were selected based on the basis of history of ALS disease infestation. Hence, the two screening areas are appropriate sites for screening common bean genotypes resistance against angular leaf spot disease. Common bean germplasms were evaluated with 2 rows of 3m length in Augmented design with randomized incomplete design (Lin, & Poushinsky,1983). The materials were evaluated under natural disease epidemics in three different environments. ALS disease severity was recorded at 55 and 66 days after planting using the 1-9 CIAT scale (Van Schoonhoven and Pastor-Corrales, 1987), where 1= no visible symptom and 9= disease covering more than 25% of foliar tissue.

2.2 Plant Materials

A total of 300 common bean germplasm including large world collections lines and varieties and standard checks known for the angular leaf spot resistance genes obtained from different countries of breeding program were included in this study.

2.3 Statistical Analysis

Mean ALS disease scoring data from each common bean germplasm were examined and used for data analysis. Data from ALS disease severity were subjected to analysis of variance using SAS version 3.2 (Lin, & Poushinsky, 1983) using PROC GLM Augmented Micro SAS procedure.

3. Result and Discussion

Existence of highly significant (<1%) variation for the disease severity among common bean germplasm tested under field conditions indicates variability for resistance among the accessions for the angular leaf spot disease under field condition (Table 1, Figure 1). The maximum disease reactions were recorded for most of germplasm. In other study over 1300 bean accessions were screened under field condition for anthracnose and angular leaf sport resistance and among the accessions few of the accessions found to be with resistance reaction (Schwartz, et al., 1982). Based on the current study 15 lines /accessions (Table 2) with resistance reactions to the angular leafspot under the field conditions were selected for further evaluation under greenhouse condition. Out of the accessions Acc#299 a small red seeded bean showed high resistance to the ALS disease at both test locations. Hence, these accessions showing resistance are potential for developing broad and durable ALS resistance

3.1 Implications in Common Bean Breeding

These day's development of cultivars with improved angular leaf spot disease resistance is one of the main goal of common bean breeding programs. The development of resistance cultivar requires best adapted parental lines with known resistance genes. This is due to continuous emergency of new ALS races, which break down disease resistance (Young et al., 1998). ALS race distribution from the bean growing areas of Ethiopia and reaction of common bean genotypes has been studied and reported by Rezene et al., (2018). A wide frequency variation in the differential cultivars were also reported by the same authors. Most of the common bean differentials were compatible to the Ethiopia angular leaf spot races. Hence, the need for continuous screening of germplasm to identify new sources of resistance that can regularly be introgressed into commercial cultivars (Young & Kelly, 1996). Therefore, the selected resistance from the current screening study will be refined and used in the common bean improvement program.

Table 1. Mean square of common bean germplasm under different environment for angular leaf spot disease

	Mean Square			
Source	**DF**	**ALSW16**	**ALSW17**	**ALSA17**	**ALSCOMB**
Block	5	0.2222NS	0.0889NS	2.2805NS	0.1343NS
Treatment	299	3.6484**	2.1019**	1.775**	1.1673**
Tests	287	2.2176**	1.71875**	1.4911**	0.8698**
Controls	11	32.7676**	11.4076**	9.3169**	6.2456**
Tests vs Controls	1	94.0444**	9.7187**	0.2007**	23.6903**
Error	55	0.2101	0.0888	0.8806	0.0891
*- significant at 5% (level of significance) NS=Non-Significant p-Value <0.05%- Significant at 5%(*), p-Value <0.01-Sigifican 1%(**)					
R-square	0.99	0.99	0.92	0.99	
CV	8.47	5.99	19.33	5.92	
ALS Mean	5.41	4.07	4.85	5.04	

DF=degree freedom, ALSW16= angular leaf spot from Wondogenet during 2016, ALS W17 =angular leafspot from Wodogenet during 2017, ALSA17= angular leaf spot from Areka during 2017.

Table 2. Common bean germ plasm that showed resistance reaction under field condition at Wondogenet (ALSW16 &ALS17) and Areka (ALSA17), Ethiopia during 2016 and 2017 cropping seasons

		ALSW16	ALSW17	ALSA17	ALSCOM		
Accessions	Genotype	ALS Adjusted mean score (1-9)				Growth habit	Seed colour
Acc#272	KG27-13	1.8	3.1	3.2	2.1(R)	VINE	RED M
ACC#269	GCI-CAL-270A	1.8	2.4	4.2	2.6(R)	BUSH	RED M
Acc#100	NABE4	3.0	1.6	2.7.	2.7(R)	BUSH	RED M
Acc#181	AFR612	2.1	2.7	3.7	2.7(R)	BUSH	RED M
Acc#194	SAB618	3.1	2.9	2.7	2.9(R)	BUSH	RED M
ACC#98	CHEUPE	2.0	2.2	4.2	3.0(R)	BUSH	WHITE
Acc#183	AND279	2.1	1.6	5.2	3.0 (R)	BUSH	RED M
Acc#255	SUGER131	1.0	3.1	5.7	3.0(R)	VINE	CRANS
Acc#86	MISHINDI	2.0	2.9	3.7	3.1(R)	BUSH	GREY
Acc#142	AW-SPS-51	2.0	2.9	2.4	2.6(R)	BUSH	S RED
Acc#188	DAB246	3.1	4.9	1.2	3.1(R)	BUSH	RED M
Acc#268	UYOLE	3.8	3.1	5.2	3.1(R)	BUSH	RED M
Acc#185	ROZO KOBO	3.1	4.9	1.7	3.2(R)	BUSH	RED M
Acc#95	TYGER BERG	5.0	3.7	1.7	3.4(R)	VINE	CRANS
Acc#299	SPS50-HB	1.2	2.4	1.7	1.8(R)	BUSH	RED S
Standard Checks							
MEX-54		3.0	2.9	1.7	3.4(R)	VINE	Pink
AND277		1.3	2.3	3.2	2.1(R)	BUSH	REDM
CV		7.2	9.2	19.3	5.9		
LSD		5.41	4.07	4.85	5.04		

ALS disease evaluations using a 1 to 9 rating scale, where 1 = no visible symptoms of the disease and 9 = very severe symptoms (Schoonhoven & Pastor-Corrales, 1987). ALS reaction type: 1–3 = resistant (R); 4–6 = intermediate (I); 7–9 = susceptible (S), here we have selected those genotypes showing resistance(R) reactions to the angular leaf spot under field condition.

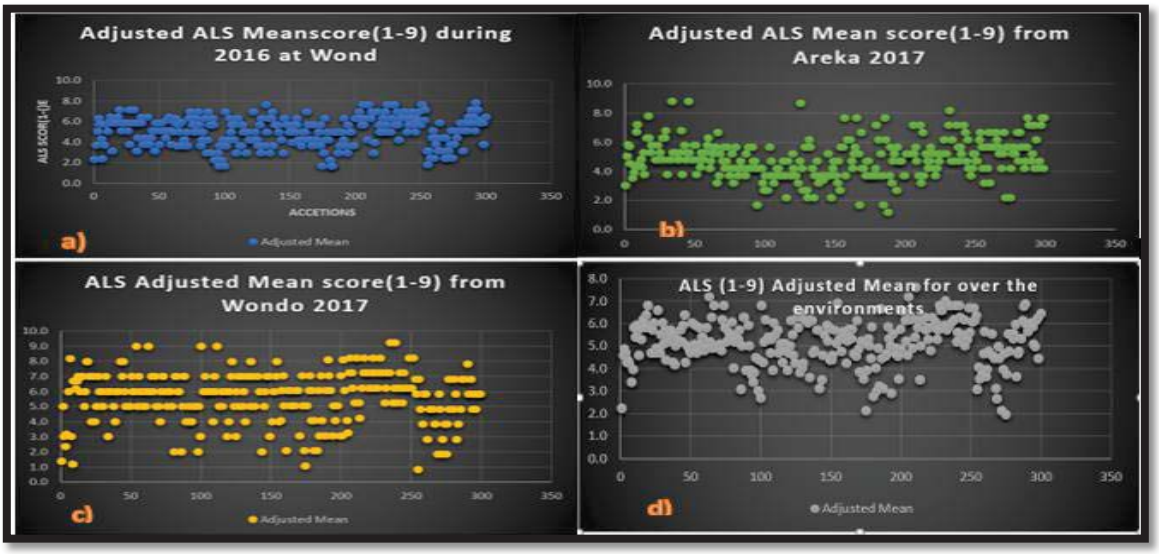

Figure 1. Adjusted mean disease severity score (1-9) for 300 common bean germplasm a) at Wondogenet 2016, b) Areka 2017, c)Wondogenet 2017 and d)the reaction across all environment. Accession their adjusted mean scores under 4 were all with resistance reaction to the naturally epidemic angular leaf spot disease

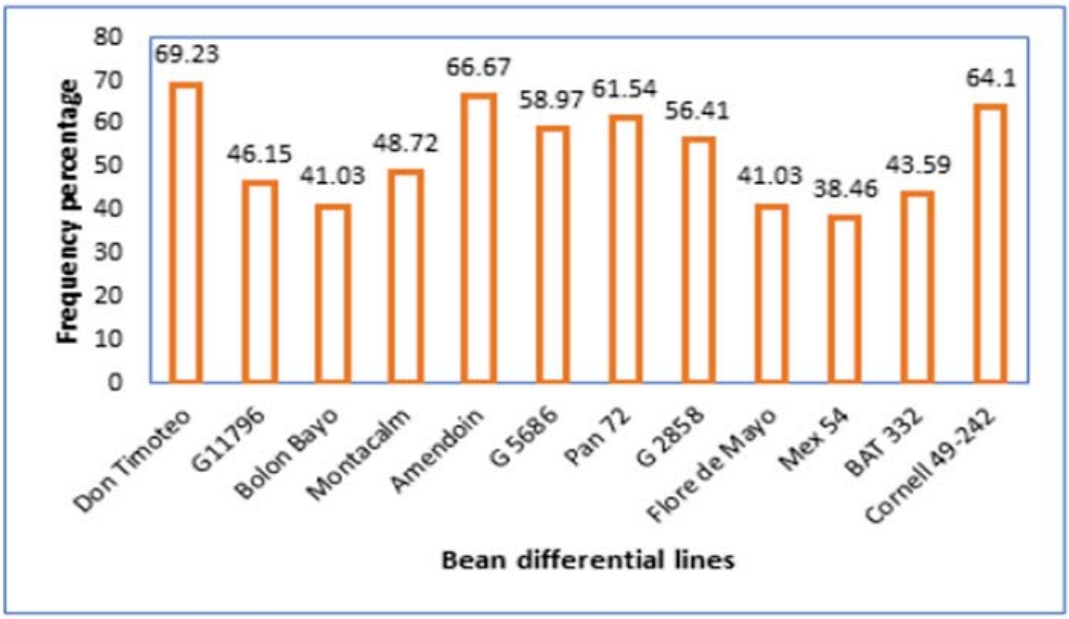

Figure 2. Frequency (%) of compatible reaction between sets of differential common bean genotypes and evaluated isolates

Source: Rezene et al., 2018

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GGE-Biplot Analysis of Multi-Environment Yield Trials of Common Bean (*Phaseolus vulgaris* L.) in the southern Ethiopia

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Abstract

The present study was conducted on thirty-six common beans (*Phaseolus vulgaris* L.) Genotypes across six contrasting environments defined for its different soil fertility status and located at the southern Ethiopia. The genotypes were arranged in 6 x 6 triple lattice design and executed for two successive main cropping seasons with the objectives to evaluate yield performance of common bean genotypes and identification of mega environments. GGE (i.e., G = genotype and GE = genotype by environment, interaction) bi-plot methodology was used for graphical presentation of yield data after subjecting the genotypic means of each environment to GGE Bi-plot software. The first two principal components (AXIS 1 and AXIS2) were used to display a two-dimensional GGE bi-plot. Thus, genotypic AXIS1 scores >0 classified the high yielding genotypes while AXIS2 scores <0 identified low yielding genotypes. Unlike genotypic AXIS1, genotypic AXIS2, scores near zero showed stable genotypes whereas large AXIS2 scores classified the unstable ones. The environmental AXIS1 were related to crossover nature of GEI while AXIS2 scores were associated with non-cross over GEI. The six test environments in the southern region were divided in to two distinct mega environments (Mega-1 and 2). Mega-1 constituted GOHF13, ARMF12 and ARLF13 while genotype 14 (SCR10) being the best winner, on the other hand, Mega-2 contained GOHF12 and while common bean genotype 20(SCR17) being the best winner. The results of this study indicated that breeding for specific adaptation should be taken as a breeding strategy in southern region to exploit positive GEI to increase production and productivity of common bean.

Keywords: GGE, Mega environment, *Phaseolus vulgaris*

1. Introduction

Common bean (*Phaseolus vulgaris L)*, also referred to as dry bean, is an annual leguminous plant that belongs to the genus, *Phaseolus,* with pinnately compound trifoliate large leaves. It is largely a self-pollinated plant though cross-pollination is possible if the stigma contacts with pollen coated bee when extended. Seeds are non-endospermic and vary greatly in size and color from the small black wild type to the large white, brown, red, black or mottled seeds of cultivars, which are 7-16 mm long (Sing *et al*., 1991; Gepts and Debouk 1991). Common bean shows variation in growth habits from determinate bush to indeterminate, extreme climbing types. The bushy type bean is the most predominant type grown in Africa (Gepts and Debouk 1991) and in Ethiopia (Asrat *et al*., 2013). Cultivation of common bean in Africa is widespread, but production (approximately 80 percent of African bean production) is concentrated in 10 countries. In terms of area, Kenya is the leading producer of common bean in Africa followed by Uganda and then Tanzania Malawi and Ethiopia rank eighth and ninth, respectively according to FAO statistics (FAOSTAT, 2016). Common bean in Ethiopia is produced in almost all the regional states with varying intensity. Production is concentrated in two regions: Oromia and the Southern National Nationality Peoples region (SNNPR), which account for more than 85 percent of the total national production (CSA, 2015). The remaining percent comes from Afar, Amhara, Tigray, Somali, Gambella and Benishangul-Gumuz (CSA, 2015).

The GGE bi-plot procedure (Yan and Tinker, 2006) consists of a set of bi-plot explanation approaches, whereby important questions regarding genotype evaluation and test-environment evaluation can be visually addressed. Increasingly, plant breeders and other agronomists have found GGE bi-plots were useful in mega-environment analysis (Dardanellia *et al*., 2006), genotype evaluation (Voltas *et al*., 2005; Kang *et al*., 2006), test-environment evaluation (Thomason and Phillips, 2006), trait-association and trait-profile analyses (Ober *et al*., 2005), and

heterotic pattern analysis (Bertoia *et al*., 2006) genotype evaluation on total starch yield in potato (Gedi *et al*. 2014), genotype environment interaction and grain yield stability in bread wheat genotypes(Mehari et al. 2015). As common bean is one of the most important legume which is produced in the southern region (Yayis *et al*., 2011; Yayis *et al*., 2012), there are limited information and knowledge (Gebeyehu *et al*. 2003; Aserat *et al*., 2008) regarding the nature and magnitude of GEI to breeders working at the southern Agricultural Research Institute, Ethiopia in order to select superior genotype across the environments, but environments vary in climate, topography, biological and edaphic factors. Understanding GEI supports plant breeders to design appropriate breeding strategy. Therefore, this study was conducted to evaluate the yield performance of each common bean genotypes in relation to each contrasting test environments, to examine possible existence of different mega environments and to identify the winning genotypes for each environment

2. Materials and Methods

2.1 Description of the Study Area

Thirty-Six Common bean (*Phaseolus vulgaris* L.) advanced genotypes (Table 3) initially introduced from CIAT together with two standard checks that were developed at SARI bean breeding program were considered for this specific contrasting environments evaluation. The experiment was conducted for two successive years at three locations known for their different soil fertility status, namely, ARLF (Areka with poor/low soil fertility status/, ARMSF (Areka with moderate soil fertility status area) and GOHF (Gofa with high/potential soil fertility status), with the objectives to evaluate the performance of common bean genotypes across different soil fertility environments and selecting best genotypes for different common bean growing areas.

2.2 Experimental Design

The experiment was executed in a lattice design with three replicates (6 x 6 triple lattice design) on a plot consisted of 4 rows of 4m length spaced x 0.4 m. Necessary agronomic management practices were applied as per the recommendation for all specific locations. Two central rows were considered for the yield and other agronomic trait data.

2.3 Data Collection and Data Analysis

The grain yield data obtained was adjusted to 10% moisture content before it was subjected to statistical analysis. Analysis of variance was conducted for experiments in each environment using the model

$$\underline{y}_{ijm} = \mu + \rho_j + \underline{b}_{m(j)} + \tau_i + \underline{e}_{ij} \qquad \text{with } \underline{b}_{m(j)} \; iid{\sim}N\left(0,\sigma_b^2\right) \; \underline{e}_{ij} \; iid{\sim}N(0,\sigma_e^2)$$

The model for a GGE biplot (Yan, 2002) based on singular value decomposition (SVD) of first two principal components is:

$$\acute{y}_{ij} - \mu_i = \sum_{k=1}^{t} \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij}$$

Where y_{ij} is the cell mean of genotype *i* in environment *j*; μ_j is the mean value in environment j; i = 1, · · · g; j = 1, · · · e, g and e being the numbers of cultivars and environments, respectively; and t is the number of principal components (PC) used or retained in the model, with $t \leq \min(e, g-1)$.

3. Results and Discussion

The analysis of variation revealed the existence of significant variation among genotypes in all environment confirming the presence of genotypic variation to be exploited by selection (Table 1 and Fig 1). The bean genotype and environment main effects were significant ($p<0.001$) as the genotype by environment was (Table 1 and Table 2). The experimental coefficient of variation (CV) were relatively low (9.9% to 15.22%) (Table 4) in individual environment indicating good experimental precision.

Table 1. Analysis of variance grain yield (kg/ha) of 36 common bean genotypes tested across six contrasting environment, southern Ethiopia

SOURCE	DF	SS	MS	F PR.
Genotypes	35	6789239	193978.3	0.007
Environments	5	48378623	9675725	<0.001
Sensitivities	35	5482295	156637	0.058
Residual	140	14828734	105919.5	
Total	215	75478892	351064.6	

The additive main effect and multiplicative interaction analysis of variance mean grain yield (kg/ha) of common bean (*Phaseolus vulgaris* L.) genotypes showed significance difference among the genotypes across the test environments (Table 2). The environment posed significant effect on the grain yield of genotypes which explained 64.1% of the total variations (G + E + GE), while the GE and G interactions explained 26.91% and 8.99% respectively.

Table 2. The additive main effect and multiplicative interaction (AMMI)

Source	DF	SS	MS.	%SS explained	F pr
Genotypes	35	6789239	193978	8.99	0.0168
Environments	5	48378623	9675725	64.1	<0.001
Interactions	175	20311029	116063	26.9	
IPCA 1	39	7205681	184761		0.0012
IPCA 2	37	4628750	125101		0.0715
Residuals	99	8476597	85622		

Table 3. Genotypes and test environments with mean grain yield (kg/ha) of 36 common bean genotypes tested across six different environments in southern Ethiopia

Genotypes		E1	E2	E3	E4	E5	E6
Code	Accessions	ARLF12	ARLF13	ARMF12	ARMF13	GOHF12	GOHF13
G1	BSF23	1416.62	2246.00	2194.60	2625.53	1983.99	2577.47
G2	BSF27	1747.25	2192.18	2022.85	2613.52	2794.68	2807.87
G3	BSF29	1483.91	2484.01	2477.45	2527.09	3577.19	3660.01
G4	BSF30	1548.18	2059.65	2161.04	2627.08	2546.50	3391.40
G5	BSF32	2025.93	2212.13	1944.15	2649.50	4093.95	3334.46
G6	BSF33	1804.87	1777.44	2201.66	2275.09	3566.66	2884.60
G7	BSF34	1971.82	2154.49	2227.40	3017.76	3883.25	3407.38
G8	BSF35	2116.48	2600.82	2174.36	2059.10	3476.53	2352.57
G9	BSF39	2388.97	1766.24	2027.14	2820.59	2849.67	3153.25
G10	BSF55	1904.51	2173.42	2740.48	2673.56	3385.27	2762.69
G11	HD	1726.58	2081.48	2732.77	2289.60	3286.24	2890.98
G12	SARI-1	1999.57	2770.33	2805.44	2784.20	3226.34	3407.40
G13	SCR1	1615.22	2329.19	2505.06	2230.26	3482.85	3614.19
G14	SCR10	2110.63	2650.95	3268.60	2505.81	3530.08	3723.42
G15	SCR12	2101.32	2438.09	2159.68	2933.07	2832.70	3237.07
G16	SCR13	2029.67	1685.47	2376.60	2989.90	3549.48	3398.86
G17	SCR14	1891.91	1713.44	2293.44	2676.63	4005.30	2514.24
G18	SCR15	1888.68	1812.07	2757.05	2946.79	3366.80	2757.10
G19	SCR16	1795.80	2228.88	1890.04	2608.09	2924.70	2815.76
G20	SCR17	2594.89	1899.49	2538.73	2977.60	4177.47	3129.69
G21	SCR18	1686.96	2487.00	2191.42	2319.06	2995.94	3156.08
G22	SCR19	1669.32	2205.90	2082.15	2792.10	2676.68	2338.33
G23	SCR20	2577.54	2111.66	2265.63	2727.86	2754.63	3087.40
G24	SCR21	1688.01	2065.62	2354.91	3147.23	3656.01	2810.92
G25	SCR22	1666.27	1864.89	1886.19	2896.88	3280.66	2960.41
G26	SCR26	1759.29	2699.58	2769.31	2801.52	3173.12	2760.92
G27	SCR27	1512.85	2152.73	2797.87	3057.30	3268.70	3066.98
G28	SCR28	1818.01	2142.23	2527.00	2564.51	2920.35	2954.37
G29	SCR29	1239.79	2395.60	1606.13	2451.05	3182.64	2496.95
G30	SCR3	2031.71	2185.06	2245.81	3008.06	2801.75	2665.21
G31	SCR31	1786.47	2171.72	2389.66	2515.31	3213.30	3639.20
G32	SCR37	1803.88	2229.48	2343.21	2758.85	2809.57	3467.70
G33	SCR4	1792.48	2150.05	2085.27	2962.24	3626.92	3514.00
G34	SCR5	2104.32	1849.37	2160.40	2572.28	2565.30	2107.36
G35	SCR7	1736.85	1973.80	2036.32	2696.01	3952.03	2724.68
G36	SCR9	2053.46	2791.04	3121.49	2284.78	3084.56	2791.46

ARLF12=Areka with low soil fertility, ARMF=Areka moderate soil fertility, GOHF=Gofa with high soil fertility, the numbers indicated year 12, 13 as 2012 & 2013

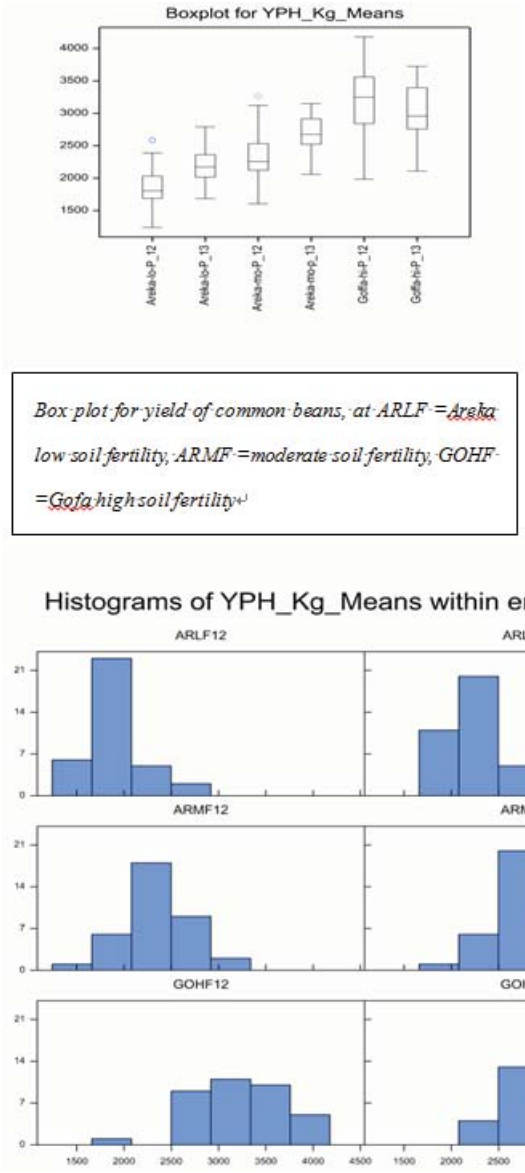

Figure 1. Box plot and Histogram for mean grain yield (kg/ha) of common bean (*Phaseolus vulgaris* L.) across six contrasting environments

Table 4. Environmental effect for the mean grain yield (kg/ha) of common bean genotypes across six contrasting environments

Code	Environment	Effect	s.e.	Mean Yield kg/ha	% CV	Rank
E1	ARLF12	-662.2	46.96	1891	13.00	6
E2	ARLF13	-377.1	46.96	2176	13.73	5
E3	ARMF12	-224.3	46.96	2329	9.90	4
E4	ARMF13	106.1	46.96	2659	15.20	3
E5	GOHF12	707.8	46.96	3261	1345	1
E6	GOHF13	449.8	46.96	3003	15.58	2

ARLF12=Areka Low soil fertility 2012, ARLF13=Areka Low soil fertility 2013, ARMF12=Areka with moderate soil fertility area 2012, ARMF13=Areka with moderate soil fertility area 2013, GOHF12=Goffa with high soil fertility area 2012, GOHF13=Goffa with high soil fertility 2013

Categorizing environments based on the values of environmental effects (Table 4) hence, based on the result

indicated in Table 4 GHF12 with higher values of environmental effects it was classified as best test environment where as ARLF12 with low values of environmental effect classified as the least test environment for tested common bean genotypes.

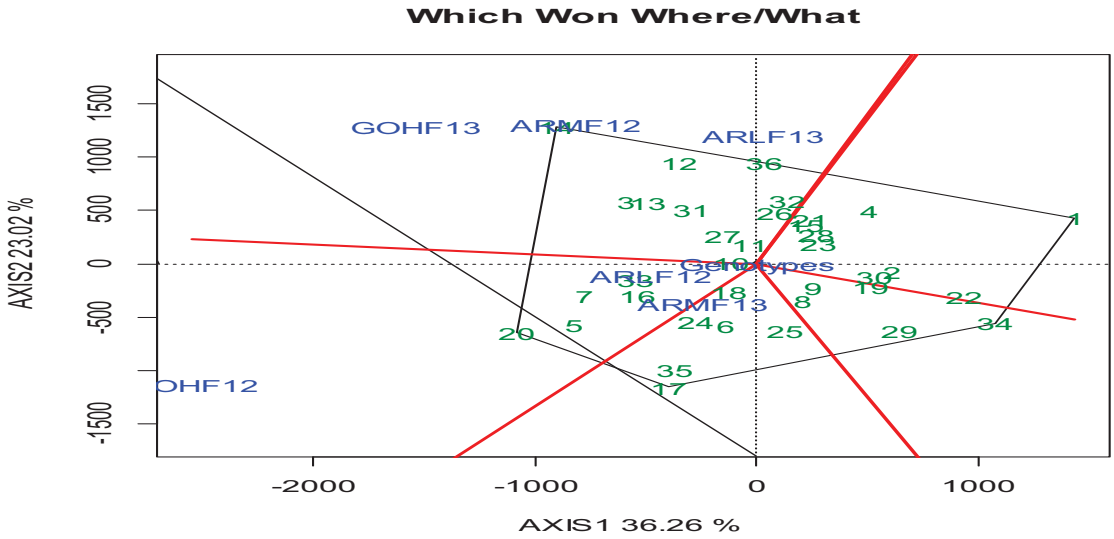

Figure 2. The "which-won-where" view of the GGE biplot based on the G × E data in Table 1. The data were not transformed ("Transform = 0"), not scaled ("Scaling = 0"), and were environment centered ("Centering = 2"). The biplot was based on environment-focused singular value partitioning ("SVP = 2") and therefore is appropriate for visualizing the relationships among environments

3.1 Which Won Where

One of the smartest features of a GGE biplot is its ability to show the which-won-where pattern of a genotype by environment dataset (Fig 2). Many researchers find the use of a biplot exciting, as it graphically addresses important concepts such as crossover GE, mega-environment differentiation, specific adaptation, etc (Kassaye et al., 2017). The "which-won-where" function of a GGE biplot is an extended use of the "pair-wise comparison" function described above. The polygon classified all environments in to two mega environments (Fig 2) the polygon drawn on genotypes (1, 34, 17 and 20) that were furthest from the biplot origin so that all other genotypes are retained in within the polygon. The perpendicular lines to each side of the polygon were drawn, starting from the biplot origin. Hence, genotype 14 (SCR10) were uniquely adapted in environments ARMF13, GOHF13 and ARLF13, whereas genotype 20 (SCR17) won on GOHF12 environment. On the other hand, genotypes 20 & 17 (SCR17 and SCR14) gives similar yield in ARMF12 environments. Ashango et al., (2016) and Kassaye et al, (2017) in their reports also indicated identification of four mega environments and specifically adapted common bean verities.

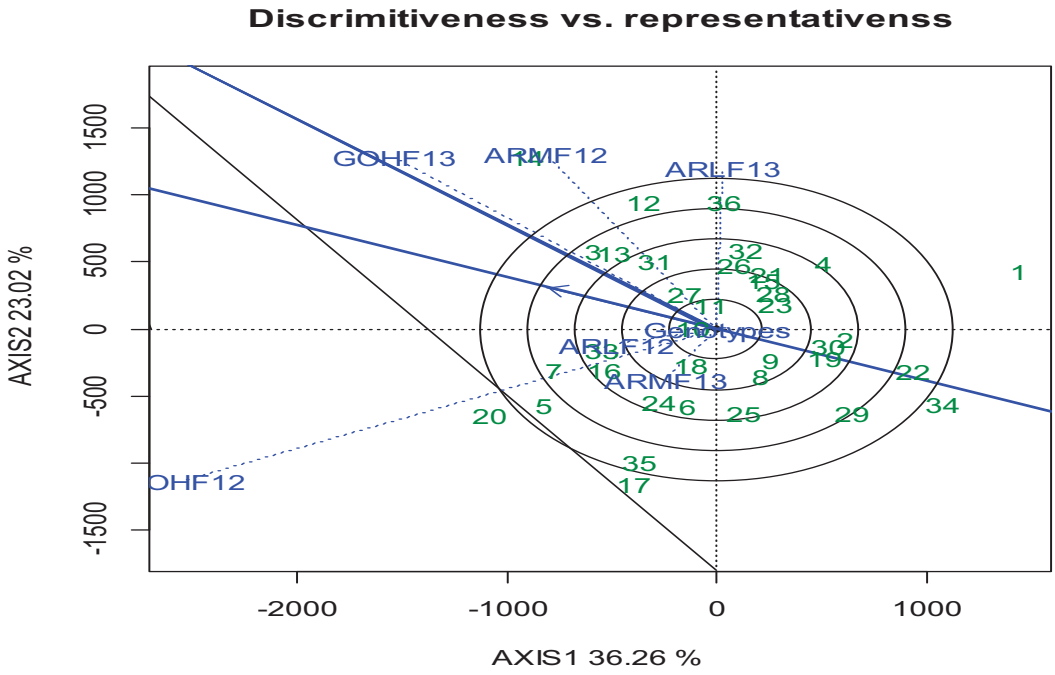

Figure 3. The discriminability and representativeness view of the GGE-biplot to show the discriminating ability and representativeness of the test environments

3.2 Discriminability and Representativeness

Average Environment Axis (AEA) is the line that passes through the average environment (represented by small circle) and biplot origin (Fig 3). The average environment has the average coordinates of all test environments. A test environment that has a smaller angle with the AEA is more representative of other test environments according to Yan and Tinker (2006). Thus, GOHF13 were the most representative environment, whereas ARLF13 and ARMF13 with the large deviation from AEA were the least representative. Test environments that are both discriminating and representative is good test environment for selecting generally adapted genotypes (Yan and Tinker, 2006; Mehari et al., 2015; Yayis et al., 2015; Ashango et al., 2016).

Hence, GOHF13 were good test environment for selecting widely adapted genotypes. Testing environments that are discriminating but non-representatives are useful for selecting specifically adapted genotypes if the target environment is divided in to mega environments (Yan and Tinker, 2006). Hence, ARLF13 (Areka low soil fertility) was useful for selecting specifically adapted genotypes. Non-discriminating testing environments are those with very short vectors and are less useful (Yan and Tinker, 2006). The ideal test environment (the center of concentric circles) should be both highly discriminating and most representatives of the target environments (Kaya *et al*., 2006; Yan and Tinker, 2006; Mehari et al., 2915; Yayis et al., 2015, Kassaye et al., 2017). Under natural condition such environment does not exist but could be used relatively as a reference. Thus, the ideal test environment was GOHF13 (Fig 3) and it is an environment in which best genotypes could be most easily identified. Yan *et al*. (2001), In his report, indicated that favorable test environments must have large PC1 scores (more discriminating genotypes) and near zero PC2 scores (more representative of an average environment).

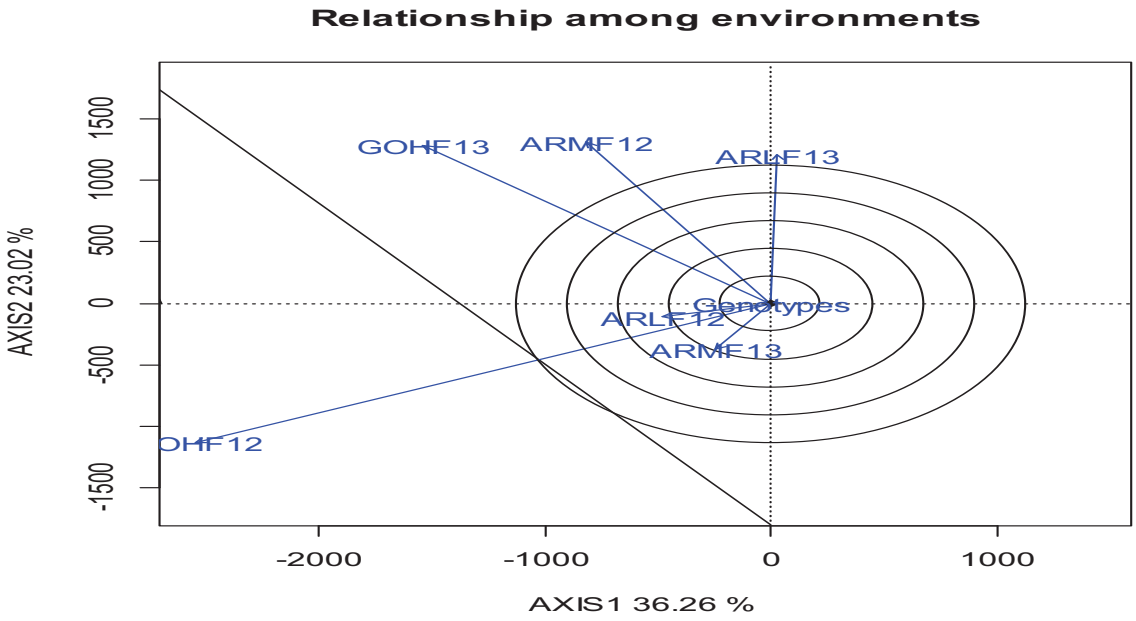

Figure 4. The environment vector of GGE-biplot is based on environment centered (centering=2) without any scaling scaling=0) and its environments metrics preserving (SVP=2). The bi plot explained 59.28% of the total variation environment based G by E table

3.3 Relationship among Environment

The lines that connect the test environments to the biplot origin are called environment vectors. According to Yan, 2001, the cosine of the angle between the vectors of two environments approximates the correlation between them. For example, environments GOHF13 and ARMF12 were positively correlated (an acute angle), ARLF13 and GHF12 with an obtuse angle were highly negatively correlated, whereas, ARMF13 and GOHF13 with a right angle were not correlated. The presence of wide obtuse angles (i.e., strong negative correlations) among test environments which is an indication of strong crossover GE. Here the largest angle is larger than 90° (between ARMF13 and ARLF13), suggesting that the GE is large. The presence of close associations among test environments suggests that the same information about the genotypes could be obtained from fewer test environments, and hence the potential to reduce testing cost. If two test environments are closely correlated consistently across years, one of them can be dropped without loss of much information about the genotypes.

The concentric circles on the bi-plot help to visualize the length of the environment vectors, which is proportional to the standard deviation within the respective environments (Yan and Tinker 2006) and is a measure of the discriminating ability of the environments. Therefore, among the six environment GOHF12 was most discriminating (informative) and ARLF12 least discriminating (Fig. 4). Test environments that are consistently non-discriminating (non-informative) provide little information on the genotypes and, therefore, should not be used as test environments.

4. Conclusion and Recommendations

The GGE Biplot analysis has evolved into an important technique in crop improvement and agricultural research. GGE biplot analysis provided genotype by environment data analysis for different contrasting environment in the southern regions of Ethiopia, which has been a challenge to plant breeders, geneticists, and agronomists. In this specific research GGE-biplot proved to be very useful in assessing the performance of genotypes in different test contrasting environment. Hence, showed the selection of winning genotypes in each specific mega environment. The genotypes and environments main effects and GEI effects were significant for common bean genotypes studied in the southern regions of Ethiopia with contrasting test environments. Thus, the bean breeding program of the southern Ethiopia should consider those two-mega environments separately when developing common bean varieties for specific and wider adaptation.

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