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Review

Utilization of wild relatives for maize (*Zea mays* L.) improvement

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Experimentally induced introgression and selection during domestication and maize (*Zea mays* L.) improvement involved selection of specific alleles at genes controlling morphological and agronomic traits, resulting in reduced genetic diversity relative to unselected genes. The plant breeder would have to extend crosses to the wild relatives to introduce novel alleles and diversify the genetic base of elite breeding materials. The use of maize wild relatives (Teosintes and *Tripsacum*) genes to improve maize performance is well established with important examples dating back more than 60 years. In fact, Teosintes and *Tripsacum* are known to possess genes conferring tolerance to several biotic and abiotic stress including chlorotic dwarf virus, downy mildew, *Fusarium, Striga hermonthica,* rootworms, drought and flooding. This review provides an overview of the application of these wild relatives and demonstrates their roles on the development of stress tolerant maize plants. It also highlights the use of Teosintes and *Tripsacum* to improve selected quantitative traits such as yield.

Key words: Maize (*Zea mays* L.), Teosintes, *Tripsacum*, stress tolerance, maize improvement.

INTRODUCTION

Maize (*Zea mays* L.) is one of the oldest domesticated plants dating back to as far as 7,000 years ago in Central Mexico by Mesoamerican natives. The crop seems to have developed as a result of gradual selection upon primitive annual teosinte (*Zea mexicana*), an ancient grass found in Mexico and Guatemala (Doebley, 1990a). Although a rapid boost in maize production has being achieved as a result of using single, double and three-way crosses, the hybrid technology has also posed a challenge on meeting the target growth in maize production due to narrowing down of genetic variability (Aditya and Jitendra, 2014). In fact, domestication has led to a severe reduction in genetic diversity within most cultivated crops including maize when compared to their wild relatives (Olsen and Gross, 2008). To meet the challenges of the future, plant breeders will need all the genetic diversity that they can get. Some of this diversity can be found in landraces and heirloom varieties that are still being cultivated by farmers around the world. However, a much wider spectrum of diversity can be found in the genomes of crop wild relatives (Hannes et al., 2014). Wild crop relatives have been playing enormously important roles both in the depiction of plant genomes and the genetic improvement of their cultivated

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counterparts (Brar, 2005; Hajjar and Hodgkin, 2007; Pickering et al., 2006; Canci and Toker, 2009; Miller and Seiler, 2003). They have contributed immensely to resolving several fundamental questions, particularly those related to the origin, evolution, phylogenetic relationship, cytological status and inheritance of genes of an array of crop plants; provided several desirable donor genes for the genetic improvement of their domesticated counterparts; and facilitated the innovation of many novel concepts and technologies while working on them directly or while using their resources (Bai et al., 1995; Clifford, 1995; Kamala et al., 2002; Nevo et al., 2002 ; Nevo, 2004; Raskina et al., 2002, 2004; Sharma et al., 2005; Price et al., 2005, 2006; Dillon et al., 2005, 2007; Peleg et al., 2005, 2007; Petersen et., 2006; Salina et al., 2006; Matsuoka and Takumi, 2007; Bennetzen et al., 2007; Gill et al., 2007; Feldman and Kislev, 2007; Oliver et al., 2008; Loskutov, 2008; Gavrilova et al., 2008; Kuhlman et al., 2008; Xu et al., 2009; Wang et al., 2009; Ashraf et al., 2009; Nevo and Chen, 2010; Chittaranjan, 2011). For example, a wild rice (*Oryza officinalis*) has recently been used to change the time of flowering of the rice cultivar Koshihikari (*Oryza sativa*) to avoid the hottest part of the day (Ishimaru et al., 2010).

In maize, alien introgression has been accomplished for improvement of kernel composition, yield and yield related traits including kernel weight, kernel row number (KRN), kernel area and kernel length using sexual hybridization (Gallinat, 1984; William et al., 2007; Wang et al., 2008; Liu et al., 2016a, b; Karn et al., 2017). Cohen and Gallinat (1984) suggested improvement of maize inbreds with respect to quantitative traits like yield via introgression of alien chromatin segments both from teosintes (closely related species, *Zea mays spp.*) and *Tripsacum* (distantly related genus).

These wild relatives of maize have also long been recognized for their remarkable ability to withstand pests and various abiotic stresses including chlorotic dwarf virus, downy mildew, *Fusarium*, *Striga hermonthica*, rootworms, drought and flooding and thus a potentially rich source of beneficial genes (Reeves and Dockholt, 1964; De Wet, 1979; Kindiger and Beckett, 1990; Leblanc et al., 1995; Savidan et al., 1995; Berthaud et al., 1995, 1997; Masanori et al., 2005; Eubanks, 2006; Mano et al 2007; Amusan et al., 2008; Prischmann et al., 2009).

In this review, we describe the wild relatives of maize (teosintes and *Tripsacum*) and discuss the results of the introduction of genes from these alien germplasm into cultivars of maize.

CHARACTERISTICS OF WILD *ZEA* SPECIES

Maize belongs to the family Poaceae and tribe Maydeae which comprises seven genera, viz. Coix (2n = 10 or 20), Chionachne (2n = 20), Sclerachne (2n = 20), Trilobachne (2n = 20), Polytoca (2n = 20), *Zea* and *Tripsacum* (Aditya and Jitendra, 2014). The genus *Zea* consists of four species of which only *Z. mays* L. (2n = 20) is economically important. The other *Zea* sp., referred to as teosintes, are largely wild grasses native to Mexico and Central America (Doebley, 1990b).

Teosintes

The teosintes are annual and perennial grasses native to Mexico and Central America. Most of these wild *Zea* species and subspecies are distributed across narrow ranges and can only be found in some tropical and subtropical areas of Mexico, Guatemala, Nicaragua, and Honduras (Chittaranjan, 2011; Aditya and Jitendra, 2014). Among teosintes, the nearest teosinte relative to *Zea mays* is *Zea mays* ssp. *mexicana* (Schrader) Iltis, which grows in central highlands of Mexico. It possesses the same diploid chromosome number as maize (2n = 20) and their chromosomes are known to generally pair and recombine with the chromosome of maize. The other teosintes include perennial teosintes, viz. *Zea diploperennis* (2n= 20) and *Zea perennis* (2n= 40), distributed in Jalisco, Mexico. The annual teosintes include *Zea luxurians* from southeastern Guatemala, *Zea mays* spp. *parviglumis* of southern and western Mexico and *Zea mays* spp. *huehuetenangensis* from the western highlands of Guatemala (Reeves and Mangelsdorf, 1942; Hitchcock, 1951; Iltis et al., 1979; Iltis and Doebley, 1980; Doebley, 1990b; Watson and Dallwitz, 1992; Aditya and Jitendra, 2014).

As the wild ancestor of modern maize, the plant architecture and general growth forms of teosintes are similar to maize. A typical teosinte plant usually has a main stalk that typically contains a series of nodes and elongated lateral branches at most nodes. The internodes can reach up to 20 to 30 cm in length. The ears occur in clusters of 1 to 5 (or more) at each node along the branch (Chittaranjan, 2011). The main morphological differences between teosinte and maize are their branches and inflorescences. Teosinte plants contain more branches and smaller female inflorescences than maize. For wild *Zea* species, the inflorescences can only form 5 to 10 triangular or trapezoidal black or brown seeds with a hard fruitcase. By comparison, maize usually has 100 or more naked seeds.

Tripsacum

The genus *Tripsacum* is comprised of about 12 perennial and warm season species that are mostly native to Mexico and Guatemala but are widely distributed throughout warm regions in the USA and South America, with some species present in Asia and Southeast Asia. Species of economic importance to agriculture in the

genus are *Tripsacum dactyloides* (L., 2n = 72) (Eastern gama grass), *T. laxum* Scrib and Merr (2n = 36). Other species include *T. andersonii* (2n = 64), *T. latifolium* (2n = 36), *T. lanceolatum* (2n = 72), *T. floridanum* (2n = 36) and *T. manisuroides* (2n = 72) (De Wet and Harlan, 1972; De Wet et al.,1972; De Wet et al., 1983; Talbert et al., 1990; Watson and Dallwitz, 1992; Aditya and Jitendra, 2014).

For example, De Wet et al. (1972) obtained hybrids with diploid *T. floridanum* (2n = 36), as well as both diploid and tetraploid races of *T. dactyloides*, *T. lanceolatum* and *T. pilosum*, using maize as the female parent. The reciprocal cross was also successful with both diploid and tetraploid *Tripsacum*, but only when the cytologically unreduced female gamete functioned sexually. Further repeated backcrossing with maize results in rapid elimination of *Tripsacum* chromosomes, and eventually plants with 20 *Zea* chromosomes only are obtained. The vast majority of these plants are pure maize. However, a few individuals with 2n = 20 *Zea* chromosomes have inherited from *Tripsacum* a tillering habit, flag leaf development, habit of producing several cobs on each stem, and probably several other less obvious tripsacoid characteristics (De Wet et al.,1972) .

Tripsacum has higher chromosome numbers (2n = 36; 64 or 72), than maize and hybridizes with it only under special circumstances. The genus, like maize, is monoecious but like teosinte differs from maize in having distichous spikes, solitary, sessile pistillate spikelets, and kernels enclosed in hard shells consisting of segments of the rachis and lower glumes (Mangelsdorf, 1961). However, genomic instability and sterility of hybrids between maize and *Tripsacum dactyloides* have limited direct genetic transfer of valuable traits into maize (Stalker et al., 1977; De Wet, 1979; Kindiger and Beckett, 1990). But fortunately, Eubanks (2006) reported a genetic bridge that permitted movement of *Tripsacum* genes into maize with conventional breeding methods by crossing the *Tripsacum* with *Zea diploperennis.*

PESTS AND DISEASE RESISTANCE

Plant breeders have been exploiting wild relatives for introgressing resistance against biotic stresses for over a century. Over 80% of the beneficial traits conferred by wild relatives involve pest and disease resistance (Harinder et al., 2014).

Disease resistance

Findley et al. (1982) introgressed resistance against maize chlorotic dwarf virus (MCDV) into maize from *Z. diploperennis*. The hybrid between maize and *Z. diploperennis* exhibited sterility, hence backcross generations were generated which revealed resistance to MCDV. Another teosinte was used to confer resistance to downy mildew in maize. In fact, the introgression of resistant genes from *Z. mays* ssp. *mexicana*, *Z. diploperennis,* and *Z. perennis* into maize were carried out by Ramirez (1997) using sexual hybridization. Moreover, introgression of resistance against *Fusarium* was reported in F1 and F2 generations of crosses between maize and *Z. mays* ssp. *mexicana* (Pásztor and Borsos, 1990). Similarly, Bergquist (1979) reported introgression of resistance from distant relatives, viz. *T. dactyloides*, where sexual mating is difficult, against *Colletotrichum graminicola*, *Helminthosporium turcicum*, *H. maydis*, *Erwinia stewartii* and *Puccinia sorghi* by backcrossing into various maize genotypes. In BC5–BC10 generations, resistance to each of the pathogens appeared to be dominant; however, a gradual breakdown of qualitative traits, including resistance, occurred in later generations. Later, Bergquist (1981) successfully transferred a dominant gene *RpTd* conferring resistance against rust pathogen of corn *Puccinia sorghi*, from *T. dactyloides*. Similarly, *T. floridanum* was used to introgress resistance gene *Ht* into the genetic background of maize (Hooker and Perkins, 1980). In another study, Zhou et al. (1997) conducted the distant hybridization involving maize × teosinte *(Z. diploperennis* L.) in order to introduce novel genetic variability. They reported fourteen inbred lines resistant to diseases, insects and environmental stress after eighth-generation selfing and selection. The best crossing of these 14 lines with normal testers produced 1,000 hybrids which showed strong heterosis. On the basis of the success of maize × teosinte (*Z. diploperennis* L.) crosses for introgression of desirable traits, *Z. diploperennis* was suggested as one of the potential sources for widening germplasm pool of maize and to overcome the static situation of maize production in China. Likewise, the alloplasmic inbred lines derived from maize × *Z. diploperennis* interspecific hybrids were reported to exhibit resistance against *H. turcium* and *H. maydis* (Wei et al., 2003).

Parasitic weed resistance

The parasitic weed *Striga* (*Striga* spp.) threatens cereal grain production in tropical and subtropical regions of Africa and Asia. Striga infests 40% of the cereal-producing areas of sub-Saharan Africa (Lagoke et al., 1991). In West Africa, Striga is believed to infest over 50 million ha (Lagoke et al., 1991), and the weed continues to expand its range.

Recently, the utility of wild relatives of maize (teosintes and *Tripsacum dactyloides*) for developing genetically improved maize was well illustrated by Rich and Ejeta (2008) in terms of resistance to the ‘witch weeds’ (*Striga* species), which are particularly prevalent in Africa. While there appears to be paucity of *Striga* resistance genes among maize landraces in Africa, although some

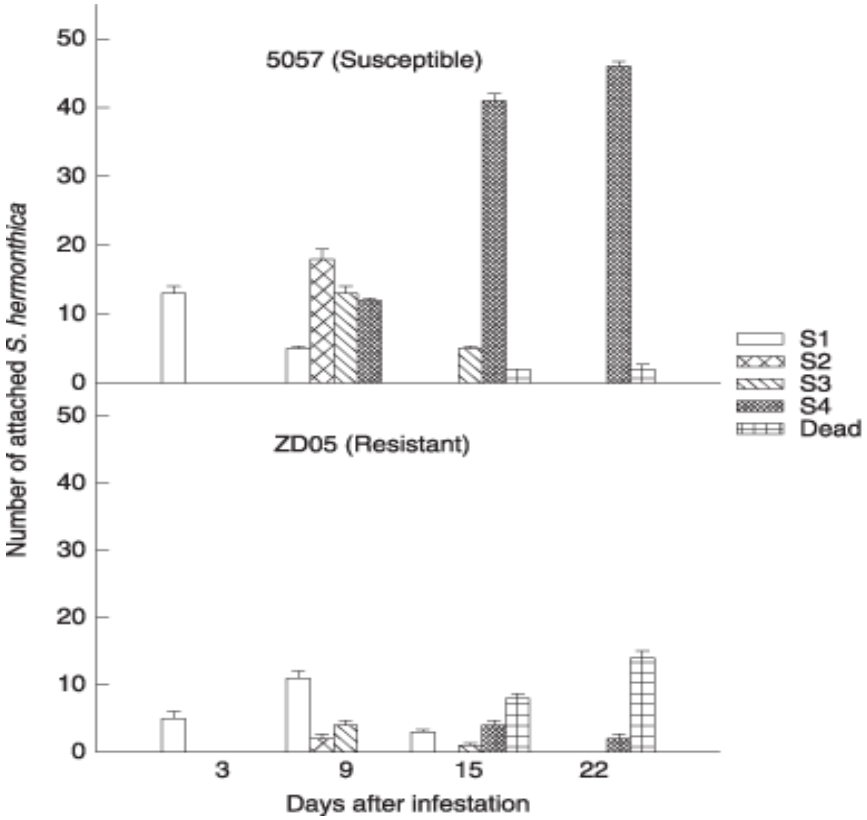

Figure 1. Development of *Striga hermonthica* on the roots of 5057 (susceptible) and ZD05 (resistant) maize genotypes at 3, 9, 15 and 22 days after infestation. Stages of development were defined as follows: S1, attached *Striga* with seed coat intact; S2, emergence of first leaf primordial; S3, attached *Striga* had three or four leaf pairs; S4, attached *Striga* having five or more leaf pairs; dead, attached *Striga* died, evident from tissue discoloration or withering (Amusan et al., 2008). Bars, +1SE.

resistance sources have been identified (Kim et al., 1999); both perennial teosintes (*Z. diploperennis*) and *T. dactyloides* showed relatively higher levels of resistance (Lane et al., 1997; Gurney et al., 2003). In addition, through a long-term breeding effort, researchers from the International Institute of Tropical Agriculture (IITA) developed a *Striga hermonthica*-resistant inbred, ZD05 (Figure 1); this inbred has in its pedigree a *Z. diploperennis* accession as well as tropical maize germplasm (Menkir et al., 2006; Amusan et al., 2008). The resistant ZD05 and the susceptible 5057 differed in root morphology. The resistant inbred had fewer, thin branched roots in the upper profile compared with the susceptible maize (Figure 2). However, further genetic studies are needed to determine the mode of inheritance as well as loci involved in the expression of this trait.

Insect resistance

Another beneficial trait conferred by wild relatives is insect-pest resistance. In fact, insect-pests cause huge yield losses by inducing direct damage to plants and by rendering the grains unfit for human and animal consumption. The major insect-pests of corn are stem and cob borers, rootworms and aphids which are generally polyphagus and damage almost all corn varieties (Aditya and Jitendra, 2014). The wild relatives of maize, viz. *Z. mays* ssp. *mexicana*, Z. *mays* ssp. *diploperennis* and *Z. mays* ssp. *perennis*, have resistance against a number of insect-pests, and these species were used to impart resistance against Asiatic corn borer (Ramirez, 1997). Pásztor and Borsos (1990) reported certain degree of resistance in the maize × Z. *mays* ssp. *mexicana* hybrids for corn borer (*Ostrinia nubilalis*). *T. dactyloides* exhibits resistance to corn rootworms via non-preferences and or antibiosis as reported by Branson (1971), Moellenbeck et al. (1995) and Eubanks (2001). Eubanks (1997, 2001, 2002) crossed *Tripsacum* with diploid perennial teosinte and produced viable recombinants that were cross-fertile with maize. This allowed the incorporation of *Tripsacum* genetic material into corn and development of experimental lines, some of which exhibited rootworm resistance, as evidenced in

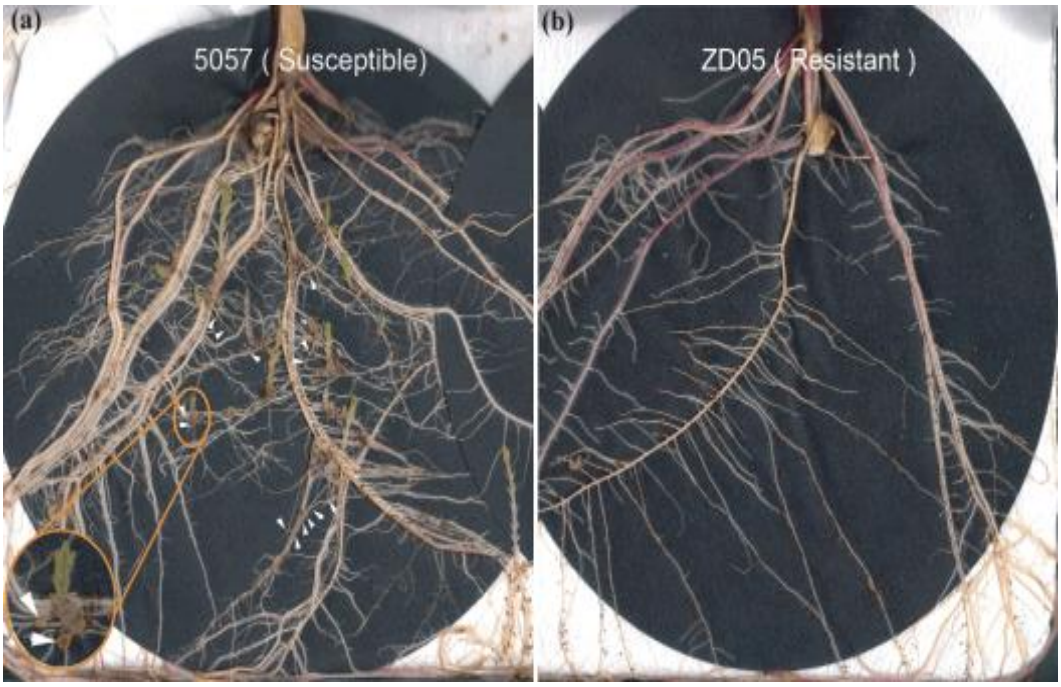

Figure 2. The root systems of 5057 (susceptible) and ZD05 (resistant) maize genotypes at 22 days after infestation with parasite *Striga hermonthica* (Amusan et al., 2008). Arrowheads, secondary haustoria.

insect bioassays and field root damage ratings (Eubanks, 2002; Eubanks, 2006). Similarly, Prischmann et al. (2009) produced *Tripsacum*-introgressed maize germplasm in breeding programmes to enhance plant resistance or tolerance to corn rootworms.

ABIOTIC STRESS RESISTANCE

Drought resistance

Drought is the most significant factor causing crop loss in hybrid maize and climate change resulting from the build-up of greenhouse gases is expected to increase its frequency and severity. The use of genetics to improve drought tolerance and provide yield stability is an important part of the solution to stabilizing global production (Sayadi et al., 2016). That is why the development of maize varieties with enhanced tolerance to drought stress and higher water use efficiency (WUE) has become a high priority goal for major breeding programs, both in the private and public sectors (Sayadi et al., 2016).

Descriptions of the anatomical and other properties of wild relatives, specially *Tripsacum* that contribute to its ability to withstand drought, come from studies of aerenchyma tissue in roots (Comis, 1997; Kemper et al., 1997), root penetration (Clark et al., 1996), and increased biomass (Risser et al., 1981). Physiological evidence suggests that superior drought tolerance in *Tripsacum* is based on high photosynthesis and WUE in leaf gas exchange analysis (Coyne And Bradford, 1985; Kemper et al., 1997). Furthermore, in another study Eubanks (2006) observed that, even under drought stress, the *Tripsacum*-introgressed SDG cultivar outperforms the maize control. *Tripsacum*-introgression appears to confer larger, more robust root systems and overall increase in grain yield.

Tolerance to flooding

Flooding damage to maize is highly dependent on the developmental stage of the plant, the length of the flooding period and the soil-air temperatures. Maize is affected most by flooding in the early stages of growth and hence is a major concern for maize growers due to huge yield losses and limited availability of flooding-tolerant lines (Aditya and Jitendra, 2014).

Although a few maize lines were reported to form adventitious roots at the soil surface during experimental flooding conditions (Mano and Omori, 2007), teosintes obtained from regions that are known to receive frequent rainfall may provide a superior genetic resource for the development of flooding-tolerant maize. The teosintes, viz. *Z*. *nicaraguensis* (Bird, 2000; Iltis and Benz, 2000), *Z*. *luxurians* and *Z*. *mays* ssp. *huehuetenangensis* (Mano et al., 2005), have been observed to exhibit a higher

capacity for adventitious root formation than some maize inbreds. Z. *mays* ssp. *huehuetenangensis* seedlings were observed to exhibit a high adaptability to flooding by developing adventitious roots above the soil surface (Mano and Omori, 2007). As a consequence, the adventitious roots of this teosinte can obtain oxygen, and this characteristic may play an important role in its adaptation to flooding conditions. Similarly, *Z. nicaraguensis* and *Z. luxurians* were reported to develop well- formed aerenchyma in adult plants (Ray et al., 1999) hence imparting tolerance to flooding conditions.

Yield and yield related traits

Yield and yield related traits are mostly governed by polygenes, and the role of alien germplasm to improve quantitative traits is less reported. The possible reason for this is a limitation in introgressing a large number of loci responsible for expression of a quantitative trait into the target host (Dela Vina et al., 1995). However, by introgression of alien chromatin segments both from teosintes and *Tripsacum*, Cohen and Gallinat (1984) suggested improvement of maize inbreds by a significant increase of yield and combining ability. In addition, Wang et al. (2008) crossed maize with *Z. mays* ssp. *mexicana* and reported that 54.6% of the hybrids had a higher yield than the superior maize hybrid checks. They also observed that the advanced backcross generations exhibited improved characters like a large number of tillers, increased height and increased 100-kernel weight. In a recent study, Liu et al. (2016b) performed joint linkage QTL analysis on each of the kernel size traits including area, perimeter, length, and width, kernel shape traits including roundness and length/width ratio (LW), weight of 50 kernels (Wt50k) and kernel density (FFD), as well as the principal component (PC) traits, in order to identify the loci responsible for kernel trait differences between teosinte and maize. They identified 43 QTL for kernel size traits, 11 QTL for kernel shape traits, four QTL for FFD, and five QTL for Wt50k. The 63 QTL were distributed only on chromosomes 1 to 8, with no QTL on chromosomes 9 and 10. For the newly defined PC traits, PC1, PC2, and PC3, the total number of QTL detected were 15, 3 and 5, respectively. The same study revealed that maize-teosinte introgression populations provide substantial power to detect pleiotropy among overlapping QTL for multiple traits. In fact, positive pleiotropy was observed between kernel weight and kernel size traits (area, perimeter, and length) and was observed among the size traits themselves. Wt50k also had positive pleiotropy with FFD. In contrast to kernel size traits, the kernel shape traits (roundness and L/W) show negative pleiotropy with each other. In another study, Liu et al. (2016a) developed 10 NIL populations derived from geographically diverse teosinte accessions by backcrossing 10 accessions into the B73 background for four generations before inbreeding. They identifed four QTL for KRN located on chromosomes 1, 2, 4 and 5, which accounted for 33.7% of the phenotypic variation. However, They were unable to calculate the effect of two alleles (Z029 allele for chromosome 4, and Z030 allele for chromosome 5) because of lack of introgression of those donors in the QTL region. Of the 38 remaining alleles, 27 alleles (71%) signifcantly decreased KRN, and no alleles increased KRN. For Wt50k, they identifed eight QTL for kernel weight located on chromosomes 1 (three QTL), 2, 3 (two QTL), 5, and 8, which accounted for 38.2% of the phenotypic variation. They were also unable to calculate the effect of nine alleles because of lack of introgression in the QTL region. Of the remaining 71 alleles, 30 alleles signifcantly decreased Wt50k, while one allele (Z036 allele for chromosome 2) significantly increased seed weight. More recently, a study done by Karn et al. (2017) revealed that teosinte can be exploited for the improvement of kernel composition traits in modern maize germplasm. In fact, teosintes near isogenic lines (NILs) were developed by backcrossing ten accessions of geographically diverse *Zea* mays ssp. *parviglumis* into the inbred B73 for four generations prior to inbreeding, creating a total of 961 NILs. They identified a total of eight QTL across the three traits: Two starch QTLs that explained 18% of the variation, three protein QTLs that explained 23% of the variation, and six oil QTLs which explained 45% of variation. The chromosome 1 QTL was significant for both protein and oil, and the chromosome 3 QTL was significant for all three traits. In addition, a total of 9 starch, 12 protein, and 25 oil teosinte significant alleles were identified. All the QTLs had a range of strong additive allelic effects, with the largest allelic effects for starch, protein, and oil QTLs being -2.56, 2.21 and 0.61% dry matter, respectively, and displayed both positive and negative additive allelic effects depending upon the trait.

CONCLUSION AND FUTURE PROSPECTS

The studies reported in this review described the continuing increase in the use of wild relatives for the production of new cultivars of maize. The ultimate value of these studies awaits demonstration that maize improvement can be advanced by interesting traits present in Teosintes and *Tripsacum*. It is specially the alleles of these wild relatives that need to be added into maize breeding programmes. Moreover, the *Tripsacum*-teosinte genetic bridge, which permits transfer of new genes into maize with conventional plant breeding methods, offers an exciting opportunity to overcome the *Tripsacum*-maize crossing barrier and confert new genetic diversity in maize breeding. The transfer of important traits such as resistance to chlorotic dwarf virus, downy mildew, *Fusarium, Colletotrichum graminicola*, *Helminthosporium turcicum*, *H. maydis*, *Erwinia stewarti*, *Puccinia sorghi, Striga hermonthica,* rootworms, drought

resistance, tolerance to flooding and increase of yield and combining ability described herein are just a few examples of a suite of valuable traits that could be targeted for maize improvement with these wild relatives.

Improving molecular technologies such as marker-assisted breeding, interspecific hybridization techniques and genetic knowledge will continue to increase the capacity to use the valuable traits found in maize wild relatives. In these circumstances, it becomes increasingly important to conserve a broad range of diversity of teosintes and *tripsacum* species, and their utilization must also increase in importance.

Further studies on phenotypic and genomic picture of introgression could greatly expand the understanding of particular alleles and genes' flow between maize and its wild relatives.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Assessment of genetic diversity among released and elite Ethiopian barley genotypes using simple sequence repeat (SSR) markers

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Barley is a major cereal grown widely and used in several food products, beverage production and animal feed. Being the fourth most important cereal crop in the world and the fifth rank in Ethiopia, it is a cash crop and used as a source of malt by the brewery industries, as food for human and feed for animals. Genetic diversity assessment is a key component in breeding programs. High level of polymorphism, codominant and multi allelic nature of simple sequence repeats (SSRs) markers make them preferable for diversity analysis in plant species. In this study, 22 SSRs markers were used to characterize the genetic diversity of 39 released and elite barley varieties collected from barley breeding program in Ethiopia. The amplification of SSRs loci were obtained for 35 primer pairs and only 22 of them showed clear polymorphic patterns which produced a total of 73 alleles with an average of 5 alleles per locus. The data generated by these informative primers were sufficient to discriminate the analysed barley genotypes. Based on the dissimilarity matrices ranging from 0.11 to 0.58, the genotypes were grouped into three major groups. The calculated polymorphism information content (PIC) values ranges from 0.17 to 0.60 with an average of 0.47 which shows the importance of the markers for future diversity analysis of barley. Locus HVACL1 and HVM36 shows higher PIC and locus HVBDHN7 shows lower PIC in this characterized barley genotype. This result will be useful for barley germplasm management and improvement in terms of biodiversity protection and design of new crosses for future breeding purpose.

Key words: Barley, elite, polymorphism information content (PIC), released, similarity, simple sequence repeats (SSRs), varieties.

INTRODUCTION

Barley (*Hordeum vulgare* L.), being the fourth most important cereal crop in the world, and ranks fifth in Ethiopia (CSA, 2012), is a cash crop and used for brewing malts, animal feed and human consumption (Hayes et al., 2002). The Ethiopian landrace barleys have been known to the botanical communities, notably

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from Vavilov's extensive collections and study. As cited by Abebe and Bjornstad (1997) Scheiemann stated that Ethiopia is considered as secondary center of diversity or center of origin for barley, which belongs to Poaceae. The diversity of barley in Ethiopia is quite high for an extended history of cultivation and variant agro-ecosystems (Eticha et al., 2010). Environmental factors such as varied soil types, altitudinal variation and climatic factors contribute to the diversity of barley manifested in Ethiopia. The entire cultivated barley of Ethiopia is a farmer variety or landrace (Hadado et al., 2010). The morphological characterizations of landraces of barley were studied (Eticha et al., 2010). The development of molecular markers makes it easy to assess genetic diversity in crops at DNA level (Reif et al., 2003). Molecular markers such as RAPD (Fernández et al., 2002; Meszaros et al., 2007), AFLP (Zhang and Ding, 2007a), ISSR (Fernández et al., 2002), STS (Meszaros et al., 2007) and SSR (Turuspekov et al., 2001; Matus and Hayes, 2002; Feng et al., 2006; Meszaros et al., 2007; Zhang et al., 2007b) can be used to estimate genetic diversity. Ramsay et al. (2000) developed SSR markers for molecular characterization and linkage mapping of barley. Molecular diversity of *H. vulgare* L. was studied using SSR markers (Wang et al., 2010; Hadado et al., 2010) and the primers were designed by Ramsay et al. (2000). Chaabane et al. (2009) also characterized barley collections of Tunisia, Syria and Denmark by SSR markers. But, the barley collections were not from Ethiopia.

SSRs are codominant, abundant, informative and their detection is very simple (Matus and Hayes, 2002). This makes them an excellent molecular marker system for analysis of genetic diversity. In this study, the authors used a set of 35 SSRs from seven linkage groups (five per each) of barley genome of which 22 were polymorphic to characterize 39 released and elite verities of barley obtained from barley breeding program of Holeta Agricultural Research Center. The objectives of this study were to assess genetic diversity and relationship of released and elite barley varieties for use in improvement and germplasm management.

MATERIALS AND METHODS

Plant materials

A total of 39 released and elite barley varieties were used in this study (Table 1). These barley varieties were provided by barley breeding program units of Holeta Agricultural Research Center.

Genomic DNA extraction

Five seeds of each genotype were sown in plastic pot of dimension (6.8 x 6.8 x 7.8 cm) and allowed to grow in greenhouse compartment in 2016, at National Agricultural Biotechnology Research Center. The soil mixture was red ash, frost soil and animal dung in the ratio of 1:1:1 and sterilized at a temperature of 150°C for 3 h. Two weeks later, the seedlings ranging from five to seven leaves were targeted and approximately, 100 mg young leaves tissues of each genotype were used for DNA extraction. DNA was extracted from each fresh and dried leaf following modified CTAB method (Doyle and Doyle, 1990).

The presence and absence of gDNA was checked in agarose gel electrophoresis (0.8% Agarose in 100 ml of 1XTAE, 5 µL of gDNA+1.5 µL of 1X Loading dye) run for 30 min at 100 V (Figure 1). DNA quality and concentration was estimated using Nano Drop Spectrophotometer (ND-8000, Thermoscitnific). DNA samples were then diluted to a concentration of 20 ng/µL using ddH_2O and stored at -20, -80 and -196°C (Yuanzheng and Angell, 2005).

Acquisitions of SSR markers and PCR amplification

SSR markers acquisition

Literature based search was done to find appropriate SSR markers for barley. Accordingly, thirty five SSR markers were found from Wang et al. (2010). All of them were screened for amplification and usefulness and 22 of them were found to be polymorphic (Table 2). The consistency of the band profiles SSR markers was assessed across the DNA samples by repeating amplifications and only the repetitive PCR products were scored.

PCR optimization, primer screening and PAGE

Polymerase chain reaction was optimized starting from the reaction set up described in Wang et al. (2010). Accordingly, PCR was carried out in a 25-µL final volume containing 2 µL of 20 ng/µL genomic DNA templates, 2.5 µL of 1X PCR buffer containing 15 mM Mg^{2+}, 0.5 µL of 15 mM dNTP mixture (2.5 mM of each), 1.25 µL of 5 u/µL of Taq DNA polymerase, and 0.25 µL of 10 µM forward and reverse primers and 1.6 ng/µL of gDNA (20 ng/µL of stock) for amplification. Depending on the primer pair used, DNA amplification was performed using master cycler (Pros, eppendorf), with a thermo cycler program of 1 cycle 4 min at 94°C of initial denaturation, followed by 35 cycles 30 s denaturation at 94°C, 30 s annealing (specific for each primer) (Table 2) and 30 s of extension at 72°C. The final extension was for 10 min at 72°C with final holding at 4°C. For primer Bmac0032, gradient PCR between 45-65°C was applied to get an optimum annealing temperature (Table 2). The success of the PCR and the associated yield was assessed in 2% agarose gel (2 g agarose in 50 ml of 1xTAE, 5 µL of gDNA+1.5 µL of 1X Loading dye with gel red (mix (1000:1)) and run for 30 min at 100 V. Once the optimization is over, the same PCR setup (as described above) was applied for amplification of SSRs with all the 22 primers across the entire barley genotypes studied. Microsatellite allele separation was carried out using polyacrylamid gel electrophoresis also called native DNA PAGE with a dual vertical electrophoresis apparatus (Cleaver, CS500 volt). The recipes used were polyacrylamide gel (5 µL of 10x TBE, 22 ml of 40% (29:1, acrylamid: bis acrylamid solution), 66 µL of TEMED, 80 µL of Nuclease free water) and 5 µL of PCR product +3µL of 1X Loading dye) run at 150 V for 1:30 h. PAGE picture (Figure 2) was captured using gel documentation system (3uv bench top, M-20 transilluminator).

Data analysis

The number of alleles detected by each SSR marker was estimated for each genotype and all SSR marker loci were scored as described by Struss and Plieske (1998). Data obtained from SSR analysis were scored as presence (1) or absence (0) of fragments for each barley genotype. Polymorphism information content (PIC), number of allele, allele frequency and gene diversity were

Table 1. List of released and elite barley varieties.

S/N	Varieties	Type	Row number	Maturity category	Seed source/pedigree*
1	HB-120	Malt	2	late High land potential	BSM2012
2	HB 52	Malt	2	High land potential	BSM2012
3	HB1533	Malt	2	High land potential	BSM2012
4	Holker	Malt	2	High land potential	BSM2012
5	Beka	Malt	2	High land potential	BSM2012
6	M-12	Malt	-	- ---	BSM2012
7	EH1847	Malt	2	Late High land	BSM2012
8	IBON174/03	Malt	2	Mid high land	BSM2012
9	Bekoji1	Malt	2	Late high land	BSM2012
10	Sabini	Malt	2	Mid high land	BSM2012
11	Bahati	Malt	2	Late high land	BSM2012
12	Ferie Gebes	Malt	2	Late high land	NMBADT 2012 P#2,15
13	HB1307	Food	6	Medium to late	Breeder seed
14	Shegie	Food	6	Medium to late	Breeder seed
15	HB42	Food	6	Medium to late	Breeder seed
16	Ardu1260B	Food	6	Medium to late	Breeder seed
17	Dimtu	Food	1r	Medium to late	Breeder seed
18	Cross 41/98	Food	6	Medium to late	Breeder seed
19	EH1493	Food	-	Medium to late	Breeder seed
20	Yedogit	Food	6	Medium to late	FBADT2012,-LS P#10
21	Estayish	Food	6	Medium to late	FBADT2012-LS P#5
22	Tiret	Food	6	Medium to late	FBADT2012-LS P#12
23	Shedeho	Food	6	Medium to late	FBADT2012-LS P#15
24	Hardu	Food	6	Medium to late	FBADT2012-LS P#6
25	Agegnehu	Food	6	Medium to late	FBADT2012-LS P#9
26	Tolose	Food	-	Medium to late	Seed Stock
27	Abdane	Food	-	Medium to late	FBADT2012-LS P#7
28	Baleme	Food	-	Medium to late	Seed stock
29	Dribie	Food	6	Early	FBADT2012-LS P#4
30	Tila	Food	6	Early	FBADT2012-LS P#5
31	Abay	Food	6	Early	FBADT2012-LS P#8
32	Biftu	Food	6	Early	FBADT2012-LS P#1
33	Dafo	Food	6	Early	FBADT2012-LS P#6
34	Dinsho	Food	2	Early	FBADT2012-LS P#12
35	Mulu	Food	ir	Early	FBADT2012-LS P#9
36	Setegne	Food	6	Early	FBADT2012-LS P#11
37	Misrach	Food	6	Early	FBADT2012-LS P#7
38	Basso	Food	6	Early	FBADT2012-LS P#2
39	Mezezo	Food	6	Early	FBADT2012-LS P#10

*BSM: Breeder seed maintenance; NMBADT: National Malt Barley Adaptation Trial; FBADT: food barley adaptation trial.

calculated using Power Marker V3.25 (Liu and Muse, 2005) (Table 3). Dendrogram was constructed using Darwin 6.0 software (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006) based on the dissimilarity matrices and neighbour joining (NJ) clustering method.

RESULTS

PIC statistics and SSR analysis

The PIC values of markers can provide an estimate of discrimination power in a set of accessions by taking not only the number of alleles, but also the relative frequencies of each allele (Smith et al., 2000). Based on this, the PIC value of this study was calculated using Power marker v3.25 and found to range from 0.17 to 0.60 and provide an estimate of discrimination power in a set of released and elite barley accessions. Similarly, number of allele, allele frequency and gene diversity was calculated using power marker v3.25, and resulted in minimum and maximum values of 2, 4 for number of

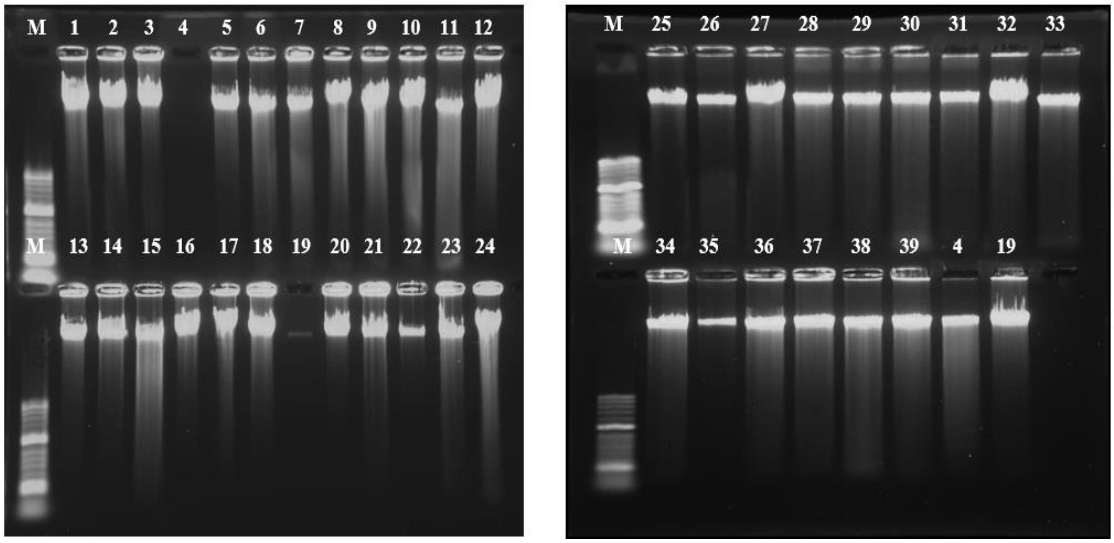

Figure 1. Genomic DNA extracted from the 39 barley cultivar (last two are repetitions) following CTAB method and loaded in 0.8% Agarose Gel concentration; M is size marker.

Table 2. Primer sequences, fragment sizes and repeat types of 22 barley SSR markers.

Locus	Chromosome	Repeat motif	Primer sequence (5′→3′)	Ta (°C)	Expected size (bp)
HVBDG	5(1H)	(CT)6	GAGAGAGAAAGAGAATGGCAGG AAAAAACTGCACCCAATCACTT	60	145
HVDHN7	7(5H)	(AAC)5	TTAGGGCTACGGTTCAGATGTT ACGTTGTTCTTCGCTGCTG	58	177
HVRCABG	4(4H)	(GA)6	TTTAAAAGAAAAGTGAATGGC TAATGAAGAATGAGGAGAAGC	55	123
HVM40	4(4H)	$(GA)_6(GT)_4(GA)_7$	CGATTCCCCTTTTCCCAC ATTCTCCGCCGTCCACTC	55	160
HVACL1	7(5H)	$(AT)_7$	TTTGGAATTATTCTGTGGGACC GGGATTCAATCAAGTATTCGGA	60	150
HVM36	2(2H)	$(GA)_{13}$	TCCAGCCGACAATTTCTTG AGTACTCCGACACCACGTCC	55	114
HVLEU	7(5H)	$(ATTT)_4$	TTGGAAGTGTACAGCAATGGAG TGAAAGGCCCCACAAGATAG	60	166
Bmag0006	3(3H)	$(AG)_{17}$	TTAAACCCCCCCCCTCTAG TGCAGTTACTATCGCTGATTTAGC	58	174
Bmag0217	1(7H)	$(AG)_{19}$	AATGCTCAAATATCTATCATGAA GGGGCTGTCACAAGTATATAG	58	196
Bmag0853	3(3H)	$(GA)_{15}$	ACAAGTATCCTGCAAACCTAA CGACCTTCTTAATGGTTAGTG	55	183
Bmag0905	3(3H)	$(TC)_{14}$	TTTATCTCCCCCTAGATAGAAG TCTCCGTATATTTAGGAAACG	55	177
Bmag0508A	3(3H)	$(AG)_{14}$	TCTCCGTATATTTAGGAAACG TATCTCCCCCTAGATAGAAGG	55	175
Bmag0807	6(6H)	$(TC)_{18}$	GGATATAAGGGTCCATAGCA AATTACATCAAATAGGCTCCA	55	111
Bmag0375	4(4H)	$(AG)_{19}$	CCCTAGCCTTCCTTGAAG TTACTCAGCAATGGCACTAG	58	135
Ebmag0793	2(2H)	$(GT)_{13}(AG)_{36}$	ATATATCAGCTCGGTCTCTCA AACATAGTAGAGGCGTAGGTG	55	177
HVBKASI	2(2H)	$(C)_{10}(A)_{11}$	ATTGGCGTGACCGATATTTATGTTCA CAAAACTGCAGCTAAGCAGGGGAACA	60	197

Table 2. Contd.

Bmac0032*	5(1H)	$(AC)_7T(CA)_{15}(AT)_9$	CCATCAAAGTCCGGCTAG GTCGGGCCTCATACTGAC	53.4	215
Bmac0209	3(3H)	$(AC)_{13}$	CTAGCAACTTCCCAACCGAC ATGCCTGTGTGTGGACCAT	58	176
Bmac0216	2(2H)	$(AC)_5$	GTACTATTCTTTGCTTGGGC ATACACATGTGCAAAACCATA	55	190
EBmac0501	5(1H)	$(AC)_{13}$	ACTTAAGTGCCATGCAAAG AGGGACAAAAATGGCTAAG	58	151
EBmac0679	4(4H)	$(AC)_{22}$	ATTGGAGCGGATTAGGAT CCCTATGTCATGTAGGAGATG	55	148
Bmac0577	4(4H)	$(AC)_{12}$	TCATACAGAAGCCCACACAG TGCATGTTCATTCTAGACAGG	53	146

Source; Wang et al. (2010); *For that primer, annealing temperature is the result of optimization in this study.

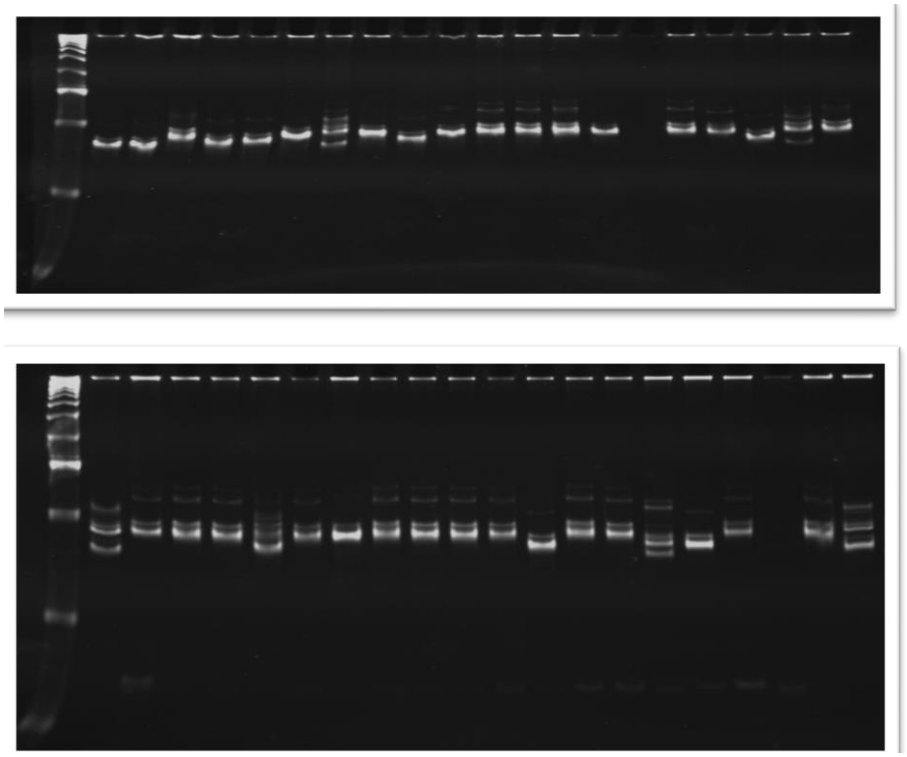

Figure 2. An example of marker profile of barley cultivars with the SSR marker HVM40. Where the first lane in both A and B is ladder size marker and the rest of the lanes represent the 39 barley genotypes except the 15th lane in A which is a gap.

allele, 0.38, 0.90 for allele frequency and 0.18, 0.67 for gene diversity, respectively (Table 3).

Dendrogram obtained with SSR markers

Dendrogram obtained from application of Darwin 6.0 using the dissimilarity matrices (Table 4), grouped the genotypes into three major groups. Cultivar Misrach and Mezezo showed greater genetic distance as compared to Cultivar Dafo, and HB120 with HB52 which showed lower genetic distance (Table 4).

DISCUSSION

SSR markers in barley genetic diversity analysis

In this study, 22 SSR markers were chosen for 39 released and elite barley genetic diversity analysis and they were from Chr. 1, Chr. 2, Ch. 3, Chr. 4, Chr. 5, Chr. 6 and Ch7 (Table 2). As far as genome coverage is

Table 3. PIC, gene diversity, major allele frequency and number of alleles generated from 22SSR markers across the genome of 39 released and elite barley cultivars.

S/N	Marker	Major allele frequency	Allele no.	Gene diversity	PIC
1	Bmac0032	0.54	4	0.62	0.57
2	HVBDG	0.64	2	0.46	0.35
3	HVBDHN7	0.90	2	0.18	0.17
4	HVRCABG	0.54	3	0.52	0.41
5	HVM 40	0.62	4	0.54	0.47
6	HVACL1	0.49	4	0.65	0.60
7	HVM36	0.38	4	0.67	0.60
8	Bmac0209	0.62	4	0.51	0.42
9	Bmac0216	0.77	3	0.37	0.31
10	Bmag0006	0.51	3	0.60	0.53
11	EBmac0501	0.46	4	0.62	0.55
12	EBmac0679	0.46	3	0.59	0.50
13	Bmag0217	0.62	4	0.52	0.45
14	Bmag0853	0.49	3	0.63	0.56
15	HVLEU	0.56	2	0.49	0.37
16	Bmag0905	0.59	3	0.52	0.43
17	Bmag-0508	0.49	4	0.57	0.48
18	HABKASI	0.74	3	0.39	0.33
19	EBmag0793	0.72	4	0.45	0.42
20	Bmac0577	0.49	3	0.55	0.44
21	Bmag0807	0.62	4	0.52	0.45
22	Bmag0375	0.74	3	0.39	0.33
	Mean	0.57	5	0.54	0.47

concerned, it may be arguable that the number of selected markers is low for barley genetic diversity study. However, we still obtained many alleles, and most of them were polymorphic. Although, some barley germplasms were not discriminated by the cluster analysis (Figure 3), the general classification was informative. It indicated that the genetic structure of barley germplasms in the study was high, which mainly attributed to difference in the genetic background of the studied barley cultivar. Therefore, it is inferred that the SSR marker used were relatively of high efficiency for barley genetic analysis and could reveal the genetic differences of barley germplasms as described in previous studies (Maroof et al., 1994; Struss and Plieske, 1998; Turuspekov et al., 2001; Matus and Hayes, 2002; Feng et al., 2006; Zhang et al., 2007b; Mikel and Kolb, 2008).

Genetic diversity for barley germplasm protection and barley breeding

With the cluster analysis, we were to identify relatively fewer number of genotype group (Figure 3) instead of total discerning of the whole genotypes in many possible groups. Two possible reasons may be attributed to the obtained result. One of the reasons could be some of the studied materials were duplications of the others or might have been developed from very closely related sister lines. In each case, the obtained result is justified. The other could be the smaller number of SSR markers used leading to smaller genome coverage which otherwise could be a good source of additional discrimination power used. In either case, we optimistically consider the SSR markers appear to provide an optimal platform to identify duplicated materials in the barley germplasm collection (Struss and Plieske, 1998), and they are helpful in managing the barley collections for subsequent barley improvement programs.

However, the result suggests that a more comprehensive result could also be obtained if more representations of germplasms and number of good genome coverage are considered future barley diversity study.

SSR markers in new variety protection

As a general knowledge and fact, molecular fingerprinting is an effective and accurate way to identify crop varieties (Nandakumar et al., 2004). In this study, it was found that

Table 4. Dissimilarity matrices for 39 studied barley varieties.

Units	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
2	0.15																																					
3	0.33	0.31																																				
4	0.33	0.31	0.24																																			
5	0.26	0.18	0.21	0.26																																		
6	0.38	0.32	0.31	0.35	0.23																																	
7	0.24	0.26	0.24	0.29	0.26	0.31																																
8	0.31	0.33	0.22	0.32	0.29	0.29	0.16																															
9	0.42	0.35	0.34	0.34	0.31	0.31	0.43	0.37																														
10	0.30	0.36	0.26	0.35	0.37	0.40	0.31	0.24	0.39																													
11	0.36	0.33	0.37	0.32	0.24	0.33	0.37	0.44	0.32	0.42																												
12	0.41	0.34	0.38	0.24	0.35	0.38	0.38	0.36	0.38	0.38	0.31																											
13	0.45	0.38	0.38	0.33	0.31	0.34	0.42	0.41	0.38	0.38	0.26	0.28																										
14	0.37	0.34	0.33	0.43	0.35	0.43	0.33	0.36	0.47	0.39	0.41	0.37	0.32																									
15	0.36	0.38	0.37	0.46	0.43	0.46	0.32	0.25	0.50	0.24	0.53	0.45	0.41	0.31																								
16	0.49	0.42	0.37	0.46	0.39	0.33	0.46	0.35	0.46	0.38	0.44	0.41	0.31	0.36	0.40																							
17	0.54	0.47	0.38	0.42	0.39	0.33	0.47	0.41	0.38	0.47	0.35	0.46	0.41	0.47	0.54	0.45																						
18	0.29	0.21	0.34	0.29	0.31	0.44	0.39	0.42	0.34	0.35	0.27	0.33	0.33	0.33	0.46	0.42	0.38																					
19	0.34	0.27	0.35	0.31	0.32	0.36	0.35	0.33	0.31	0.32	0.33	0.30	0.30	0.25	0.29	0.42	0.38	0.33	0.15																			
20	0.41	0.34	0.38	0.29	0.35	0.30	0.38	0.36	0.33	0.38	0.41	0.18	0.41	0.42	0.49	0.41	0.41	0.38	0.30																			
21	0.38	0.32	0.31	0.31	0.23	0.27	0.31	0.42	0.39	0.40	0.24	0.30	0.20	0.34	0.46	0.33	0.43	0.35	0.32	0.34																		
22	0.38	0.31	0.25	0.34	0.22	0.26	0.34	0.37	0.34	0.31	0.37	0.33	0.29	0.33	0.37	0.32	0.42	0.38	0.31	0.29	0.22																	
23	0.44	0.37	0.26	0.31	0.29	0.28	0.36	0.43	0.41	0.37	0.29	0.35	0.31	0.35	0.43	0.34	0.03	0.36	0.37	0.31	0.18	0.12																
24	0.47	0.45	0.44	0.49	0.42	0.45	0.44	0.49	0.49	0.43	0.43	0.47	0.53	0.43	0.51	0.43	0.57	0.49	0.53	0.47	0.45	0.39	0.42															
25	0.43	0.32	0.31	0.35	0.32	0.32	0.35	0.38	0.35	0.40	0.33	0.30	0.25	0.24	0.38	0.33	0.43	0.31	0.27	0.34	0.22	0.28	0.41															
26	0.46	0.43	0.38	0.33	0.40	0.29	0.33	0.41	0.47	0.43	0.46	0.05	0.00	0.47	0.50	0.05	0.00	0.47	0.47	0.47	0.42	0.43	0.38	0.35	0.49	0.39												
27	0.41	0.34	0.33	0.33	0.31	0.20	0.38	0.36	0.33	0.43	0.36	0.37	0.32	0.46	0.45	0.41	0.04	0.42	0.38	0.37	0.34	0.38	0.39	0.39	0.30	0.03	0.37											
28	0.46	0.36	0.44	0.39	0.41	0.32	0.04	0.44	0.40	0.42	0.04	0.38	0.38	0.47	0.42	0.42	0.47	0.35	0.32	0.38	0.32	0.31	0.32	0.36	0.32	0.43	0.30											
29	0.38	0.26	0.30	0.34	0.32	0.26	0.30	0.37	0.38	0.31	0.37	0.33	0.38	0.38	0.32	0.37	0.46	0.34	0.35	0.29	0.26	0.16	0.17	0.31	0.22	0.33	0.29	0.17										
30	0.46	0.40	0.35	0.39	0.41	0.32	0.04	0.44	0.04	0.43	0.04	0.36	0.38	0.47	0.42	0.42	0.43	0.39	0.40	0.43	0.36	0.26	0.23	0.36	0.27	0.43	0.30	0.17										
31	0.41	0.34	0.42	0.38	0.39	0.34	0.38	0.41	0.05	0.45	0.04	0.45	0.38	0.34	0.32	0.38	0.29	0.35	0.39	0.30	0.42	0.32	0.25	0.19	0.31	0.22	0.33	0.29	0.25									
32	0.45	0.39	0.34	0.43	0.40	0.26	0.38	0.41	0.42	0.49	0.41	0.04	0.46	0.46	0.35	0.29	0.35	0.26	0.32	0.39	0.26	0.38	0.29	0.26	0.31	0.26	0.38	0.29	0.26	0.33								
33	0.51	0.49	0.35	0.40	0.46	0.41	0.49	0.51	0.44	0.04	0.49	0.55	0.47	0.55	0.53	0.55	0.51	0.48	0.49	0.45	0.34	0.45	0.35	0.37	0.58	0.45	0.44	0.47	0.45	0.39	0.41	0.51	0.35					
34	0.34	0.36	0.30	0.40	0.32	0.31	0.03	0.47	0.43	0.03	0.47	0.43	0.03	0.38	0.43	0.44	0.04	0.45	0.43	0.41	0.39	0.37	0.46	0.03	0.45	0.31	0.36	0.03	0.44	0.05	0.45	0.36	0.34	0.44	0.54			
35	0.28	0.25	0.24	0.24	0.26	0.34	0.29	0.36	0.38	0.34	0.26	0.28	0.32	0.03	0.24	0.46	0.02	0.25	0.37	0.03	0.29	0.31	0.39	0.02	0.42	0.03	0.24	0.30	0.03	0.37	0.33	0.43	0.03	0.24	0.30	0.37		
36	0.42	0.39	0.34	0.34	0.41	0.44	0.04	0.46	0.04	0.46	0.04	0.50	0.43	0.05	0.50	0.50	0.47	0.03	0.48	0.03	0.36	0.04	0.40	0.03	0.46	0.04	0.35	0.03	0.38	0.04	0.46	0.03	0.35	0.04	0.38	0.34	0.44	0.24
37	0.53	0.46	0.46	0.54	0.47	0.42	0.05	0.46	0.04	0.49	0.04	0.49	0.05	0.49	0.03	0.45	0.04	0.37	0.04	0.50	0.04	0.41	0.04	0.47	0.04	0.42	0.03	0.54	0.04	0.40	0.03	0.56	0.04	0.47	0.04	0.41		
38	0.47	0.45	0.40	0.44	0.42	0.41	0.04	0.40	0.04	0.38	0.04	0.44	0.04	0.43	0.04	0.47	0.03	0.40	0.04	0.26	0.04	0.41	0.04	0.35	0.04	0.42	0.04	0.46	0.03	0.31	0.04	0.49	0.03	0.39	0.03	0.26	0.03	0.31	0.21
39	0.38	0.35	0.39	0.39	0.36	0.39	0.02	0.42	0.03	0.39	0.03	0.37	0.03	0.38	0.04	0.60	0.54	0.05	0.47	0.04	0.54	0.05	0.47	0.03	0.43	0.03	0.35	0.03	0.38	0.03	0.35	0.03	0.38	0.03	0.35	0.03	0.34	0.03	0.39	0.02	0.56	0.04	0.40	0.02	0.39	0.03	0.36	0.02	0.39	0.03	0.25

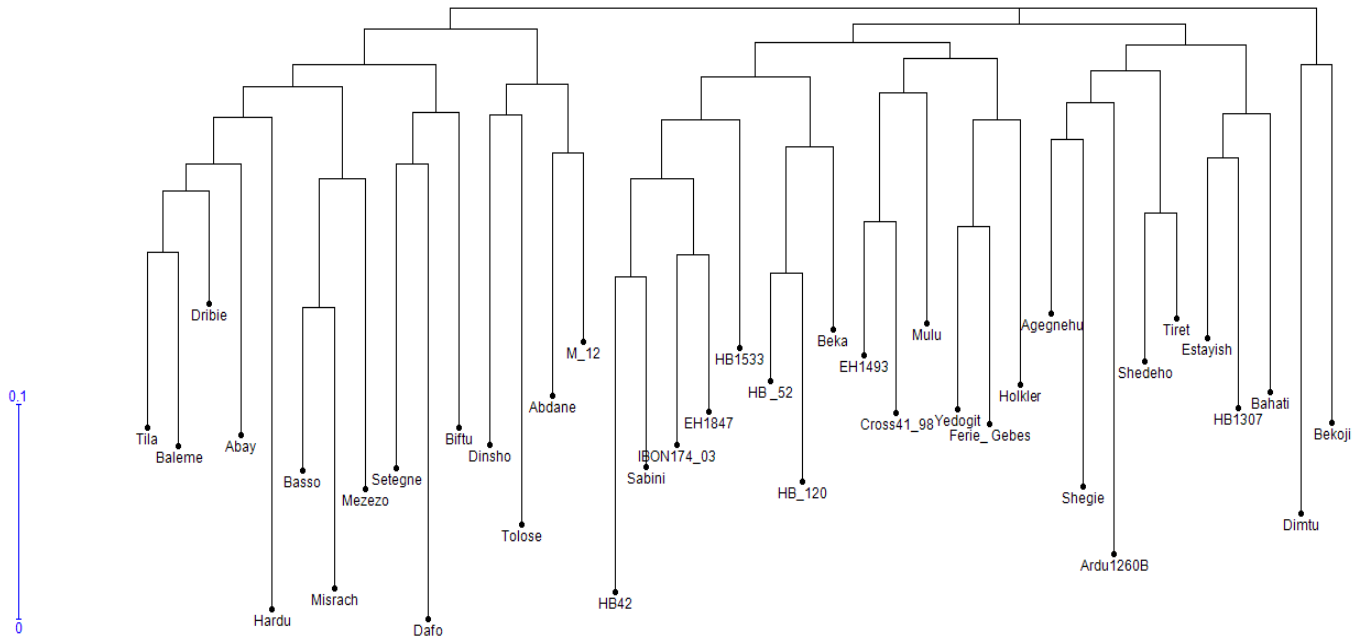

Figure 3. Dendrogram constructed using 22 SSR markers across 39 barley cultivars.

the SSR marker have at least distinguished the released barley cultivars from other elite barley cultivars. This suggests that we can protect the breeder rights of the released barley cultivars by using SSR markers. Similarly, we can identify all barley landraces by SSR fingerprinting which intern allows us to find out whether there are varietal duplications or mistakes in a given germplasm collection.

Conclusions

In conclusion, genetic variation is a raw material for plant breeding and assessments of existing similarities or differences in any crop germplasm pool. It plays a great role in a predictable area to improve agricultural production and productivity, to solve food insecurity in developing world. This study was conducted to determine the levels of genetic variation in released and elite Ethiopian barley materials. The good information content of the markers used, estimated extent of diversity and limited clustering among the studied barley materials are basic outcomes of this study upon which a more comprehensive study can be built. Relatively speaking however, the results can still be used for the consumption of barley breeding programs where breeders should think of the distinctness of the varieties already released and their future plans to release new ones. Finally, diversity study such as this is useful for the establishment of genetic relatedness and molecular characterization of barley germplasm. This in turn benefits barley breeding programs to make choice of the genotypes to be used in crosses and will facilitate the germplasm management.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Rhizobium inoculation and sulphur fertilizer improved yield, nutrients uptake and protein quality of soybean (*Glysine max* L.) varieties on Nitisols of Assosa area, Western Ethiopia

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A greenhouse experiment was conducted to study the effect of inoculation of *Rhizobium* strain and sulphur fertilization on seed and straw yields, nutrients uptake and seedquality protein of two soybean (*Glysine max* L.) varieties. The experiment consisted of four levels of S (0, 20, 30 and 40 kgha^{-1}), three *Rhizobium* strains (MAR-1495, SB-6-1-A$_2$and TAL-379) and two soybean varieties (Belessa-95 and Wollo) combined factorially in complete randomized design (CRD) with three replications. Grain and straw yield and nutrients uptake increased by inoculation of *Rhizobium* strain whether used alone or in combination with S. Application of strain and S further increased seed and straw yield and nutrients uptake. Seed yields of Bellessa-95 inoculated with MAR-1495 at 30 and 40 kg S ha^{-1} were estimated to be 3864.1 and 3893.8 kg ha^{-1}, corresponding to 112 and 114% increase; but Wollo was with seed yields of 3633.3 and 3709.2 kg ha^{-1}, corresponding to 160 and 166% increase, respectively over control. Estimated soil N balance was maximum for S application along with inoculation of strains, ranged from -29.25 kg ha^{-1} in control to 80.74 kg ha^{-1} for Bellessa-95 and from -21.76 kg ha^{-1} to 84.66 kg N ha^{-1} for Wollo, respectively. Efficient seed nutrients uptake due to combined application further caused significant ($p<0.001$) increase in protein yield of soybean. With strain MAR-1495 protein yield varied from 26% in the control to 41.5% for Belessa-95 at 30 kg S ha^{-1}, while from 28% in the control to 44% for Wollo at 40 kg S ha^{-1}, respectively. The results clearly suggested that balanced application of S along with *Rhizobium* strain can affect grain and straw yield and nutrients uptake of soybean varieties, enhance their growth and improve grain quality protein as well as benefit on soil N balance in S-deficient soils.

Key words: Sulphur, strain, nutrients use efficiency, quality protein, soil nitrogen balance.

INTRODUCTION

Soybean (*Glycine max* L.) is one of grain legumes and it is grown for its edible bean, an important source of inexpensive and high quality protein (40%) and oil (20%) around the world (Laswai et al., 2005). In Ethiopia, soybean is an important food crop widely produced in high rainfall areas, in west and southwestern parts (such

as Assosa) and it is recently integrated into the cropping systems and serves as a cash crop for smallholder farmers of the area (Nigussie et al., 2009). Legumes including soybean are able to fix atmospheric N_2 in association with rhizobia. In this symbiosis they partly supply their own N needs and also provide some nutrients left over to succeeding crops through decomposition of their nodule, roots and biomass (Chianu et al., 2011). The practices appeared to be very useful for smallholder farmers as it is cost effective to improve the N requirement of legumes and succeeding crops (Graham et al., 2004). Furthermore, N_2 fixing soybean crop is of considerable interest for more sustainable agriculture and particularly in organic farming systems (Cazzato et al., 2012).In view of this, biological nitrogen fixation (BNF), a renewable N fertilizer source, holds great promise for smallholder farmers in sub-Saharan legume crops rank second after cereals, with their 12% contribution to national food production and occupy 18% of the total cultivated area in Ethiopia. In recent years, production of haricot bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.) has increased as they are exportable and cash earning commodities (Abera et al., 2013).

Declining soil fertility particularly low soil N availability is often the major factor resulting in decreased crop plant yields and recognized as a major problem to continue cereal cropping in soils of Ethiopia, specifically in soils of Assosa area (Zeleke et al., 2010). Due to low soil fertility status in the country grain legumes are generally grown in sever soil conditions which are inherently low in fertility including S and low soil pH especially in western Ethiopia (ATA, 2013). Fertility situation is further deteriorated by nutrient depletion by crops and other related processes, such as leaching and removal of crop residues in the area (Zeleke et al., 2010). The Ethiopian Soil Information Service is currently involved in mapping the entire country for all nutrients, and has found extensive areas of S, Zn, and B deficiency (Vanlauwe et al., 2015).

Sulphur is an essential nutrient for plant growth accounting to about 10% of the total N content (Anandham et al., 2007) and legume crops such as soybean generally require it in a similar quantity or more than that of phosphorus for high yield and quality (Jemal et al., 2010). But reductions in S sources from organic matter and less S returned with inadequate use of crop residues and rare addition of manure which often deplete soil organic matter (Habtemichial et al., 2007). In other words, in countries like Ethiopia where subsistence farming is practiced, the turnover of S through SOM is usually insufficient even to meet the small requirement associated with the small yields. Organic S pool which is the large proportion of soil S highly affected by long term cultivation in the tropics (Solomon et al., 2001) and further aggravated through removing plant residue. In intensive crop rotation S uptake can be very high, especially when the crop residue is removed from field along with the product (Fismes et al., 2002). In addition, less S returned with inadequate use of crop residues and rare addition of manure which often deplete soil organic matter in Ethiopia (Habtemichial et al., 2007).

In addition, using of S-free mineral fertilizers is decreasing soil S levels and threatens the adequate fertilization of most crops (Khalid et al., 2011). Application of N containing fertilizers year after year on S deficient soils can make its deficiency worse because of widen N:S ratio. Mineral fertilizers that are used in Ethiopia such as di-ammonium phosphate (DAP) and urea contain no S. Despite the important roles of S in agriculture, research pertaining to its status in soils and its response in crops are almost nonexistent in Ethiopia (Habtemichial et al., 2007). Sulphur fertilization of soil has significant potential of increasing the amount of N fixed by legumes and their grain yield, thus improving fertility status of soil (Cazzato et al., 2012). Nitrogen fixing capacity of leguminous plants can be increased by the supply of adequate amounts of nutrients such as S. S is a vital part of the ferredoxin, an iron-S protein occurring in the chloroplasts. Ferredoxin has a significant role in NO_2 and sulphate reduction, the assimilation of N by root nodule bacteria and frees living N-fixing soil bacteria (Scherer et al., 2008). A study revealed lower N accumulation and a yield reduction of legumes when S was limiting (Scherer et al., 2006) and also recognized as a limiting factor not only for crop growth and seed yield but also for quality of products (Jemal et al., 2010). Because S is a main component of the amino acids methionine, cysteine, and cystine, coenzymes, thioredoxine and sulfolipids and hence improves protein quality (Jemal et al., 2005). Sharma and Sharma (2014) also reported S-containing amino acids such as methionine and cysteine increased significantly by combined application of N and S and indicated improvement in soybean nutritional value. This is because N and S are both involved in plant protein synthesis, a process that may determines yield of crops (Habtemicheal et al., 2013). Therefore, the most important constraints to soybean crop growth may be those caused by the shortage of plant nutrients such as S and N. In addition, there are not many studies available about the effect of S in presence of seed inoculation with effective strains of *Rhizobium* for successful soybean cultivation in Ethiopia. Therefore, a greenhouse trial was conducted to evaluate the effect of S fertilization and inoculation of *Rhizobium* strain on yield and nutrients uptake of grain and straw, grain quality protein content and estimated soil N balance of two soybean varieties.

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MATERIALS AND METHODS

Description of the study area

A greenhouse experiment was conducted using Dystric Nitiosols (AsARC, 2007) at Assosa Agricultural Research Center (EIAR) to investigate the effects of integrated application of S and *Rhizobium* strains on grain and straw nutrients yield and grain quality protein content of two soybean varieties (Belessa-95 and Wollo). Assosa is located about 670 km west of Addis Ababa, the capital city of Ethiopia. It is a capital city of 'Benishangul Gumuz' Region State of Ethiopia and lies on altitude of 1,480 m above sea level, and located at 09°58'41.7" N, 034°38'09.5" E coordinates. The study soil was slity clay loam in texture with clay 33%; silt 25%; sand 42%, acidic in pH (5.30), medium in soil organic carbon (OC,1.90%), medium in soil nitrogen content (TN, 0.12%), very low in available P (14.55 ppm) and low in CEC (14.7, milliequivalents per 100 g soil).Available S was low (2.97 ppm) can be categorized under S-deficient soil.

Experimental set up

Prior to the experiment, 32 surface (0-20 cm) random sub samples were collected in a zigzag walk from different villages of Assosa district. The measurement of soil pH was performed to identify and select the experimental soil with acidic soils pH range of 5.1 to 5.5. Thirty-two farmers' fields were considered from sampling villages with known soil acidity problems based on past and present management and production of soybean and with no previous history of *Rhizobium* inoculation. Surface soil samples (0-20 cm) from Megele-32 was air dried, passed through a 0.5 cm sieve and filled in 5 kg soil pots containing holes at the bottom to ensure free drainage with saucers placed under the pots to prevent losses of nutrients. The experiment consisted of four levels of S (0, 20, 30, and 40 kg ha^{-1} S) and three strains of *Rhizobium* (*MAR-1495*, *SB-6-1-A*$_2$, and *TAL-379*) along with uninoculated control and two soybean varieties (Belessa-95 and Wollo) that was arranged in a Completely Randomized Design (CRD) with three replications in greenhouse conditions. For the purpose of assessing BNF, a non-N fixing reference crop (wheat, variety called Digalu) was grown in similar environmental condition with soybean.

Soil fertilization and sowing

Sulphur was applied as potassium sulfate (K_2SO_4) in solution form. In addition to sulphur, other nutrients, such as starter dose of N fertilizer at 18 kg N ha^{-1} as Urea (Solomon et al., 2012) and phosphorus fertilizer as TSP at recommended dose of 23 kg P ha^{-1} were applied at sowing for each treatment pots (5 kg of soil). Since sulphur fertilizer was applied as K_2SO_4, the disproportionate addition of K in different treatments was counter balanced by the addition of proportionate amount of potassium chloride. Basal nutrients were also added to each pot to prevent deficiency of other nutrients. These include 5 kg ha^{-1} Mg as $MgCl_2$, 10 kg ha^{-1} Zn as $ZnCl_2$, 1 kg ha^{-1} Mo as $Na_2MoO_{24}{\cdot}2H_2O$. S and other basal nutrients were dissolved in deionized water and applied to each pot in required amounts. Soils were then mixed thoroughly, and deionized water was added to raise the soil moisture to pF 2.5.

Seed treatment with *Bradyrhizobuium* strains

Soybean seeds were selected based on size and healthiness (able to shoot). Then the seeds were weighed and surface sterilized by soaking them first with 70% (v/v) ethanol for 10 s and 4% (v/v) sodium hypochlorite (NaOCl) solution for five minutes and later washed five times with sterilized water as indicated in Somasegaran and Hoben (1994). Each strain was applied at the rate of 10 g peat-based powder inocula per 100 g of seed. In order to ensure that all the applied inoculum stick to the seed, the required quantity of inoculants was suspended in 1:1 10% sugar solution. The sugar slurry was gently mixed with dry seed and then with Carrier-based inoculant so that all the seeds received a thin coating of the inoculant. Then *Bradhyrhizobium* inoculum was mixed thoroughly with these seeds. For each inoculation, separate plastic bag was used and care was taken to avoid contamination of the inoculated and uninoculated seeds. Seeds were allowed to air dry for a few minutes and were then sown at the required rate and spacing. Pots with uninoculated seeds were planted first to avoid contamination. Seeds were sown at 3-4 cm depth of soil. Five seeds per pot were sown, and plant populations was maintained by thinning at four to six leaf stages (that is, 15 days after germination) into three plants per pot and maintain plant distance of 5 cm. Soil N balance (Nba) considering the aboveground biomass (straw) was calculated by subtracting N output from N input using Equation 1 (Habtemichial et al., 2007). Roots were not removed from the soil, and hence the calculated potential N benefits are conservative estimates, as they do not include root N.

$$Nba = (N_f + N_2 \text{ fixed}) - N_g \quad (1)$$

Where: N_f = Applied N fertilizer (kg ha^{-1}), N_g = N removed by soybean grain.

Chemical analysis

At physiological maturity, plants were harvested and partitioned into grain and straw and samples from each treatment were collected for analysis of N, P, K and S. Each plant part was dried in an oven at 70°C for 48 h, ground and sieved with 0.5 mm mesh for analysis of nitrogen, sulphur, phosphorus and potassium. N was determined by Micro Kjeldahl's method (Nelson and Sommers, 1973). After samples were digested with di-acid mixture (HNO_3 and $HClO_4$), P was determined using spectrophotometric vanadium phospho-molybdate method, K using digested solution on a flame photometer and S by turbidimetric, Barium sulfate precipitation by turbidimetric, Barium sulfate precipitation method of estimating available S adapted from Motsara and Roy (2008). N, P, K and S uptakes in the grain and straw was determined quantitatively by multiplying N, P, K and S content of the seed and straw with that of seed and straw yield, respectively.

Statistical analysis

The analysis of variance was carried out using SAS statistical software version 9.00 (SAS, 2004) after parameters taken were converted into kg ha^{-1}. Three-factor analysis of variance also were performed to evaluate the effects of treatments (*Rhizobium* strain, S rate and variety) and their interactions on grain and straw yield and N, P, K and S uptake. Means were separated using Tukey's procedure ($P < 0.05$). A correlation analysis between grain and straw yield and nutrients uptake were also carried out.

RESULTS

Seed and straw yields and nutrients uptake of soybean

Seed and straw yields, and nutrient uptake (N, P, K and S) were significantly ($P<0.05$) varied between the two

Table 1. Seed and straw yield, estimated soil N balance and protein yield of soybean varieties as affected by S fertilizer rates and *Rhizobium* strains.

Variations	Seed yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Nba (kg ha^{-1})	Protein yield (%)
		Variety		
Belessa-95	2493.21^{a}	4195.26^{b}	21.30^{b}	31.98
Wollo	2158.58^{b}	4403.22^{a}	37.26^{a}	32.37
LSD	52.19	110.36	3.30	NS
		S (kg ha^{-1})		
0	1972.42^{c}	4487.79^{a}	18.27^{b}	31.31^{b}
20	2323.04^{b}	4507.17^{a}	13.27^{b}	31.82^{b}
30	2540.12^{a}	4032.97^{b}	41.25^{a}	32.04^{b}
40	2468.01^{a}	4169.05^{b}	44.33^{a}	33.52^{a}
LSD	97.46	206.09	6.17	1.02
		Rhizobium strain		
Uninoculated	1493.63^{d}	4181.67^{b}	-14.43^{c}	24.55^{d}
TAL-379	2020.77^{c}	3935.12^{c}	44.06^{a}	31.14^{c}
MAR-1495	3342.15^{a}	4855.62^{a}	37.55^{a}	39.10^{a}
SB-6-1-A_2	2447.05^{b}	4224.57^{b}	49.95^{a}	33.89^{b}
LSD	97.46	206.09	6.17	1.02

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

soybean varieties. The straw and seed yields and their respective N, P, K and S uptakes of soybean were significantly (P<0.001) improved by S fertilization and *Rhizobium* inoculation (Tables 1 and 3). Seed yield of soybean was increased by 28.8 and 25.2%, when 30 and 40 kg ha^{-1} S was applied as compared to control (no S application, S_0) (Table 1).

Even though most parameters were significantly (P < 0.05) affected by main effect and two-way interaction of the factors, but three-way interactions significantly (P<0.001) affected all seed and straw yields, and nutrients (N, P, K and S) uptake more than their respective two way interaction and/or main effects (Tables 2 and 4). The combined application of S (at 30 and 40 kg S ha^{-1}) along with inoculation of MAR-1495 further showed highest seed yield for Bellessa-95 and Wollo, respectively (Table 2). For Bellessa-95, seed yields of 3864.1 and 3893.8 kg ha^{-1} were obtained, corresponding to 112% and 114% increases at 30 and 40 kg S ha^{-1}, respectively with strain MAR-1495. For Wollo, seed yields of 3633.3 and 3709.2 kg ha^{-1} were obtained, corresponding to 160 and 166% increases for S at 30 and 40 kg ha^{-1}, respectively with strain MAR-1495. While, S application at the rate of 30 kg S ha^{-1} was significantly at par with 40 kg S ha^{-1} for both varieties.

In addition, application of different doses of S increased straw yield of soybean significantly (p < 0.001) (Table 1). The highest straw yield of soybean (4507 kg ha^{-1}) was recorded with 20 kg S ha^{-1} which was statistically at similar content with S_0 treatment (4487 kg ha^{-1}), whereas, the lowest straw yield were recorded in the highest S rates (30 and 40 kg S ha^{-1}). Consequently, straw yield was higher in the inoculated treatments than uninoculated, with and/or without S application (Table 1).

N, P, K and S uptakes in soybean seed and straw were increased significantly with application of S application and inoculation of *Rhizobium* strains individually as well as in combination (Tables 3 and 4). The maximum increase in N uptake (3-folds) was obtained for Wollo at 40 kg S ha^{-1} along with inoculation of MAR-1495, while at 30 kg S ha^{-1} for Bellessa-95 inoculated with MAR-1495 was obtained an N uptake (256.6 kg ha^{-1}) increased by two and half folds over control. The same is true for P and K seed uptakes which were increased significantly by addition of combined treatments of S fertilizer and inoculation of *Rhizobium* strain. Like N uptake, significantly the highest increase in S uptake (seventeen folds) was obtained for Wollo at 40 kg S ha^{-1} along with inoculation of MAR-1495, while at 30 kg S ha^{-1} for Bellessa-95 inoculated with MAR-1495 induced an S uptake of 39.02 kg ha^{-1}, increased by about fifteen folds over control (Table 4).

Seed protein yield

Significant variation (p<0.001) was observed in the seed protein yield of soybean with different doses of S application, resulted increased with increasing S rates (Table 2). Application of S at 40 kg ha^{-1} resulted with significantly the highest protein content, ranged from 31.3% for unfertilized treatment to 33.5%, corresponding

Table 2. Interaction effects of S rates, *Rhizobium* strains and soybean varieties on seed and straw yield, estimated soil N balance and grain protein yield.

S (kg ha^{-1})	Strain	Variety	SY (kg ha^{-1})	StY (kg ha^{-1})	Nba (kg ha^{-1})	Protein content (%)
0	Uninoc.	Belessa-95	1819.5^{ijk}	4120.0^{efghi}	-29.25^{p}	25.99^{jklm}
	MAR-1495		2782.5^{de}	4259.4^{defghi}	24.32^{hijkl}	37.89^{bcdef}
	SB-6-1-A_2		2185.5^{fghij}	4249.3^{defghi}	23.03^{hijklm}	27.91^{ijk}
	TAL-379		1926.2^{ghijk}	3981.2^{efghi}	32.97^{ghijk}	34.63^{defg}
	Uninoc.	Wollo	1390.5^{lmn}	4672.0^{bcdefg}	-21.76^{nop}	27.96^{ijk}
	MAR-1495		2535.7^{ef}	5374.1^{ab}	55.20^{bcdefg}	37.23^{bcdef}
	SB-6-1-A_2		1896.7^{ghijk}	3705.3^{ijk}	21.43^{hijklm}	29.12^{ijk}
	TAL-379		1242.8^{n}	5801.1^{a}	40.25^{efghij}	33.89^{fgh}
20	Uninoc.	Belessa-95	1840.5^{hijk}	4374.7 ^{cdefgh}	-37.60^{p}	27.03^{ijk}
	MAR-1495		3184.7^{cd}	5218.7^{abc}	7.49 ^{klm}	38.35^{bcde}
	SB-6-1-A_2		2788.5^{de}	4400.0^{cdefgh}	18.41^{ijklm}	34.86^{defg}
	TAL-379		2178.2^{fghij}	3840.0^{ghij}	24.75^{hijkl}	28.33^{ijk}
	Uninoc.	Wollo	1794.0^{jkl}	4228.0^{defghi}	-28.81^{op}	23.40^{lm}
	MAR-1495		3134.0^{cd}	4716.0^{bcdef}	14.59^{jklm}	39.23^{bc}
	SB-6-1-A_2		2007.0^{ghijk}	4640.0^{bcdefg}	41.42^{defghi}	30.92^{ghi}
	TAL-379		1657.5^{klm}	4643.0 ^{bcdefg}	65.92^{abcde}	28.41^{ijk}
30	Uninoc.	Belessa-95	1336.5^{mn}	3953.3^{efghi}	4.85^{lm}	25.28^{klm}
	MAR-1495		3864.1^{a}	4594.7 ^{bcdefg}	34.60^{fghij}	41.52^{ab}
	SB-6-1-A_2		3356.4^{bc}	4067.2^{efghi}	57.70^{bcdefg}	34.29^{efgh}
	TAL-379		2473.5^{ef}	3445.3^{ijk}	15.36^{ijklm}	26.54^{ijk}
	Uninoc.	Wollo	1386.0^{lmn}	3705.3^{hij}	0.903^{lmn}	22.72^{lm}
	MAR-1495		3633.3^{ab}	4827.1^{bcde}	84.13^{a}	40.21^{ab}
	SB-6-1-A_2		2247.7^{fgh}	4654.7^{bcdefg}	72.21^{abc}	35.61^{cdef}
	TAL-379		2023.5^{ghijk}	3016.0^{jk}	60.29^{abcdef}	30.16^{hij}
40	Uninoc.	Belessa-95	1233.0^{n}	4162.7^{efghi}	-2.62 ^{mno}	22.05^{m}
	MAR-1495		3893.8^{a}	4761.7^{bcde}	39.74^{efghij}	34.50^{defgh}
	SB-6-1-A_2		2808.7^{de}	3869.3^{fghij}	80.74^{ab}	44.00^{a}
	TAL-379		2219.9^{fghi}	3826.7^{ghij}	46.40^{cdefgh}	28.56^{ijk}
	Uninoc.	Wollo	1149.0^{n}	4237.3^{defghi}	-1.153^{lmn}	22.05^{m}
	MAR-1495		3709.2^{ab}	5093.3^{abcd}	40.34^{efghij}	43.90^{a}
	SB-6-1-A_2		2286.0^{fg}	4470.7^{cdefgh}	84.66^{a}	34.50^{defgh}
	TAL-379		2444.5^{ef}	2930.7^{k}	66.57^{abcd}	38.64^{bcd}
	LSD		413.82	875.02	26.21	4.34

Non-inoculated (0)=Uninoc.; SY-Seed yield; StY-Straw yield; Nba- Estimated soil N balance.

to an average increase of 7.0%. In addition, inoculation of MAR-1495 significantly increased protein content, ranged from 24.5% for uninoculted treatment to 39.1%, corresponding to an average increase by 59.2% over control. But, results showed that combined application of S with *Rhizobium* strain further increased protein yield for the two soybean varieties, resulted with highest protein content (Table 2). Consequently, Wollo inoculated with MAR-1495 along with S application at 40 kg ha^{-1} showed the highest seed protein content (43.9%) which was statistically at similar content with Wollo inoculated with SB-6-1-A_2 at similar S rate (40 kg ha^{-1}). While Belessa-95 produced higher percentage of protein when inoculated with MAR-1495 along with S at 30 kg ha^{-1}. Whereas, the lowest protein content of soybean (27.9 and 25.9%) were recorded in the uninoculated unfertilized (R_0S_0) treatment combination for Wollo and Belessa-95, respectively.

Estimated soil N balance (Nba)

Nitrogen fixed by legumes represents a key contribution

Table 3. Seed and straw nutrients (N, P, K and S) uptake of soybean varieties as affected by S fertilizer rates and *Rhizobium* strains.

Variations	Seed nutrient uptake (kg ha^{-1})				Straw nutrient uptake (kg ha^{-1})			
	N	P	K	S	N	P	K	S
Variety								
Belessa-95	133.46^{a}	17.88^{b}	64.66^{a}	14.50^{a}	30.82^{b}	5.93	51.20^{b}	11.68^{b}
Wollo	118.42^{b}	19.62^{a}	49.82^{b}	12.13^{b}	42.89^{a}	6.11	58.05^{a}	12.44^{a}
LSD	3.34	0.963	1.56	0.614	2.13	NS	2.37	0.625
S (kg ha^{-1})								
0	102.16^{c}	15.17^{c}	45.23^{d}	6.45^{d}	43.06^{a}	6.25^{ab}	47.68^{c}	4.45^{d}
20	120.89^{b}	18.61^{b}	55.74^{c}	11.02^{c}	35.57^{b}	6.48^{a}	54.53^{b}	14.88^{b}
30	138.71^{a}	19.09^{b}	67.67^{a}	18.53^{a}	34.84^{b}	5.79^{ab}	59.40^{a}	11.42^{c}
40	141.98^{a}	22.13^{a}	60.32^{b}	17.25^{b}	33.95^{b}	5.55^{b}	56.89^{ab}	17.48^{a}
LSD	6.24	1.79	2.92	1.14	3.73	0.743	4.42	1.16
Rhizobium strain								
Uninocu.	59.13^{d}	8.87^{c}	38.89^{d}	7.52^{c}	32.17^{bc}	4.11^{c}	51.95^{b}	12.85^{b}
TAL-379	100.38^{c}	16.55^{b}	54.07^{c}	11.27^{b}	35.87^{b}	6.10^{b}	55.01^{ab}	8.37^{c}
MAR-1495	209.69^{a}	24.65^{a}	76.70^{a}	22.44^{a}	48.78^{a}	6.86^{a}	59.21^{a}	12.31^{b}
SB-6-1-A_2	134.54^{b}	24.93^{a}	59.30^{b}	12.03^{b}	30.60^{c}	7.00^{a}	52.28^{b}	14.70^{a}
LSD	6.24	1.79	2.92	1.14	3.99	0.743	4.42	1.16

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

Table 4. Interaction effects of S rates, *Rhizobium* strains and soybean varieties on seed and straw nutrients (N, P, K and S) uptake.

S (kg ha^{-1})	Strain	V	Seed nutrient uptake (kg ha^{-1})				Straw nutrient uptake (kg ha^{-1})			
			N	P	K	S	N	P	K	S
0	Uninoc.	B	75.5^{mnop}	12.4^{jklmn}	51.64^{ijkl}	2.46^{qr}	27.43^{ij}	4.03^{fgh}	50.93^{defg}	4.12^{k}
	MAR-1495		168.7^{fg}	18.80^{ghijk}	59.24^{ghij}	12.2^{fghijk}	44.4^{cdefgh}	6.47^{abcdefgh}	25.28^{i}	3.34^{k}
	SB-6-1-A_2		97.53^{klm}	12.4^{jklmn}	49^{ijklmn}	7.49^{klmnop}	20.56^{j}	6.55^{abcdefgh}	31.55^{hi}	4.75^{jk}
	TAL-379		106.6^{ijk}	16.06^{hijkl}	47^{jklmno}	6.08^{opqr}	35 ^{cdefghij}	5.80^{bcdefgh}	42.17^{ghi}	3.05^{k}
	Uninoc.	W	62^{nopqr}	11.29^{klmn}	29.71^{p}	1.90^{r}	28.58^{hij}	3.60^{h}	61.49^{bcdef}	5.03^{jk}
	MAR-1495		151.1^{gh}	15.32^{ijklm}	51.44^{ijkl}	9.5^{ijklmno}	91.01^{a}	6.87^{abcdefg}	53.45^{defg}	5.55^{jk}
	SB-6-1-A_2		88.2^{klmn}	25.31^{cdefg}	43^{lmno}	7.35^{lmnop}	25.99^{ij}	7.57^{abcde}	50.01^{defgh}	2.97^{k}
	TAL-379		67.3^{nopq}	9.62^{lmn}	29.35^{p}	4.56^{pqr}	70.56^{b}	9.15^{a}	66.54^{bcd}	6.84^{ijk}
20	Uninoc.	B	79.6^{lmno}	9.41^{lmn}	50^{ijklm}	11^{fghijklm}	36^{cdefghij}	3.74^{gh}	50.21^{defgh}	16.9^{cdef}
	MAR-1495		195.4^{de}	23.28^{defgh}	71.95^{def}	12^{fghijkl}	45.64^{cdefg}	8.41^{abc}	63.25^{bcde}	15.8^{cdefg}
	SB-6-1-A_2		155.57^{g}	28.88^{bcde}	66.46^{fgh}	10^{ghijklmn}	24.47^{ij}	6.85^{abcdefg}	49.93 ^{defgh}	20.51^{bc}
	TAL-379		98.37^{klm}	13.3^{jklmn}	61.58^{fghi}	12.44^{fghij}	24.87^{ij}	8.16^{abc}	51.55^{defg}	5.84^{jk}
	Uninoc.	W	67.1^{nopq}	9.99^{lmn}	45.5^{klmno}	12.98^{fghij}	35^{cdefghij}	4.86^{defgh}	53.87^{defg}	16.9^{cdef}
	MAR-1495		196.6^{de}	26.67^{bcdef}	64.87^{fgh}	15.71^{defg}	49.89^{cd}	7.00^{abcdef}	58.17^{bcdefg}	11.28^{ghi}
	SB-6-1-A_2		98.97^{klm}	27.97^{bcde}	46.1^{klmno}	6.62^{nopqr}	30.9^{efghij}	7.56^{abcde}	50.69^{defg}	22.36^{b}
	TAL-379		75.4^{mnop}	9.33^{lmn}	38.7^{mnop}	5.61^{opqr}	30.9^{efghij}	5.32^{cdefgh}	58.55^{bcdefg}	9.33^{hij}
30	Uninoc.	B	54.0^{opqr}	7.42^{n}	37.73^{nop}	8.2^{jklmnop}	30.60^{ghij}	3.95^{fgh}	48.87^{defgh}	14.8^{defg}
	MAR-1495		256.6^{ab}	33.23^{ab}	102.46^{a}	39.02^{a}	29.55^{ghij}	8.68^{ab}	51.27^{defg}	6.98^{ijk}
	SB-6-1-A_2		184.16^{ef}	19.11^{fghij}	92.12^{ab}	19.92^{cd}	27.29^{ij}	6.47^{abcdefgh}	62.46^{bcdef}	13.28^{fgh}
	TAL-379		105.0^{ijkl}	21.32^{efghi}	79.02^{cde}	18.68^{cde}	22.87^{ij}	4.95^{defgh}	57.47^{bcdefg}	12.73^{fgh}
	Uninoc.	W	50.40^{pqr}	8.25^{mn}	36.53^{op}	8.8^{ijklmnop}	30.68^{fghij}	3.55^{h}	46.43^{efgh}	14.3^{efgh}

Table 4. Contd.

40	MAR-1495	B	233.7^{bc}	26.3^{bcdefg}	84.71^{bc}	26.73^{b}	51.22^{c}	6.53^{abcdefgh}	89.80^{a}	6.87^{ijk}
	SB-6-1-A_2		127.84^{hi}	21.56^{efghi}	56.88^{hijk}	14.16^{efgh}	47.78^{cde}	7.75^{abcd}	74.32^{abc}	17.6^{bcdef}
	TAL-379		97.75^{klm}	15.48^{ijklm}	51.94^{ijkl}	12.59^{fghij}	44.5^{cdefgh}	4.44^{efgh}	44.62^{efgh}	4.75^{jk}
	Uninoc.		43.50^{qr}	5.94^{n}	30.47^{p}	7.00^{mnopq}	32.9^{defghij}	4.33^{fgh}	54.27^{defg}	15.0^{defg}
	MAR-1495		214.9^{cd}	23.40^{defgh}	97.73^{a}	29.13^{b}	30.9^{efghij}	5.95^{bcdefgh}	75.40^{ab}	19.67^{bcd}
	SB-6-1-A_2		198.0^{de}	25.6^{bcdefg}	69.53^{defg}	20.72^{c}	28.39^{hij}	5.65^{bcdefgh}	43.67^{fghi}	18.6^{bcde}
	TAL-379		101^{jklm}	15.3^{ijklmn}	66.86^{efgh}	13.69^{fghi}	30.9^{efghij}	4.86^{defgh}	60.94^{bcdefg}	11.33^{ghi}
	Uninoc.		40.54^{r}	6.15^{n}	28.84^{p}	6.93^{mnopq}	35^{cdefghij}	4.85^{defgh}	49.53^{defgh}	15.5^{defg}
	MAR-1495	W	260.35^{a}	30.20^{bcd}	81.21^{bcd}	35.01^{a}	47.59^{cdef}	4.96^{defgh}	57.10^{bcdefg}	29.01^{a}
	SB-6-1-A_2		125.9^{hij}	38.51^{a}	50.2^{ijklm}	9.0^{ijklmnop}	39.4^{cdefghi}	7.63^{abcd}	55.66^{cdefg}	17.5^{bcdef}
	TAL-379		151.1^{gh}	31.92^{abc}	57.7^{ghijk}	16.51^{cdef}	26.35^{ij}	6.17^{abcdefgh}	58.59^{bcdefg}	13.07^{fgh}
	LSD		26.51	7.63	12.42	4.87	16.94	3.15	18.8	4.95

B- Belessa-95; W- Wollo. Note: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

to nutrient cycling in legume-based farming systems by increasing soil organic N status. Soil N balance (Nba) was negative in the control treatments. Application of S had significantly increased soil N balance over the control (unfertilized) except S at 20 kg ha^{-1} (Table 1). On the other hand, response of inoculation of *Rhizobium* strain was accompanied by significant increase in estimated soil N balance compared to uninoculated control.

Inoculation of SB-6-1-A_2 had showed maximum increase in N balance with corresponding variation from -14.43 kg N ha^{-1} (uninoculated) to 50.0 kg N ha^{-1}. Further benefit of estimated N in soil increased with both S fertilization and inoculation *Rhizobium* strain for the two soybean varieties (Table 2). Deficit N balance (Nba) of soybean was lowest (-29.25 kg ha^{-1}) in control and maximum (80.74 kg ha^{-1}) for Bellessa-95 treated with S application at 40 kg ha^{-1} along with inoculation of SB-6-1-A_2. For Wollo, lowest (-21.76 kg ha^{-1}) in control and maximum (84.66 kg N ha^{-1}) along with S application at 40 kg ha^{-1} and inoculation of SB-6-1-A_2 (Table 2).

DISCUSSION

The results revealed that the interactive positive effect of S fertilization and inoculation of *Rhizobium* strain on grain and straw yield and nutrients uptake and quality protein of soybean varieties, grown as a major crop on Nitisols of Assosa area, Ethiopia. Application ofS with *Rhizobium* strains plays an important role in physiological and developmental processes in plant life and the favorable effect of these important nutrients combination (N and S) might accelerate the growth processes, which ultimately resulted in increased seed yield and quality of the crop. Increased in root nodulation due to *Rhizobium* inoculation and S fertilization resulted in absorption of higher concentration of mineral nutrients from soil and hence increased shoot dry weight by soybean. The highest nutrient in shoot was due to total N accumulation enhanced biomass yield and showed the synergistic effect of nutrients in shoot. In addition, the positive effect of S fertilization with *Rhizobium* strain on nitrogen fixing potential and shoot dry weight attributed to seed yield production. *Rhizobium* strain treatments to soybean significantly increased seed weight per plant either alone or in combination with S. Each *Rhizobium* strain has own synergetic effect on production of seed weight as seed weight also increased when *Rhizobium* strains (significantly greater for MAR-1495) were applied individually but greater in combination with S. That shows S application is important nutritional element to get better soybean yield. Similarly, results for beneficial effect of S and *Rhizobium* strain application on yield and yield attributing characteristics have also been recorded by other workers (Habtemichial et al., 2007; Scherer et al., 2008).

This study revealed that *Rhizobium* inoculation with S fertilizer increased sufficient yield of quality soybean seed. The synergistic effect of N and S may be due to utilization of high quantities of nutrients through their well-developed root system and nodules which might have resulted in better growth and yield. These results confirm the earlier findings reported by Hussain et al. (2011). The application of S might have increased the availability of nutrient to soybean plant due to improved nutritional environment, which in turn, favorably influenced the energy transformation activation of enzymes, chlorophyll synthesis as well as increased carbohydrate metabolism (Dhage et al., 2014). It constitutes the main element of

Table 5. Correlation analysis between grain and straw yield and nutrients uptake and protein yield of soybean.

Correlation	SY	Nba	Protein	Seeds Nup	Seeds Pup	Seeds Kup	Seeds Sup	Straw Nup	Straw Pup	Straw Kup	Straw Sup
SY	0.19^{NS}	0.38***	0.76***	0.96***	0.70***	0.93***	0.84***	0.08^{NS}	0.34***	0.27**	0.20*
SY	1	0.02^{NS}	0.32**	0.26**	0.004^{NS}	0.03^{NS}	0.07^{NS}	0.61***	0.40***	0.41***	0.18^{NS}
Nba		1	0.57***	0.44***	0.51***	0.32**	0.32**	0.22*	0.40***	0.26*	0.05^{NS}
Protein			1	0.88***	0.71***	0.60***	0.60***	0.34***	0.46***	0.18^{NS}	0.12^{NS}
SeedsNup				1	0.73***	0.85***	0.83***	0.18^{NS}	0.37***	0.25*	0.21*
SeedsPup					1	0.61***	0.55***	-0.03^{NS}	0.46***	0.16^{NS}	0.27***
SeedsKup						1	0.85***	-0.09^{NS}	0.24*	0.28**	0.18^{NS}
SeedsSup							1	0.003^{NS}	0.18^{NS}	0.30**	0.32**
Straw Nup								1	0.31**	0.27**	-0.08^{NS}
Straw Pup									1	0.21*	-0.07^{NS}
Straw Kup										1	0.22*

NS- non significant; *, **, *** significant at 0.05, 0.01 and 0.001, respectively.

amino acids such as cysteine and methionine, which are of essential nutrient value and can increase seed yield. Therefore, soybean protein quality could be significantly improved by increasing the concentration of the sulfur-containing amino acids through S fertilization. The yield and quality of legume seeds are limited by the amount of S (S) partitioned to the seeds (Tan et al., 2010). The role of S in the seed production of soybean has also been reported by (Jamal et al., 2005). Dhage et al. (2014) found that soybean seed yield and straw yield increased significantly due to application of 60 kg ha^{-1} S followed by 40 kg ha^{-1} S over control.

Each *Rhizobium* strain has its own synergetic effect on seed yield and nutrients uptake increased substantially when applied in combination with S. Generally, the two soybean varieties, Belessa-95 and Wollo showed highest seeds N,P, K and S uptakes when inoculated with MAR-1495 at S rate of 30 and 40 kg ha^{-1}, respectively, indicated that balanced S application is important nutritional aspect for increasing soybean yield and nutrients uptake efficiency along with *Rhizobium* strain. Islam et al. (2012) also reported that S fertilization enhanced crop yield and uptake of macronutrients such as nitrogen, phosphorus and potassium especially nitrogen. According to Cazzato et al. (2012), S fertilization rates were increased nutrient levels in lupin seeds when compared with the control unfertilized treatment. Seed yield and N, P, K and S uptakes of seeds were significantly improved by *Rhizobium* inoculation and S fertilization. Results indicated a significant positive correlation (Table 5) between seed yield and N, P, K and S uptakes (r^2=0.96, r^2=0.70, r^2=0.93, and r^2=0.84, respectively) showing the importance of nutrients uptake on improvement in growth and seed yield of soybean. The magnitude of response on straw yield of soybean was more in case of inoculated with or without S fertilization. This may be due to the enhanced shoot nutrient uptake and N_2 fixation due to combined application of *Rhizobium* strain with S during the vegetative growth; therefore most of the absorbed forms are re-translocated in seed than to straw during the reproductive stages. Therefore, S input modifies N allocation more to storage organs (seeds) than to straw.

Results also revealed that *Rhizobium* strain alone or combined with S application increased protein content, while inoculation of strain with S increased the protein content up to 44.0%. The increased in protein content with increasing level of S may be because in the absence of S, amino acids cannot be transformed into proteins, which results in reduced N acquisition (Zuber et al., 2013). Because of central role of S and N in the synthesis of proteins, the supplies of these nutrients in plants are highly inter-related (Jamal et al., 2005), which suggested that an insufficient S supply can affect yield and quality of crops. In fact, there is close link between S supply and N requirement of plant in addition. There was accumulation of one nutrient in plant when other nutrient was lacking and accumulated nutrient was used in protein synthesis when treatments were reversed (Jamal et al.,2010).The least protein content was produced in the uninoculated plants (Tables 1 and 2); resulted with decreasing protein content with increasing S application. This may be due to nutrient imbalance between the two nutrients. Fismes et al. (2000) also found that their interactions, reflected by plant uptake, are synergistic at optimum rates and antagonistic at excessive levels of one of the both. But when applied in combination known to create a more synergism, which was helpful in improved plant growth, including nutrient uptake in the plants and the improved protein content. In legumes, previous studies showed that S deficiency decreases N assimilation and fixation (Scherer et al., 2006). In addition, modifies seed protein composition by decreasing the abundance of storage proteins with the highest content of S–containing amino acid, while increasing the level of S–poor globulins (Zuber et al., 2013). Similarly, Sharma and Sharma (2014) also reported that a significant increase in S-containing amino acids such as methionine and cysteine

obtained by combined application of N and S for soybean. Therefore, the results of this study indicated that S fertilization is required to improve N-use efficiency and thereby maintaining protein quality (Fismes et al., 2000). The increase in seed nutrients uptake was correlated with the enhancement of N_2 fixation due to S increased the nitrogenenase activity when applied with *Rhizobium* inoculation. A significant positive correlation between seed protein content and N, P, K and S uptakes of seeds (r^2=0.88, r^2=0.71, r^2=0.60, and r^2=0.60, respectively) may be due to fertilization of S facilitated the growth of plants by improving the uptake of nutrients in shoot and seeds and stimulating seeds and straw production. Accordingly, the present observations strongly support the view that seed yield, nutrients uptake and quality protein of soybean varieties are improved with combined application of *Rhizobium* strain and S-fertilization.

Amount of N taken by plant from soil and fertilizer was significantly higher due to inoculation and S application as compared to control. Even though the estimation only considers the chemical nature of the plant residues, it should be further estimated considering the magnitude and timing of N and S release from residues, and any subsequent immobilization. The subsequent decomposition of N rich residues replenish N removed by harvesting without the addition of fertilizer N and contribute fixed N to subsequent crops (Jensen and Hauggaard, 2003). Nitrogen inputs from grain legumes are highly dependent on the crop N harvest index, that is, the proportion of total above-ground N production removed as grain (Ravuri and Hume, 1993). Some studies show a positive effect of grain legumes on the soil N balance due to S fertilization and *Rhizobium* inoculation (Habtemichial et al., 2007), whereas other study show a negative effect (Hussain et al., 2011). However, most of these N balances have probably underestimated the below-ground input of fixed N by legumes due to problems of root sampling and quantifying root exudates or rhizodeposition (Khan et al., 2003). Negative N balance in the uninoculated and unfertilized control may be due to the fact that nitrogen input (starter N at 18 kg ha^{-1} fertilizer) was not enough to meet crop demand. Therefore, large amounts of N moved away from the soil for uninoculated as well as unfertilized control. (Amanuel et al., 2000) also reported that N balance after legume harvest is positive when crop residues are returned to soil and only seed or grain is removed, which was not included in this study. Habtemichial et al. (2007) reported positive soil N balance in range of 12 to 52 kg ha^{-1} after harvest of faba bean crop in Northern Ethiopia, but major difference was that crop residues were returned to soil. Higher positive soil N balance estimated with S application and inoculation might be due to the fact that amount of N fixed increased from 22.4 to 300 kg ha^{-1} and 28.3 to 273.2 kg ha^{-1} for Wollo and Belessa-95, respectively. Therefore, the estimated soil nitrogen balance became more positive, because the estimation only considers the chemical nature of the plant residues (straw nutrients content) that soybean straw can be a benefit for soil nutrients replenishment. Therefore, soybean inoculation with S fertilizer can be beneficial to farmers whose aim is to increase N balance in the soil in addition to maximize soybean yields since most of the N is removed in seeds as well as in straw harvested. Because, the amount of straw N in soybean varieties depended on how completely N was translocated to seed, total amount of N remaining in the field after soybean has been harvested for seed (Ravuri and Hume, 1993).

Conclusion

In this experiment the two soybean varieties performed better when inoculation of *Rhizobium* strain was applied with S fertilization than when both were applied alone. The combined application increased the availability and uptake of N, P, K and S by seeds and straw, and the growth and yield of the two varieties of soybean. The most beneficial effect of S with inoculation on N_2 fixation, plant growth and yield can be understood given the fact that the experimental soil was severely deficient in available S as well as *Rhizobium* strain, which can be considered major limiting factors for quality soybean production on acidic soils of Assosa area. Hence, the integrated application of *Rhizobium* strain with S could be a viable strategy to improve the yield and quality of soybean, in soils containing suboptimal S and N.

The two varieties responded to the combined application of *Rhizobium* strain MAR-1495with S fertilizer at 30 and 40 kg ha^{-1}. Even though with the highest yield achieved at the highest S rate (40 kg ha^{-1}) and indicated that the yield of soybean can still be improved at further higher S rate, but were significantly at par for most parameters. Therefore, S at 30 and 40 kg ha^{-1} with MAR-1495was the most profitable interaction between inorganic S levels and *Rhizobium* strain treatments for N_2 fixation, yield and quality attributes of the two soybean varieties. In addition, strain SB-6-1-$A_2$with S fertilization also suggesting a promising way for enhancing the growth and yield of soybean. Therefore, proper fertilization programs including S integrated with inoculation of *Rhizobium* strain should be implemented to improve the productivity of food legumes and thereby increase total food production, enhance the supply of good quality proteins in Ethiopia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Response of *Cyperus papyrus* productivity to changes in relative humidity, temperature and photosynthetically active radiation

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Ecosystem development is related to climatic conditions. To assess the effect of seasonal changes in climate factors on papyrus ecosystem development, community biomass was estimated non-destructively and climate factors recorded in the wetland. Absolute aerial biomass and productivity were not significantly different between wet and dry seasons. Although there was significant vapour pressure deficit change over the seasons, the impact on net primary productivity was not significant, but exhibited positive association during dry season. Primary productivity was positively related to photosynthetically active radiation during both dry and wet seasons. Except for the dry season that accounted for 26.1% of primary productivity, that of the wet season was independent (0%) of measured weather variables.

Key words: *Cyperus papyrus,* photosynthetically active radiation, productivity, relative humidity, temperature, wetlands.

INTRODUCTION

Although photosynthesis is fundamental to plant growth and estimated as net biomass accumulation, other environmental factors modify its magnitude (Hall and Long, 1993; Page et al., 2011). The understanding of how environmental changes influence plant growth is critical for the assessment of potential impacts of climate change on ecosystems.

Weather variability measured as changes in rainfall, temperature, solar radiation, humidity and wind characteristics interact with plant and influence processes such as photosynthesis, respiration, transpiration and translocation (Beadle, 1993; Jones, 1993). Changes in

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weather variables (rainfall pattern, wind speed, temperature and evaporation rate) during the year in Lake Victoria basin in East Africa region has been reported (Hughes and Hughes, 1992; Spigel and Coulter, 1996; Yin et al., 2000; Oyugi, 2010).

Knowledge of the effects of such changes are necessary for improving the accuracy of global climate carbon models within the region, and also provide support to the program of improving wetland management and policy for climate change mitigation and carbon accounting. The new policy to reduce greenhouse gas emission through reforestation in developing countries have highlighted the prospects of conserving tropical wetlands by negotiating carbon offset and trading agreements (Murdiyarso et al., 2010). This study investigated the contribution made by tropical papyrus wetlands to carbon stock.

The influence of weather variability on *Cyperus papyrus* ecosystem productivity is yet unclear. However, modeling results of micro-climate and light interception on *C. papyrus* bracteole (leaf) photosynthesis was done (Jones, 1988; Humphries and Long, 1995). Model prediction and productivity measurements suggest that increasing temperatures may result in substantial decrease in carbon sequestration and inputs to stored carbon in the underlying papyrus peat deposits (Humphries and Long, 1995; Saunders et al., 2007).

Saunders et al. (2007) estimated the carbon sequestration of tropical papyrus wetlands from measurement of net ecosystem exchange by eddy covariance. Maximum rates of CO_2 assimilation were associated with peaks in both air temperature and photosynthetically active radiation (PAR) flux with lesser vapour pressure deficit (VPD) effect. The study involved analysis of diurnal CO_2 flux produced from the wetland as a result of decomposition, respiratory growth and maintenance respiration.

Temperature influence and other weather factors that directly or indirectly influence papyrus productivity measured as absolute biomass change has not been fully investigated. The standing biomass is important because individual dying shoots in papyrus wetlands are replaced through recruitment. This takes care of the population dynamics within wetland systems, and provides empirical data for biomass quantification that can be used to upscale to larger wetland area using accurate papyrus area estimation by satellite imagery (Maclean et al., 2006).

In this study, it is hypothesized that weather factors drive pattern of papyrus development. Although the quality and quantity of stream inflows into and out of wetlands, macrophyte harvesting and characteristics human settlement around the wetland would have influence through nutrients input into the wetland systems that would ultimately influence productivity and its variability, they were considered as integral constant factor in this model.

MATERIALS AND METHODS

Study area

The study was conducted in Lubigi wetland, a monotypic *C. papyrus* wetland in Kampala District, Uganda. The wetland covers total area of 2.96 km^2 (Namakambo, 2000). Daily average temperature of the area ranges between 17 to 27°C during the year. This is a typical tropical wetland conditions, characterized with wet and dry climate. Rainy seasons are from September to December and March to May of each year. Erratic changes have been observed in the onset and end of the different seasons in the recent past.

Site selection for the metallic tower in the study area

The selection of the site for mounting the metallic tower was in the monotypic stand of papyrus plants located about 170 m from the wetland edge. The bottom sediment of the location was approximately 4.5 m deep. The tower was used for the attachment of meteorological unit (Figure 1). The meteorological unit was located at approximate 1,165 m above sea level at 0°24´ N and 32°31´ E. Further anchorage of the tower in the papyrus mat was done by forcing longer pieces of timber across the width of rectangular frame that rested on the papyrus mat. This gave the tower structural firmness on the papyrus mat in order to support additional weight. A ladder walk way (transect) was constructed in the wetland using eucalyptus poles to allow easy movement into and out of the wetland (Figure 2).

Papyrus biomass and primary productivity assessment

Biomass and primary productivity assessments were done in quadrats (3 x 3 m^2), and limited to the aerial growth of the monotypic stand of papyrus. The assumption was that growth in papyrus wetlands only occur as turnover rate of the aerial shoots for fully developed rhizome structures. Eight replicate quadrats located along the access transect at distance of 100 m were used for the investigation. Each quadrat was 2 m away from the center line of the transect. All live culms within the quadrats were identified and culms girth measured during each visit. The assessment covered eleven months (October, 2010 to August, 2011) at intervals of three (3) weeks. Live culms whose girth were measured included those with un-opened umbels, partially open and fully open green umbels without signs of senescence (< 40% of the umbel brown and no clear evidence of senescence). The regression equation $Log\ (y) = 2.832Log(x) - 0.5241$ of biomass (y) against culm sizes (girth) (x) was used (Figure 3) to estimate *C. papyrus* biomass after measurements of culm-girth of different papyrus population, and life cycle within the quadrats. Productivity was computed from biomass difference between sampling intervals divided by number of days in each interval. Biomass and productivity values were reported per unit area.

Weather factors in the surface of *C. papyrus* wetland canopy

Temperature and relative humidity (RH) in the surface of *C. papyrus* wetland canopy were monitored using inbuilt sensors in the Skye Data Hog 2 logger, type SDL 5260 and changes in photosynthetically active radiation (PAR) was recorded using SKP 215/S/D/I 36961 pyranometer. These were used as indicators of critical weather factors. All weather variables (values) were averaged on 30 min interval from October, 2010 to August 2011. The stored weather data was regularly downloaded to a lap top

Figure 1. Mounted metallic tower in Lubigi wetland. On top are Data Hog 2 logger, anemometer and pyranometer. The pyranometer and anemometer are obscured by papyrus umbel structures.

Figure 2. Constructed access transects into Lubigi wetland.

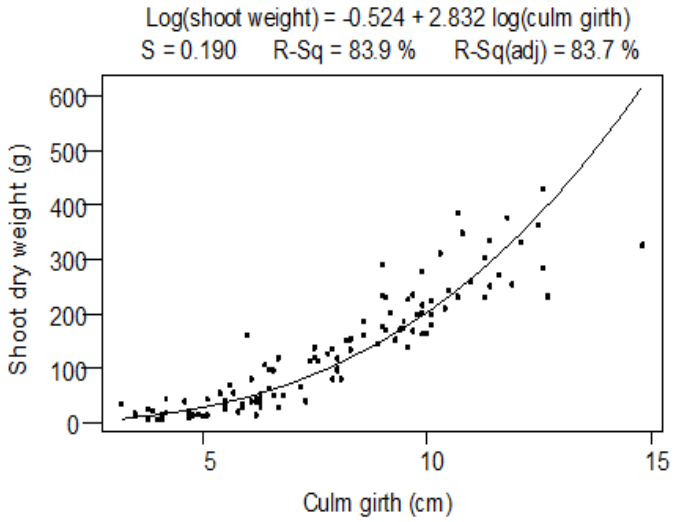

Figure 3. Relationship between culm-girth and dry weight of aerial shoots of *C. papyrus* in Lubigi wetland (n = 120) (Adopted from Opio et al., 2014).

computer using Skye software after every two weeks interval. The vapour pressure deficit (VPD) in papyrus canopy was calculated based on formula of FAO (1998). Minimum and maximum values of temperature and RH were used in the calculations to avoid lower estimation of mean saturation vapour pressure. A standard value for the atmospheric pressure (kPa) and psychrometric constant ($\mathrm{kPa}\,^{0}\mathrm{C}^{-1}$) as a function of altitude was used.

Mean saturated vapour pressure (e_s) ***of the air***

Saturated vapour pressure is related to air temperature and was calculated from the air temperature;

$$e_s = \{e^0\,T_{max} + e^0\,T_{min}\}/2$$

Where *T* = Daily air temperature (°C).

$$e^0(T) = 0.6108\,exp\{17.27T\,/\,(T + 237.3)\}$$

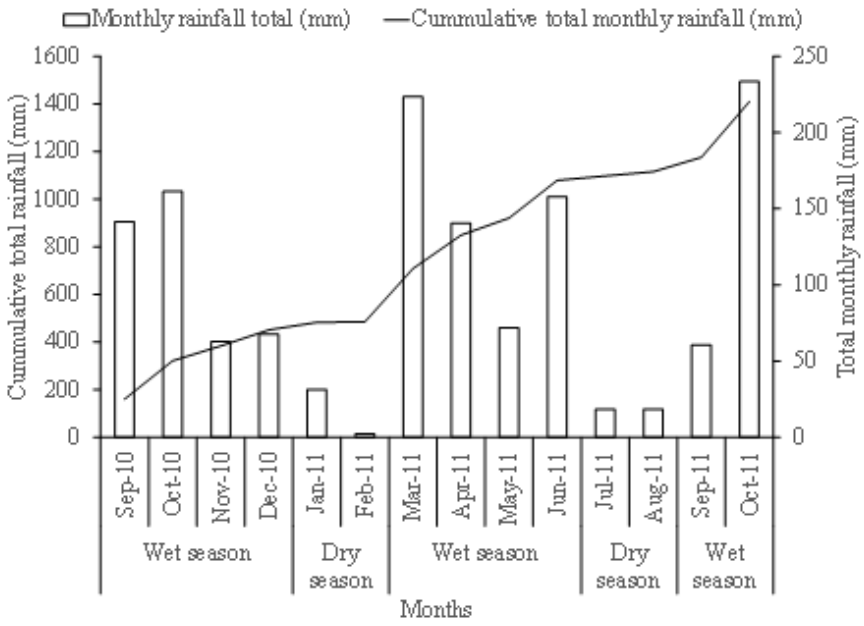

Figure 4. Total monthly rainfall and cumulative total rainfall distribution in Lubigi area.

$exp^{\{0\}}$ = 2.7183 (base of natural logarithm) raised to the power { }.
$e^0(T)$ = Saturated vapour pressure at the air temperature T($kPaC^{-1}$).

Actual vapour pressure (e_a) ***derived from relative humidity data of the air***

Relative humidity (RH) was expressed as the degree of saturation of the air as a ratio of the actual (e_a) to the saturated vapour pressure $e^0(T)$ at the same temperature (T). It constituted the ratio between the actual amount of water, the ambient air had and that it could hold at the same temperature. Although the actual vapour pressure was considered relatively constant throughout the day, the relative humidity was considered a maximum near sunrise and minimum around early afternoon. Therefore, the calculation of vapour pressure was based on the mean RH data.

$$e_{a\ =(RH_{mean}\ )/100\ \{e^0\ (Tmax\ )+e^0\ \ (T_{min}\ )\}/2}$$

Where

e_a = Actual vapour pressure (kPa)
RH_{mean} = Average relative humidity, defined as the average between RH_{max} and RH_{min}
RH_{max} = Maximum relative humidity (%).
RH_{min} = Minimum relative humidity (%).
$e^0\ (T_{max})$ = Saturated vapour pressure at daily maximum temperature (kPa).
$e^0\ (T_{min})$ = Saturated vapour pressure at daily minimum temperature (kPa).
Vapour pressure deficit $(e_s - e_a)$ of the air (VPD) was the difference between the saturated (e_s) and actual vapour pressure(e_a) of the air. Total monthly rainfall data was also obtained from Makerere University weather station located about 2 km from Lubigi wetland. Cumulative monthly total rainfall was plotted to generate the seasonal characteristics. Ogallo (1989) observed that during rainfall periods, much of the rainfall volume is accumulated and therefore the curve reaches its maximum curvature. Therefore, onset of the rainy seasons is determined from the curves as the first points of the maximum curvature.

Data analysis

Statistical analysis was done using Minitab software, Release 13 for windows. Non-parametric Kruskal-Wallis test was used to assess differences of biomass, productivity and weather values between dry and wet season. Multiple regression analysis was done to assess the relationship between independent variables (RH, temperature, PAR) and the dependent variable (productivity). Homogeneity of variance in data sets during the multiple regression analysis was automatically tested by Durbin - Watson statistics to ensure uniformity. All statistical values were considered significant at less than 0.05.

RESULTS

Monthly total and cumulative total rainfall distribution

The monthly total rainfall and cumulative distribution showed longer wet season compared to the dry seasons (Figure 4). The percentage rainfall in the dry season was below 50% of the minimal monthly rainfall during the wet season.

Papyrus aerial standing biomass and primary productivity (NPP) in Lubigi wetland

Biomass range was 1.80 to 6.69 kg m^{-2} and 3.43 to 6.76

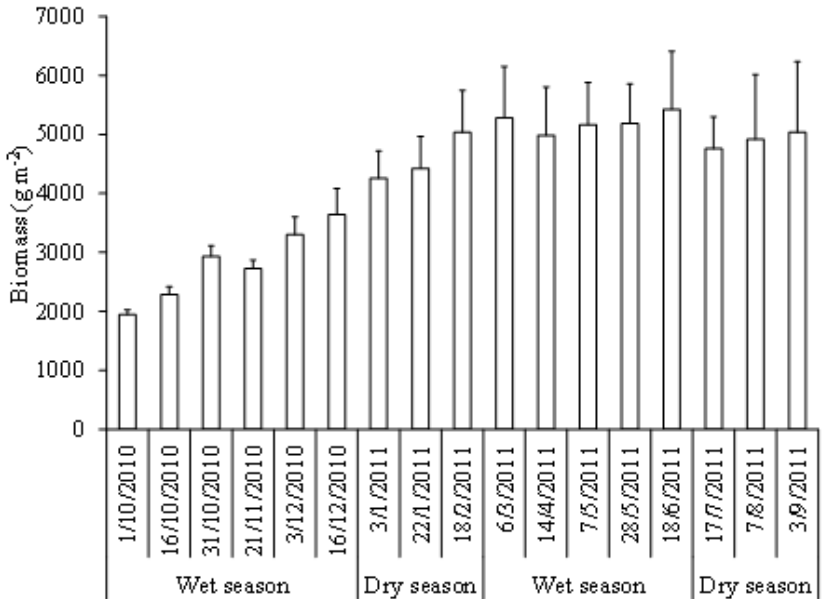

Figure 5. Mean aerial biomass of *C. papyrus* in Lubigi wetland. Error bars are standard error of means (n = 8).

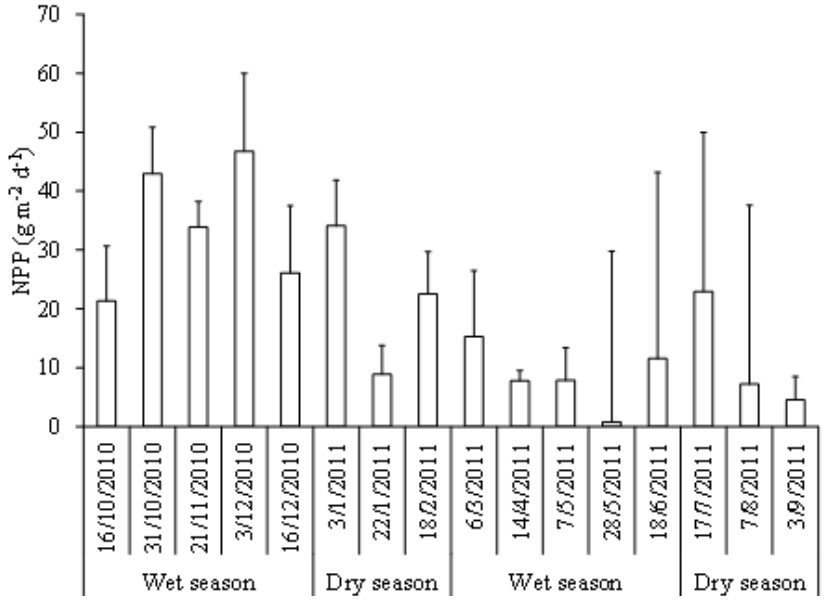

Figure 6. Mean primary productivity (NPP) of papyrus in Lubigi wetland. Error bars are standard errors of means (n = 8).

kg m^{-2} during wet and dry season respectively (Figure 5). Kruskal-Wallis test of seasonal biomass differences was not significant (H = 31.15, DF = 15, p = 0.983). The increased trend of papyrus biomass up to February 2011 indicated a recovering ecosystem which is attributed to the effect of past massive papyrus harvesting. During the establishment of the quadrats, the papyrus culms appeared well established. NPP were not significantly different between the seasons with mean values of 28.96±3.99 g m^{-2} d^{-1} and 25.99±6.35 g m^{-2} d^{-1} during wet and dry seasons respectively. NPP changes coincided with the months of biomass changes. The high variability during May, 2011 and August, 2011 (Figure 6) is attributed to some of the experimental quadrats that were covered by a climber, *Ipomoae purpurea*. The climber emerged in the wetland during the investigation period.

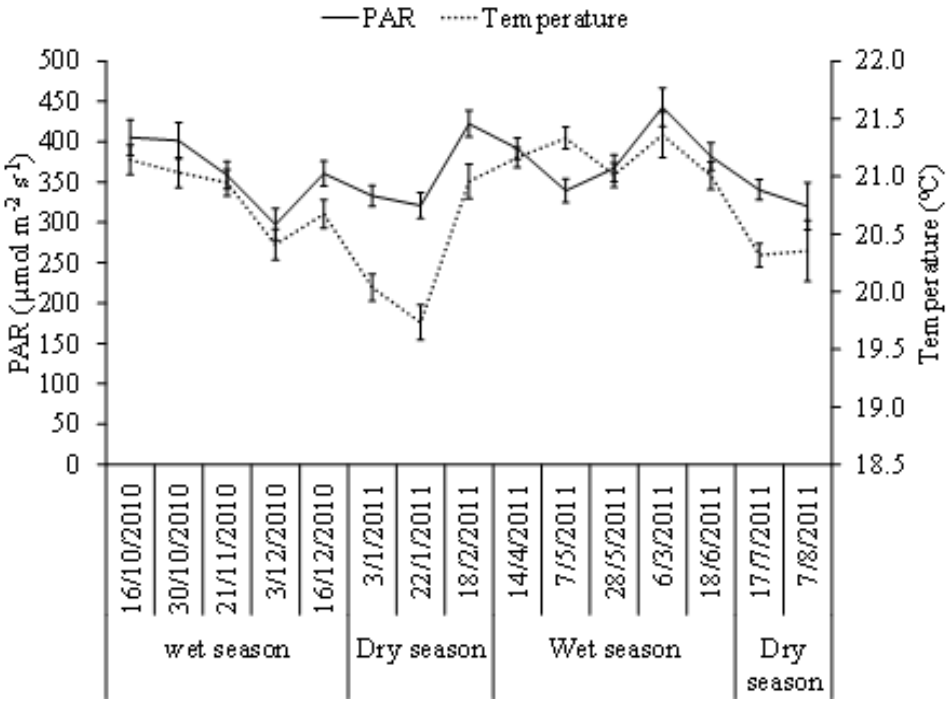

Figure 7. Mean temperature and photosynthetically active radiation (PAR) in papyrus surface canopy during the days of the year. Error bars are standard error of means.

The variability could also be related to the effect of the strong flow regime that disturbed the quadrats. Kruskal-Wallis test of NPP during the days of the year was not significant (H = 22.37, DF = 15, p = 0.099) except for the wet season from October, 2010 to December, 2010, and the dry months of February, 2011 and July, 2011 that were above the critical value (H) of 22.37 g m^{-2} d^{-1} which corresponded to the significance level.

Weather changes in *Cyperus papyrus* surface canopy

Temperature pattern was similar to PAR (Figure 7), and had higher range during wet season (12.7 to 30.6°C) compared to the dry season (11.2 to 31.1°C). Temperature changes were not significantly different since all values were below the critical value (H) (H = 61.18, DF = 1, p = 0.00) that corresponded to the significance level. Diurnal PAR range was 0 to 2332.6 µmol m^{-2} s^{-1} and 0 to 2031.6 µmol m^{-2} s^{-1} during wet and dry season respectively. Highest PAR coincided with the months of September and March. Although mean PAR was higher during the dry season, comparison of means indicated non-significant difference between seasons (H = 0.54, DF = 1, p = 0.464). VPD decreased to minimum values in the dry season, and increased to maximum values during the wet season (Figure 8). Seasonal comparison of VPD indicated significant difference during the days of the year (p = 0.037).

Relationship of aerial papyrus productivity with VPD, temperature and PAR

The overall relationship between weather variables and productivity was not significant during both dry and wet seasons (p = 0.409 and p = 0.882 respectively) (Table 1). The seasonal regression models for the relationships were:

NPP (dry season) = 0.34PAR + 1.24VPD – 1.39 Temperature + 17.2
NPP (wet season) = 0.01PAR -3.3VPD – 1.75 Temperature + 69.7

Overall, weather variables explained productivity variance by 26.1% during the dry season, and that of the wet season was independent of the weather variables (0%).

DISCUSSION

The estimated NPP in Lubigi wetland were within the range reported by many authors (Thompson et al., 1979; Muthuri et al., 1989; Jones and Muthuri, 1997; Boar, 2006; Bakari et al., 2007). The non-significant difference in seasonal NPP conforms to the study by Muthuri et al. (1989) in Naviasha wetland, Kenya. However, NPP values were slightly higher than 22.1 g m^{-2} d^{-1} (Saunders et al., 2007) and 21 g m^{-2} d^{-1} (Muthuri et al., 1989). The

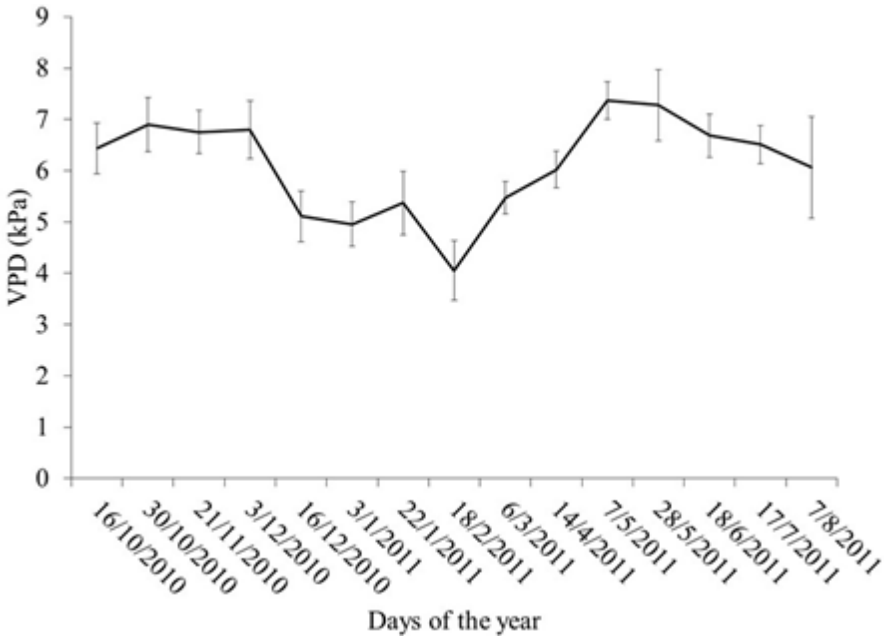

Figure 8. Mean vapour pressure deficit (VPD) in papyrus canopy during the days of the year. Error bars are standard errors of the means.

Table 1. Relationships between *Cyperus papyrus* aerial productivity with temperature, photosynthetically active radiation (PAR) and vapour pressure deficit (VPD) during the dry and wet seasons.

Variable	Productivity in dry season		Productivity in wet season	
	Regression coefficient	p-value	Regression coefficient	p-value
Temperature (°C)	-1.39	0.600	-1.75	0.869
PAR (µmol m^{-2} s^{-1})	0.34	0.312	0.01	0.791
VPD (kPa)	1.24	0.405	-3.30	0.473

variability of 3.40 to 64.42 g m^{-2} d^{-1} and 3.12 to 74.65 g m^{-2} d^{-1} during wet and dry seasons respectively were broader compared to 24.7 to 34.1 g m^{-2} d^{-1} range (Muthuri et al., 1989). Productivity differences in papyrus wetlands are attributed to structural differences which affect the amount of carbon that can be assimilated by the plant community (Jones, 1988). Beadle (1993) reported older or more complex community normally exhibit decreased productivity.

Mitsch and Jørgensen (1989) reported development of plants as a function of initial conditions and the strength of the conditions on the plants. The effects of dead biomass accumulation, high senescence and culm production contribution to the lowering of NPP have been investigated in plant (Leoni et al., 2009). Therefore, natural regeneration capacity in papyrus wetlands contributes to structural changes with effect on growth (Bakari et al., 2007). This is because papyrus wetlands exhibit establishment, growth and mortality concurrently (Muthuri et al., 1989), which imply a balanced trade-off of resources characteristic of tropical environment. However, environmental factors have been reported to affect magnitude of growth, shift carbon allocation, change productivity and shift mode of reproduction (Hunt, 1982; Beadle, 1993; Fennessy et al., 1994; Jones, 1993). Coletti et al. (2012) indicated that short term and long term vegetation responses may also be counter-intuitive due to adaptability in water uptake strategy of the vegetation community partially decoupling biomass from water availability. Lowest ratios of measured transpiration and photosynthesis in plants in the middle of dry season at a time when stomata responded strongly during optimum of photosynthesis at high temperatures were reported (Schulze et al., 1975). Therefore, the feedback mechanism in carbon sequestration involves synergy of processes, where an initial process triggers changes in a second process that in turn influences the initial process. A positive feedback intensifies the original process, and a negative feedback reduces it.

In papyrus dominated wetlands, changes in seasonal

growth were thought to occur independent of relatively constant weather parameters (Saunders et al., 2007). In this study, although the variation of VPD was significant over the period; the relationship with productivity was not significant. High NPP was associated with increased PAR during both seasons. Increasing VPD reduced NPP during the wet season but was associated with the rising NPP during the dry season. Overall, impact variance of weather variables on NPP was zero during the wet season; imply productivity change is caused by other environmental factors. These include nutrient concentrations that were reported to increase papyrus growth (Kansiime et al., 2003; Mugisha et al., 2007; Kanyiginya et al., 2010).

The 26% productivity variance could be explained by weather variables during the dry season although the association was not significant. Efficiency of photosynthesis increases to a maximum with temperature rise and then decline (Saunders et al., 2007, 2012). While the rate of respiration continue to increase more or less up to the point that a plant dies (Turral et al., 2011). Therefore, temperature effect is related to the influence of physiological changes (Pospišil et al., 2000). Related study of carbon dioxide flux model prediction and growth measurements suggest that increasing temperatures may result in substantial decrease in carbon sequestration and inputs to stored carbon in the underlying papyrus peat deposits (Humphries and Long, 1995). In this study, temperature was also negatively associated with productivity during both dry and wet seasons.

Conclusion

NPP was not significantly different between wet and dry season, and was not significantly influenced by the changes in temperature, VPD and PAR.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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African Journal of Plant Science

Full Length Research Paper

Variation of leaf and fruit characteristics of *Vitellaria paradoxa* (shea tree) according to agronomical performance along south-north climatic gradient in Mali

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Shea trees identified for agronomical performances were compared through leaf and fruit characteristics. Seventy adult trees were selected using purposive sampling from seven sites along a south-north climatic gradient covering four agro-climatic zones. The effects of two factors (type of performance and site) on leaf and fruit parameters were investigated. Results showed significant effect of the type of the performance as shea trees performant for pulp production had longer petiole and laminar, wider laminar basis and top and had longer, wider and heavier fruits with more abundant pulp as compared to those performant for butter production. The factor site was found significant for all leaf and fruits parameters but the effect of climatic gradient was rare. The leaves in Siby and Kaniko had the longest laminar and the widest laminar basis, but those in Kaniko and Noumoudama had the widest laminar as compared to all other sites. The leaves in Noumoudama had the shortest petiole as compared to the rest. The leaves in Nampossela, Zanzoni, Solosso and Noumoudama had the widest laminar top as compared to those of Kaniko, Siby and Fougatiè. The longest fruits were observed at Nampossela, while the widest and heaviest fruits as well as the most abundant pulp were observed at Kaniko and the heaviest nuts were observed at Siby. The smallest and slightest fruits were observed at Noumoudama and Solosso in more arid zones, but often, not significantly different from some sites in wettest zones regarding certain fruit parameters. This study highlighted phenotypic descriptors of agronomical performance through the leaves and the fruits of this tree species.

Key words: Agronomical performance, climatic gradient, leaf and fruit characteristics, Mali, *Vitellaria paradoxa.*

INTRODUCTION

Morphological traits of plants are usually influenced by genetic and environmental factors. The variation of local environmental conditions such as light, water, temperature, wind, etc., causes modifications in the development and the growth of plants. Hence, according to their environment, individuals of the same species may

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display different morphological traits as well as a similar response could be displayed by different plants species growing in the same environment. Anthropogenic activities could also influence plants' morphological traits. For instance, human horticultural practices (crossing, selection, grafting, etc.) may affect directly the phenotypic traits of domesticated plants species like *Vittelaria paradoxa* (*V. paradoxa*).

V. paradoxa (Shea butter tree, *karité* in french), is a semi-domesticated forest tree species covering a wide geographical area in Mali. This economically valuable species faces environmental condition effects and human practices, which certainly, have an impact on its development, particularly on the morphological traits of organs like leaves, flowers, fruits and nuts. Thus, an important variation of trunk, leaves, fruits and nuts of this species and strong correlation between several morphological traits were observed in several countries like Mali, Burkina Faso, Cameroon, Ghana and also Nigeria (Enaberue et al., 2012).

Most of studies concerning shea trees species were focused on populations located in various agro ecological zones because of the economical and socio-cultural value of the species due to the several uses of shea butter nowadays becoming very important in the international market (Divine et al., 2014).

Another shea products becoming internationally important is shea pulp which is very nutritious and plays an important alimentary role for rural populations (Aguzue et al., 2013; Divine et al., 2014; Fernande et al., 2014). These two products could be considered as the most important agronomical products of shea tree.

Unfortunately, little is known about shea trees performant for pulp production. This is why the project "Projet d'Appui aux Filières Agricoles (PAFA)" aiming to improve shea parklands and shea products by identifying and promoting performant vegetal material, was interested in these shea trees and aimed to base investigation on local populations knowledge. It is important also to investigate if shea trees performant for pulp production were different from those performant for butter production, regarding leaf and fruit characteristics but also if this difference varies according to environmental conditions.

The characterisation of a forest tree species based on its agronomical performance is useful in the objective of genetic material improvement and in improved material dissemination. Also, taking into account local knowledge of rural populations (men and women), which are the main and direct users of the resource, is one of the first steps in identifying varieties or individuals of a plant species.

The study was conducted by investigating the variation of morphological traits of leaves and quantitative traits of fruits of individual trees performing well in the production of abundant fruits with high fat content and individual trees performing well in the production of fruits with abundant and succulent pulp. The objective of the study was to determine the variation of morphological traits of leaves and that of quantitative traits of fruits for these trees with the aim of finding marker variables for these two types of agronomical performance of shea trees, to contribute to facilitate identification and choice of plus trees for multiplication and promotion of performant vegetal material.

MATERIALS AND METHODS

Study sites

The study was conducted in seven sites, selected along the south-north climatic gradient (Map 1) and belonging to four agro climatic zones (Table 1). Sites were identified in concert with local forest office technicians and local community authorities, based on the abundance of the resource in villages' territories and the presence of known associations or groups of people collecting, processing and commercialising shea products.

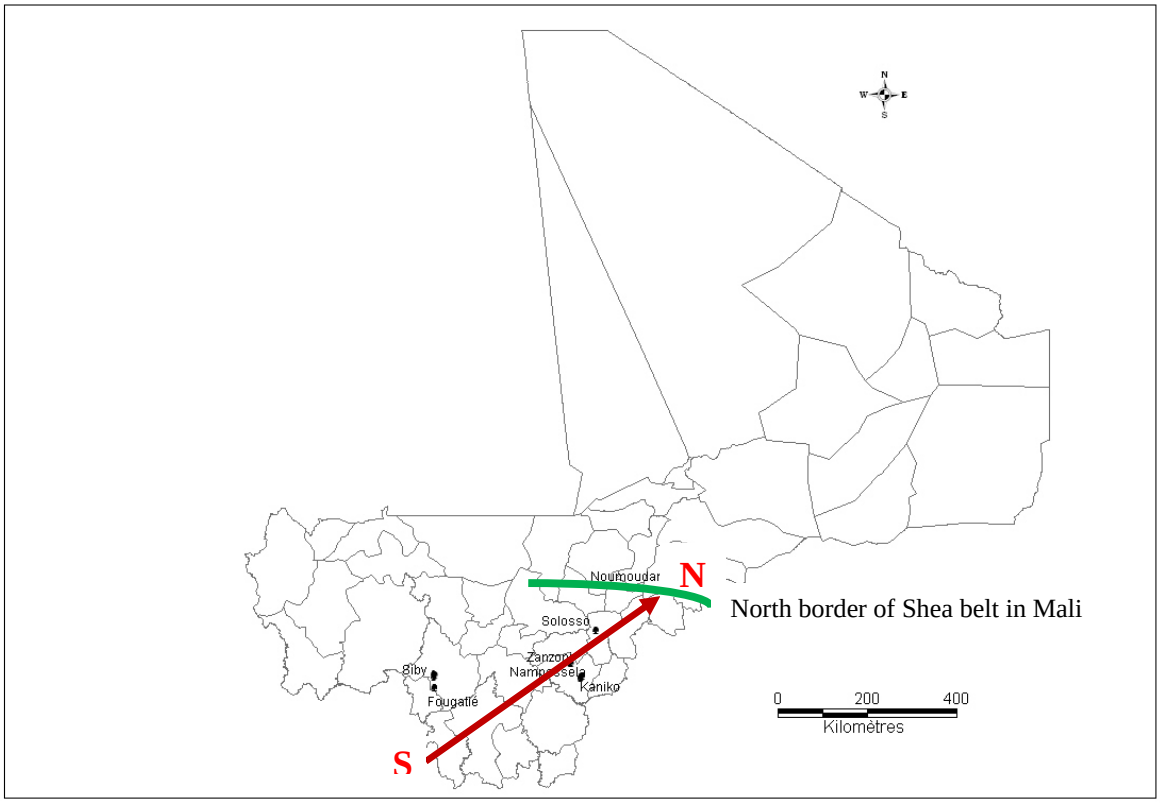
Experimental procedures

Once the sites were identified, local populations were met to collect their knowledge of shea trees performant for butter and pulp production through a survey. In each site, three to four women and three men were designated to respond to the questions and the interview allowed each person interviewed to freely give his point of view. Persons interviewed were suggested on consensus by the villagers based on their experience and knowledge of addressed issue. After interviews, guides (men and women) were designated for the localization of shea trees performant for the two products. The choice of guide people was done on consensus by the villagers in the same way as the people chosen for the interview. The number of guides varied from two to six persons according to site.

Localised shea trees, identified as performant for butter and for pulp production, were marked with yellow paint in all the seven sites in October 2010 when fruiting period was over. GARMIN GPS 72 was used to determine the geographical position of each marked tree which bears also an identity code formed with initials of the site, the type of product (butter, pulp) and tree number. At each site, ten adult shea trees (five trees per type of performance) with diameter at breast height (dbh) varying from 30 to 118 cm giving a total of 70 trees (35 per type of performance) were identified and localised.

Data collection and statistical analysis

Fruits and leaves were harvested in July 2011 from all marked trees in all sites. For leaf characterisation, 50 leaves were sampled per tree and the following variables were measured: laminar and petiole length, laminar width, laminar basis and top width. For fruit quantitative traits, on the same trees (except those of Fougatiè because fruiting has ended when study started), 50 fresh ripe fruits were harvested. Fruits were weighed using electronic balance AND GR 202 and their length and width were measured using Vernier calliper SUNRISE. The pulps of collected fruits were removed on the same day and nuts washed with tap water. Fresh nuts were also weighed using the same balance and the pulp weight was calculated as a difference between the fresh fruit weight and fresh nut weight. Analysis of variance was used to determine the effect of studied factors in comparing LSD means at 5% significance level. The studied factors were the agronomical performance with two levels (shea trees performant for butter production, shea trees



Map 1. Geographical distribution of study sites (from south to north sites: Fougatiè, Siby, Kaniko, Nampossela, Zanzoni, Solosso and Noumoudama as shown in Table 1).

Table 1. Study sites in different agro-climatic zones.

Administrative regions	Sites	Agro-climatic zones	Site coordinates
Sikasso	Fougatiè	Guinean North (GN) (Isohyets >1100 mm)	11°07.777N; 008°22.063W
Koulikoro	Siby		12°22.686N; 008°20.258W
Sikasso	Kaniko	Sudanian South (SS) (Isohyets 800-1100 mm)	12°19.115N; 005°22.007W
	Nampossela		12°19.879N; 005°20.456W
	Zanzoni		12°36.541N; 005°34.204W
Ségou	Solosso	Sudanian North (SN) (Isohyets 500-800 mm)	13°14.408N; 005°02.525W
Mopti	Noumoudama	Sahelian (Sa) (Isohyets 200-500 mm)	14°06.480N; 003°30.246W

performant for pulp production) and site with seven levels as described above (Table 1). The statistical package SYSTAT9 for WINDOWS was used for analyses.

RESULTS

Effect of studied factors on leaf morphological traits

Table 2 shows statistics of leaf variables and the analysis of variance showed significant effects of studied factors. A part from laminar width, the factor type of performance was significant for all leaf variables (Table 2). Shea trees performant for pulp had longer leaf petiole and laminar, wider laminar basis and top as compared to the performant for butter.

The factor site was significant for all leaf variables (Table 2). The leaves in Siby and Kaniko (zone SS) had the longest laminar and the widest laminar basis, but

Table 2. Leaf parameters per type of performance of shea trees and per site.

Factors	Laminar length (cm)	Laminar width (cm)	Petiole length (cm)	Laminar basis width (cm)	Laminar top width (cm)
Performance					
Butter	14.35^{b}	4.29^{a}	7.76^{b}	1.24^{b}	1.51^{b}
Pulp	14.88^{a}	4.26^{a}	7.94^{a}	1.72^{a}	1.62^{a}
Signification levels	***	ns	**	***	***
Sites					
Fougatiè (GN)	15.05^{ab}	4.04^{c}	8.29^{b}	1.15^{c}	1.50^{cd}
Siby (SS)	15.27^{a}	4.13^{c}	8.69^{a}	1.31^{a}	1.42^{d}
Kaniko (SS)	15.24^{a}	4.56^{a}	7.93^{c}	1.37^{a}	1.39^{d}
Nampossela (SS)	14.37^{bc}	4.31^{b}	7.77^{c}	1.27^{ab}	1.75^{a}
Zanzoni (SS)	14.67^{b}	4.45^{ab}	7.97^{c}	1.20^{bc}	1.66^{ab}
Solosso (SN)	13.71^{d}	3.99^{c}	7.39^{d}	1.00^{d}	1.57^{bc}
Noumoudama (Sa)	14.03^{cd}	4.47^{a}	6.92^{e}	1.13^{c}	1.69^{ab}
Signification levels	***	***	***	***	***

LSD means not significantly different at P = 0.05 were indicated by same character. *** = Very highly significant at P ≤ 0.001, ** = highly significant at P ≤ 0.01, * = significant at P ≤ 0.05, ns = non-significant at P = 0.05. GN = Guinean north. SS = Sudanian south. SN = Sudanian north. Sa = Sahelian.

Table 3. Leaf parameters of shea trees performant for butter production in different sites.

Sites	Laminar length (cm)	Laminar width (cm)	Petiole length (cm)	Laminar basis width (cm)	Laminar top width (cm)
Fougatiè (GN)	14.79^{ab}	4.21^{b}	8.34^{ab}	1.35 b	1.43^{c}
Siby (SS)	14.59^{ab}	3.94^{c}	8.37^{a}	1.18^{c}	1.16^{d}
Kaniko (SS)	14.77^{ab}	4.57^{a}	7.89^{ab}	1.60^{a}	1.40^{c}
Nampossela (SS)	14.14^{bc}	4.49^{b}	7.74^{b}	1.18^{c}	1.45^{c}
Zanzoni (SS)	14.87^{a}	4.47^{a}	7.88^{b}	1.36^{b}	1.78^{b}
Solosso (SN)	13.76^{c}	4.17^{b}	7.49^{b}	1.05^{d}	1.04^{d}
Noumoudama (Sa)	13.55^{c}	4.49^{a}	6.58^{c}	0.98^{d}	2.33^{a}
Grand mean	**14.35**	**4.29**	**7.76**	**1.24**	**1.51**
Coefficient of Variation in %	17.3	18.5	22.5	41.2	52.5
Signification levels	***	***	***	***	***

LSD means not significantly different at P = 0.05 were indicated by same character; *** = Very highly significant at P ≤ 0.001; GN = Guinean north. SS = Sudanian south. SN = Sudanian north. Sa = Sahelian.

those in Kaniko (zone SS) and Noumoudama (zone Sa) had the widest laminar as compared to all other sites. The leaves in Noumoudama (zone Sa) had the shortest petiole as compared to the rest. The leaves in Nampossela, Zanzoni (zone SS), Solosso (zone SN) and Noumoudama (zone Sa) had the widest laminar top as compared to those of Kaniko, Siby (zone SS) and Fougatiè (zone GN).

Variation of leaf morphological traits for shea trees performant for butter production

Table 3 shows the variation of leaf morphological traits for shea trees performant for butter production according to site. For all leaf variables of shea trees performant for butter production, sites were found very highly significantly different (Table 3).

The variation of leaf laminar length displayed climatic gradient effect as sites of Guinean zone (Fougatiè) and south Sudanian zone (Zanzoni, Kaniko and Siby) had the longest leaf laminar as compared to those of north Sudanian and Sahelian zones. More obvious climatic gradient effect was observed for petiole length and laminar top wide as the site of Sahelian zone (Noumoudama, the most arid site) showed the shortest petiole length and the widest laminar top. For leaf laminar wide, climatic gradient effect was less obvious as sites of

Table 4. Leaf parameters of Shea trees performant for pulp production in different sites

Sites	Laminar length (cm)	Laminar width (cm)	Petiole length (cm)	Laminar basis width (cm)	Laminar top width (cm)
Fougatiè (GN)	15.31^{a}	3.87^{c}	8.25^{b}	0.95^{d}	1.56^{bc}
Siby (SS)	15.94^{a}	4.32^{b}	9.00^{a}	1.44^{a}	1.68^{b}
Kaniko (SS)	15.71^{a}	4.55^{a}	7.98^{b}	1.14^{c}	1.38^{c}
Nampossela (SS)	14.59^{b}	4.43^{ab}	7.80^{b}	1.37^{ab}	2.04^{a}
Zanzoni (SS)	14.48^{b}	4.39^{ab}	8.05^{b}	1.03^{cd}	1.53^{bc}
Solosso (SN)	13.66^{c}	3.81^{c}	7.29^{c}	0.96^{d}	2.10^{a}
Noumoudama (Sa)	14.50^{b}	4.44^{ab}	7.25^{c}	1.28^{b}	1.06^{d}
Grand mean	14.88	4.26	7.94	1.72	1.62
Coefficient of Variation in %	17.2	18.9	22.9	46.2	50.3
Signification levels	***	***	***	***	***

LSD means not significantly different at P = 0.05 were indicated by same character; *** = very highly significant at P ≤ 0.001 GN = Guinean north; SS = Sudanian south; SN = Sudanian north. Sa = Sahelian.

south sudanian zone and sahelian zone were found not to be significantly different. The widest laminar basis was found at Kaniko (zone SS), but no climatic gradient effect was displayed for this variable.

Variation of leaf morphological traits for shea trees performant for pulp production

Table 4 shows the variation of leaf morphological traits for shea trees performant for pulp production according to site. Like shea trees performant for butter, sites were found to be very highly significantly different for all leaf variables of shea trees performant for pulp production.

For most variables (laminar length and wide, petiole length, laminar basis wide), results observed for shea trees performant for pulp production (Table 4) were similar to those observed for shea trees performant for butter (Table 3) regarding the trend of the difference between sites relative to agro climatic zones. However, contrary to Shea trees performant for butter, an effect of climatic gradient was not displayed for laminar top wide of shea trees performant for pulp.

From these results, it appears that the trend of the variation according to sites of the leaf morphological traits does not differ very much for the two types of agronomical performance of shea trees. The study showed globally that, independently to the type of performance, shea trees of sites located at the southern part of study area (zones GN and SS) had longer leaf laminar and longer petiole, while those located at the northern part (zone SN and Sa) had wider leaf laminar, wider laminar top and shorter petiole (Table 2). For all performances confounded (Table 2) and per type of performance (Tables 3 and 4), a sort of transitional zone (composed of few sites) was found between the extreme south (Fougatiè, Siby) and the extreme north (Noumoudama, Solosso) regarding some variables, particularly the petiole length.

Effect of studied factors on fruit quantitative traits

Table 5 shows statistics for fruit variables and the analysis of variance showed significant effects of studied factors. A part from nut weight, the type of performance was significant for all other fruit variables. Shea trees performant for pulp had longer, wider and heavier fruits with more abundant pulp as compared to those performant for butter.

The factor site was significant for all fruit quantitative traits. The longest fruits were observed at Nampossela (zone SS), while the widest and heaviest fruits as well as the most abundant pulp were observed at Kaniko (zone SS) and the heaviest nuts were observed at Siby (zone SS). The smallest and slightest fruits were observed at Noumoudama (zone Sa) and Solosso (zone SN) which are the more arid zones, but often, not significantly different from some sites of south Sudanian zone regarding certain fruit variables (Table 5).

Variation of fruit quantitative traits for shea trees performant for butter production

Table 6 shows the variation of fruit quantitative traits according to site for shea trees performant for butter production. For all fruits quantitative traits of shea trees performant for butter production, sites were found to be very highly significantly different (Table 6).

The longest and widest fruits were observed at Solosso (zone SN) and Nampossela (zone SS), while the heaviest fruits were observed at Zanzoni, Siby (zone SS) and Solosso (zone SN). The most abundant pulp was still

Table 5. Fruit parameters per type of performance of shea trees and per site.

Factors	Fruit length (cm)	Fruit width (cm)	Fresh fruit weight (g)	Fresh nut weight (g)	Pulp weight (g)
Performance					
Butter	3.75[b]	3.19[b]	24.50[b]	10.72[a]	13.80[b]
Pulp	4.14[a]	3.53[a]	33.83[a]	11.09[a]	22.74[a]
Signification levels	***	***	***	ns	***
Sites					
Siby (SS)	3.75[d]	3.40[b]	29.06[bc]	12.33[a]	16.73[c]
Kaniko (SS)	4.13[b]	3.50[a]	32.71[a]	10.56[bc]	22.15[a]
Nampossela (SS)	4.39[a]	3.36[bc]	31.44[b]	10.90[bc]	20.54[b]
Zanzoni (SS)	4.04[c]	3.45[b]	31.47[b]	11.30[b]	20.17[b]
Solosso (SN)	3.99[c]	3.31[bc]	26.95[cd]	10.20[c]	16.74[c]
Noumoudama (Sa)	3.66[d]	3.24[c]	26.19[d]	10.18[c]	15.95[c]
Signification	***	***	***	***	***

LSD means not significantly different at P = 0.05 were indicated by same character. *** = Very highly significant at P ≤ 0.001, ns = non-significant at P = 0.05. GN = Guinean north. SS = Sudanian south. SN = Sudanian north. Sa = Sahelian.

Table 6. Fruit parameters of shea trees performant for butter production in different sites

Sites	Fruit length (cm)	Fruit width (cm)	Fresh fruit weight (g)	Fresh nut weight (g)	Pulp weight (g)
Siby (SS)	3.66[c]	3.26[a]	26.89[a]	11.98[a]	14.90[b]
Kaniko (SS)	3.86[b]	3.06[b]	22.54[b]	9.59[d]	12.94[c]
Nampossela (SS)	3.90[ab]	2.94[b]	23.23[b]	9.992[cd]	13.31[bc]
Zanzoni (SS)	3.76[bc]	3.35[a]	28.79[a]	10.78[bc]	18.00[a]
Solosso (SN)	4.02[a]	3.36[a]	26.66[a]	11.52[ab]	15.13[b]
Noumoudama (Sa)	3.26[d]	2.98[b]	18.67[c]	10.07[cd]	8.59[d]
Grand mean	**3.75**	**3.19**	**24.52**	**10.72**	**13.80**
Coefficient of Variation in %	15	15.8	36.6	30.2	53.6
Signification levels	***	***	***	***	***

LSD means not significantly different at P = 0.05 were indicated by same character. *** = Very highly significant at P ≤ 0.001; GN = Guinean north. SS = Sudanian south. SN = Sudanian north. Sa = Sahelian.

observed at Zanzoni but the heaviest nuts were observed at Siby and Solosso. For this variable (nut weight), many homogenous groups were displayed (Table 6) yielding a non-obvious trend of difference between sites and it was also the only one for which the two types of performance (all sites confounded) were found not significantly different (Table 5). The variation of the difference between sites did not display any climatic gradient effect for all fruit quantitative traits. However, the site of Noumoudama (zone Sa) had the least of all fruit quantitative traits. In few cases, it was found not to be significantly different from certain sites (Table 6).

Variation of fruit quantitative traits for shea trees performant for pulp production

Table 7 shows the variation of fruit quantitative traits according to site for shea trees performant for pulp production. For all fruits quantitative traits of shea trees performant for pulp production, sites were found to be very highly significantly different (Table 7).

Results showed that fruits from Kaniko (zone SS) were longest, widest, heaviest and with most abundant pulp, while those from Siby in the same zone had the heaviest nut weight. Only for fruit length, the site of Kaniko formed the same group with the site of Nampossela (very close). For nut weight, Siby was not significantly different from Kaniko and Zanzoni in the same zone (Table 7). For shea trees performant for pulp production, like shea trees performant for butter production, the variation of the difference between sites did not display any climatic gradient effect for all fruit quantitative traits.

When comparing Tables 6 and 7, one can also observed that the trend of the difference between sites

Table 7. Fruit parameters of shea trees performant for pulp production in different sites.

Sites	Fruit length (cm)	Fruit width (cm)	Fresh fruit weight (g)	Fresh nut weight (g)	Pulp weight (g)
Siby (SS)	3.82^{d}	3.51^{b}	30.88^{c}	12.62^{a}	18.25^{c}
Kaniko (SS)	4.58^{a}	4.22^{a}	53.72^{a}	12.56^{ab}	41.16^{a}
Nampossela (SS)	4.68^{a}	3.60^{b}	35.91^{b}	11.43^{bc}	24.47^{b}
Zanzoni (SS)	4.21^{b}	3.52^{b}	33.30^{bc}	11.65^{ab}	21.65^{b}
Solosso (SN)	3.96^{cd}	3.26^{c}	27.24^{d}	8.88^{d}	18.36^{c}
Noumoudama (Sa)	3.99^{c}	3.46^{b}	32.42^{c}	10.28^{c}	22.14^{b}
Grand mean	**4.14**	**3.56**	**33.83**	**11.09**	**22.74**
Coefficient of variation in %	14.7	16	39.1	37	50.4
Signification	S P=0.000***	S P=0.000***	S P=0.000***	S P=0.000***	S P=0.000***

LSD means not significantly different at P = 0.05 were indicated by same character; *** = Very highly significant at P ≤ 0.001; GN = Guinean north. SS = Sudanian south. SN = Sudanian north. Sa = Sahelian.

was different for the two types of performance and the trend displayed for shea trees performant for pulp production was similar to that displayed for all performance confounded (Table 5). This result suggested that, the trend displayed for all performance confounded regarding the variation of the difference between sites (Table 5), reflected the trend due to shea trees performant for pulp production which appears to be more marked than that due to shea trees performant for butter production.

DISCUSSION

Variation of leaf morphological traits

Shea trees identified for two agronomical performances (butter and pulp) were found significantly different according to leaf morphological traits. Results indicated that shea trees performant for pulp had longer leaf petiole and laminar, wider laminar basis and top. According to this result, leaf morphological traits could be used to distinguish shea trees of different agronomical performances. Kelly et al. (2011) reported that farmers (men and women) were able to recognise shea trees through leaf characteristics. According to farmers, shea trees performant for butter production have thin and smooth leaves while those performant for pulp production have broad and shiny leaves.

Sites also were found significantly different and various trends were observed regarding climatic gradient effect as two variables displayed evident climatic gradient effect, two others displayed less evident climatic gradient effect and one variable did not displayed any climatic gradient effect.

Regarding variables having evident climatic gradient effect (petiole length and laminar top wide), leaves of shea trees from more arid zones (Sa and SN) had shorter petiole and wider laminar top as compared to those of shea trees from more humid zones (SS and NG). It was observed that petiole length decrease progressively from the GN (Fougatiè) to the Sa (Noumoudama). This variable seems to be an interesting morphological descriptor allowing discriminating shea tree according to the climatic gradient. Shorter petiole observed at more arid zones could be an adaptation strategy. This result suggests an important role played by this organ (petiole) in shea plant physiology and in its adaption to local environmental conditions. It was also observed that leaves of shea trees from these more arid zones had wider laminar top. The result suggests that shea trees vary in the dimension of their organs, therefore resist and survive in their environment.

Similar results were observed by Nyarko et al. (2012) who found significant difference between shea trees from three zones (Sudan Savanna, Guinea Savanna and the transitional belt of the Northern Savanna and the Southern Forest) in northern Ghana and observed wider laminar and shorter petiole for more arid zone (Sudan Savanna zone). Nyarko et al. (2012) explained their results as possible effects of climatic conditions, soils characteristics and environmental mutation and stated that, probably, the short petiole of the leaves made them to be positioned at angles that allow maximum interception of sunlight for photosynthesis. The wider laminar top that we observed for arid zone may also play the role of intercepting maximum sunlight for better photosynthesis and better predisposition to other vital and agronomical performance functions like the role that Nyarko et al. (2012) reported for the short petiole.

Regarding variables that displayed less obvious climatic gradient effect (laminar length and width), leaves of shea trees from more humid zones had longer laminar, while those of shea trees from more arid zones had wider

laminar. Shea leaves with longer laminar were observed for more humid zone by Nyarko et al. (2012), strengthening the present study results particularly in the case of shea trees performant for butter production. The broad leaves in more arid area could be explained by a greater need of shea trees in photosynthesis and/or in the regulation of transpiration/respiration mechanism in order to withstand the harsh climatic conditions. This result suggests that shea trees would, in view of the climatic conditions, developed an adaptation strategy controlled by the leaf surface which, through the mechanisms of photosynthesis and respiration/transpiration, would play an important role in the physiology of trees.

The manifestations of the climatic gradient effects suggest that climatic conditions only would not explain the variance for these variables according to the sites. Other factors (management practices, individuals age, genetic, etc.), would contribute to their variation. Djekota et al. (2014) observed variation within and between sites regarding the length and the width of the shea tree leaves in the region of Mandoul in Chad but did not identify the sources of this variation.

Leaf laminar basis width was the variable that did not display any climatic gradient effect. Like the variation of leaf laminar length and width, other factors including the genetic, could explain its variation according to the sites. Gwali et al. (2012) did not observe any climatic gradient effect in the study of morphological variation of shea tree in Uganda.

Variation of fruits quantitative traits

Shea trees performant for butter and pulp production were also found to be significantly different according to fruit quantitative traits. Results indicated that shea trees performant for pulp had longer, wider, heavier fruits with more abundant pulp. These results confirmed that, farmers in designating shea trees performant for pulp production had good appreciation of this agronomical performance of shea trees. It is important to note that the size of shea fruit is the apparent characteristic and the major criteria used by farmers in selecting trees. Studies discriminating shea trees based on their agronomical performance are rare. This study reveals, however, that several fruit quantitative traits differentiate shea trees performant for pulp production from those performant for butter production. Hence, the length, width and weight of the fruits of shea trees are good indicators and descriptors of shea tree performant for pulp.

Sites were also found to be significantly different for all fruit variables (Table 5). The variation of the length and width of the fruit according to the site did not display climatic gradient effect. Other factors that influence the variation of these traits include management practices and/or the size and age of the trees. Highest fruit sizes were found in sites from the old cotton belt, because of the intensification of cotton culture, there is a problem of land which resulted in a long use of the fields and a reduction, or even the disappearance of fallow. Shea trees therefore are beneficial to cultivation and fertilization activities. Elias (2013) reported shea trees benefit from farmers' management practices of agroforestry parklands.

Observed fruit weights in this study were higher than those observed by Nyarko et al. (2012) and the variability in this study was also higher than that observed by Gwali et al. (2012) who did not observe an effect of climatic gradient in the variation of the weight of shea fruits, but noted a strong positive correlation between the weight of the fruit and the pulp ($r = 0.963$). This correlation would explain the similar variations of these two variables observed. Fresh nut weights were also higher than those observed by Nyarko et al. (2012). Like other variables, the weight of the nuts did not display a climatic gradient effect.

The current study did not clearly reveal climatic gradient effect on shea fruit quantitative traits like the results reported by Nyarko et al. (2012) according to which the fruits of the Sudanian savanna zone (dryer), were longer, wider and heavier than those of wetter zones. According to Nyarko et al. (2012), variation among sites could also be explained by farmers' selection criteria, based mainly on morphological traits of fruits and influenced by several factors including social, cultural, economic, biotic and abiotic. They are also referred to environmental mutations as a potential cause of the difference between sites, and also the edaphic conditions that might explain the lower performance of the wettest sites as compared to dryer sites. Ugese et al. (2010) did not observe a climatic gradient effect in comparing nine sites covering three agro ecological zones (Sudanian savanna, Northern Guinea savanna and southern Guinea savanna) in Nigeria, despite the considerable diversity of shea fruit and nut morphological traits. These authors have noted a strong influence of some environmental variables on phenotypic traits of the shea tree fruits. The effect of environment would explain the result observed for Noumoudama (zone Sa) at the extreme north of shea area in Mali. Fruits from this site had the least of all fruit parameters and it is believed that this is an adaptation strategy of the species in the environment.

Conclusion

This study, which is the first regarding description of shea trees according to agronomical performance, shows approaches for shea trees characterization according to shea agronomical performance, highlighting some phenotypic descriptors of agronomical performance through the leaves, but also and especially through the fruits.

The effect of the type of performance varied according to the studied organs and variables. This factor was significant for most of the leaf variables and for all fruit variables. Thus, leaf morphological traits allowed distinguishing shea trees performant for different products and so are agronomical performance descriptors of shea at least for the two types of performance considered by this study. Fruit quantitative traits were found as excellent descriptors by distinguishing clearly shea trees for pulp production (fruits of larger dimensions and a more abundant pulp) from those for butter production.

Site effect was significant for all leaf and fruit variables. Climatic gradient effect was only displayed for few leaf variables, suggesting a possible effect of other factors (management practices, environment, genetics, biology, etc.) which in addition to the climate, would influence leaf and fruit characteristics, regardless of the agronomical performance of shea trees.

This study highlighted the importance of local knowledge in identification and selection of plus trees since all indicated trees were found performant for the criterion of selection. Hence, any improvement and multiplication program must take into account, this aspect for a greater chance of success. The study also confirmed results of several previous studies on the conjunction of several factors which would be the basis for the variability observed in shea leaf and fruit morphological traits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Fall management of fleabane based on glyphosate+2, 4-D, MSMA and glufosinate applied isolated or in tank mixture with residual herbicides

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Fleabane (*Conyza* spp.) has spread in no-tillage areas of Paraná State (PR), Brazil, and currently represents one of the main challenges related to weed control, particularly during off-season period. For this purpose, two experiments were carried out in Campina da Lagoa (PR) and Floresta (PR) with the aim of assessing the efficacy of herbicide treatments applied to fleabane areas during the off-season period between maize harvest (June to July) and soybean sowing (November). Treatments consisted of herbicide treatments (glyphosate + 2, 4-D; glufosinate, MSMA) targeting the control of fleabane plants in POST, tank-mixed with residual herbicides (metsulfuron, chlorimuron, diclosulam, imazethapyr, imazaquin, flumioxazin, metribuzin, amicarbazone and isoxaflutole) to control the emergence and growth of new flushes in PRE. Applications were performed when fleabane plants reached a height of 2 cm. Evaluation on both efficacy and residual weed control was accomplished from the first day of application day to 75 days after application (soybean crop sowing). Glyphosate + 2, 4-D mixture was efficient for burndown of C*onyza* spp. in all situations. During 75 day off season period, diclosulam and chlorimuron were the best options for controlling fleabane emergence when mixed with any other options of herbicide treatments, for the control in POST. Mixtures of glyphosate + 2, 4-D with metribuzin and glufosinate with flumioxazin, metribuzin and isoxaflutole maintained good fleabane control throughout the whole 75 days off season period. Fall management was an effective option for fleabane control.

Key words: *Conyza* spp., herbicide resistance, management, residual effect, weeds.

INTRODUCTION

Fleabane (*Conyza* spp.) ranks among the top ten main weeds distributed around the world. Different species in this genus stand out in this ranking due to their ability to develop resistance to herbicides of different mechanisms

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of action, making its control even more difficult (Trainer et al., 2005). In the United States, the controlling failures were observed after only three years of continued use of glyphosate in post-emergence (POST).

In 2001, the first case of *Conyza canadensis* resistant to glyphosate was reported Vangessel (2001). In Brazil, glyphosate-resistant *Conyza bonariensis* and *C. canadensis* were first reported in 2006 both in citrus orchards located in São Paulo State (Moreira et al., 2007) and in grain-producing areas in Rio Grande do Sul (Vargas et al., 2007). Besides that, resistant biotypes of *Conyza sumatrensis* were also reported in Paraná State (Santos et al., 2014).

Depending on rain availability and fall/winter temperatures, two consecutive cropping cycles can be achieved within the same agricultural year in some regions of Brazil. The first and usually more important growing season starts in early spring (September to October), and ends in the mid-summer (February to March). This is the most favorable growing season, due to abundant rain and warm temperatures. The second growing season ("safrinha") starts as soon as the main growing season ends which usually finish in June to August, depending on crop cycle and climate. The second growing season is characterized by a higher risk of low temperatures (South and Southeast regions) or water deficit (Southeast and West Central regions).

The control of winter annual emerged with weed species which may not ensure an adequate control throughout the next summer crop sowing, once, new flushes of weeds may emerge after herbicide application, infesting the area again. Thus, the addition of residual herbicides such as acetolactate synthase inhibitors, photosystem II inhibitors or PROTOX inhibitors (Armel et al., 2009) may help to extend residual weed control until crop sowing (Owen et al., 2009).

The concept of fall burndown is related to the group of off-season weed control strategies, that is, those weed control methods adopted in the period of time between crop harvest and next crop sowing, when such period is relatively long. In Brazil, such situation will likely happen after the second growing season (June/August to September/October) (Constantin et al., 2012).

Fall burndown as a fleabane management strategy meets one of the basic assumptions on hard-to-control weed management, that is, to intervene with a control measure when the weed is most susceptible. In field conditions, fleabane emergence peak occurs from June through September. Shortly after emergence, plants are usually in a stage in which chemical control is easier due to increased susceptibility of fleabane plants, coinciding with the period when fall management can be performed.

The length of that period is directly related to when second maize is harvested, and may last from 45 to 90 days. In those areas, maize harvest can be divided into so called early harvest maize when harvest is performed not later than the first half of July (off-season longer than 60 days) and late harvest maize when harvest is performed after July 15 (off-season shorter than 60 days). This division is important because each harvest demands exclusive management practices in off-season time. For the first harvest season, fleabane emergence peak generally occurs after maize harvest, whereas for second harvest, it occurs also during the end of maize cycle. Thus, because of the length of off-season period, different approaches as related to herbicide residual control may be demanded.

The aim of this research was to evaluate the efficiency of three chemical options for burndown (glyphosate + 2, 4-D, glufosinate or MSMA) applied either isolated or in tank mixtures with residual herbicides for PRE and POST fleabane control.

MATERIAL AND METHODS

Two field experiments were performed from June to October, 2009. The first one was carried out at Campina da Lagoa (PR) (24°34'45.44"S, 52°41'47.95" W, altitude = 618 m) and the second one in Floresta (PR) (23°36'44.82"S, 52°05'22.79" W, altitude = 380 m). Both areas were farmed for the previous last years with a soybean-second maize crop succession and herbicide failures *Conyza* spp. history.

Soils from experimental areas were identified as Red Oxisoil eutrophic (Embrapa, 2013), clay texture. In Campina da Lagoa, main soil properties included 60.0 % clay, 27.0 % sand, pH H_20 = 6.0, and 28.58 g dm^{-3} of organic carbon (OC), while in Floresta, 61.0 % clay, 29.0 % sand, pH H_20 = 6.0, and 20.89 g dm^{-3} OC. Regional climate is subtropical with rainy summers and dry winters (Cfa, according to Köppen climate classification). Monthly rainfalls observed during the period of time when experiments were in the field are presented in Figure 1. At each location, the experiment was initiated right after second maize harvest (July 08, 2009 in Campina da Lagoa and July 17, 2009 in Floresta). Those maize harvest dates provided an off-season period longer than 60 days, so both experiments were therefore considered as first-season maize experiments.

After maize harvest, there was a 15-days interval before the treatments application, due to the need to stabilize crop residues on soil surface, in order to enable both burndown efficacy and uniform distribution of residual herbicides on the soil. Treatments were identical for both experiments. They comprised three chemical treatments (glyphosate + 2,4-D 960 + 536 g ha^{-1}, MSMA 2370 g ha^{-1} and ammonium-glufosinate 400 g ha^{-1}) applied either isolated or in tank mixed with nine herbicide treatments with residual activity in soil (metsulfuron 3.6 g ha^{-1}, chlorimuron 20 g ha^{-1}, diclosulam 33.6 g ha^{-1}, imazethapyr 100 g ha^{-1}, imazaquin 180 g ha^{-1}, flumioxazin 125 g ha^{-1}, metribuzin 480 g ha^{-1}, amicarbazone 420 g ha^{-1} and isoxaflutole 56.25 g ha^{-1}) with an additional treatment and no herbicide application. The combination of MSMA + imazaquin was replaced by amicarbazone (560 g ha^{-1}) due to tank mixture incompatibility. Both experiments were arranged in a completely randomized block design, with four replicates.

Treatments with glyphosate + 2,4-D, glyphosate, MSMA and glufosinate were used to control emerged plants of *Conyza* spp. at the moment of application and were considered as without or limited residual treatments, as compared to the tank mixture, which were considered as soil residual treatments. Herbicide applications were done when fleabane plants were in a very early development stage (≤ 2 cm). That stage was chosen due to both the increased sensitivity to herbicides as compared to more developed plants, and to the lower sprouting capacity in early stages of fleabane

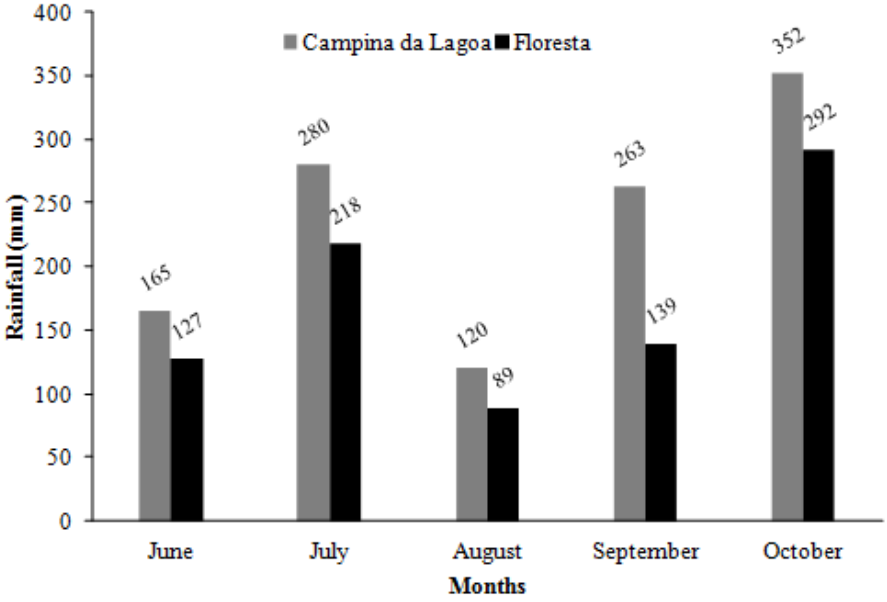

Figure 1. Monthly rainfall during the time of conducting experiments in two locations in Paraná.

Table 1. Climatic conditions, site description and characterization of *Conyza* spp. densities and stage of development at the dates of spraying of treatments in two locations in Paraná.

Specifications	Campina da Lagoa	Floresta
Application date	08/07/2009	14/07/2009
Temperature (°C)	20	21
Relative air humidity (%)	80	82
Soil moisture	Moist	Partly moist
Wind (km h^{-1})	2.0	0.0
Cloud	Partly cloud	Sunny
Soil cover residues (t ha^{-1})	10.6	10.1
Soil cover (%)	85	65
Conyza spp. density (plants m^{-2})	22	12
Conyza spp. average height (cm)	2	2
Conyza spp. infestation (%)	85	70

development (Vangessel et al., 2009; Moreira et al., 2010; Oliveira Neto et al., 2010).

Herbicide applications were made with a backpack CO_2 sprayer calibrated to deliver 200 L ha^{-1} using 207 kPa as CO_2 pressure and five XR-110.02 nozzles. Climatic conditions, weed densities and stages in each location are shown in Table 1. Visual weed control ratings were collected at 15 and 30 days after applications (DAA). The ratings were based on a 0 to 100% scale, where 0 means no control and 100 correspond to weeds completely dead or absence of weeds.

Density of emerged *Conyza* spp. plants was determined fortnightly starting at 30 DAA up to soybean planting (75 DAA). Fleabane densities were counted using four randomized sampling frames of 0.25 m^2 of inner area, each were counted per experimental unit. In these evaluations, fleabane height was also measured from root collar to its apical growing region. Data from each experiment were submitted to the F-test variance analysis and means compared by Scott-Knott test ($p<0.05$).

RESULTS AND DISCUSSION

In Campina da Lagoa (PR) treatments with glyphosate + 2, 4-D and glufosinate provided >90% control for fleabane, while MSMA presented the lowest efficacy (81%) among burndown options. However, for this particular option, tank mixing these herbicides with residual herbicides increased control to >87% (data not shown). Improved levels of weed control might be related to the POST effect promoted by residual herbicides, once

metsulfuron, chlorimuron and diclosulam are considered efficient for both PRE and POST fleabane control (Vargas et al., 2007). Tank mixing of residual herbicides with glufosinate improved control of *C. canadensis* when compared to glufosinate alone (Steckel et al., 2006).

An efficient burndown is essential for fleabane fall burndown success, since eventual escaped plants begin to grow again freely during off-season time, when there is no soil cover until next summer crop sowing. When a summer crop is planted in areas where fleabane is already established, its competitive capacity is affected, once a very low fleabane density is enough to cause significant crop yield losses (Trezzi et al., 2015).

In Floresta (PR), except for MSMA alone, all the other herbicide treatments provided ≥82% of weed control after burndown. In other circumstances, this level of weed control could be satisfactory; however in fleabane areas this is not enough once non-controlled plants continue to grow during off-season and become even more serious problem afterwards.

Glyphosate + 2, 4-D-based treatments were the most efficient in weed control after burndown (data not shown). Although 2,4-D has proven to be an efficient post-emergent alternative in fleabane glyphosate-resistant biotypes (Oliveira Neto et al., 2010), this should not be seen as the only herbicide option, not only for its narrow weed spectrum control but also for the potential selection of resistant biotypes. Differential tolerance levels for 2, 4-D have been detected among *C. canadensis* populations in studies conducted by Kruger et al. (2008).

Residual herbicide treatments have proved to be more effective in decreasing densities of new emerged fleabane plants, especially from 45 to 75 DAA, in Campina da Lagoa (PR) (Table 2). Tank mixing with diclosulam provided the lowest *Conyza* spp. densities until summer crop pre-sowing assessment (75 DAA) (Table 2). However, due to the long residual period, diclosulam should be used in a rational way in order to avoid damage to sensitive crops. According to Dan et al. (2011), residual activity of this herbicide may cause negative effects in crops considered to be sensitive such as millet, maize, sunflower, sorghum and brassicas.

Herbicide treatments with glyphosate + 2,4-D + chlorimuron, glyphosate + 2,4-D + flumioxazin, glyphosate + 2,4-D + isoxaflutole, MSMA + flumioxazin, glufosinate + chlorimuron, glufosinate + flumioxazin and glufosinate + isoxaflutole provided low fleabane densities until summer crop pre-sowing evaluation (Table 2). Norsworthy et al. (2009) found that glufosinate + dicamba + flumioxazin mixture were efficient in reducing *C. canadensis* density for up to six weeks after herbicide application.

Tank mixing metribuzin and amicarbazone applied with glyphosate + 2, 4-D, MSMA and glufosinate were ranked as an intermediate category by Scott-Knott test at 75 DAA (Table 2). However, these treatments provided good performance till 60 DAA thus, that can be considered as efficient management alternatives in situations where off-season period does not exceed 60 days between the first application and summer crop sowing. Eubank et al. (2008) observed that glyphosate, glufosinate and paraquat tank mixed with metribuzin (420 g ha^{-1}) reduced *C. canadensis* density for 7 weeks.

In Floresta (PR), regarding weed emergence during off-season, all herbicide treatments resulted in low fleabane densities, regardless of whether treatments contained residual herbicides or not. This reflects the fact that at this site, there were virtually no new flushes of fleabane emergence after the application of fall burndown (Table 3). The evaluation performed at summer crop pre-sowing (75 DAA), treatments with glufosinate, MSMA + imazethapyr, MSMA + amicarbazone and glufosinate + imazethapyr produced the highest fleabane densities; all the other herbicide mixtures were efficient on controlling fleabane and presented densities ≤5.3 plants m^{-2}.

Long diclosulam residual activity in soil provided excellent performance on new emergence flushes of fleabane, since no emerged plants were found in treatments containing this herbicide. This result is similar to that found in Campina da Lagoa and demonstrates the efficacy of diclosulam on fleabane in fall burndown application.

Plant density is an important variable component to both efficiency and residual activity of herbicides. However, when analyzed isolated, it is not appropriate for the decision-making process regarding fleabane plants management. The best strategy to evaluate fleabane control should consider not only the number of emerged plants but also plant height right when next crop is about to be planted, since the effectiveness of another pre-sowing burndown application will depend on that. Fleabane height is probably the best single predictor to settle the best strategy for fleabane control in no-tillage areas, since it can be used to decide when applications should be done and how many will be needed.

Eubank et al. (2008) concluded that mixtures of non-selective herbicides with auxin-derived herbicides provided inconsistent *C. canadensis* control for plants with >15 cm. Also, 16 cm was the upper size limit to ensure an effective control of fleabane plants in POST applications (Blainski, 2011). Based on that assumption, all herbicide treatments were considered as effective tools to manage fleabane at 30 and 45 DAA, since plants were not taller than 16 cm in Campina da Lagoa (PR) (Table 4).

Therefore, for those situations when the period of time between fall burndown and summer crop sowing is close to 45 days, treatments with no-residual herbicides might be recommended, once the new flushes of fleabane will still be within a growing stage that provides adequate weed control by a second, pre-sowing application.

At 60 DAA, three treatments had plants >16 cm in Campina da Lagoa (PR): glufosinate, MSMA and MSMA + imazethapyr (23, 23 and 20 cm, respectively) (Table 4).

Table 2. Density of glyphosate-resistant fleabane at 30, 45, 60 and 75 days after application (DAA) of herbicides in fall burndown. Campina da Lagoa (PR), 2009. [1/] DAA – days after application of fall burndown.

Treatments	Fleabane density (emerged plants m^{-2})							
	30 DAA[1/]		45 DAA		60 DAA		75 DAA	
Glyphosate+2,4-D	0.0	f	24.5	d	13.3	b	16.8	b
Glufosinate	0.3	f	17.5	e	15.0	b	13.0	b
MSMA	0.5	f	38.8	b	15.8	b	15.5	b
Glyphosate+2,4-D+mesulfuron	0.5	f	16.8	e	9.0	c	5.5	d
Glyphosate+2,4-D+chlorimuron	0.5	f	0.3	g	1.0	e	2.5	e
Glyphosate+2,4-D+diclosulam	0.5	f	0.5	g	0.3	e	0.3	f
Glyphosate+2,4-D+imazethapyr	0.5	f	17.0	e	11.8	c	10.8	c
Glyphosate+2,4-D+imazaquin	0.5	f	14.0	f	12.3	c	10.5	c
Glyphosate+2,4-D+flumioxazin	1.0	f	1.3	g	2.3	d	2.5	e
Glyphosate+2,4-D+metribuzin	1.3	f	3.5	g	4.3	d	8.0	d
Glyphosate+2,4-D+amicarbazone	1.3	f	3.0	g	4.3	d	7.0	d
Glyphosate+2,4-D+isoxaflutole	1.5	f	3.5	g	5.3	c	4.8	e
MSMA+mesulfuron	1.8	f	23.5	d	11.0	d	8.3	d
MSMA+chlorimuron	2.0	f	7.0	g	5.5	e	6.0	d
MSMA+diclosulam	2.3	f	0.5	g	0.3		1.0	f
MSMA+imazethapyr	2.3	f	29.5	c	15.3	b	10.0	c
MSMA+flumioxazin	2.5	f	0.8	g	5.3	d	4.8	e
MSMA+metribuzin	3.5	e	3.3	g	5.0	d	7.3	d
MSMA+amicarbazone	3.8	e	6.5	g	5.0	d	6.8	d
MSMA+isoxaflutole	4.0	e	4.0	g	8.8	c	6.3	d
Amicarbazone	2.3	f	7.5	g	7.5	d	7.8	d
Glufosinate+mesulfuron	4.3	e	9.5	f	13.3	b	9.3	c
Glufosinate+chlorimuron	5.0	e	4.8	g	2.0	e	3.3	e
Glufosinate+diclosulam	5.8	e	0.3	g	0.3	e	0.0	f
Glufosinate+imazethapyr	8.5	d	10.5	f	8.8	c	7.0	d
Glufosinate+imazaquin	9.3	d	13.0	f	11.8	c	9.3	c
Glufosinate+flumioxazin	9.3	d	1.8	g	5.0	d	3.8	e
Glufosinate+metribuzin	10.8	d	3.5	g	5.8	d	5.5	d
Glufosinate+amicarbazone	12.5	d	3.5	g	3.3	d	6.0	d
Glufosinate+isoxaflutole	17.3	b	4.8	g	4.0	d	4.5	e
Untreated	35.5	a	56.3	a	42.5	a	28.8	a
CV (%)	40.1		34.4		34.6		32.8	
F	52.7		48.5		31.1		20.7	

Means followed by the same latter do not differ according to Scott-Knott test (p<0.05).

It is important to mention that glyphosate + 2, 4-D mixture performed well up to 60 DAA. Although there has been a high level of fleabane that emerged after fall burndown, plants were kept within a suitable size (<16 cm) to be managed at pre-sowing burndown. Thus, taking into account the evaluations performed up to 60 DAA, there was a large number of herbicide mixtures which might be considered effective for fall management (Table 4).

At 75 DAA (pre-sowing), there was an increment of plant heights when compared to the previous evaluation, but treatments based on diclosulam or chlorimuron maintained good performance. Glyphosate + 2, 4-D + metribuzin, glufosinate + flumioxazin, glufosinate + metribuzin and glufosinate + isoxaflutole mixtures also provided efficient fleabane growth suppression for such a long off-season period (Table 4). Although herbicide combinations which presented plants ranging from 17 to 20 cm at 75 DAA were considered as inadequate tools for fall management, they should not be disregarded within fleabane integrated management. Fleabane with 20 cm height were effectively controlled with summer burndown options tank mixing with glyphosate + 2, 4-D (Oliveira Neto et al., 2010).

Under Campina da Lagoa conditions, longer residual herbicides such as diclosulam, chlorimuron and flumioxazin performed best in terms of reduction of new

Table 3. Density of glyphosate-resistant fleabane at 30, 45, 60 and 75 DAA. Floresta (PR), 2009. [1/] DAA – days after application of fall burndown.

Treatments	Fleabane density (emerged plants m^{-2})							
	30 DAA[1/]		45 DAA		60 DAA		75 DAA	
Glyphosate+2,4-D	2.3	e	1.0	f	2.25	e	2.0	d
Glufosinate	6.8	c	8.8	c	11.25	c	6.3	b
MSMA	8.8	b	17.2	b	17.25	b	1.3	d
Glyphosate+2,4-D+mesulfuron	1.3	f	3.5	d	2.00	e	0.8	d
Glyphosate+2,4-D+chlorimuron	0.0	f	0.0	f	1.25	f	0.8	d
Glyphosate+2,4-D+diclosulam	0.0	f	0.0	f	0.00	f	0.0	d
Glyphosate+2,4-D+imazethapyr	0.8	f	0.8	f	2.50	e	2.3	c
Glyphosate+2,4-D+imazaquin	0.5	f	0.5	f	1.50	f	1.8	d
Glyphosate+2,4-D+flumioxazin	0.3	f	1.3	e	2.75	e	3.5	c
Glyphosate+2,4-D+metribuzin	0.3	f	0.0	f	1.25	f	1.3	d
Glyphosate+2,4-D+amicarbazone	0.3	f	0.3	f	1.25	f	1.0	d
Glyphosate+2,4-D+isoxaflutole	0.0	f	0.3	f	1.25	f	2.5	c
MSMA+mesulfuron	1.3	f	2.3	e	2.75	e	1.0	d
MSMA+chlorimuron	2.5	e	1.0	f	1.00	f	0.3	d
MSMA+diclosulam	1.3	f	0.8	f	0.50	f	0.0	d
MSMA+imazethapyr	1.8	e	4.3	d	6.25	d	10.2	a
MSMA+flumioxazin	4.5	d	5.5	e	7.50	d	5.3	c
MSMA+metribuzin	1.3	f	0.5	f	2.50	e	1.5	d
MSMA+amicarbazone	2.0	e	2.0	e	3.00	e	11.0	a
MSMA+isoxaflutole	0.5	f	0.8	f	2.25	e	2.8	c
Amicarbazone	1.0	f	0.8	f	3.25	e	3.3	c
Glufosinate+mesulfuron	1.0	f	1.5	e	2.50	e	2.3	c
Glufosinate+chlorimuron	0.3	f	0.0	f	1.00	f	1.0	d
Glufosinate+diclosulam	0.0	f	0.3	f	0.00	f	0.0	d
Glufosinate+imazethapyr	3.0	e	4.0	d	7.00	d	6.0	b
Glufosinate+imazaquin	2.8	e	1.5	e	3.00	e	2.3	c
Glufosinate+flumioxazin	0.5	f	1.3	e	4.00	e	4.5	c
Glufosinate+metribuzin	0.5	f	0.8	f	1.00	f	0.8	d
Glufosinate+amicarbazone	2.3	e	1.8	e	4.00	e	3.5	c
Glufosinate+isoxaflutole	0.5	f	2.0	e	2.25	e	3.8	c
Untreated	28.3	a	35.0	a	27.75	a	12.3	a
C.V (%)	54.7		33.2		38.8		48.9	
F	59.6		163.9		51.1		18.3	

Means followed by the same latter do not differ according to Scott-Knott test (p<0.05).

fleabane flushes and, or growth suppression of emerged plants. This is presumably due to the intense fleabane emergence after fall burndown application, provided by climatic conditions (temperature around 20°C and adequate soil moisture conditions).

ALS-inhibitor herbicides diclosulam and chlorimuron were efficient in controlling fleabane. However, herbicides with this mechanism of action should be rationally used, because their continuous use can lead to the selection of resistant biotypes. Studies carried out by Trainer et al. (2005) reported the existence of *C. canadensis* biotypes which is resistant to chlorimuron and cloransulam. Chlorimuron-resistant fleabane biotypes in Brazil were first found by Santos et al. (2014). In order to ensure long-term use of ALS-inhibitors, it is important to plan a rational mechanism of action rotation and/or herbicide mixtures with different mechanisms of action.

Long-term residual activity herbicides such as diclosulam and chlorimuron can potentially cause carryover effects on sensitive crops like maize, bean, sorghum, sunflower and cotton, so crop succession should be carefully planned before deciding the best herbicide options for off-season fall management.

Fleabane plants were kept under a stage of development suitable for chemical control up to 45 DAA, for all herbicide treatments in Floresta (PR) (Table 5). At

Table 4. Height of glyphosate-resistant fleabane at 30, 45, 60 and 75 DAA. Campina da Lagoa (PR), 2009. [1/] DAA – days after application of fall burndown.

Treatments	Fleabane Height (cm)			
	30 DAA[1/]	45 DAA	60 DAA	75 DAA
Glyphosate+2,4-D	3	6	13	26
Glufosinate	2	4	23	40
MSMA	5	10	23	40
Glyphosate+2,4-D+mesulfuron	<1	2	11	21
Glyphosate+2,4-D+chlorimuron	<1	<1	<1	<1
Glyphosate+2,4-D+diclosulam	<1	<1	<1	<1
Glyphosate+2,4-D+imazethapyr	<1	2	15	32
Glyphosate+2,4-D+imazaquin	<1	2	10	20
Glyphosate+2,4-D+flumioxazin	<1	2	7	20
Glyphosate+2,4-D+metribuzin	<1	2	5	14
Glyphosate+2,4-D+amicarbazone	<1	2	7	26
Glyphosate+2,4-D+isoxaflutole	<1	2	3	18
MSMA+mesulfuron	3	8	12	40
MSMA+chlorimuron	2	2	7	16
MSMA+diclosulam	<1	2	4	<1
MSMA+imazethapyr	4	8	20	42
MSMA+flumioxazin	<1	2	9	25
MSMA+metribuzin	2	3	8	20
MSMA+amicarbazone	2	2	7	28
MSMA+isoxaflutole	<1	5	2	25
Amicarbazone	<1	2	5	25
Glufosinate+mesulfuron	<1	5	13	34
Glufosinate+chlorimuron	<1	2	7	4
Glufosinate+diclosulam	<1	2	<1	<1
Glufosinate+imazethapyr	<1	4	12	34
Glufosinate+imazaquin	<1	7	9	26
Glufosinate+flumioxazin	<1	2	14	16
Glufosinate+metribuzin	<1	2	4	16
Glufosinate+amicarbazone	<1	2	6	21
Glufosinate+isoxaflutole	2	3	7	16
Untreated	6	17	27	55

60 DAA, fleabane plants treated with MSMA exceeded the desirable maximum height of 16 cm, but all other treatments resulted in plants with appropriate height. Such results are similar to those observed in Campina da Lagoa, demonstrating that, when the off-season time is no longer than 60 days, there are a larger number of alternatives that can be employed in fall management. At 75 DAA, treatments with MSMA, glyphosate + 2,4-D + imazethapyr, MSMA + imazethapyr, MSMA + amicarbazone, MSMA + isoxaflutole and amicarbazone resulted in plant heights that exceeded the maximum desirable for chemical control, which may compromise the efficacy of any burndown treatment performed at pre-sowing time.

Due to the limited emergence of new fleabane flushes at Floresta site, traditional burndown treatments with no residual activity such as glyphosate + 2,4-D and glufosinate were considered effective to control emerged fleabane, which was enough to keep plants within the adequate size necessary to achieve efficacy with the chemical control at pre-sowing (Table 5).

Conclusion

Glyphosate + 2, 4-D was effective for emerged fleabane burndown in all situations in this study. For a 75 day off-season period, diclosulam and chlorimuron were the best options for burndown plus fleabane residual control when combined with glyphosate + 2, 4-D, MSMA and glufosinate. Besides that, glyphosate + 2, 4-D + metribuzin, glufosinate with flumioxazin, metribuzin and

Table 5. Height of glyphosate-resistant fleabane at 30, 45, 60 and 75 DAA. Floresta (PR), 2009. [1/] DAA – days after application of fall burndown.

Treatments	Fleabane Height (cm)			
	30 DAA[1/]	45 DAA	60 DAA	75 DAA
Glyphosate+2,4-D	<1	3	8	12
Glufosinate	4	7	13	16
MSMA	9	13	20	40
Glyphosate+2,4-D+mesulfuron	<1	3	6	6
Glyphosate+2,4-D+chlorimuron	-	-	2	5
Glyphosate+2,4-D+diclosulam	-	-	-	-
Glyphosate+2,4-D+imazethapyr	<1	2	8	20
Glyphosate+2,4-D+imazaquin	<1	2	3	11
Glyphosate+2,4-D+flumioxazin	<1	2	5	7
Glyphosate+2,4-D+metribuzin	<1	<1	2	10
Glyphosate+2,4-D+amicarbazone	<1	2	15	15
Glyphosate+2,4-D+isoxaflutole	-	2	2	5
MSMA+mesulfuron	<1	4	10	14
MSMA+chlorimuron	<1	2	11	12
MSMA+diclosulam	<1	2	<1	-
MSMA+imazethapyr	<1	6	12	20
MSMA+flumioxazin	6	13	12	10
MSMA+metribuzin	3	3	2	10
MSMA+amicarbazone	<1	7	8	24
MSMA+isoxaflutole	<1	3	9	24
Amicarbazone	<1	7	12	20
Glufosinate+mesulfuron	<1	8	13	13
Glufosinate+chlorimuron	<1	<1	10	10
Glufosinate+diclosulam	-	2	-	-
Glufosinate+imazethapyr	<1	10	9	15
Glufosinate+imazaquin	5	4	7	7
Glufosinate+flumioxazin	3	5	10	10
Glufosinate+metribuzin	<1	10	6	9
Glufosinate+amicarbazone	<1	8	15	15
Glufosinate+isoxaflutole	4	4	11	11
Untreated	9	20	31	40

isoxaflutole mixtures maintained good performance for fleabane fall management for a 75 days off-season period. The study data demonstrates that, fall management practices are effective option for fleabane control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Ethnobotanical survey of medicinal plants used for treating preschool children anemia in an urban setting, Douala-Cameroon

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Anemia is one of the most widespread public health problems which mainly affect preschool children. Its prevalence is 60% in Cameroon and due to poverty, many patients turn towards medicinal plants for treatment. This study was designed to compile plants used for the treatment of anemia in the Littoral Region (Douala) of Cameroon and classify them based on their use. An ethnobotanical survey was carried out in December 2015. A total of 32 herbalists and 40 mothers of children under 5 years were interviewed by means of questionnaires. Results showed that malaria (88%) was the main cause of anemia. Twenty-six plant species belonging to 17 families were identified. The most used plant was *Eremomastasx speciosa*. Three families stand out as the most used: *Acanthaceae* (11.5%), *Asteraceae* (11.5%) and *Euphorbiaceae* (11.5%). Seventeen of them have been therapeutically described. Most of the reported species were shrubs. The most used plant parts were leaves. The herbal remedies are administrated in aqueous form and usually orally (83%) or anally. The survey provides the preliminary information on some medicinal plants having anti-anemic properties. Further investigations should be conducted so that the use of these plants can be an alternative to the population.

Key words: Ethnobotanical survey, infantile anemia, medicinal plants, Douala-Cameroun.

INTRODUCTION

According to the WHO, anemia is defined as a lowering in blood hemoglobin level. A child is referred to as anemic when hemoglobin level is below the normal rate of 11 g/dl. This disease is of multifactorial nature. The

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most serious consequence on health, lies in an increased mortality risk which is 3 to 4 times higher among anemic children (OMS/ UNICEF, 2005). In addition, anemia also reduces physical capacity, working capacity, growth and immune status (Abdullah et al., 2011).

Child mortality is a core indicator for child health. Between 1990 and 2012, half of all deaths of children under 5 years worldwide (6.6 million) were in Africa (UNICEF, 2014). The main causes of deaths include diarrhea, infections, malaria and above 50% under nutrition (UNICEF, 2015). These diseases share anemia in their physiopathology in individuals and especially in children under five. Globally, an estimation of 273 million (43%) of preschool children are affected by anemia and Africa accounts for most of the cases (62.0%) according to the latest WHO estimates (WHO, 2015). In Cameroon, 6 out of 10 children under five suffer from anemia. Almost half suffer from moderate anemia. Children living in rural areas are more frequently affected than those living in urban areas (EDS-MICS, 2011). This deficiency is a significant risk of morbidity in resources-limited countries (English et al., 2002).

Iron deficiency which is the leading cause of anemia is the first nutritional deficiency in the world (El Hioui et al., 2009); it is the most common public health problem. Man has long used medicinal plants to manage its health problems. These traditional practices are still relevant since modern medicine is costly and thus is not affordable to populations which are resources-constrained (Bhushan, 2005). Besides, many studies have already confirmed the efficiency of some wild plants and identified the active compound for some diseases (WHO, 2009). Africa and especially Cameroon own an amazing rich and diverse flora harboring many plants used in traditional medicine. These plants could be used instead of conventional medicines by growing population. This is due to the fact that these drugs are expensive and sometimes inaccessible (Dibong et al., 2011). Besides, absent or inadequate health infrastructures require the mothers of developing countries to turn towards traditional medicine for the treatment of children. Therefore, development and search of novel and effective anti-anemic agents have become very important issues.

The present study sets out to identify potential anti-anemic medicinal plants used for the treatment of anemia on preschool children and to determine the therapeutic pattern habits, in order to better promote this pharmacopoeia.

METHODOLOGY

Site of the study and justification

Douala is located in the southern part of western of Cameroon (04°03'N, 009°41'E). It features a tropical monsoon climate (Köppen climate classification *Am*), with relatively consistent temperatures throughout the course of the year, though the city experiences somewhat cooler temperatures in July and August. It rains very much in Douala during the year. The town experiences an average precipitation of about 3.600 mm per year (World Meteorological Organization, 2016). The average temperature is 26.7°C. Some ecosystems such as humid dense forest, mangrove and swamps coexist there. This town is a cosmopolitan site where people from several areas and cultures stay. Other reasons are environmental degradation, development of malaria vectors and food insecurity that foster the development of anemia. The high density of the population and poverty experienced by people in this area promote the practicing of herbal medicine as a valid source of income.

Informed consent

The purpose of the study was explained to the local traditional herb sellers and mothers of children under 5 years who use plants and natural products to treat anemia. Informed consent was obtained from each of the participants.

Ethnobotanical survey

An ethnobotanical survey of medicinal plants used in the treatment of anemia was conducted in December 2015 in the city of Douala. The targeted population was the actors of traditional medicine, sellers of medicinal plants and mothers of preschool children's. The raids were made first in the most populous markets namely "Central market", "Nkoulouloun market", "Dakar's market" for traditional healers (Figure 1) and other units in residential areas for mothers of children. Subsequent interaction with the traditional healers and mothers included interviews and field collection of some samples. They were asked to provide information on preschool children anemia (causes and symptoms), herbs they use to treat infantile anemia, plant parts used, modes of preparation and administration, other plants or ingredients used in association, storage and reasons for use. A sample of each plant was collected, corresponding to the amount needed for the preparation of one litre of potion. This sample was then weighed.

Identification of plants

Voucher specimens of plants were collected and their identification was made by botanists of the Faculty of Sciences of the University of Douala, confirmed by the National Herbarium of Cameroon, Yaoundé.

Data analysis

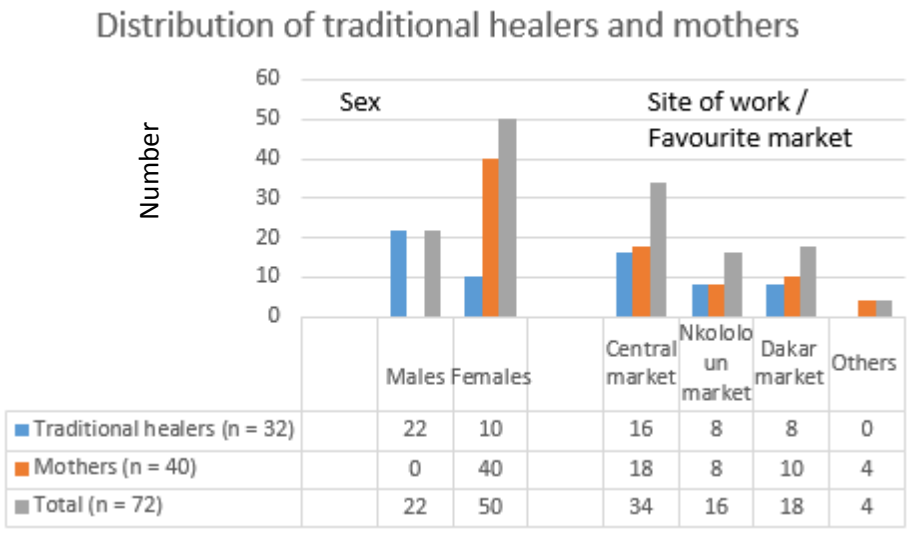
Data generated from the field survey on the ethnobotanical survey of anemia was subjected to descriptive statistics using percentages and quantity. Microsoft office Excel was used for each parameter.

RESULTS

Distribution of informants

A total of 72 participants including 32 traditional healers and 40 mothers were included in the study (Figure 2). The Central Market of Douala is the most favourite because it is the most federating market in Douala. Despite the existence of many peripheral markets, the

Figure 1. Markets location in Douala-Cameroon (Nkoulouloun market: 4.036366, 9.708485; Central market: 4.036666, 9.705953 Dakar market: 4.028063, 9.735645).



	Males	Females	Central market	Nkololoun market	Dakar market	Others
Traditional healers (n = 32)	22	10	16	8	8	0
Mothers (n = 40)	0	40	18	8	10	4
Total (n = 72)	22	50	34	16	18	4

Figure 2. Distribution of traditional healers and mothers.

Central Market draws crowds from all districts of Douala. All the traditional healers sell plants or potions. Mothers of children were interviewed at home (Akwa, Ndogbong, Mboppi, New bell, PK14, PK12, Cité-sic and Nkomondo). Twenty-five percent of mothers were married. There were house wives (55%), students (15%), tailors (10%), gendarmes (5%) and office secretaries (5%). Fifty-two percent were between 30 and40 years old.

Knowledge on anemia

Healers and mothers concerned with this survey identified infantile anemia based on signs and symptoms that included tiredness, loss of appetite, abdominal swelling, fever, thinness, pale eyes, pale palms and pale soles. The causes known were: malaria (88%), splenomegaly (28%), jaundice (16%), sickle cell disease (16%), typhoid fever (11%), measles (8%), malnutrition (5%), intestinal worms (5%) and yellow fever (3%). The mothers get plants principally in markets, often in fields, forests and home gardens. The herbalists get their supplies from fields and forests.

Botanical characteristics of plants

Twenty-six plant species of 17 families were identified and listed in Table 1 in the order of most used. Three families stand out as the most used, Acanthaceae (11.5%), Asteraceae (11.5%) and Euphorbiaceae (11.5%). Of all the plants, 10/26 were trees, 10/26 herbs, 05/26 shrubs and 01/26 creepers.

Collection of plants

Majority (80%) of the plants were collected in markets, either from traditional healers or food sellers (*Solanum lycopersicum* and *Beta vulgaris).* Some were collected in fields and home gardens with the aid of mothers. Those that had only common name were identified with the help of botanist of University of Douala.

Reasons for use

The reasons for using these plants for the treatment of anemia are diverse. The main justifications are very effective (64%), cheered children up (55%) and lack of money (55%). The availability of plants is mention in spite of scarcity of some of them during the dry season.

Ethnopharmacology of plants

Treatment descriptions of seventeen plants are presented in Table 2. The plant part most cited is the leaves but barks, fruits, flowers and roots are also used. Water is the main solvent. The most used pharmaceutical form of recipe was decoction (76%). The preservation is either at room temperature or in a refrigerator. The conservation is recommended not to exceed 5 days. The decoction is usually administered orally (83%) or anally. The average time of treatment is 3 to 7 days. Herbal practitioners and mothers who were interviewed identified improvement of children when they become physically well and strong, regain appetite and play.

DISCUSSION

This survey shows that infantile anemia is present in the population of Douala. It also indicates the use of medicinal plants for the treatment of anemia on preschool children. Many plant species are used. Majority of these plants are available in their immediate environment as also observed by Dibong et al. (2011). The preferential use of one or another plant is a function of the availability. Some are not found in the markets and sometimes are seasonal. In this case, people will harvest in forest and fields. This could justify the frequency of use of some plants as compared to others.

Fresh leaves are the most used part; this may be justified by its availability and accessibility to local people, and by its high content of anti-anemic agents. This fact may explain the short time of preservation of leaves and preparations. Herbal preparations in this study are administrated orally. The treatment by anal route is less frequent (1/week) than oral route (overall 7 days). Only fresh bark decoctions are administrated by anal route.

To elaborate some medicinal recipes, different plants or substances are blended. The mixing depends on the type of preparation for the same plant and the interviewee's origin. This problem, associated with dosage prescriptions in the use of herbal remedies in traditional medicine have been highlighted by a number of authors (Agbor and Naidoo, 2015).

Certain plants have several therapeutic activities as mentioned by respondents in addition to their anti-anemic activity (94%). It has been shown that some of these plants have anti-microbial properties: *Eremomastax speciosa* (*Salmonella typhi* and *Escherichia coli*) (Okokon et al., 2007), *Alchornea cordiflia* (*Helicobacter pylori*, *Salmonella typhi, Salmonella enteritidis, Shigella flexneri* and *Escherichia coli*) (Adeleye et al., 2008), *Tectona grandis* (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella pneumoniae*) (Nayeem and Karvekar, 2011). *Bidens pilosa* has anti-malarial properties (Bilanda et al., 2004). *Eremomastax speciosa* also has an anti-diarrheal effect (Oben et al., 2006) and anti-anemic effect (Okokon et al., 2007). These activities could justify the improvement in the condition of anemia in children because it has been shown that the origin of anemia can result from malnutrition and microbial infections (parasitic, bacterial and virological) (OMS/UNICEF, 2005; Ami et al., 2012; Kobto, 2012). Many of these plants have not yet been the subject of scientific studies. Efforts should be made to improve this situation in order to better promote our traditional medicine.

Conclusion

The ethnobotanical survey has shown 26 species of medicinal plants used for the treatment of anemia in preschool children in the city of Douala. The exploitation

Table 1. List of medicinal plants used in the treatment of anemia in children aged 2 to 5 years.

Species common name vernacular name	Family	Nature of plant	Parts used
1. *Eremomastasx speciosa* (Hochst.) Cufod Two sides, one side red	Acanthaceae	Shrub	Fresh leaves
2. *Alchornea cordifolia* (Schum. &Thonn.)Müll. Arg. Dry leaves Bobondji (Bassa)	Euphorbiaceae	Tree	Dry leaves
3. *Manihot esculenta* Crantz Kpwem (Ewondo)	Euphorbiaceae	Shrub	Fresh leaves
4. *Solanum lycopersicum* Linn. Tomatoe	Solanaceae	Herbs	Fresh fruit or box
5. *Tectona grandis* Linn. Ken-teck (Bassa)	Lamiaceae	Tree	Fresh bark
6. *Beta vulgaris* Linn. Beet	Amaranthaceae	Herbs	Root/whole plant/ Fresh leaves
7. *Dicliptera laxata* C.B. Clarke Green blood	Acanthaceae	Shrub	Fresh leaves
8. *Mimosa pudica* Linn. Mouko iyo (Duala)	Mimosaceae	Herbs	Whole plant
9. *Sacoglottis gabonensis* (Bail.) Urb Bidou (Beti)	Humiriaceae	Tree	Fresh bark
10. *Hibiscus sabdarifa* Linn. Foléré (Foufouldé)	Malvaceae	Herbs	Dry flowers
11. *Gnetum africanum* Welw. Eru, Okok	Gnetaceae	Creepers	Fresh leaves
12. *Ricinodendron heudelotii* (Bail.) Pierre ex Pax Djanssang (Bassa)	Euphorbiaceae	Tree	Fresh bark
13. *Eucalyptus sailgna* Smith	Myrtaceae	Tree	Fresh leaves
14. *Justicia ladanoides* Lam.	Acanthaceae	Shrub	Fresh leaves
15. *Anthocleista vogelli* Planch. Bopolopolo (Duala)	Loganiaceae	Tree	Fresh bark
16. *Entandrophragma candollei* Harms Koh-jock (Bassa)	Meliaceae	Tree	Fresh bark
17. *Bidens pilosa* Linn. Ward of poor	Asteraceae	Herb	Fresh leaves
18. *Cassia alata* Linn. Lonkana (Bassa)	Caesalpiniaceae	Shrub	Fresh leaves
19. *Moringa oleifera* Lam.	Moringaceae	Tree	Fresh/dry leaves or seed
20. *Amaranthus cruentus* Linn.	Amaranthaceae	Herb	Fresh leaves
21. *Ageratum conyzoides* Linn. Roi des herbes	Asteracaea	Herb	Fresh leaves
22. *Vernonia stellulifera* (Benth.) C. Jeffrey Ndolè sucré	Asteracaea	Herb	Fresh leaves
23. *Platostoma africanum* P. Beauv. Ewuda bie (Duala)	Lamiaceae	Herb	Fresh leaves and sterm
24. *Alstonia boonei* De Wild. Kokmot (quinine)	Apocynaceae	Tree	Fresh bark
25. *Dacryodes edulis* (G.Don.) H. J. Lam. Prunier Sa'a (Beti)	Burseraceae	Tree	Fresh bark/leaves
26. *Ipomea* sp. Linn.	Convolvulaceae	Herb	Fresh leaves

Table 2. Traditional medicine plants with anti-anemic potentiality in Douala Cameroon.

Scientific name	Association	Quantity/L solvent	Method of Preparation	Preparation Time (min)	Dosage	Others therapeutic activities
Alchornea cordifolia (Schum. &Thonn.)Müll. Arg.	Green blood, red red	110 g	Decoction	15-30	½ Glass, 2 to 3 times daily for 5-7days	Dental analgesic
	Red red, tutle bark	120 g	Infusion	10-20	½ Glass, 3 times daily for 3 Days	
Anthocleista vogelli Planch.	-	500 g	Decoction	30	01 full teaspoon, 3 times daily for 5 Days	-
Beta vulgaris Linn.	Carrots	700 g	Decoction	20-30	½ Glass, 2 times daily for 1-2 Weeks	-
	Grape fruit		Maceration	1-2h	½ Glass once a day for 2 to 3 Days	
	Lemons		Grated	-	01 full teaspoon unlimited a twill until improvement.	
Bidens pilosa Linn.	04 lemons	300 g	Infusion	30	¼ Glass, 2 times daily for 7 days	Anti-jaundice Anti-malaria Anti-tiphoid Anti-viral (Yellow fever)
Dicliptera laxata C.B. Clarke	Two sides	300 g	Decoction	20-30	¼ or ½ Glass, 2 times daily for 5 to 7 Days	
	-	300 g	Infusion	15	¼ Glass, 3 times daily for 14 Days	Antiviral (Meales)
Entandrophragma candolei Harms	Tol (Bassa) and bark djanssang	01 kg	Decoction	20	Morning purge (150 ml), 01/Week for 02 Week	Anti-typhoid Anti-inflammatory Antibiotic
Eremomastax speciosa (Hochst.) Cufod	Bobondji Foléré Beet Fresh leaves grapefruit tree	250 g	Decoction	10-15	¼ or ½ Glass*, 3 times daily for 7-14 Days	Antibiotic (diaper rash)
	-	500 g	Infusion	10-15	¼ Glass, 3 times daily for 4-5 Days	
	-	250 g	Cruch	10	01 Full teaspoon, 2 times daily for 7days	
Eucalyptus sailgna Smith	-	300 g	Decoction	20-30	01 Full teaspoon l unlimited, 6 to 12 Days	Anti-typhoid Anti-jaundice
Gnetum africanum Welw.	Foléré	100 g	Infusion	10	½ glass, 3 times daily for 01 Days	Antibiotic
	01 small can of condensed milk	250 g	Crush			
Hibiscus sabdariffa Linn.	01 pineapple	150 g	Décoction	30	02 Full teaspoon, 2 times daily for 7 Days	-
Justicia ladanoides Lam.	-	300 g	Decoction	30	¼ Glass, 2 times daily for 7 Days	-
Manihot esculenta Crantz	Beet	300-500 g	Crush	20	½ Glass, 2 times daily for 3 to 7 Days	Anti-stomach ache

*Glass volume: 200 mL, -: no information.

Table 2. Contd.

Mimosa pudica Linn.	-	250 g	Decoction	10-15	01 Full teaspoon or 01 full tablespoon, 2 to 3 times daily for 7 Days	
			Infusion	10	01 Full teaspoon, 2 times daily for 2 Days	Anti-typhoid
Ricinodendron heudelotii (Bail.) Pierre ex Pax	-	01 kg	Infusion	10-15	¼ Glass, 3 times daily for 7 Days	Anti-splenomegaly
Sacoglottis gabunensis Bail.) Urb	Dry leaves of Bidou	01 kg	Decoction	15	Morning purge (150ml), 01/Week for 03 Week	Anti-malaria Anti-splenomegaly
Solanum lycopersium Linn.	Lemons /oranges + 01 small can of milk + 01 egg	400 g or 01 small box	Mix	-	¼ glass, 2 times daily for 7 Days	-
Tectona grandis Linn.	Red Koh-jock, Mouressi, Bobondji (Bassa)	01 kg	Decoction	20-30	Morning purge (150ml), 01/Week for 03 Week	Anti-malaria Anti-kidney pain
	Bark djanssang	500 g	Infusion	15-30	¼ or ½ Glass, 2 times daily 7 Days	Poison antidote

of medicinal plants by the population could be encouraged and valued. Valorization of these plants could help reduce the prevalence of severe anemia in urban and rural areas. It could also contribute to management of recurrent diseases of pre-school children among indigenous populations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Sucker multiplication in plantain using chicken manure as a substrate supplement

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This study was carried out to evaluate the response of chicken manure (CM) as a substrate supplement for sucker multiplication and its effect on varietal differences in two subgroups of plantain (Apantu, a False Horn and Apem, a French plantain), using well-developed sword suckers. The research was laid out in a 2 x 2 factorial randomized complete block design with two substrates (chicken manure/sawdust mix and sole sawdust substrate) and the two groups of plantain with three replications. Data were collected on temperature changes within the substrates, number of plantlets sprouted, physical growth of the plantlets, root formation of plantlets and their correlation with plantlet establishment in the nursery. It was observed that plantlet/substrate-types had no significant effect on number of plantlets sprouted. The corms of Apantu showed a higher positive response to all the treatments applied than those of Apem. Plantlets from CM/SD mix recorded better physical parameters but had lower survival rate as compared to the SD treatments. There was low positive correlation (26.79%) between rooted sprouts and survival rates of regenerated plantlets, which might be due to differences in environmental conditions. Generally, Apantu produced more vigorous sprouts than Apem, which may be due to differences in maturity period and varietal effect. Planting-material-type did not have any effect on plantlet acclimatization, while media-type showed greater impact on number of plantlets and acclimatization. CM/SD mix influenced higher sprouts but plantlets were poorly acclimatized to the environment.

Key words: Chicken manure, sawdust, 'Apantu' (False horn), 'Apem' (French plantain), cultivar, media, plantlets.

INTRODUCTION

Research and development in the plantain sub-sector is needed to improve its cultivation and increase productivity. Diverse approaches have been used to overcome the inadequate supply of good quality planting

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materials, where the only source for subsequent cultivation is the farmers' fields (Quain and Dzomeku, 2013; Lagoda, 2013). Tissue manipulation technology has become one of the effective methods of multiplying plantain planting materials aside from tissue culture, where sawdust is used as the main substrate for this technology. This is mainly due to the fact that sawdust is easy to apply, long lasting with high moisture conservation efficiency, increases water absorption and penetration, reduces evaporation and maintains more uniform substrate temperature (Overholser, 1955; Boss, 2009). However, like with other highly carbonaceous organic matter, the low digestibility, low protein content and high lignin content of sawdust under certain conditions can cause a deficiency of available nutrients that retards plantlet growth (Overholser, 1955; Tripathy et al., 2011). According to the literature, sawdust has a moisture content of about 6.03 to 23.7%, particle sizes ranging from 0.07 to 3.5 mm, % nitrogen of 0.2 to 0.7, % phosphorus of 0.03 to 0.11, % potassium of 0.45 to 0.55, % organic carbon of 47 to 56, pH of 7.03 to 7.21 and C/N ranging from 150 to 351 depending on the tree species (Mitchual, 2014; Osibe and Chiejina, 2015; Sarpong, 2014). These low chemical properties of sawdust are inadequate for good performance as a substrate.

Chicken manure can be used to supplement sawdust to improve the yield of plantain plantlet regeneration under sucker multiplication technology. Chicken manure contains all the essential plant nutrients that are required by plants (Amanullah et al., 2010). With % N of 2.13 – 2.43, % P of 0.52 - 1.23, % K of 0.73 - 1.62, %, organic carbon of 35.30 – 55.85, pH of 6.80 – 8.0, about 70 - 92 % moisture and C/N of 11.30 – 13.80 (Aziz, 2010; Boateng et al., 2006; Quansah, 2010), chicken manure could be a valuable source of crop nutrients and organic matter which can improve biophysical conditions including moisture, nutrient retention, root development, temperature fluctuation minimization and biological activities of substrates (Aba et al., 2011). It is considered to be highest in demand for crop production among all animal manures because of its high composition of phosphorous, nitrogen and potassium (Alexander and Knutson, 2000). Chicken manure increases the moisture holding capacity of substrates and improves lateral water movement, thus improving irrigation efficiency and decreasing the general droughtiness of substrates (Amunulla et al., 2010). It also increases the number, diversity and activities of soil microorganisms which consequently enhances soil physical properties, particularly in sandy conditions (Aziz, 2010; Quansah, 2010; Boateng et al., 2006).

In a study by Aba et al. (2011), the use of chicken manure with increased manure rates reduced days to plantain harvesting by over 30 days with significant yield improvement. In addition to this, there was an increase in plant stature, suckering, leaf chlorophyll content, non-spotted leaves index, crop cycling and total biomass resulting from increased chicken manure rate. Similarly, Odoemena (2006) also reported significant increase in tomato yield due to chicken manure application. Therefore in this study, chicken manure was applied to supplement sawdust as a substrate for plantlet regeneration to evaluate the response of sword suckers of Apantu and Apem cultivars to suckering.

METHODOLOGY

The experiment was laid-out in a 2 x 2 factorial randomized complete block design (RCBD) with three replications. The factors and their levels studied were: Type of substrates [(i) sawdust (SD) and (ii) chicken manure plus sawdust (CM/SD Mix) in a 2 : 1 ratio by weight] and cultivars [(i) Apem, and (ii) Apantu].

Well-developed sword suckers with an average weight of 1.3g ± 0.3 of the two cultivars were used as planting materials. Structure of sprouting chamber and planting materials through to data processing and analysis were done as described by Mensah et al. (2017). Two sprouting chambers were constructed and provided with 50% shade. Chamber one was filled with CM/SD mixed substrates to 30 cm thickness, while chamber two was treated the same way with SD substrate. Each chamber was divided into three subplots for the replications and then built air-tight with translucent poly-ethylene sheets. Healthy sword suckers of the two cultivars of plantain were prepared and treated with nematicide (Fura 3g) at a rate of 10 g/m^2. Sixteen corms of the sword suckers were used for each replication. Sprouted plantlets were harvested three weeks after planting. These were carefully detached from the main underground stem using kitchen knife.

Data collected included temperature changes within the sprouting chamber, rate of sprouting of plantlets, number of plantlets sprouted, height, width and number of leaves of harvested plantlets. Data were subjected to standard analysis of variance using GenStat Release 10.3DE (PC/Windows 7). The means were separated using least significant difference (LSD) at $p < 0.05$.

RESULTS

In general, the CM/SD Mix recorded higher diurnal temperatures than the sole sawdust treatment (Table 1). The mid-day temperatures of the two chambers were higher than the evening and the morning hours. The average daily temperature of the environment observed within the sprouting chambers was 32.8±6.9°C in the SD chamber and this was slightly lower than 33.7±6.9°C in the CM/SD Mix chamber. The average diurnal temperature within the SD substrate was 31.7±3.7°C, which was lower than the CM/SD Mix with a temperature of 37.4±2.3°C (Table 1).

The medium and cultivar effects revealed highly significant differences ($P < 0.05$) on the quality of sprouted plantlets, while all the treatments studied had no significant differences on number of leaves of plantlets harvested (Table 2). The two media used for this work did not record any significant differences on total number of plantlets sprouted from each treatment, average number of plantlets sprouted per corm and number of leaves of harvested plantlets. However, the cultivars had significant effect on all the characteristics studied except number of

Table 1. Average daily temperature (°C) in the sprouting chamber.

Time of the day	Sprouting chamber		Within the substrate	
	SD	CM/ SD Mix	SD	CM/SD Mix
Morning (6 to 8 h)	25.7± 0.5	26.7±0.5	27.4±0.7	34.8±1.1
Mid-day (12 to 14 h)	39.6±1.3	40.5±1.1	34.1±1.1	38.7±0.5
Evening (16 to 18 h)	33.0± 1.5	33.3±1.2	33.6±0.5	38.9±0.7
Average	32.8±6.9	33.7±6.9	31.7±3.7	37.4±2.3

SD– Sawdust; CM/SD Mix– chicken manure and sawdust mixture.

Table 2. Analysis of variance of effect of medium and genotype on plantlets characteristics.

Variance	1st day sprouted	Total no. sprouted	Average no. sprouted	Weight	Height	Girth	No. of leaves	% No. rooted	% survival
Media (M)	**	na	na	**	**	**	na	**	**
Cultivar (C)	**	**	**	**	**	**	na	**	**
M*C	*	na	na	na	na	na	na	na	*

Where * and **, significantly different at P< 0.05, respectively; na: not significantly different.

Table 3. Response to sprouting of corms as affected by medium and genotype.

Treatments	First day sprouted	Total number of sprouts (60 DAP)	Average number of sprouts per corm
Cultivar			
Apantu	39	145	9
Apem	26	83	6
Media			
SD	28	113	7
CM/SD Mix	37	115	8
$Lsd_{0.05}$	5	27	2
%Cv	4	10	11

DAP– Days after planting.

leaves produced.

The average sprouting time for the two plantain cultivars were 39 and 26 days, respectively (Table 3). Corms planted in the SD medium took an average of 28 days to sprout, while those in the CM/SD Mix took 37 days to sprout. Apantu recorded a significantly higher number of 145 sprouts in 60 days after planting with an average of 9 sprouts per corm. No significant differences were observed between the number of plantlets sprouted and the two different media evaluated.

Plantlets from Apantu recorded significantly higher physical parameters than plantlets from Apem. However, 56% of plantlets harvested from the Apem cultivar had developed roots as compared to Apantu which had only 30.8% harvested plantlets that had developed roots (Table 4). The SD medium influenced the highest rooting percentage of 67.7 but recorded significantly the least physical parameters such as plant height, stem diameter and plantlets weight among the two media studied (Table 4).

Plantlets harvested from the SD medium recorded 95.6% survival as against 81.7% of plantlets harvested from CM/SD Mix (Table 5). Apantu plantlets also recorded 92.8% survival which was significantly higher than 84.5% of Apem plantlets.

Low positive correlation of 25.76% existed between number of plantlets rooted within the sprouting media and the number of plantlets survived at nursery after forty days of transplanting (Figure 1).

DISCUSSION

This study showed that the two media, SD and CM/SD

Table 4. Physical parameters of sprouted plantlets.

Treatments	Fresh weight of plantlets (g)	Height of plantlets(cm)	Girth of plantlets (cm)	% Number of plantlets rooted
Cultivar				
Apantu	120.60	67.20	2.50	30.80
Apem	38.10	40.30	1.20	56.00
Media				
SD	68.60	45.60	1.70	67.70
CM/SD Mix	90.10	61.90	2.00	19.90
$Lsd_{0.05}$	15.60	7.60	0.14	7.53
%Cv	3.60	6.50	7.80	10.00

Table 5. Percentage (%) survival of plantlets at nursery and forty days after transplanting.

Treatments	Apantu	Apem	Av. media (M)
SD	93.4	93.8	95.6
CM/SD Mix	88.3	75.2	81.7
Average cultivar (C)	92.8	81.7	

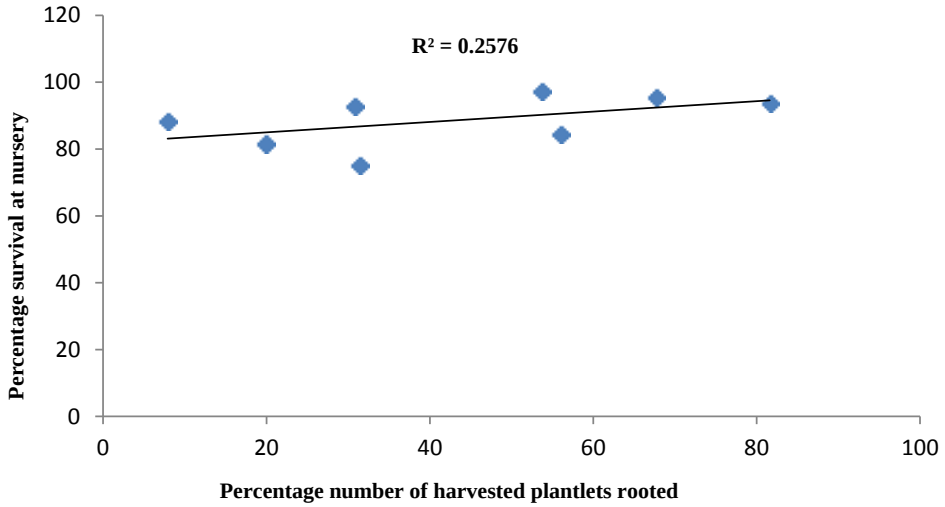

Figure 1. Correlation between number of plantlets rooted in sprouting media and number of plantlets survived at nursery.

Mix substrates, supported the growth of the sprouted plantlets of the two plantain cultivars, Apantu and Apem. The sprouting rate of the suckers of both cultivars grown in the two media was similar, indicating that the crop relies more on the stored food in the corm to initiate sprouting which corroborates with previous studies by Rajan and Markose (2007). After sprouting, the availability of nitrogen in the CM/SD Mix substrate promoted the growth and development of the sprouted plantlets which led to significant increases in height, girth and weight as compared to those grown in SD substrate. These findings were similar to those using oil palm seedlings whereby the usage of chicken manure as substrate aided in the metabolic processes of the release of hormones, which stimulated plant growth and nutrient adsorption (Owolabi et al., 2013).

Although the plantlets grown in CM/SD Mix took a longer period to sprout as compared to those grown in SD substrate, there was higher number of plantlets per corm. A study by Aba et al. (2011) on impact of chicken manure on growth, response and yield attributes of two plantain genotypes indicated that the high rate of

application of chicken manure enhanced plant suckering and this is due to suitable production of warmth to the plants which help to facilitate enzymatic activities resulting in sprouting of the lateral buds. Warmth is one of the three main conditions necessary for germination of most of the tropical plants and it affects the rate of enzymatic actions and facilitation of the activities of growth hormones (Schutz et al., 2001; Serrano- Bernardo et al., 2007; Ali et al., 2011; Njukwe et al., 2007).

Between the two cultivars studied, Apantu significantly produced more sprouts than Apem. Similarly, plantlets from Apem recorded higher number of rooting than those from Apantu. This might be due to the regeneration capacity effects among families, species and even within genotypes from the same species (Yildiz, 2012). Similar genotypic differences in yield have been reported by Khadiga et al. (2009) using *in vitro* micropropagation of potato (*Solanum tuberosum* L) and as such, the varying degrees of *in vitro* bud proliferation of endogenous growth regulators differ among genotypes (Vuylsteke, 1989). Beeds et al. (2008) observed positive correlation between mother plants and the growth of its suckers for most plantain cultivars and also proved that, the fast-growing mother plants had well-developed suckers.

Moreover, the plantlets grown in SD substrate had greater root system than those under CM/SD Mix and similar trend was observed in using Bougainvillea *glabra*, *Ixora coccinea* and *Rosa chinensis* (Fagge and Manga, 2011). This was due to enhanced root penetration and porosity resulting from increased aeration and drainage. Also, using sawdust, sand and combination of the two as rooting media, *Prunus africana* produced a greater percentage of roots when grown in sawdust as compared to sand alone or a mixture with sawdust (Tchoundjeu et al. 2002). Although, sawdust substrate provides good aeration, moisture and physical support to the plantlets which facilitates root growth and development (Luna et al., 2009), lesser root system was observed in sheanut tree (*Vitellaria paradoxa* C. F. Gaertn) (Akakpo et al., 2014). Furthermore, there was low correlation between the percentage of rooted plantlets in the sprouting chamber and the survival of plantlets in the nursery. The ability of plantlets to form roots when grown in the sprouting chamber may not influence their establishment in the nursery. Thus, most of the old roots of the plantlets decay when transferred to the nursery and hence the development of new roots. This indicates that rooting within sprouting media does not have strong effect on root establishment of plantlets in the nursery.

Plantlets grown in CM/SD Mix had a significantly higher death rate than those grown in SD substrate as a result of higher transpiration rate due to profuse growth which might be caused by higher nitrogen content in the substrate, which may have produced the shocking effect when plantlets were transferred to the nursery (Krogdahl and Dahlsgard, 1981; Cooke et al., 2005; Amanullah et al., 2010). High nitrogen content in the CM/SD Mix enhanced faster shoot growth rate at the expense of root growth and development, thereby inhibiting the maintenance of water balance and survival rate of newly transplanted seedlings (Grossnickle, 2005). This may have led to the poor acclimatization of the plantlets to the environment.

Conclusion

In general, the Apantu cultivar produced more vigorous sprouts than Apem, due to the differences in maturity period and varietal effect. The planting-material-types had no impact on the acclimatization of the plantlets when transplanted to the nursery. On the other hand, the media used in this study had effect on the growth and development of the plantlets in the nursery. For instance, the CM/SD Mix greatly enhanced the production of sprouts but plantlets poorly acclimatized to the environment when transplanted to the nursery. However, plants grown in SD medium acclimatized well at the nursery though the number of sprouts was lesser. The addition of chicken manure to sawdust seems very promising due to the increased production of sprouts. However, further studies may be needed, especially on the acclimatization of plantlets in the nursery.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Pod yield stability and adaptation of groundnut (*Arachis hypogaea* L.) genotypes evaluated in multi-environmental trials in Zimbabwe

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Twenty-five groundnut genotypes were evaluated to identify the types of Genotype-Environment-Interaction (GEI) for pod yield. Genotypes were evaluated under multi-environmental yield trial conducted in 2013/14 season at five environments. The objectives of the experiment were to: identify genotypes with high pod yield stability, to identify genotypes with specific/wide adaptation, identify groundnut mega environments and identify an ideal environment. ANOVA was performed using GenStat Version 14. The results of the ANOVA indicate that there was GEI. The environments (E) and the interaction between the genotype and the environment were significant. GGE biplot analysis for yield data was the performed. The partitioning of GGE through GGE bi-plot analysis indicated that principal coordinate 1 and 2 (PC1 and PC2) explained 59.22 and 20.17% of GGE sum of squares, respectively, explaining 79.39% of the total variation. This large percentage variability of GGE (79.39%) accounted by the bi-plot indicates that there was complex GEI. The environment and genotype explained 58.8 and 6.1% respectively of the total treatment variance, while the genotype by environment interaction accounted for 35.1%, indicating that the environment had huge influence on genotype performance. The results revealed the existence of mega-environments, most ideal environment and genotypes with specific and others with wide adaptation. The results indicate that certain genotypes may be released for commercial production in specific environments based on their performance.

Key words: Groundnut, genotypes, pod-yield, multi-environmental trial, genotype x environment interaction, discriminating, representative.

INTRODUCTION

Groundnuts does not only provides high quality edible oil (48 to 50%), easily digestible protein (26 to 28%), nearly half of the 13 essential vitamins (e.g. Vitamin E, K and B) and seven of the essential minerals necessary for normal

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human growth but it also produces high quality fodder for livestock (Monyo et al., 2012), and is also the richest source of thiamine and niacin which is low cereals, it is thus important for nursing mothers, babies and pregnant women. It thus plays a significant role in the livelihoods of marginal farmers through income and nutritional security (ICRISAT, 2006 and Monyo et al., 2012). Unsaturated fatty acids such as linoleic and oleic acids are also abundant in groundnuts.

The haulms are processed into groundnut cake, which is a high protein source for livestock. Peanuts are rich in energy; one pound of peanuts provide approximately the energy value of 2 Ib of beef, 1.5 Ib of Cheddar cheese, 9 pints of milk or 36 medium size eggs (Carley and Stanely, 1993). Peanuts are sold fresh as a vegetable, canned, frozen, roasted in shells, toasted and salted, used in more than 50 confections and bakery products and are ground into butter for use in more than 100 recipes. In actual fact, every part of the peanut plant is used for one purpose or another like for food, feed or agribusiness; the halls for fuel, mulch, feed and industrial uses. The leaves and stems for feed, soil conditioning, soil nutrients, and possible protein extraction for special diets; the roots are essential for soil enrichment through atmospheric nitrogen fixation and fibre; and the oil from seeds for food, lubrication and motor fuel. As much as breeding all other crops is important, this is by far one of the most crucial crops to put focus on, as it has a lot of uses than many other crops.

Groundnuts are an important crop in Zimbabwe, grown by a large proportion of smallholder farmers (36%); groundnuts are second after maize in terms of area coverage. Groundnuts can provide an important source of food and nutrition, feed and soil amendment, as well as income (Homann-Kee Tui et al., 2015).

Breeders throughout the world want to present data for candidate cultivars to the cultivar release panels for their products to be commercially recognized. In Zimbabwe data from at least five sites and at least two seasons is required. This goal is achieved by conducting a series of multi-environmental trials (MET) annually for all major crops to identify superior genotypes for the target locations (Kang, 1998). It has generally been accepted that the measured grain/pod yield for each cultivar in each test environment is in fact a measure of the environment main effect (E), the genotype main effect (G), and the genotype × environment (GE) interaction. The GE interaction results from the differences in responses of genotypes at different study locations (environments) (Gauch and Zobel, 1997; Yan et al., 2000; Yan, 2002).

This study was designed to (i) identify groundnut genotypes with high pod yield stability under different environments (ii) identify groundnut genotypes with specific or wide adaptations to certain environments (iii) examine the possible existence of mega environments among the environments which were used in this study (iv) identify most discriminating and best representative environment, that is, ideal environment.

Genotypes that always give high average yields with minimum G x E interaction have been gaining importance over increased yields whenever trials are conducted at many different environments or locations (Gauch and Zobel, 1997; Ceccarelli, 1989; Kang, 1998; Xing-Ming et al., 2007). The analysis of G x E interaction is closely related with the quantitative estimation of phenotypic stability of genotypes over different environments (Kang, 1998; Mohammadi and Haghparast, 2010). In the case that significant G x E interactions are observed, the effects of genotypes and environments are statistically non-additive, this implies that the differences between genotypes are due to the environment and not genotypes themselves. G x E interactions may, but not all the time, lead to different rank orders of genotypes in different environments, that is, they result in non-crossover and or crossover interactions (Sharma et al., 2009). Yield stability analysis is usually performed using many different models including GGE biplot analysis whenever there is a presence of G x E interaction in multi environment trials (Yan and Tinker, 2006). Many authors have described yield stability in many different ways over the years and there have also been different concepts of stability tests (Lin et al., 1986). According to Becker and Leon (1988), many researchers use the terms adaptation, phenotypic stability and yield stability in different ways. Chahal and Gosal (2002) noted that stability indicates consistency in performance that would mean minimum variation among environments for a particular genotype.

GGE biplot (Yan et al., 2000) is one of the best and very important tools for graphical analysis of multi-environment trials (MET) data. GGE denotes genotypic main effect (G) plus the interaction of the genotype and the environment (G x E interaction). These have been considered to be the two main sources of variation that are important to assessment of genotype performance across different locations. The biplot is constructed by plotting the first two principal components (PC1 and PC2) and these are also referred to as primary and secondary effects respectively. The PC1 and PC2 values are derived from singular value decomposition (SVD) of the environment-centered data. The GGE biplot analysis is used to identify some of the most and the least discriminating locations and representative test locations as well as the non-representative locations (Fan et al., 2006). The GGE biplot analysis methodology is a very important tool for categorizing sites that lead to optimum cultivar performance and efficient utilization of limited resources available for most of the breeding and other testing programmes (Fan et al., 2006).

The main genotype effect (G) and the genotype x environment interaction effect (GEI) is shown by the GGE bi-plot. The GGE bi-plot shows the first 2 principal components (PC1 and PC2) that are derived from subjecting environment centered yield data to singular

Table 1. Pedigree information and source of Spanish groundnut genotypes of medium seed size.

Variety/Line Code	Pedigree	Breeding status	Origin
G1	297/7/29	Intermediate line	C.B.I
G2	302A/6/2	Intermediate line	C.B.I
G3	401/92/14	Intermediate line	C.B.I
G4	262/4/3	Intermediate line	C.B.I
G5	AB/5/11	Intermediate line	C.B.I
G6	321/5/15	Intermediate line	C.B.I
G7	9607/5/14	Intermediate line	C.B.I
G8	9503/6/11	Intermediate line	C.B.I
G9	267/6/13	Intermediate line	C.B.I
G10	9607/5/10	Intermediate line	C.B.I
G11	9607/5/22	Intermediate line	C.B.I
G12	294/5/16	Intermediate line	C.B.I
G13	9503/6/5	Intermediate line	C.B.I
G14	294/5/16	Intermediate line	C.B.I
G15	374/92/16	Intermediate line	C.B.I
G16	9607/5/11	Intermediate line	C.B.I
G17	296/5/4	Intermediate line	C.B.I
G18	295/5/8	Intermediate line	C.B.I
G19	H97/3F7/1	Intermediate line	C.B.I
G20	H97/14F7/1	Intermediate line	C.B.I
G21	267/6/6	Intermediate line	C.B.I
G22	Falcon	Released	C.B.I
G23	Tern	Released	C.B.I
G24	Jesa	Released	C.B.I
G25	Ilanda	Released	C.B.I

value decomposition (Yan et al., 2001). PC1 scores of both genotypes and environments are then plotted against their respective PC2 scores. This methodology has been widely used to determine grain yield stability and identify superior, identify superior, specifically adapted, and generally adapted genotypes as well as identifying groundnut mega environments (Yan et al., 2007).

MATERIALS AND METHODS

A total of 25 genotypes (4 commercially released varieties and 21 experimental lines) were tested in 2013/14 season. All the check varieties and the intermediated experimental lines were obtained from Crop Breeding Institute (C.B.I). Ilanda and Tern are the highest yielding short season groundnut varieties in Zimbabwe and for that reason they were included as check varieties. More details on genotypes and the information on their breeding status are highlighted in Table 1.

Study site

The project was conducted at five locations; Harare Research Station (HRS), Panmure Experimental Station (PES), Gwebi Variety Testing Centre (GVTC), Save Valley Experimental Station (SVES) and Kadoma Research Station (KRS). Two of the locations belong to high veld (Harare Research Station and Gwebi VTC, the other two to middle veld (Kadoma Research Station and Panmure Experimental Station) and one belongs to the low veld (Save Valley Experimental Station). More details on the testing sites and the agro-ecological characteristics for all the locations used are shown in Table 2.

Management

The seeding rate was 100 kg/ha for all environments because the seed that was planted were Spanish varieties that have medium seed size. Compound D was applied at planting at a general recommended blanket rate of 300 kg/ha. A special request was done at all the sites that the crop was planted in a field where the previous crop was maize, and that was accomplished. Gypsum was also applied during first flowering (7 to 8 weeks after planting) at a general recommended rate of 300 kg/ha. Harvesting was done manually, were 2.4 m (0.3 m from either sides of the row) of the 3 m rows were harvested as net plot by way of hand pulling as well as hand plucking. Pod yield was then recorded after drying the groundnut pods to 12.5% moisture content by exposing the pods to the sun and moisture content was measured using the moisture meter. All other recommended groundnut production practices such as weed, pest and disease management were followed and practiced.

Table 2. Description for the sites used on the multi-environmental groundnut yield trials in 2014.

Code	Location	Soil properties	Latitude	Longitude	Altitude (masl)	Rainfall data (mm)
E1	Harare	Clay	17° 48 S	31° 03 E	1506	660
E2	Gwebi VTC	MG/SCL	17° 41 S	30° 32 E	1448	880
E3	Kadoma	Clay	18° 19 S	29° 53 E	1149	818
E4	Panmure	MG/SCL	17° 16 S	31° 47 E	881	796
E5	Save Valley	Sandy-loam	20° 48 S	33° E	450	500

Experimental design

The trials were laid in a Complete Randomized Block Design (CRBD) at all the sites. Each of the twenty-five treatments with 3 replicates and that translated to seventy-five plots in total. The plot sizes were 5.4 m^2 with 5 rows of 3 m long with spacing of 0.45 m between rows. The net plot sizes were 2.16 m^2, 1 row from both sides and 0.3 m from either side were discarded.

Data collection

Data collection includes, days to 50% flowering, days to 75% maturity, diseases scores, insect pest scores, pod size, seed size, shelling percentage and pod yield. For the sake of this study, only pod yield was considered for statistical analysis. Pod yield was recorded on the net plot basis. After drying and cleaning, the weights of the pods per plot were recorded and converted to t/ha using a formula (yield in grammes × 10 000/ (Net plot × 1000 for kg × 1000 for tonnes).

Analysis of variance

Analysis of variance (ANOVA) for pod yield data was conducted using GenStat 14th Edition software to determine the G, E and GEI effects. The effects of the genotypes, environments as well as their interaction were determined from ANOVA analysis.

Yield stability analysis

To determine pod yield stability and identify superior and well adapted genotypes across locations and ideal site(s) (most discriminating and representative) as well as determining mega-environments, (GGE) bi-plots (Yan, 2001) was done using GGE bi-plot software. The GGE bi-plot methodology is composed of two concepts, the bi-plot concept and the GGE concept.

GGE bi-plot analysis

Genotype + Genotype x Environment (GGE) bi-plots were conducted using GGE bi-plot software in GenStat 14.1 to determine pod yield stability and identify superior, specifically adapted, generally adapted genotypes as well as identifying groundnut mega environments. The GGE bi-plot methodology is composed of two concepts, the bi-plot concept and the GGE concept (Yan et al., 2001). GGE biplots were also used to compare genotypes performance with a reference genotype (ideal genotype). The ideal genotype is usually an imaginary genotype that will be stable and have the highest average mean value among the genotypes. Correlation coefficients among environments were also conducted. Which-one-where pattern of multi environment yield trial was visualized using symmetric scaling within the GGE biplots (Hunt and Yan, 2002).

Discrimination ability, representativeness, mega-environmets and relationships among test environments

The relationship among environments and comparing among a set of environments with discriminating ability and representativeness was conducted using the GGE bi-plot analysis. The correlation between two environments can be approximated by the cosine of the angle between the vectors of two environments. Any two environments can be positively, negatively or not correlated if the angles between their vectors are less than 90°, more than 90° or equal to 90° respectively (Sharma et al., 2009).

RESULTS AND DISCUSSION

ANOVA and mean yield performance

Analysis of variance at 5% significance level was performed and the results indicated that genotypes (G) were not significant ($p = 0.153$), but environments (E) and genotype x environment interactions (GEI) were highly significant both at same significance level ($P < 0.001$) on pod yield of twenty-five groundnut genotypes (Tables 3 and 4). The presence of significant interaction between the genotype and the environment lead the researcher to perform pod yield stability and adaptation analysis of the different genotypes using GGE biplot analysis with the results that are presented below.

Pod yield stability and adaptability analysis

The GGE biplot methodology has been used to evaluate test environments in soybean (Yan and Rajcan, 2002), cotton (Blanche et al., 2008), and common bean (Kang et al., 2006). Using the same methodology, Ober et al. (2005) managed to evaluate physiological traits as indirect selection criteria for drought tolerance. The results on GE main effects and the first principal component scores of the interactions summary information for both genotypes and environments are shown in Figure 1. The partitioning of GGE through GGE bi-plot analysis indicated that principal coordinate 1 (PC1) and principal coordinate (PC2) significantly explained

Table 3. Analysis of variance for pod yield (t/ha) of twenty-five groundnut genotypes evaluated across five locations over a season.

Source	DF	SS	MS	% total sum of squares
Rep stratum	2	0.8646	0.4323	
Genotype	24	29.2839	1.2202	4.1
Environment	4	281.8406	70.4602***	39.7
Genotype.Environment	96	168.4375	1.7546***	23.7
Residual	248	229.8636	0.9269	
Total	374	710.2903		

Coefficient of variation (%CV) = 18.1%.

Table 4. ANOVA table for AMMI model.

Source	df	SS	MS	Percentage total sum of squares	Percentage treatment	% G x E
Total	374	710.3	1.899			
Treatments	124	479.6	3.867***	67.5		
Genotypes	24	29.3	1.22	4.1	6.1	
Environments	4	281.8	70.46***	39.7	58.8	
Block	10	9.9	0.989			
Interactions	96	168.4	1.755***	23.7	35.1	
IPCA I	27	100.4	3.719***			59.6
IPCA II	25	39.6	1.584*			23.5
Residuals	44	28.4	0.646			16.9
Error	240	220.8	0.92	31.1		

Genotype + (Genotype x Environment) (GGE) biplot analysis.

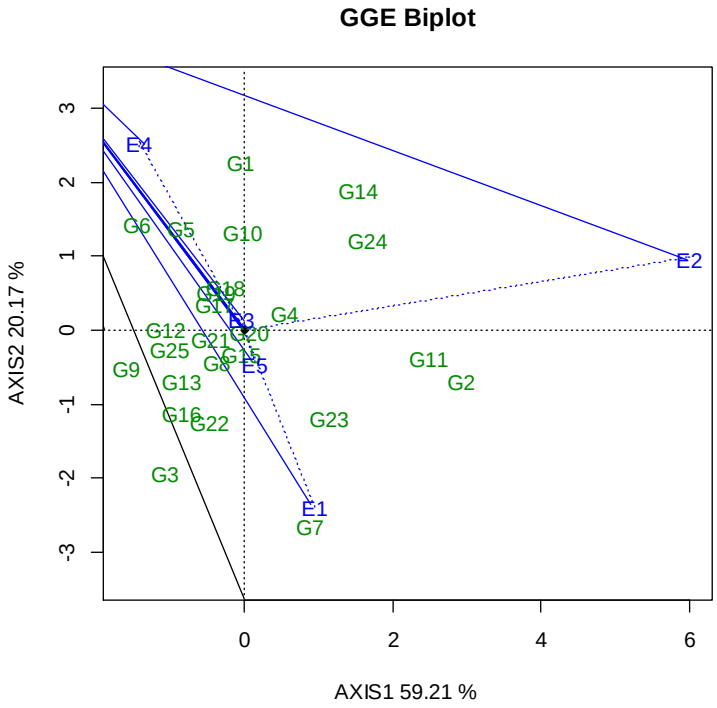

Figure 1. The discriminating ability and relationship among 5 environments based on 25 groundnut genotypes.

59.22 and 20.17% of GGE sum of squares, respectively, explaining a total of 79.39% variation. This moderate percentage variability of GGE (79.39%) accounted by the bi-plot indicates that there is strong and complex GE interaction in this multi-environment yield trial data. The presence of GEI resulted in differential pod yield performance among the groundnut genotypes across five testing environments that were used in this study (Crossa et al., 1991).

Genotypes or environments with high positive PC1 scores are high yielding or high potential locations respectively. In the biplot (Figure 1) G2 and G11 had the largest positive PC1 score indicating that they were high yielding and E2 (Gwebi VTC) had the largest PC1 score indicating that it is a high potential environment. On the other hand, genotypes or environments that had PC1 less than zero scores were identified as lower yielding or low potential locations respectively, for instance, according to the biplot (Figure 1) G9, G3 and G6 were the lowest yielders and E4 (Panmure) was a low potential environment.

The PC2 is associated with genotypic stability or instability across environments. Genotypes with low positive or low negative PC2 scores (scores near zero) are more stable than those with large PC2 scores (Yan and Tinkler, 2006). In this study, there are a lot of genotypes located on the negative side of PC1 and they included 2 check varieties (G22 and G25) as well as many experimental genotypes implying that these genotypes were low yielding. There are promising elite breeding lines that bear potential as candidate genotypes for release. Genotypes G2, G11, G14, G7, G4, G20, G15 and G10 were generally high yielding with G2 (moderately stable) being the overall best (largest PC1 score). The results agreed with (Sharma et al., 2009) who stated that higher yielding genotypes are not always the most stable across environments. On the other hand, the genotypes G9, G6, G25, G12, G3, G5, G13, G16, G22, G21, G17, G19 G8 AND G18 were generally low yielding, with G3, G6 and G5 being part of the most unstable genotypes (Figure 1). Genotypes that are not stable are not desirable as this has a negative effect on farmers' income and, in the case of staple and legume crops, contributes to food and nutrition insecurity at household and national level (Simmonds, 1991). The genotypes G12 and G21 were consistently poor performing hence the high stability. Even though G12 was most stable genotype, it was the least performing genotype (highest negative PC1 score) with low yields in different environments.

The genotypes formed at least four groups on the bi-plot (Figure 1): 'G25, G8, G9, G13, G17, G18 and G19' generally low yielding, and moderately stable (near zero PC2 scores); 'G6, G5, G16, G22, G3 and G1'generally low yielding and highly unstable (variable) across environments (high positive and negative PC2 scores). Genotypes 'G15, G2 and G11' were generally high yielding, and moderately stable across environments (high positive PC1 scores); 'G20 and G4' were generally high yielding, and stable genotypes (absolute PC2 scores near zero) across environments (positive PC1 scores). This indicates that these genotypes may be suitable for growth in a wide range of environments. The genotypes 'G7, G10, G14, G24 and G23' formed the other group which consisted of generally high yielding and unstable genotypes across environments. The superior, high yielding and stable experimental genotypes 'G20 and G4' can be used to crossing with other genotypes (especially those that are high yielding and unstable, because only traits of being stable need to be introduced) to improve pod yield and stability across environments. This is important and appropriate especially considering that the superior genotypes differed in their genotypic background; and therefore probably there are high chances that these genotypes could provide opportunities for genetic gain through recombination of superior alleles.

Genotypes G12, G21 and G20 had the most consistent performance, being high stable with G20 being high yielding and G12 and G21 being low yielding because their absolute PC2 scores were almost at the zero line (Figure 1). Therefore, these three genotypes had little interaction across environments indicating that G20 had broad adaptations and G12 and G21 are well adapted to the low potential environments (Akcura et al., 2011). According to Akcura et al. (2011), genotypes with PC2 values near zero would have had little interactions across environments and vice versa for environments.

Projected lines perpendicular to the AEA measures the stability of the genotypes in either direction. Genotypes with smallest perpendicular lines with AEA are called stable cultivars (Yan and Tinker, 2006). Genotypes G20 and G4 were the most stable and productive genotypes in the different environments (shortest perpendicular line) (Figure 2). G12, G21 and G25 were also very stable genotypes but low yielding. Genotypes, G10 and G23 were moderately stable in the environments (moderately shorter perpendicular lines with AEA). G1 and G7 were the most unstable high yielding genotypes across the environments (longer per perpendicular lines to the AEA). The rest of the genotypes were low yielding, among them some were unstable, moderately and some highly stable (Figure 2). The following genotypes yielded above average: G2, G11, G14, G24, G23, G4, G7, G1, G10 and G20 (according to their ranking order) with G2 and G11 being the highest yielders (Figure 2).

An ideal genotype (centre of the smallest concentric circle), is usually an imaginary genotype that is both high yielding and high stable across all the environments (Yan and Tinker, 2006). The best genotypes are identified basing on the concentric circles like those in Figure 3. According to the GGE biplot above G11 (highlighted in blue circle) was found to be the best genotype as it fell on the circumference of the smallest inner concentric circle. This implies that this genotype was the most stable and

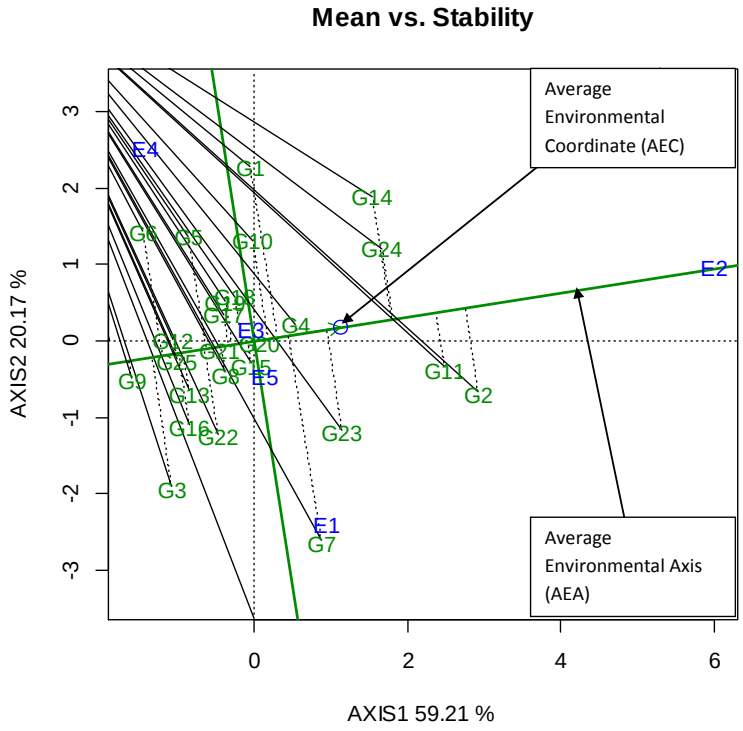

Figure 2. Ranking plot based on mean performance and stability.

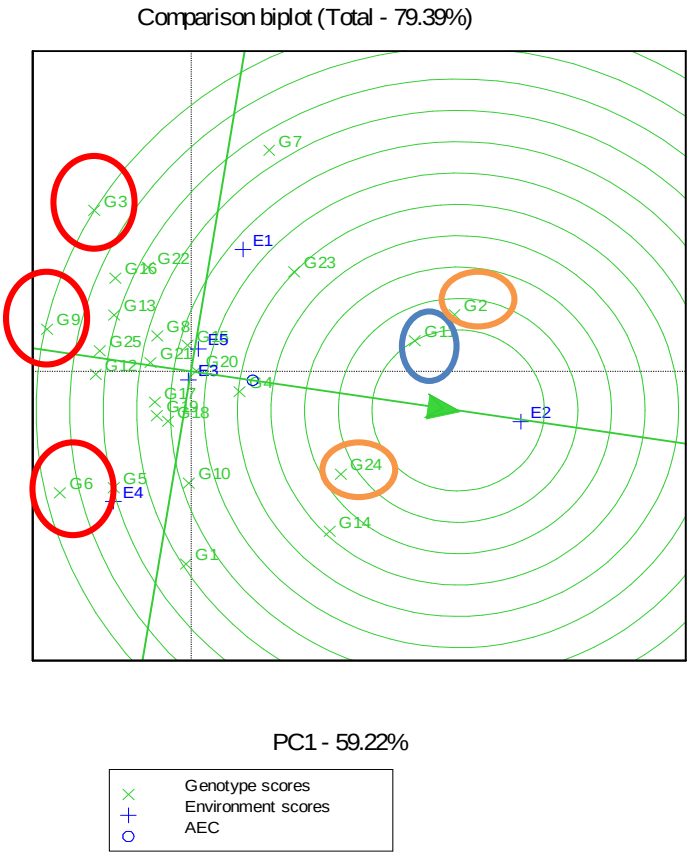

Figure 3. Comparison Plot for genotypes (relative to the ideal genotype).

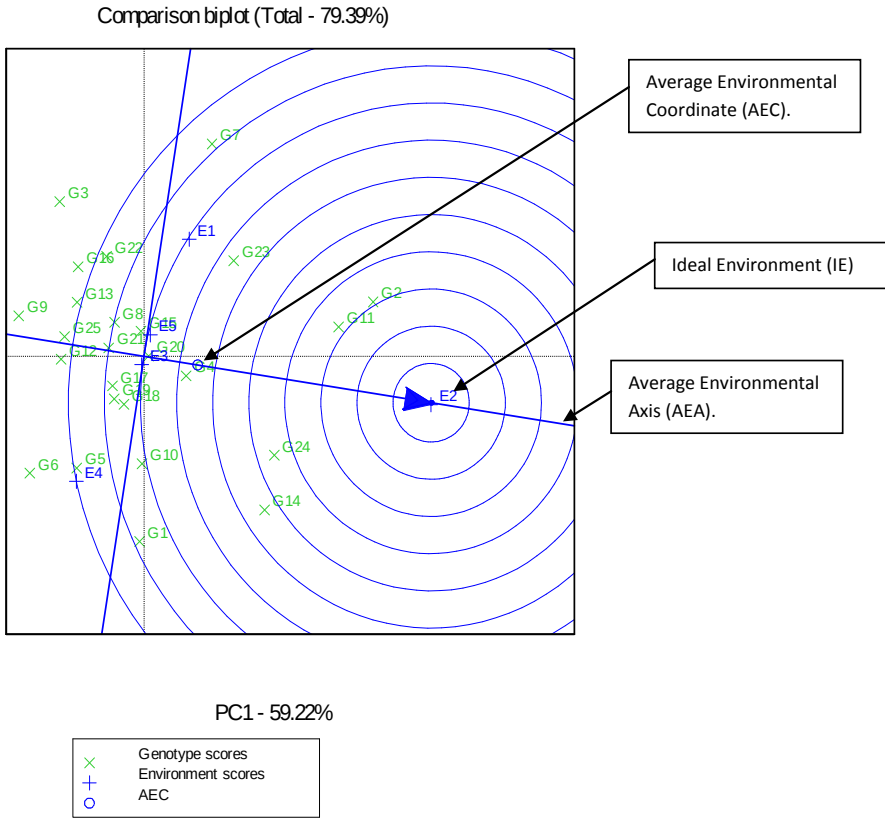

Figure 4. Discriminating ability vs. representativeness of test environments; average environment.

high yielding at the same time (Yan and Tinker, 2006). The best genotype according to this study may not be the most stable cultivar. Genotypes G2 and G24 (highlighted in oranges circles) were the next best genotypes as they lied on the circumference of the second concentric circle and just outside the second concentric circle respectively. The implication is that these two genotypes failed to strike a balance between high yielding and stability as G11 did (Yan and Tinker, 2006). According to the biplot (Figure 3), G9, G3 and G6 (highlighted in red circles) were the most unfavourable and most undesirable genotypes as they lie in the last outer concentric circle.

Best test environment for groundnut genotypes

GGE biplot is a data visualization tool, which graphically shows a GxE interaction in a two way table (Yan et al., 2000). The analysis of GGE biplot is useful for: 1. mega-environment identification (e.g. “which-won-where” pattern), that help to recommend specific genotypes to their suitable mega-environment 2. Evaluation of genotypes performance (genotypic stability) and 3. The environmental evaluation (the power to discriminate among genotypes in target environments) (Yan and Kang, 2003; Yan and Tinker, 2006). Lines that connect the environment to the origin of the biplot are known as the environmental vectors (EV). The length of the environmental vectors is proportional to their standard deviation which is a measure of discriminating ability of a particular environment (Yan and Tinker, 2006). In this particular study, environment E2 (Gwebi VTC) had the longest environmental vector, implying that, it was most discriminating environment (Figures 1 and 4). Environments E4 (Panmure) and E1 (Harare) also had long environmental vectors, meaning that they also have the capacity to discriminate genotypes according to their genotypic performance. Environments E5 (Save Valley) and E3 (Kadoma) had the shortest environmental vectors (Yan and Tinker, 2006), implying their inability to discriminate varieties basing on their genotypic performance (Figures 1 and 4). Any two environments can be positively, negatively or not correlated if the angles between their vectors are less than 90°, more than 90° or equal to 90° respectively (Sharma et al., 2009) respectively. Each environment was connected to the bi-plot origin using a vector to determine the discriminating ability of test environments. Environments with longer vectors are known to be more discriminative of the genotypes than those with short vectors discriminative (Sharma et al., 2009). An environment with a small angle to the average environment axis (AEA) is

more representative of other test environments. Ideal test environments should have near zero PC2 scores (more representative of the average environment) (Yan et al., 2001). Therefore, the biplot shows that E1 (Harare) and E5 (Save Valley), E3 (Kadoma) and E4 (Panmure) are positively correlated, E1 (Harare) and E2 (Gwebi VTC) are slightly positively correlated, E3 (Panmure) and E2 (Gwebi VTC) are not correlated and the same applies for E2 (Gwebi VTC) and E4 (Panmure) (the angle is about 90°). Environments E1 (Harare) and E4 (Panmure), E4 (Panmure) and E5 (Save Valley) as well as E1 (Harare) and E3 (Kadoma) are negatively correlated, E1 (Harare) and E5 (Save Valley) are negatively correlated to E3 (Kadoma) and E4 (Panmure) shown by the biplot (the angle between the environmental vectors is more than 90°). Environments E2 (Gwebi VTC) and E5 (Save Valley) as well as E2 (Gwebi VTC) and E3 (Kadoma) are not correlated. Similarity of the environments in their discriminating ability is obtained when there is a combination of similar environmental vector length and an acute cosine angles between the vectors (Yan and Tinker, 2006). Therefore, there was dissimilarity in discriminating ability among all the environments; this is shown in the biplot (Figure 1) by the combination of different lengths in the environmental vectors and the large angles between the environments with similar lengths of the environmental vectors (Yan and Tinker, 2006).

The GGE methodology has been used to target cultivars to specific environments in rice (Samonte et al., 2005), that is, specific adaptation. The length of the environmental vectors is proportional to their standard deviation which is a measure of discriminating ability of a particular environment (Yan and Tinker, 2006). An environment whose environmental vector has a smaller angle with the Average Environmental Axis (AEA) is known to be representative. Being representative is the ability of the environment to allow the genotypes to perform more or less the same as they would do in any other environment in the study. In this study, environment E2 (Gwebi VTC) is an ideal environment because it is both representative (smaller angle to the AEA) and highly discriminating (longer EV). This environment is an ideal environment because it lies at the centre of the first inner concentric circle (Figure 4). The same figures shows that, environments E1 (Harare) and E4 (Panmure) were only discriminating (longer EV) but not representative (larger angles to the AEA). These environments can be used to select for specifically adapted genotypes. E3 (Kadoma) and E5 (Save Valley) were neither discriminating (short environmental vectors) nor representative (large angle with the AEA) (Figures 1 and 4) (Yan and Tinker, 2006).

Mega-environment identification (which-won-where pattern)

A polygon connects all the furthest (the highest yielder in one or more environments) genotypes (Yan and Tinker, 2006). The GGE biplot (Figure 5) shows that there are 3 mega environments, of which two of the mega environments were intersecting at the biplot origin implying that they belong to one complex mega-environment. These results coincide with the conclusion that was reached by Rukuni et al. (2006) when they said Zimbabwe is a very small country but is very diverse, hence the reason why it is divided into 5 agro-ecological zones. In this study, genotypes G2, G14, G1, G6, G9, G3 and G7 were the highest yielders in one or more sectors, hence the furthest points on the polygon (Yan and Tinker, 2006). Perpendicular lines divide the polygon into sectors; hence in this case there are seven sectors. Sectors are the ones that are used to visualize mega environments. In view of the GGE biplot (Figure 5) based on G x E data exhibits crossover interaction, that is, there are different genotypes winning in different sectors. According to the GGE biplot Figure 5, for the locations that were used in the study there are three groundnut mega environments. Environment E2 (Gwebi VTC) was allocated its own mega environment; hence this was the only environment that was both discriminating and representative. Gwebi VTC is generally a high potential location, and according to Souta (2012) and Ceccarelli and Grando, (1997), high potential environments are usually highly discriminating and best representative. Environment E3 (Kadoma) and E4 (Panmure) were clustered into same mega-environment. Environments E1 (Harare) and E5 (Save Valley) were also clustered into the same environment. Harare is generally high potential location that under normal conditions it was not supposed to have been clustered (but could have been clustered with Gwebi VTC) into the same mega environment with Save Valley which is inherently a low potential area due to its unfavourable climatic conditions and type of soil. Ideally high potential locations are generally highly discriminating and representative (Yan and Tinker, 2006). The reason for this phenomenon is the delay in planting that transpired at Harare, leading to the crop spending most of its growing time exposed to the decreasing temperatures in this environment. The crop at Harare also experienced mid-season drought during flowering, pegging periods as well as early podding, hence the reduction in the pods that where set and automatically achieved low yields that match those at Save Valley. Winning genotypes for each sector are located at the vertex. Genotypes G2, G14, G1, G6, G9, G3 and G7 are the ones that are on the vertex meaning that they the winning genotypes of those particular sectors.

Conclusion

GGE biplot analysis made the reseacher to understand that there were high levels of interaction between the environments and the genotypes. Environment explained much of the variation amoung the genotypes, and this

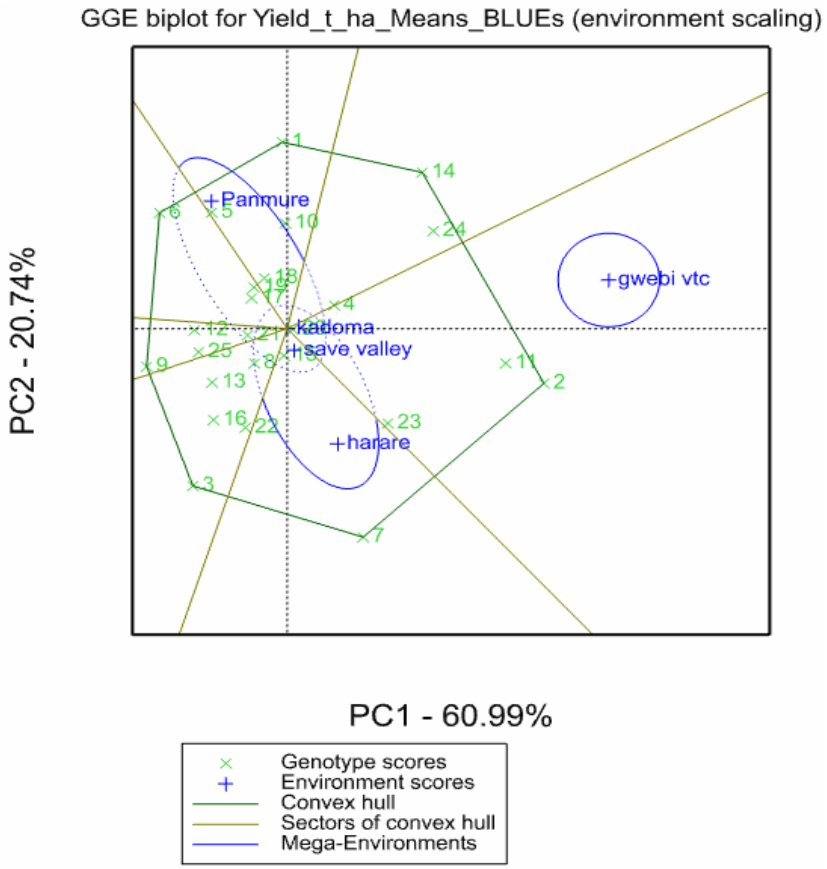

Figure 5. Which-Won-Where pattern.

was evidenced by the high percentage contribution to the total sum of squares.

Widely adapted genotypes were identified to be G20 and G4, with G4 being the most productive. These two genotypes are then recommended for further testing and released to be grown across all the environments. G12, G21 and G25 were also highly stable but cannot really be recommended for production across the environments since they were yielding below average. On the other hand G2 and G11 were identified to be the most productive genotypes but lacked stability. The two genotypes were recommended for further testing and released specifically for high potential environments because they were more adapted in those environments, since government policy allows this kind of decisions to be made.

Ideal environment was identified to be Gwebi VTC. The implication of this is that this environment is both highly discriminating therefore can be used in the early generation genotypic screening. It also shows that this environment is representative of others; so as to save resources this location can be used with a few other locations to acquire data for release. In the event that there are no enough resources to establish trials at all the sites, either Kadoma or Save Valley can be chose since they represent each other. Three mega environments were identified and these were: i. E2 (Gwebi VTC), ii. E1 (Harare) and E5 (Save Valley) and iii. E3 (Kadoma) and E4 (Panmure). There is need to validate of this information through the use of more sites and seasons is recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Abbreviations

GEI, Genotype-environment-interaction; **E**, environment; **G**, genotypes; **PC1**, principal coordinate 1; **PC 2**, principal coordinate 2; **ANOVA**, analysis of variance.

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