

African Journal of Plant Science

Volume 11 Number 5, May 2017

ISSN 1996-0824



*Academic
Journals*

ABOUT AJPS

The **African Journal of Plant Science (AJPS)** (ISSN 1996-0824) is published Monthly (one volume per year) by Academic Journals.

African Journal of Plant Science (AJPS) provides rapid publication (monthly) of articles in all areas of Plant Science and Botany. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in AJPS are peer-reviewed.

Contact Us

Editorial Office: aips@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: <http://www.academicjournals.org/journal/AJPS>

Submit manuscript online <http://ms.academicjournals.me/>

Editor

Prof. Amarendra Narayan Misra

*Center for Life Sciences, School of Natural Sciences,
Central University of Jharkhand,
Ratu-Lohardaga Road, P.O. Brambe-835205,
Ranchi, Jharkhand State,
India.*

Associate Editors

Dr. Ömür Baysal

*Assoc. Prof.
Head of Molecular Biology and Genetic Department,
Faculty of Life Sciences,
Mugla Sıtkı Koçman University,
48000 -Mugla / TURKEY.*

Dr. Pingli Lu

*Department of Biology
416 Life Sciences Building
Huck Institutes of the Life Sciences
The Pennsylvania State University
University Park, PA 16802
USA.*

Dr. Nafees A. Khan

*Department of Botany
Aligarh Muslim University
ALIGARH-202002, INDIA.*

Dr. Manomita Patra

*Department of Chemistry,
University of Nevada Las Vegas, Las Vegas,
NV 89154-4003.*

Dr. R. Siva

*School of Bio Sciences and Technology
VIT University
Vellore 632 014.*

Dr. Khaled Nabih Rashed

*Pharmacognosy Dept.,
National Research Centre,
Dokki, Giza, Egypt*

Dr. Biswa Ranjan Acharya

*Pennsylvania State University
Department of Biology
208 Mueller Lab
University Park, PA 16802.
USA*

Prof. H. Özkan Sivritepe

*Department of Horticulture Faculty of
Agriculture Uludag University Görükle
Campus Bursa 16059
Turkey.*

Prof. Ahmad Kamel Hegazy

*Department of Botany, Faculty of Science,
Cairo University, Giza 12613,
Egypt.*

Dr. Annamalai Muthusamy

*Department of Biotechnology
Manipal Life Science Centre,
Manipal University,
Manipal – 576 104
Karnataka,
India.*

Dr. Chandra Prakash Kala

*Indian Institute of Forest Management
Nehru Nagar, P.B.No. 357
Bhopal, Madhya Pradesh
India – 462 003.*

African Journal of Plant Science

Table of Content: Volume 11 Number 5, May 2017

ARTICLES

- Utilization of wild relatives for maize (*Zea mays* L.) improvement** 105
Abdoul-Raouf Sayadi Maazou, Ju Qiu, Jianyu Mu and Zhizhai Liu
- Assessment of genetic diversity among released and elite Ethiopian barley genotypes using simple sequence repeat (SSR) markers** 114
Abebaw Misganaw, Sisay Kidane and Kalkidan Tesfu
- Rhizobium inoculation and sulphur fertilizer improved yield, nutrients uptake and protein quality of soybean (*Glycine max* L.) varieties on Nitisols of Assosa area, Western Ethiopia** 123
Zerihun Getachew, Girma Abera and Sheleme Beyene
- Response of *Cyperus papyrus* productivity to changes in relative humidity, temperature and photosynthetically active radiation** 133
Opio A., Jones B. M., Kansiime F. and Otiti T.
- Variation of leaf and fruit characteristics of *Vitellaria paradoxa* (shea tree) according to agronomical performance along south-north climatic gradient in Mali** 142
Bokary Allaye Kelly and Oumar Senou
- Fall management of fleabane based on glyphosate+2, 4-D, MSMA and glufosinate applied isolated or in tank mixture with residual herbicides** 151
Antonio Mendes de Oliveira Neto, Jamil Constantin, Rubem Silvério de Oliveira Júnior, Naiara Guerra, Eder Blainski, Hugo de Almeida Dan and Diego Gonçalves Alonso
- Ethnobotanical survey of medicinal plants used for treating preschool children anemia in an urban setting, Douala-Cameroon** 160
Suzanne Sandrine Beack Bayengue, Mathieu Ndomou, Luther Martin Koanga Mogtomo, Rosalie Annie Ngono Ngane and Clergé Tchiegang

Review

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

Abdoul-Raouf Sayadi Maazou, Ju Qiu, Jianyu Mu and Zhizhai Liu*

College of Agronomy and Biotechnology, Southwest University, Chongqing, China.

Received 25 January, 2017; Accepted 3 March, 2017

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 been 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

*Corresponding author. E-mail: liuzhizhai@126.com. Tel: (+86) 13883880102.

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 al., 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; Gavriloova 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. turcicum* 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

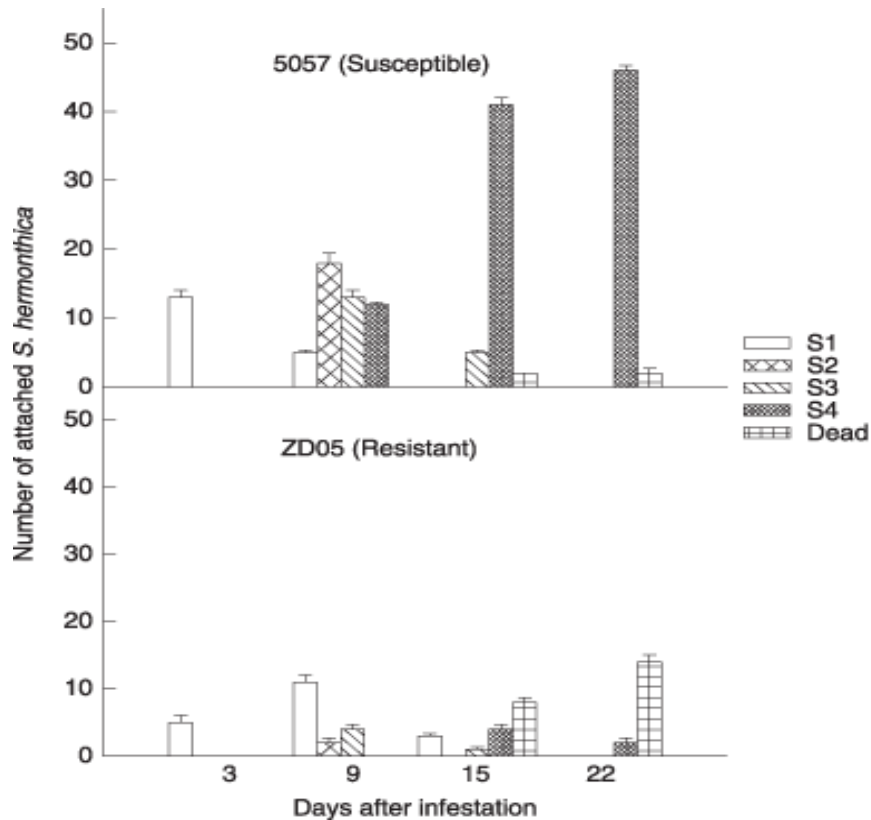


Figure 1. Development of *Striga hermonthica* on the roots of 5057 (susceptible) and ZD05 (resistant) maize genotypes at 3, 9, 15 and 22 days after infestation. Stages of development were defined as follows: S1, attached *Striga* with seed coat intact; S2, emergence of first leaf primordial; S3, attached *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 polyphagous 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 LW) 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 identified 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%) significantly decreased KRN, and no alleles increased KRN. For Wt50k, they identified 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 significantly 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 confer 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 stewartii*, *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.

ACKNOWLEDGEMENTS

Financial supports from the General Project of Natural Science Foundation of Chongqing (cstc2016jcyjA0344), the Scientific Innovation Program of Southwest University (20162602005) and Maize Germplasm Resources Protection Project (2016NWB036-04-10) are thankfully acknowledged.

REFERENCES

- Aditya P, Jitendra K (2014). Alien Gene Transfer in Crop Plants, Volume 2: Achievements and Impacts. Springer Science & Business Media.
- Amusan IO, Patrick JR, Abebe M, Thomas H, Gebisa E (2008). Resistance to *Striga hermonthica* in a maize inbred line derived from *Zea diploperennis*. *New Phytol.* 178:157-166.
- Ashraf M, Ozturk M, Ahar HR (eds) (2009). Salinity and water stress: improving crop efficiency. Springer, Berlin
- Bai D, Scoles GJ, Knott DR (1995). Rust resistance in Triticum cylindricum Ces. (4x, CCDD) and its transfer into durum and hexaploid wheats. *Genome* 38:8-16.
- Bennetzen JL (2007). Patterns in grass genome evolution. *Curr. Opin. Plant Biol.* 10:176-181.
- Bergquist RR (1979). Selection for disease resistance in a maize breeding programme. II. Introgression of an alien genome from *Tripsacum dactyloides* conditioning resistance in *Zea mays*. Proceedings of the tenth meeting of the Maize and Sorghum Section of Eucarpia, Varna, Bulgaria. Pp. 200-206.
- Bergquist RR (1981). Transfer from *Tripsacum dactyloides* to corn of a major gene locus conditioning resistance to *Puccinia sorghi*. *Phytopathology* 71:518-520.
- Berthaud J, Savidan Y, Barré M, Leblanc O (1997). *Tripsacum*. In: D. Fuccillo, Sears L, Stapleton P, Eds., Biodiversity in Trust. Cambridge University Press, Cambridge. Pp. 227-233.
- Berthaud J, Savidan Y, Leblanc O (1995). *Tripsacum*: diversity and
- Bird RMK (2000). A remarkable new teosinte from Nicaragua: growth and treatment of progeny. *Maize Genetics Cooperation Newsletter* 74:58-59.
- Branson TF (1971). Resistance in the grass tribe Maydeae to larvae of the western corn rootworm. *Ann. Entom. Soc. Am.* 64:861-863.
- Brar DS (2005). Broadening the gene pool of rice through introgression from wild species. In: Toriyama, K., Heong, K.L., Hardy, B., ed., *Rice is life: Scientific perspectives for the 21st century*, Proceedings of the World Rice Research Conference, Tokyo and Tsukuba, Japan, November 4-7, 2004.
- Canci H, Toker C (2009). Evaluation of annual wild Cicer species for drought and heat resistance under field conditions. *Genet. Resour. Crop Evol.* 56:1-6.
- Chittaranjan K (2011). Wild crop relatives: Genomic and breeding resources: Cereals. Springer Science & Business Media.
- Clark RB, Alberts EE, Zobel RW, Sinclair TR, Miller MS, Kemper WD, Foy CD (1996). Eastern gamagrass (*Tripsacum dactyloides*) root penetration and chemical properties of claypan soils. In: JE Box Jr, Ed., *Root Demographics and Their Efficiencies in Sustainable Agriculture, Grasslands and Forest Ecosystems*. Kluwer Acad. Pub., Dordrecht, The Netherlands. Pp. 191-211.
- Clifford BC (1995). Diseases, pests and disorders of oats. In: Welch RW (ed) *The oat crop: production and utilization*. Chapman & Hall, London, UK, Pp. 252-278.
- Cohen JL, Gallinat WC (1984). Potential use of alien germplasm for maize improvement. *Crop Sci.* 24:1011-1015.
- Comis D (1997). Aerenchyma: lifelines for living underwater. *Agric. Res.* 45:4-8.
- conservation. In: S. Taba., Ed., *Maize Genetic Resources. Maize Program Special Report. CIMMYT, Mexico, D.F.* Pp. 74-85.
- Coyne PJ, Bradford JA (1985). Comparison of leaf gas exchange and water-use efficiency in two Eastern gamagrass accessions. *Crop Sci.* 25:65-75.
- De Wet JMJ (1979). *Tripsacum* introgression and agronomic fitness in maize (*Zea mays* L.). *Proc. Conf. Broadening Genet. Base Crops, Pudoc, Wageningen.*
- De Wet JMJ, Harlan JR (1972). Origin of maize: tripartite hypothesis. *Euphytica* 21:271-279.
- De Wet JMJ, Brink DE, Cohen CE (1983). Systematics of *Tripsacum* section Faciculata (Gramineae). *Am. J. Bot.* 70:1139-1146.
- De Wet JMJ, Harlan JR, Lambert RJ, Engle LM (1972). Introgression from *Tripsacum* into *Zea* and the Origin of Maize. *Caryologia* 25(1):25-31.
- Dela Vina AC, Mendoza ACA, Eagle LM, Ramirez DA (1995). Inheritance of selected morphological characters in *Zea* l. *Zea mays* ssp. *mays* x *Zea mays* ssp. *mexicana* and *Zea mays* ssp. *mays* x *Zea diploperennis*. *Philipp. J. Crop Sci.* 20:94-107.
- Dillon SL, Lawrence PK, Henry RJ (2005). The new use of Sorghum bicolor-derived SSR markers to evaluate genetic diversity in 17 Australian Sorghum species. *Plant Genet. Res* 3(1):19-28.
- Dillon SL, Shapter FM, Henry RJ, Cordeiro G, Izquierdo L, Lee LS (2007). Domestication to crop improvement: genetic resources for Sorghum and Saccharum (Andropogoneae). *Ann. Bot.* 100:975-989.
- Doebley JF (1990a). Molecular systematics of *Zea* (Gramineae). *Maydica* 35:143-150.
- Doebley JF (1990b). Molecular evidence for gene flow among *Zea* species. *Bioscience* 40:443-448.
- Eubanks MW (1997). Molecular analysis of crosses between *Tripsacum dactyloides* and *Zea diploperennis* (Poaceae). *Theor. Appl. Genet.* 94:707-712.
- Eubanks MW (2001). The origin of maize: evidence for *Tripsacum* ancestry. In: Janick J, Ed., *Plant breeding reviews*. John Wiley & Sons, Inc., New York 20:15-66.
- Eubanks MW (2002). Investigation of novel genetic resource for rootworm resistance in corn. In: NSF (ed) *Proceedings of the NSF design, service and manufacturing conference*. Iowa State University, San Juan, Puerto Rico, Pp. 2544-2550.
- Eubanks MW (2006). A genetic bridge to utilize *Tripsacum* germplasm in maize improvement. *Maydica* 51:315-327.
- Feldman M, Kislev ME (2007). Domestication of emmer wheat and evolution of free-threshing tetraploid wheat. *Isr. J. Plant Sci.* 55:207-221.
- Findley WR, Nault LR, Styer WE, Gordon DT (1982). Inheritance of maize chlorotic dwarf virus resistance in maize x *Zea diploperennis* backcrosses. *Maize News Lett.* 56:165-166.
- Gavrilova O, Gagkaeva T, Burkin A, Kononenko G, Loskutov I (2008). Susceptibility of oat germplasm to Fusarium infection and mycotoxin

- accumulation in grains. In: Proceedings of the 8th international oat conference, 27 June–2 July 2008, Minneapolis, MN, USA, Poster V-2a.
- Gill BS, Li W, Sood S, Kuraparthi V, Friebe SKJ, Zhang Z, Faris JD (2007). Genetics and genomics of wheat domestication-driven evolution. *Isr. J. Plant Sci.* 55:223-229.
- Gurney AL, Grimanelli D, Kanampiu F, Hoisington D, Scholes JD, Press MC (2003). Novel sources of resistance to *Striga hermonthica* in *Tripsacum dactyloides*, a wild relative of maize. *New Phytol.* 160: 557-568.
- Hajjar R, Hodgkin T (2007). The use of wild relatives in crop improvement : A survey of developments over the last 20 years. *Euphytica* 156:1-13.
- Hannes D, Ruth JE, Luigi G, Colin KK, Jonas VM, Jane T (2014). Adapting Agriculture to Climate Change: A Global Initiative to Collect, Conserve, and Use Crop Wild Relatives. *Agroecology Sustain. Food Syst.* 38(4):369-377.
- Harinder KC, Vineeta K, Shoukat AR (2014). Maize.. In: Aditya P, Jitendra K. *Alien Gene Transfer in Crop Plants, Volume 2 Achievements and Impacts.* Springer. Pp. 27-50.
- Hitchcock AS (1951). *Manual of grasses of the United States*, Second edition, revised by A. Chase. U. S. Government Printing Office, Washington, DC.
- Hooker AL, Perkins JL (1980). *Helminthosporium* leaf blights of corn the state of the art. Proceedings of the annual Corn and Sorghum Research Conference, 35:68-87.
- Iltis HH, Benz BF (2000). *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. *Novon* 10:382-390.
- Iltis HH, Doebley JF (1980). Taxonomy of *Zea* (Gramineae). II. Subspecific categories in the *Zea mays* complex and a generic synopsis. *Am. J. Bot.* 67:994-1004.
- Iltis HH, Doebley JF, Guzman RM, Pazy B (1979). *Zea diploperennis* (Gramineae): a new teosinte from Mexico. *Science* 203:186-188.
- Ishimaru T, Hirabayashi H, Ida M, Takai T, San-Oh YA, Yoshinaga S, Ando I, Ogawa T, Kondo M (2010). A genetic resource for early-morning flowering trait of wild rice *Oryza officinalis* to mitigate high temperature-induced spikelet sterility at anthesis. *Ann. Bot.* 106:515-520.
- Kamala V, Singh SD, Bramel PJ, Manohar Rao D (2002). Sources of resistance to downy mildew in wild and weedy sorghums. *Crop Sci.* 42:1357-1360.
- Karn A, Gillman JD, Flint-Garcia SA (2017). Genetic analysis of teosinte alleles for kernel composition traits in maize. G3 (Bethesda) pii: g3.117.039529.
- Kemper WD, Alberts EE, Foy CD, Clark RB, Ritchie JC, Zobel RW (1997). Aerenchyma, acid tolerance, and associative N fixation enhance carbon sequestration in soil. In: R Lal, JM Kimble, RF Follett, BA Stewart., Eds., *Management of Carbon Sequestration in Soil.* CRC Press, Boca Raton, FL. Pp. 221-234.
- Kim SK, Akintunde AY, Walker P (1999). Responses of maize inbreds during development of *Striga hermonthica* infestation. *Maydica* 44:333-339.
- Kindiger BK, Beckett JB (1990). Cytological evidence supporting a procedure for directing and enhancing pairing between maize and *Tripsacum*. *Genome* 33:495-500.
- Kuhlman LC, Burson BL, Klein PE, Klein RR, Stelly D, Price HJ, Rooney WL (2008). Genetic recombination in *Sorghum bicolor* × *S. macrospermum* interspecific hybrids. *Genome* 51:749-756
- Lagoke STO, Parkinson VO, Agunbiade RM (1991). Parasitic weeds and control methods in Africa. In: Kim SK, ed. *Combating Striga in Africa*, proceedings of the international workshop organized by IITA, ICRISAT, and IDRC. Ibadan, Nigeria: IITA, 3-14.
- Lane JA, Child DV, Moore THM, Arnold GM, Bailey JA (1997). Phenotypic characterisation of resistance in *Zea diploperennis* to *Striga hermonthica*. *Maydica* 42:45-51.
- Leblanc O, Grimanelli D, Gonzalez DLD., Savidan Y (1995). Detection of the apomixis mode of reproduction in maize *Tripsacum* hybrids using maize RFLP markers. *Theor. Appl. Genet.* 90:1198-1203.
- Liu Z, Cook J, Melia-Hancock S, Guill K, Bottoms C, Garcia A, Ott O, Nelson R, Recker J, Balint-Kurti P, Larsson S, Lepak N, Buckler E, Trimble L, Tracy W, McMullen MD, Flint-Garcia SA (2016a). Expanding maize genetic resources with pre-domestication alleles: Maize-teosinte introgression populations. *Plant Genome* 9:1.
- Liu Z, Garcia A, McMullen MD, Flint-Garcia SA (2016b). Genetic analysis of kernel traits in maize-teosinte introgression populations. *G3* 6(8): 2523-2530.
- Loskutov IG (2008). On evolutionary pathway of *Avena* species. *Genet. Resour. Crop Evol.* 55:211-220.
- Mangelsdorf PC (1961). Introgression in Maize. *Euphytica* 10:157-168.
- Mano Y, Muraki M, Fujimori M, Takamizo T (2005). Varietal difference and genetic analysis of adventitious root formation at the soil surface during flooding in maize and teosinte seedlings. *Jpn. J. Crop Sci.* 74:41-46.
- Mano Y, Omori F (2007). Breeding for flooding tolerant maize using "teosinte" as a germplasm resource. *Plant Root* 1:17-21.
- Mano Y, Omori F, Takamizo T, Kindiger B, McK BR, Loaisiga CH, Takahashi H (2007). QTL mapping of root aerenchyma formation in seedlings of a maize × rare teosinte "*Zea nicaraguensis*" cross. *Plant Soil* 295:103-113.
- Masanori Y, Maud IT, Irie VB, Steve GS, Hector SV, John FD, Brandon SG, Michael DM (2005). A large-scale screen for artificial selection in maize identifies candidate agronomic loci for domestication and crop improvement. *Plant Cell* 17:2859-2872.
- Matsuoka Y, Takumi S (2007). Natural variation for fertile triploid F1 hybrid formation in allohexaploid wheat speciation. *Theor. Appl. Genet.* 115:509-518.
- Menkir A, Kling JG, Badu-Apraku B, Ibikunle O (2006). Registration of 26 tropical maize germplasm lines with resistance to *Striga hermonthica*. *Crop Sci.* 46:1007-1009.
- Miller JF, Seiler GJ (2003). Registration of five oilseed maintainer (HA 429-HA 433) sunflower germplasm lines. *Crop Sci.* 43:2313-2314.
- Moellenbeck DJ, Barry BD, Darrach LL (1995). *Tripsacum dactyloides* (Gramineae) seedlings for host plant resistance to the western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 88:1801-1803.
- Nevo E (2004). Evolution of genome dynamics under ecological stress. In: Parisi V, De Fonzo V, Alluffi-Pentini F (eds) *Dynamical genetics.* Research Signpost, Kerala, India, ISBN 81:7736-231-3.
- Nevo E, Chen G (2010). Drought and salt tolerances in wild relatives for wheat and barley improvement. *Plant Cell Environ.* 33:670-685.
- Nevo E, Korol AB, Beiles A, Fahima T (2002). Evolution of wild emmer and wheat improvement: population genetics, genetic resources, and genome organization of wheat's progenitor *Triticum dicoccoides*. Springer, Berlin, 364 p.
- Oliver RE, Cai X, Wang RRC, Xu SS, Friesen TL (2008). Resistance to tan spot and *Stagonospora nodorum* blotch derived from relatives of wheat. *Plant Dis.* 92:150-157.
- Olsen KM, Gross BL (2008). Detecting multiple origins of domesticated crops. *Proceedings of the National Academy of Sciences of the United States of America*, 105:13701-13702.
- Pásztor K, Borsos O (1990). Inheritance and chemical composition in inbred maize (*Zea mays* L.) × teosinte (*Zea mays* subsp. *mexicana* (Schröder) Iltis) hybrids. *Növénytermelés* 39:193-213.
- Peleg Z, Fahima T, Abbo S, Krugman T, Nevo E, Yakir D, Saranga Y (2005). Genetic diversity for drought resistance in wild emmer wheat and its ecogeographical associations. *Plant Cell Environ.* 28:176-191.
- Peleg Z, Fahima T, Saranga Y (2007). Drought resistance in wild emmer wheat: physiology, ecology, and genetics. *Isr. J. Plant Sci.* 55:289-296.
- Petersen G, Seberg O, Yde M, Berthelsen K (2006). Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the A, B, and D genomes of common wheat (*Triticum aestivum*). *Mol. Phylogenet. Evol.* 39:70-82.
- Pickering R, Ruge-Wehling B, Johnston PA, Schweizer G, Ackermann P, Wehling P (2006). The transfer of a gene conferring resistance to scald (*Rhynchosporium secalis*) from *Hordeum bulbosum* into *H. vulgare* chromosome 4HS. *Plant Breed.* 125:576-579.
- Price HJ, Dillon SL, Hodnett G, Rooney WL, Ross L, Johnston JS (2005). Genome evolution in the genus *Sorghum* (Poaceae). *Ann. Bot.* 95:219-227.
- Price HJ, Hodnett GL, Burson BL, Dillon SL, Stelly DM, Rooney WL (2006). Genome dependent interspecific hybridisation of *Sorghum bicolor* (Poaceae). *Crop Sci.* 46:2617-2622.
- Prischmann DA, Dashiell1 KE., Schneider DJ, Eubanks MW (2009).

- Evaluating *Tripsacum*-introgressed maize germplasm after infestation with western corn rootworms (*Coleoptera: Chrysomelidae*). *J. Appl. Entomol.* 133:10-20.
- Ramirez DA (1997). Gene introgression in Maize (*Zea mays* ssp *mays* L.). *Philipp. J. Crop Sci.* 22:51-63.
- Raskina O, Belyayev A, Nevo E (2002). Repetitive DNAs of wild emmer wheat (*Triticum dicoccoides*) and their relation to S-genome species: molecular cytogenetic analysis. *Genome* 45:391-401.
- Raskina O, Belyayev A, Nevo E (2004). Quantum speciation in *Aegilops*: molecular cytogenetic evidence from rDNA cluster variability in natural populations. *Proc. Natl. Acad. Sci. USA* 101:14818-14823.
- Ray JD, Kindiger B, Sinclair TR (1999). Introgressing root aerenchyma into maize. *Maydica* 44:113-117.
- Reeves RG, Bockholt AJ (1964). Modification and improvement of a maize inbred by crossing it with *Tripsacum*. *Crop Sci.* 4:7-10.
- Reeves RG, Mangelsdorf PC (1942). A proposed taxonomic change in the tribe Maydeae (family Gramineae). *Am. J. Bot.* 29:815-817.
- Rich PJ, Ejeta G (2008). Towards effective resistance to *Striga* in African maize. *Plant Signal. Behav.* 3:618-621.
- Risser PE, Birney EC, Blocker H, May S, Parton W, Weins J (1981). *The True Prairie Ecosystem*. Hutchinson Publishing Co., Stroudsburg, PA.
- Salina EA, Lim KY, Badaeva ED, Shcherban AB, Andrey B, Adonina IG, Amosova AV, Samatadze TE, Vatolina TY, Zoshchuk SA, Leitch AR (2006). Phylogenetic reconstruction of *Aegilops* section *Sitopsis* and the evolution of tandem repeats in the diploids and derived wheat polyploids. *Genome* 49:1023-1035.
- Savidan Y, Grimanelli D, Leblanc O (1995). Transferring apomixis from *Tripsacum* to maize: progress and challenges. In: Taba, S., Ed., *Maize Genetic Resources*. CIMMYT, Mexico, D.F. pp. 86-92.
- Sayadi Maazou A, Tu J, Qiu J, Liu Z (2016). Breeding for Drought Tolerance in Maize (*Zea mays* L.). *Am. J. Plant Sci.* 7:1858-1870.
- Sharma HC, Reddy BV, Dhillon MK, Venkateswaran K, Singh BU, Pampapathy G, Folkertsma RT, Hash CT, Sharma KK (2005). Host plant resistance to insects in sorghum: present status and need for future research. *J. Agric. Res.* 1:1-8.
- Stalker HT, Harlan JR, De Wet JMJ (1977). Cytology and morphology of maize-*Tripsacum* introgression. *Crop Sci.* 17:745-748.
- Talbert L, Doebley JF, Larson S, Chandler V (1990). *Tripsacum andersonii* is a natural hybrid involving *Zea* and *Tripsacum*: molecular evidence. *Am. J. Bot.* 77:722-726.
- Wang L, Xu C, Qu M, Zhang J (2008). Kernel amino acid composition and protein content of introgression lines from *Zea mays* ssp. *mexicana* into cultivated maize. *Cereal Sci.* 48:387-393.
- Wang XY, Gowik U, Tang HB, Bowers JE, Westhoff P, Paterson AH (2009). Comparative genomic analysis of C4 photosynthetic pathway evolution in grasses. *Genome Biol.* 10(6):R68.
- Watson L, Dallwitz MJ (1992). *The grass genera of the World*. CAB International, Oxon, P. 1038.
- Wei WH, Zhao WP, Song YC, Liu LH, Guo LQ, Gu MG (2003). Genomic in situ hybridization analysis for identification of introgressed segments in alloplasmic lines from *Zea mays* × *Zea diploperennis*. *Hereditas* 138:21-26.
- William HB, Michael DM, Brandon SG, John D (2007). Linkage Mapping of Domestication Loci in a Large Maize–Teosinte Backcross Resource. *Genetics* 177:1915-1928.
- Xu SS, Jin Y, Klindworth DL, Wang RRC, Cai X (2009). Evaluation and characterization of seedling resistance to stem rust Ug99 races in wheat-alien species derivatives. *Crop Sci.* 49:2167-2175.
- Zhou H, Deng Y, Li J (1997). Inbred selection from distant hybridization of maize (*Zea mays* L.) × teosinte (*Zea diploperennis* L.). *Acta Agron. Sin.* 23(3):333-337.

Full Length Research Paper

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

Abebaw Misganaw*, Sisay Kidane and Kalkidan Tesfu

Ethiopian Institute of Agricultural Research, National Agricultural Biotechnology Research Center, P. O. Box, 249/2003, Addis Ababa, Ethiopia.

Received 3 March, 2017; Accepted 1 April, 2017

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

*Corresponding author. E-mail: amisanaw68@yahoo.com.

from Vavilov's extensive collections and study. As cited by Abebe and Bjornstad (1997) Scheieman 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 agroecosystems (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 varieties 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, Thermoscientific). DNA samples were then diluted to a concentration of 20 ng/µL using ddH₂O 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 Mg²⁺, 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 (Pro, 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 (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 polyacrylamid 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	Agegehehu	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

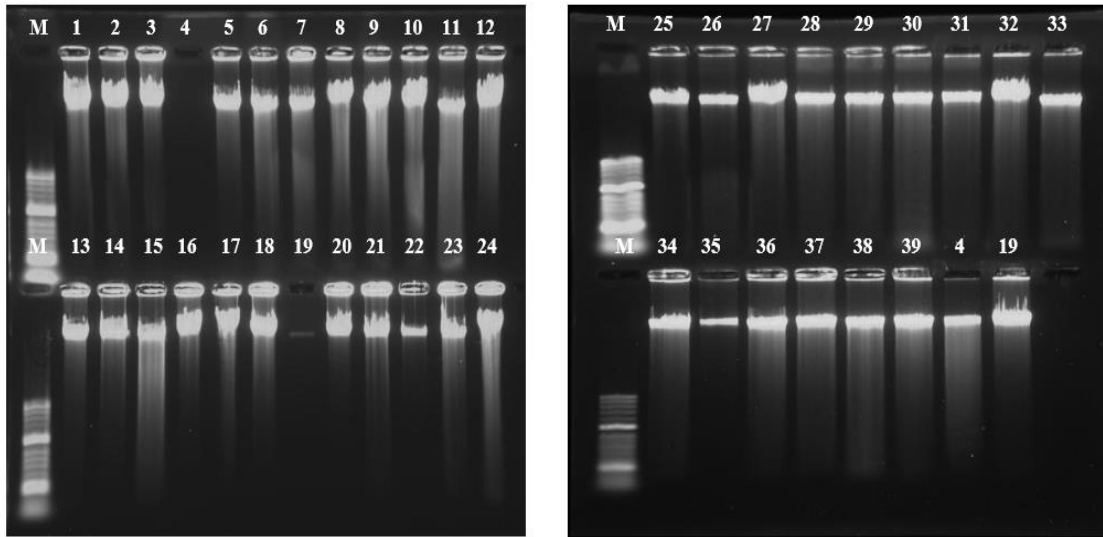


Figure 1. Genomic DNA extracted from the 39 barley cultivar (last two are repetitions) following CTAB method and loaded in 0.8% Agarose Gel concentration; M is size marker.

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	TTTGAATTATTCTGTGGGACC GGGATTCAATCAAGTATTCGGA	60	150
HVM36	2(2H)	(GA) ₁₃	TCCAGCCGACAATTTCTTG AGTACTCCGACACCACGTCC	55	114
HVLEU	7(5H)	(ATTT) ₄	TTGGAAGTGACAGCAATGGAG TGAAAGGCCCCACAAGATAG	60	166
Bmag0006	3(3H)	(AG) ₁₇	TTAAACCCCCCTCTAG 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 TACTCAGCAATGGCACTAG	58	135
Ebmag0793	2(2H)	(GT) ₁₃ (AG) ₃₆	ATATATCAGCTCGGTCTCTCA AACATAGTAGAGGCGTAGGTG	55	177
HVBKASI	2(2H)	(C) ₁₀ (A) ₁₁	ATTGGCGTGACCGATATTTATGTTCA CAAACCTGCAGCTAAGCAGGGGAACA	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	GTA CTATTCTTTGCTTGGGC 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.

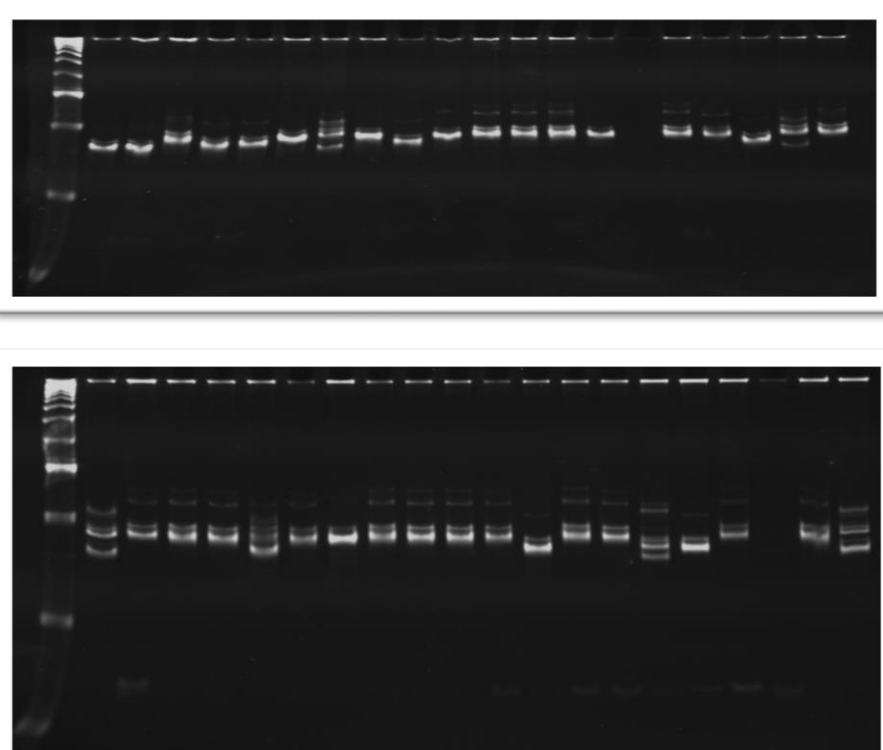


Figure 2. An example of marker profile of barley cultivars with the SSR marker HVM40. Where the first lane in both A and B is ladder size marker and the rest of the lanes represent the 39 barley genotypes except the 15th lane in A which is a gap.

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	39	
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	0.25	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	0.34	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	0.47	0.49	0.49	0.43	0.43	0.47	0.53	0.43	0.51	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	0.32	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	0.50	0.50	0.47	0.50	0.50	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	0.41	0.42	0.38	0.37	0.34	0.38	0.39	0.39	0.30	0.37														
28	0.46	0.36	0.44	0.39	0.41	0.32	0.44	0.46	0.44	0.40	0.42	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	0.44	0.46	0.44	0.36	0.38	0.43	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	0.17											
31	0.41	0.34	0.42	0.38	0.39	0.34	0.38	0.41	0.33	0.34	0.36	0.37	0.41	0.50	0.45	0.45	0.46	0.38	0.34	0.32	0.38	0.29	0.35	0.39	0.30	0.42	0.32	0.25	0.19	0.25										
32	0.45	0.39	0.34	0.43	0.40	0.26	0.38	0.41	0.43	0.43	0.45	0.38	0.41	0.42	0.49	0.41	0.46	0.46	0.35	0.29	0.35	0.26	0.32	0.39	0.26	0.38	0.29	0.31	0.26	0.26	0.33									
33	0.51	0.49	0.35	0.40	0.46	0.41	0.49	0.51	0.44	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	0.30	0.33	0.40	0.31	0.33	0.47	0.43	0.34	0.38	0.43	0.44	0.40	0.45	0.43	0.41	0.39	0.37	0.46	0.31	0.34	0.29	0.45	0.31	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	0.32	0.41	0.49	0.46	0.24	0.25	0.37	0.30	0.29	0.31	0.39	0.20	0.42	0.32	0.30	0.24	0.30	0.37	0.33	0.43	0.34						
36	0.42	0.39	0.34	0.34	0.41	0.44	0.43	0.46	0.43	0.39	0.46	0.42	0.50	0.43	0.50	0.50	0.47	0.34	0.31	0.38	0.48	0.30	0.36	0.40	0.31	0.38	0.46	0.35	0.30	0.35	0.38	0.34	0.35	0.44	0.24					
37	0.53	0.46	0.46	0.54	0.47	0.42	0.50	0.44	0.46	0.46	0.49	0.49	0.49	0.50	0.49	0.39	0.45	0.46	0.37	0.40	0.50	0.41	0.47	0.42	0.32	0.54	0.40	0.37	0.36	0.42	0.40	0.36	0.56	0.47	0.40	0.41				
38	0.47	0.45	0.40	0.44	0.42	0.41	0.40	0.38	0.44	0.36	0.43	0.43	0.39	0.43	0.47	0.39	0.40	0.26	0.39	0.41	0.35	0.42	0.46	0.31	0.49	0.39	0.26	0.31	0.36	0.34	0.35	0.50	0.41	0.29	0.30	0.21				
39	0.38	0.35	0.39	0.39	0.36	0.39	0.29	0.42	0.43	0.39	0.37	0.38	0.46	0.47	0.54	0.54	0.47	0.43	0.35	0.38	0.35	0.38	0.45	0.44	0.35	0.47	0.38	0.35	0.34	0.39	0.29	0.34	0.56	0.40	0.29	0.39	0.36	0.25		

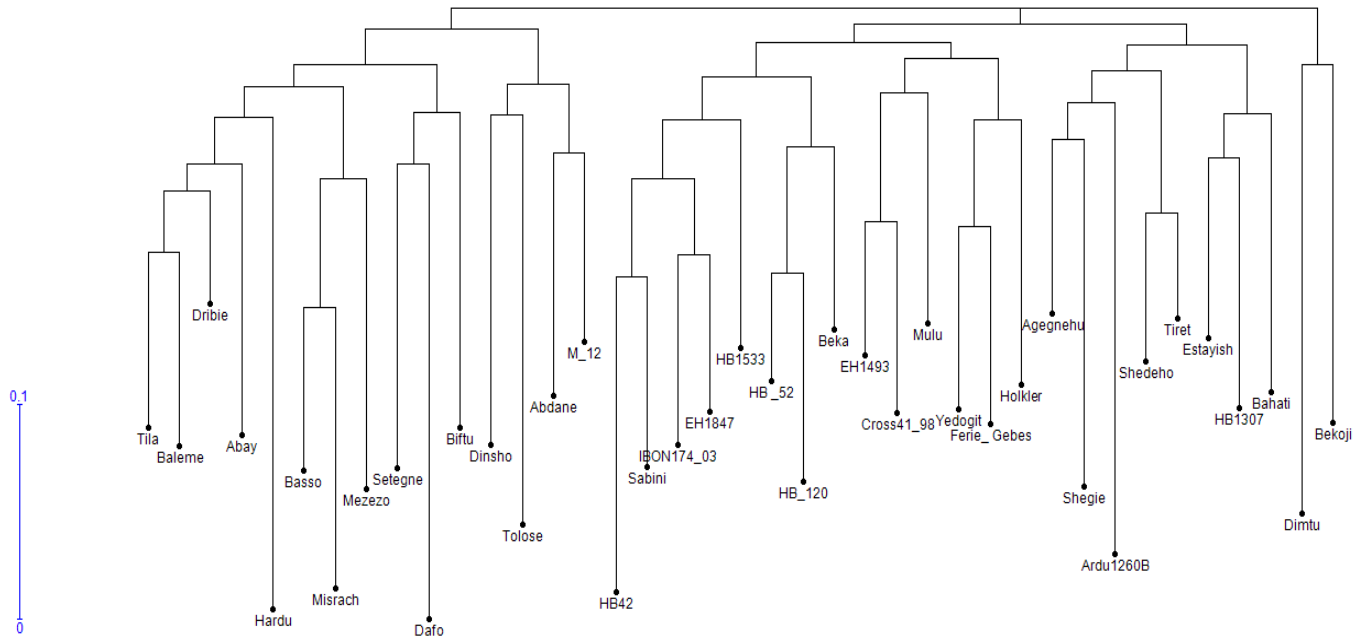


Figure 3. Dendrogram constructed using 22 SSR markers across 39 barley cultivars.

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.

ACKNOWLEDGEMENTS

The Ethiopian Institute of Agricultural Research and in particular, National Agricultural Biotechnology Research Center is gratefully acknowledged for providing finance and research facility to carry out the study. Thanks are also due to Dr. Zerihun Tadele for his kind support in providing the SSR markers and Dr. Birhane Lakew and Mr. Wondimu Fekadu from Barley Breeding Program of Holeta Agricultural Research Center for their kind support in providing planting materials. The authors are also grateful to Dr. Tesfaye Disasa and Dr. Dereje Worku, NABRC, for their help and guidance in statistical data analysis and manuscript preparation.

REFERENCES

- Abebe D, Bjornstad A (1997). Geographical, altitudinal and agro-ecological differentiation of isozymes and hordein genotypes of landraces of barleys from Ethiopia: implications for germplasm conservation. *Genet. Resour. Crop Evol.* 44:43-55.
- Chaabane R, El Felah M, Ben Salah H, Ben Naceur M, Abdelly C, Ramla D, Nada A, Saker M (2009). Molecular Characterization of Tunisian Barley (*Hordeum vulgare* L.) Genotypes using Microsatellites

- (SSRs) Markers. Euro. J. Sci. Res. 36(1):6-15.
- CSA-Central Statistics Agency (2012). Annual Statistics Bulletin. Addis Ababa, Ethiopia.
- Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus 12:13-15.
- Eticha F, Sinebo W, Gausgruber H (2010). On-farm Diversity and Characterization of Barley (*Hordeum vulgare* L.) Landraces in the Highlands of West Shewa, Ethiopia. Ethnobotan Res. Appl. 8:25-34.
- Feng ZY, Liu XJ, Zhang YZ, Ling HQ (2006). Genetic diversity analysis of Tibetan wild barley using SSR markers. Yi Chuan Xue Bao 33:917-928.
- Fernández M, Figueiras A, Benito C (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet. 104:845-851.
- Hadado TT, Rau D, Bitocchi E, Papa R (2010). Adaptation and diversity along an altitudinal gradient in Ethiopian barley (*Hordeum vulgare* L.) landraces revealed by molecular analysis. BMC Plant Biol. 10(1):121.
- Hayes PM, Castro A, Marquez-Cedillo L, Corey A, Henson C, Jones BI, Kling J, Mather D, Matus I, Rossi CSato K (2002). Genetic Diversity for Quantitatively Inherited Agronomic and Malting Quality Traits. In: Diversity Barley (Von Bothmer R, Knupfeer H, van Hintum T and Sato K, eds.). USDAARS. Chapter 10. 2003. pp. 147-169.
- Liu K, Muse SV (2005). PowerMarker: Integrated analysis environment for genetic markers data. Bioinformatics 21(9):2128-2129.
- Maroof MS, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994). Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc. Natl. Acad. Sci. U. S. A. 91:5466-5470.
- Matus IA, Hayes PM (2002). Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome 45:1095-1106.
- Meszaros K, Karsai I, Kuti C, Banyai J (2007). Efficiency of different marker systems for genotype fingerprinting and for genetic diversity studies in barley (*Hordeum vulgare* L.). S. Afr. J. Bot. 73(1):43-48.
- Mikel MA, Kolb FL (2008). Genetic diversity of contemporary North American barley. Crop Sci. 48:1399-1407.
- Nandakumar N, Singh AK, Sharma RK, Mohapatra T (2004). Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. Euphytica 136:257-264.
- Perrier X, Flori A, Bonnot F (2003). Methods for data analysis. In: Hamon P, Seguin M, Perrier X, Glaszmann JC (eds). Genetic diversity of cultivated tropical plants. Science Publishers, Montpellier, Inc. and CIRAD. pp. 31-63.
- Perrier X, Jacquemoud-Collet JP (2006). DARwin software. <http://darwin.cirad.fr/>
- Ramsay L, Macaulay M, Ivanissevich degli S, MacLean K, Cardle L, Fuller J, Edwards JK, Edwards, Tuveesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganai M, Powell W, Waugh R (2000). A Simple Sequence Repeat-Based Linkage Map of Barley. Genetics 156:1997-2005.
- Reif JC, Melchinger AE, Xia XC, Warburton ML (2003). Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. Crop Sci. 43:1275-1282.
- Smith JSC, Kresovich S, Hopkins MS, Mitchell SE, Dean RE, Woodman WL, Lee M, Porter K (2000). Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Aust. J. Crop Sci. 40:226-232.
- Struss D, Plieske J (1998). The use of microsatellite markers for detection of genetic diversity in barley populations. Theor. Appl. Genet. 97:308-315.
- Turuspekov Y, Nakamura K, Yoshikawa R, Tuberosa R (2001). Genetic diversity of Japanese barley cultivars based on SSR analysis. Breed. Sci. 51:215-218.
- Wang JM, Yang JM, Zhu JH, Jia QJ, Tao YZ (2010). Assessment of genetic diversity by simple sequence repeats markers among forty elite varieties in the germplasm for malting barley breeding. J. Zhejiang Univ. Sci. B 11(10):792-800.
- Yuanzheng Y, Angell C (2005). Clarifying the glass transition behaviour of water by comparison with hyper quenched inorganic glasses. Nature 427(6976):717-720.
- Zhang D, Ding Y (2007a). Genetic diversity of wild close relatives of barley in Tibet of China revealed by AFLP. Yi Chuan 29:725-730.
- Zhang DL, Gao HY, Li SP (2007b). Analysis of genetic diversity on beer barley varieties in China by SSR. Acta Agric. Boreali-Occidentalis Sin. 16:72-76.

Full Length Research Paper

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

Zerihun Getachew^{1*}, Girma Abera² and Sheleme Beyene²

¹Assosa Agricultural Research Center, Ethiopian Institute of Agriculture Research, Ethiopia.

²School of Plant and Horticultural Sciences, College of Agriculture, Hawassa University, Ethiopia.

Received 16 January, 2017; Accepted 2 February, 2017

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 seed quality protein of two soybean (*Glycine max* L.) varieties. The experiment consisted of four levels of S (0, 20, 30 and 40 kg ha⁻¹), three *Rhizobium* strains (MAR-1495, SB-6-1-A₂ 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 Belessa-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 Belessa-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 N₂ 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, N₂ 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 (Zelege et al., 2010). Due to low soil fertility status in the country grain legumes are generally grown in severe 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 (Zelege 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 (Habtemichael 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 (Habtemichael 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 (Habtemichael 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 NO₂ 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 (Habtemichael 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.

*Corresponding author. E-mail: zerihungetachew019@gmail.com.

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 silty 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-A₂, 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 (K₂SO₄) 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 K₂SO₄, 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 MgCl₂, 10 kg ha⁻¹ Zn as ZnCl₂, 1 kg ha⁻¹ Mo as Na₂MoO₂₄·2H₂O. 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 *Bradyrhizobium* 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 *Bradyrhizobium* 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 (HNO₃ and HClO₄), P was determined using spectrophotometric vanadium phosphomolybdate 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, S₀) (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 S₀ 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 ^{klm}
	MAR-1495		2782.5 ^{de}	4259.4 ^{defghi}	24.32 ^{hijkl}	37.89 ^{bcdef}
	SB-6-1-A ₂		2185.5 ^{fg hij}	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 ^{fg hij}	3840.0 ^{ghij}	24.75 ^{hijkl}	28.33 ^{ijk}
	Uninoc.	Wollo	1794.0 ^{kl}	4228.0 ^{defghi}	-28.81 ^{op}	23.40 ^{lm}
	MAR-1495		3134.0 ^{cd}	4716.0 ^{bcdef}	14.59 ^{klm}	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 ^{efghij}	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 ^{fg hij}	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 uninoculated 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-A₂ 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 (R₀S₀) 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 ^{mnp}	12.4 ^{klmn}	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 ^{klmn}	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 ^{ijklmno}	6.08 ^{opqr}	35 ^{cdefghij}	5.80 ^{bcddefgh}	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 ^{bcddef}	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 ^{bode}	15.8 ^{cdefg}
	SB-6-1-A ₂		155.57 ^g	28.88 ^{bcde}	66.46 ^{fgh}	10 ^{ghijklmn}	24.47 ^j	6.85 ^{abcdefg}	49.93 ^{defgh}	20.51 ^{bc}
	TAL-379		98.37 ^{klm}	13.3 ^{ijklmn}	61.58 ^{fghi}	12.44 ^{fghij}	24.87 ^j	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 ^{bcddef}	64.87 ^{fgh}	15.71 ^{defg}	49.89 ^{cd}	7.00 ^{abcdef}	58.17 ^{bcddefg}	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 ^{mnpq}	9.33 ^{lmn}	38.7 ^{mnpq}	5.61 ^{opqr}	30.9 ^{efghij}	5.32 ^{cdefgh}	58.55 ^{bcddefg}	9.33 ^{hij}
30	Uninoc.	B	54.0 ^{opqr}	7.42 ⁿ	37.73 ^{nop}	8.2 ^{ijklmnop}	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 ^{bcddef}	13.28 ^{fgh}
	TAL-379		105.0 ^{ijkl}	21.32 ^{efghi}	79.02 ^{cde}	18.68 ^{cde}	22.87 ^j	4.95 ^{defgh}	57.47 ^{bcddefg}	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.

	MAR-1495	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 ^{ghij}	44.5 ^{cdefgh}	4.44 ^{efgh}	44.62 ^{efgh}	4.75 ^{jk}
	Uninoc.	43.50 ^{qr}	5.94 ⁿ	30.47 ^p	7.00 ^{mnpq}	32.9 ^{defghij}	4.33 ^{fgh}	54.27 ^{defg}	15.0 ^{defg}
40	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 ^{ijklm}	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 ^{mnpq}	35 ^{cdefghij}	4.85 ^{defgh}	49.53 ^{defgh}	15.5 ^{defg}
	MAR-1495	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-A₂ 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-A₂. 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-A₂ (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 of S 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 N₂ 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 nitrogenase 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-1495 with 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-1495 was 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-A₂ 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.

REFERENCES

Agricultural Transformation Agency (ATA) (2013). Status of soil

- resources in Ethiopia and priorities for sustainable management. GSP for Eastern and Southern Africa. Mar 25-27, 2013, Nairobi, Kenya.
- Amanuel G, Kuhne RF, Tanner DG, Vlek PLG (2000) Biological N fixation in faba bean (*Vicia faba* L.) in the Ethiopian highlands as affected by P fertilization and inoculation. *Biol. Fertil. Soils* 32:353-359.
- Anandham R, Sridar R, Nalayin P, Poonguzhalia S, Madhaiyana M, Tongmin SAA (2007) Potential for plant growth promotion in groundnut (*Arachis hypogaea* L.) cv. ALR-2 by co-inoculation of sulfur-oxidizing bacteria and *Rhizobium*. *Microbiol. Res.* 162:139-153.
- Assosa Agricultural Research Center (AsARC) (2007) Assosa Agricultural Research Center Farming system survey. Assosa, Ethiopia.
- Cazzato E, Laudadio V, Stellacci AM, Ceci E, Tufarelli V (2012). Influence of S application on protein quality, fatty acid composition and nitrogen fixation of white lupin (*Lupinus albus* L.). *Eur. Food Res. Technol.* 235:963-969.
- Chianu JN, Nkonya EM, Mairura FS, Chianu JN Akinnifesi FK (2011). Biological nitrogen fixation and socioeconomic factors for legume production in sub-Saharan Africa: a review. *Agron. Sustain. Dev.* 31(1):139-154.
- Dhage SJ, Patil VD, Patange MJ (2014). Effect of various levels of phosphorus and sulphur on yield, plant nutrient content, uptake and availability of nutrients at harvest stages of soybean [*Glycine max*(L.)]. *Int. J. Curr. Microbiol. Appl. Sci.* 3(12):833-844.
- Fismes J, Vong, PC, Guckert A, Frossard E (2002). Influence of sulfur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus*L.) grown on a calcareous soil. *Eur. J. Agron.* 12:127-141.
- Graham PH, Tlusty B, Beyhaut E (2004). Inoculated legumes and revegetation / roadside plantings-Final Report. Department of Soil, Water, and Climate, University of Minnesota, Department of Transportation Research Services Section. pp. 1-53.
- Habtemichael KH, Singh BR, Aune JB (2007). Wheat response to N₂ fixed by faba bean (*Vicia faba*L.) as affected by sulfur fertilization and rhizobial inoculation in semi-arid Northern Ethiopia. *J. Plant Nutr. Soil Sci.* 170:412-418.
- Hussain K, Islam M, Siddique MT, Hayat R, Mohsan S (2011). Soybean growth and nitrogen fixation as affected by sulfur fertilization and inoculation under rain fed conditions in Pakistan. *Int. J. Agric. Biol.* 13:951-955.
- Islam M, Mohsan S, Ali S (2012). Effect of different phosphorus and sulfur levels on nitrogen fixation and uptake by chickpea (*Cicer arietinum*L.). *Agrociencia* 46:1-13.
- Jamal A, Fazli SI, Ahmad S, Abdin MZ, Yun SJ (2005). Effect of Sulfur and Nitrogen application on Growth Characteristics, Seed and Oil Yields of Soybean Cultivars. *Korean J. Crop Sci.* 50(5):340-345.
- Jamal A, Moon YS, Abdin MZ (2010). Enzyme activity assessment of peanut (*Arachis hypogaea*L.) under slow-release S fertilization. *Aust. J. Crop Sci.* 4(3):169-174.
- Jensen ES, Hauggaard NH (2003). How can increased use of biological N₂ fixation in agriculture benefit the environment? *Plant Soil* 252:177-186.
- Khalid R, Khan KS, Akram Z, Qureshi R, Gulfranz M (2011). Relationship of plant available S with soil characteristics, rainfall and yield levels of oilseed crops in Pothwar Pakistan. *Pak. J. Bot.* 43(6):2929-2935.
- Laswai HS, Mpagalile JJ, Silayo VCK, Ballegu WR (2005). Use of soybeans in food formulation in Tanzania. In. Myaka FA, Kirenga G, Malema B (Eds.). *Proceedings of the First National Soybean Stakeholders Workshop, 10th-11th November 2005, Morogoro, Tanzania.* Pp. 52-59.
- Motsara MR, Roy RN (2008). 'Guide to laboratory establishment for plant nutrient analysis'. Food and Agriculture Organization of the United Nations (FAO) Fertilizer and plant nutrition bulletin-19, Rome.
- Nigussie M, Girma A, Anchala C, Kirub A (Eds.) (2009). Improved technologies and resource management for Ethiopian Agriculture. A Training Manual. RCBP-MoARD, Addis Ababa, Ethiopia.
- Ravuri V, Hume DJ (1993) Soybean Straw Nitrogen Affected by Dinitrogen Fixation and Cultivars. *Agron. J.* 85:328-333.
- SAS Institute Inc. (2004) SAS/STAT User's Guide: Version 9.1th edition. SAS Institute Inc., Cary, North Carolina.
- Scherer HW, Pacyna S, Spoth K, Schulz M (2006). Sulphur supply to peas (*Pisum sativum* L.) influences symbiotic N₂ fixation. *Plant Soil Environ.* 52(2):72-77.
- Scherer HW, Pacyna S, Spoth K, Schulz M (2008). Low levels of ferredoxin, ATP and leghemoglobin contribute to limited N₂ fixation of peas (*Pisum sativum* L.) and alfalfa (*Medicago sativa* L.) under S deficiency conditions. *Biol. Fertil. Soils* 44:909-916.
- Sharma A, Sharma S (2014).Effect of nitrogen and S nutrition on yield parameters and protein composition in soybean [*Glycine max*(L.)]. *J. Appl. Nat. Sci.* 6(2):402-408.
- Solomon D, Lehmann J, Tekalign M, Fritzsche F, Zech W (2001). Sulfur fractions in particle-size separates of the sub-humid Ethiopian highlands as influenced by land use changes. *Geoderma* 102:41-59.
- Solomon T, Pant LM, Angaw T (2012). Effects of Inoculation by *Bradyrhizobium japonicum* Strains on Nodulation, Nitrogen Fixation, and Yield of Soybean (*Glycine max* L.) Varieties on Nitisols of Bako, Western Ethiopia. *ISRN Agronomy* (261475):1-8.
- Somasegaran P, Hoben HJ (1994). Handbook for Rhizobia-Methods in Legume-Rhizobium Technology. Springer-Verlag, Heidelberg, Germany. pp. 10-52.
- Tan Q, Zhang L, Grant J, Cooper P, Tegeder M (2010). Increased Phloem Transport of S-Methylmethionine Positively Affects Sulfur and Nitrogen Metabolism and Seed Development in Pea Plants. *Plant Physiol.* 15:1886-1896.
- Vanlauwe B, Descheemaeker K, Giller KE, Huisin J, Merckx R, Nziguheba G, Wendt J, Zingore S (2015). Integrated soil fertility management in sub-Saharan Africa: unravelling local adaptation. *Soil* 1:491-508.
- Zelege G, Getachew A, Dejene A, Shahidur R (2010). Fertilizer and Soil Fertility Potential in Ethiopia: Constraints and Opportunities for Enhancing the system. Working Paper, International Food Policy Research Institute. Pp. 1-20.
- Zuber H, Poignavent G, Le Signor C, Aime D, Vieren E, Tadla C, Lujan R, Maya Belghazi M, Labas V, Santoni AL, Wipf D, Julia BJ, Avice JA, Salon C, Karine GK (2013). Legume adaptation to sulfur deficiency revealed by comparing nutrient allocation and seed traits in *Medicago truncatula*. *Plant J.* 76:982-996.

Full Length Research Paper

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

Opio A.^{1*}, Jones B. M.², Kansiime F.³ and Otiti T.⁴

¹Department of Biology, Faculty of Science, Gulu University, P. O. Box 166, Gulu, Uganda.

²Department of Botany, School of Natural Sciences, Trinity College Dublin, Dublin, Ireland.

³Department of Environmental Management, College of Agricultural and Environmental Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda.

⁴Department of Physics, College of Natural Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda.

Received 19 April, 2016; Accepted 26 May, 2016

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

*Corresponding author. E-mail: alfonseopio@gmail.com.

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 (Murdiyarto 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 CO₂ 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 CO₂ 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 km² (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 m²), 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 $\text{Log}(y) = 2.832\text{Log}(x) - 0.5241$ 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.

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 (kPa °C⁻¹) as a function of altitude was used.

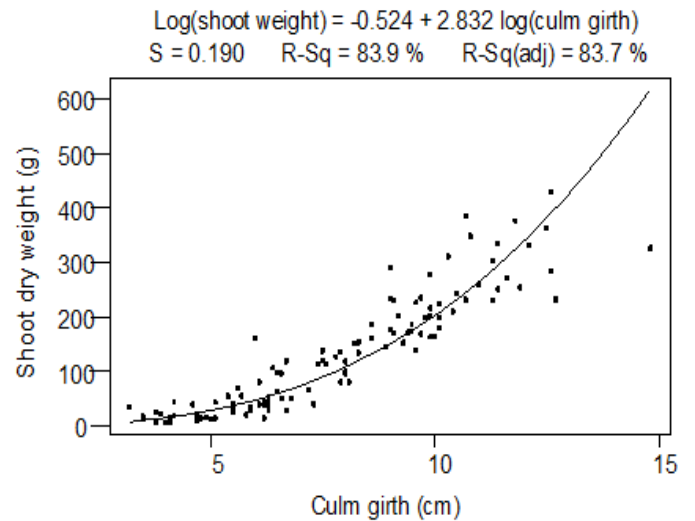


Figure 3. Relationship between culm-girth and dry weight of aerial shoots of *C. papyrus* in Lubigi wetland (n = 120) (Adopted from Opio et al., 2014).

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).

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

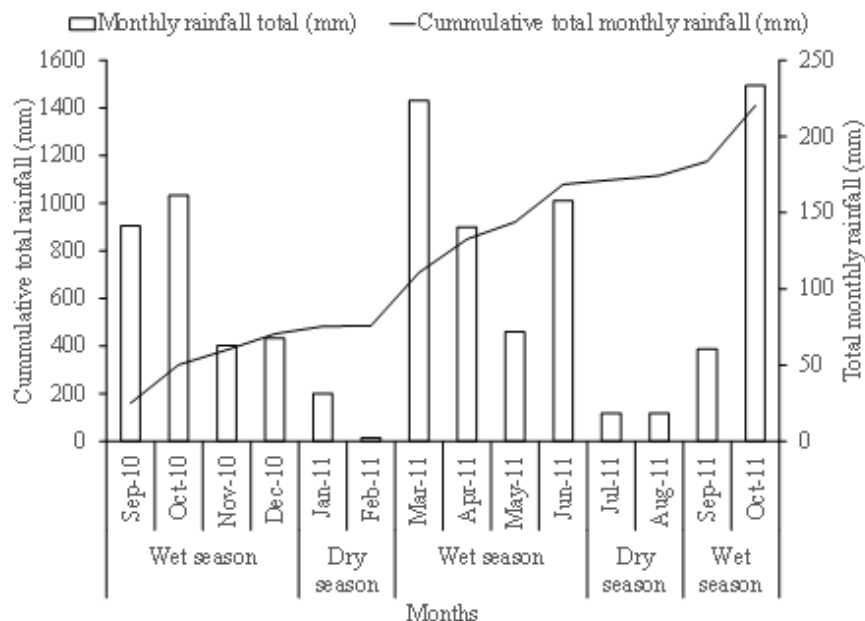


Figure 4. Total monthly rainfall and cumulative total rainfall distribution in Lubigi area.

$exp^{(0)} = 2.7183$ (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.

$$e_a = (RH_{mean}) / 100 \{ e^0(T_{max}) + e^0(T_{min}) \} / 2$$

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

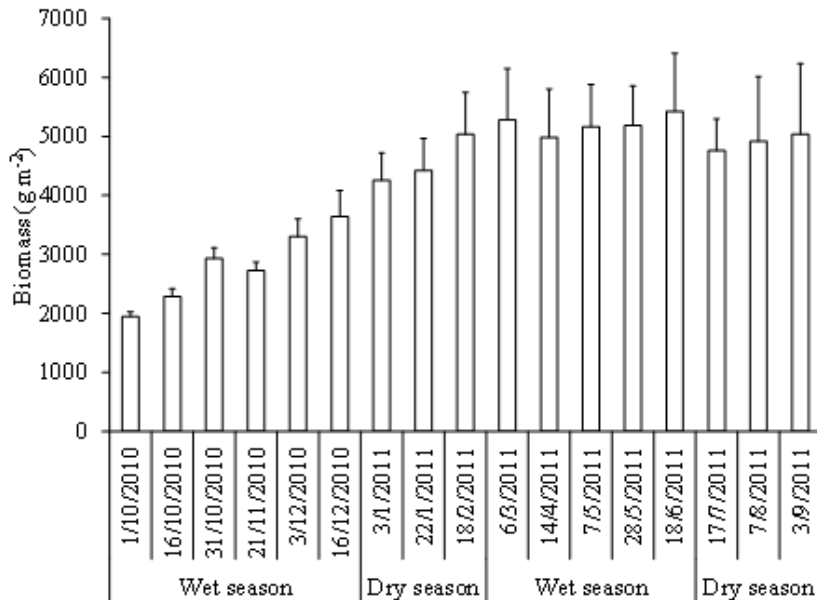


Figure 5. Mean aerial biomass of *C. papyrus* in Lubigi wetland. Error bars are standard error of means (n = 8).

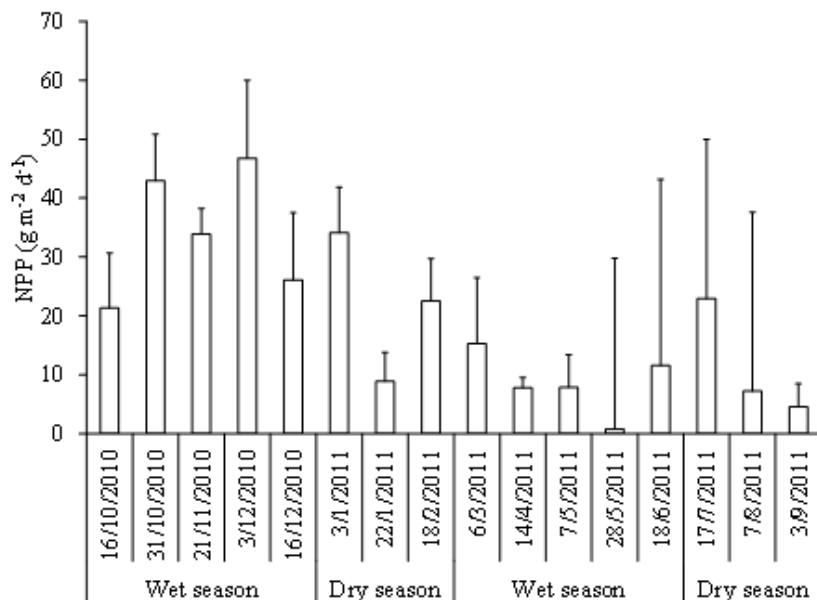


Figure 6. Mean primary productivity (NPP) of papyrus in Lubigi wetland. Error bars are standard errors of means (n = 8).

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, *Ipomoea purpurea*. The climber emerged in the wetland during the investigation period.

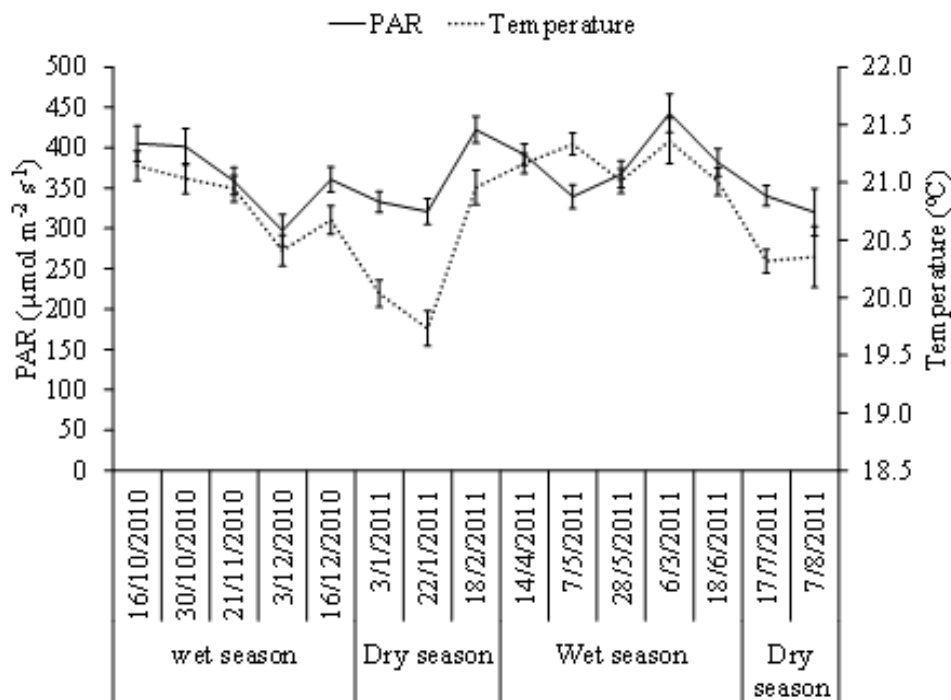


Figure 7. Mean temperature and photosynthetically active radiation (PAR) in papyrus surface canopy during the days of the year. Error bars are standard error of means.

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 $22.37 \text{ g m}^{-2} \text{ d}^{-1}$ 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 $2332.6 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ and 0 to $2031.6 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ 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:

$$\text{NPP (dry season)} = 0.34\text{PAR} + 1.24\text{VPD} - 1.39\text{Temperature} + 17.2$$

$$\text{NPP (wet season)} = 0.01\text{PAR} - 3.3\text{VPD} - 1.75\text{Temperature} + 69.7$$

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 $22.1 \text{ g m}^{-2} \text{ d}^{-1}$ (Saunders et al., 2007) and $21 \text{ g m}^{-2} \text{ d}^{-1}$ (Muthuri et al., 1989). The

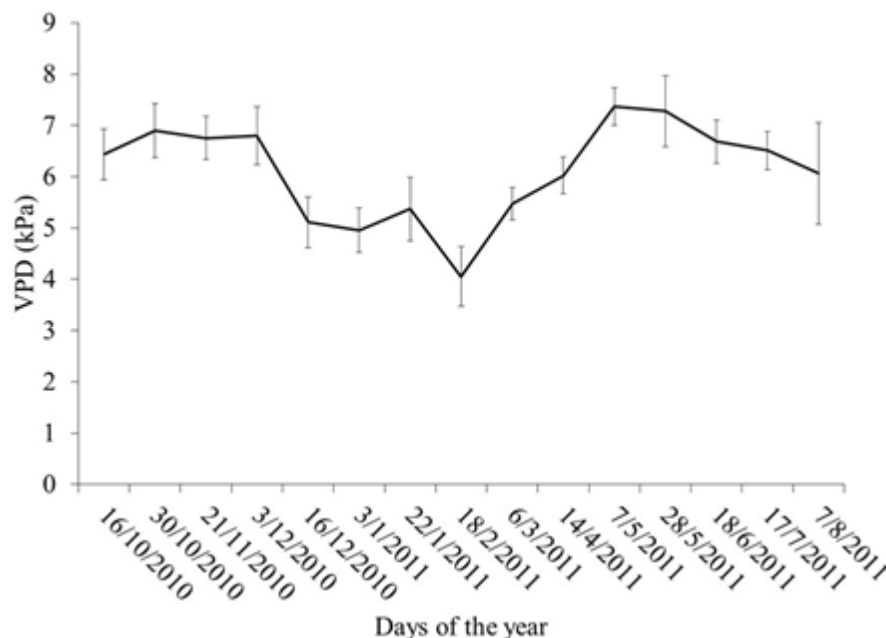


Figure 8. Mean vapour pressure deficit (VPD) in papyrus canopy during the days of the year. Error bars are standard errors of the means.

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 ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	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 $\text{g m}^{-2} \text{d}^{-1}$ and 3.12 to 74.65 $\text{g m}^{-2} \text{d}^{-1}$ during wet and dry seasons respectively were broader compared to 24.7 to 34.1 $\text{g m}^{-2} \text{d}^{-1}$ 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 (Turrall 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

The authors would like to thank Irish Aid and Trinity International Development Initiative (TIDI) for financial support. Thanks to Mr. Bright Twesigye and Mr. Joseph Kaketu Pale who assisted during the field work.

REFERENCES

- Bakari M, Takashi A, Yustina K, Elisamehe A (2007). Primary production in papyrus (*Cyperus papyrus* L.) of Rubondo Island, Lake Victoria, Tanzania. *Wetlands Ecol. Manage.* 15:269-275.
- Beadle CL (1993). Growth analysis. In: Hall D.O., Scurlock J.M.O., Bolh ar-Nordenkamp H. R., Leegood R. C., Long S. P. (eds), Photosynthesis and production in a changing environment: a field and laboratory manual. Chapman and Hall, London.
- Boar RR (2006). Responses of a fringing *Cyperus papyrus* L. swamp to changes in water level. *Aquat. Bot.* 84:85-92.
- Coletti JZ, Hinz C, Vogwill R, Hipsey MR (2012). Hydrological controls on carbon metabolism in wetlands. *Ecol. Model.* 249:3-18.
- FAO (1998). Crop evaporation - Guidelines for computing crop water requirement. FAO irrigation and drainage paper 56. ISSN 0254 - 5284. <http://www.fao.org/docrep/X0490E/X0490E00.htm>. Accessed on 15/02/2013.
- Fennessy MS, Cronk JK, Mitsch WJ (1994). Productivity and macrophyte community dynamics in created wetlands subjected to experimental hydrologic regimes. *Ecol. Eng.* 3:469-484.
- Hall DO, Long SP (1993). Photosynthesis and changing environment. In: Hall D.O., Scurlock J.M.O., Bolh ar-Nordenkamp H.R., Leegood R.C., Long S.P. (eds), Photosynthesis and production in a changing environment: a field and laboratory manual, Chapman and Hall, London.
- Hughes R, Hughes J (1992). A dictionary of African wetlands. IUCN, Gland/UNEP, Nairobi / WCMW, Cambridge.
- Humphries SW, Long SP (1995). WIMOVAC - a software package for modeling the dynamics of plant leaf and canopy photosynthesis. *Computer Applications. Biosciences* 11:361-371.
- Hunt R (1982). Plant growth analysis: second derivatives and compound second derivatives of spline plant growth curves. *Ann. Bot.* 50:317-328.
- Jones MB (1988). Photosynthetic responses of C₃ and C₄ wetland species in a tropical swamp. *J. Ecol.* 76:253-262.
- Jones MB (1993). Plant micro-climate. In: Hall D.O., Scurlock J.M.O., Bolh ar-Nordenkamp H.R., Leegood R.C., Long S.P. (eds), Photosynthesis and production in a changing environment: A field and laboratory manual. Chapman and Hall, London.
- Jones MB, Muthuri F (1997). Standing biomass and carbon distribution in a papyrus (*Cyperus papyrus* L.) swamp on Lake Naivasha, Kenya. *J. Trop. Ecol.* 13:347-356.
- Kansiime F, Nalubega M, van Bruggen JJA, Denny P (2003). The effect of wastewater discharge on biomass production and nutrient content of *Cyperus papyrus* and *Miscanthidium violaceum* in the Nakivubo wetland, Kampala, Uganda. *Water Sci. Technol.* 48:233-240.
- Kanyiginya V, Kansiime F, Kimwaga R, Mashauri DA (2010). Assessment of nutrient retention by Natete Wetland Kampala, Uganda. *Phys. Chem. Earth* 35:657-664.
- Leoni E, Altosor A, Paruelo JM (2009). Explaining patterns of primary production from individual level traits. *J. Veg. Sci.* 20:612-619.
- Macleane IMD, Hassall M, Boar RR, Lake IR (2006). Effects of disturbance and habitat loss on papyrus - dwelling passerines. *Biol. Conserv.* 131:349-358.
- Mitsch WJ, J rgensen SE (1989). Ecological engineering: an introduction to ecotechnology. Environmental Sciences and Technology. Volume 94. University of California, Wiley. 0471625590.
- Mugisha P, Kansiime F, Mucunguzi P, Kateyo E (2007). Wetland vegetation and nutrient retention in Nakivubo and Kirinya wetlands in the Lake Victoria basin of Uganda. *J. Phys. Chem. Earth* 32:1359-1365.
- Murdiyasarso D, Hergoualc K, Verchot LV (2010). Opportunities for reducing greenhouse gas emissions in tropical peatlands. *PNAS* 107(46):19655-19660.
- Muthuri FM, Jones MB, Imbamba SK (1989). Primary productivity of papyrus (*Cyperus papyrus*) in a tropical swamp; Lake Naivasha, Kenya. *Biomass* 18:1-14.
- Namakambo N (2000). Kampala. National wetlands programme. Wetlands inspection division. Litho Consult, Uganda.
- Ogallo LA (1989). The spatial and temporal patterns of the East African seasonal rainfall derived from principle component analysis. *Int. J. Climatol.* 9:145-167.
- Opio A, Jones BM, Kansiime F, Otiti T (2014). Growth and development of *Cyperus papyrus* in a tropical wetland. *Open J. Ecol.* 14:113-123.
- Oyugi D (2010). Lake Victoria basin. In: Chapman L. (Reviewer), Freshwater ecoregions of the world. Eco-region description. <http://www.feow.org>. Retrieved on 02/11/2012.
- Page SE, Rieley JO, Banks CJ (2011). Global and regional importance of the tropical peatland carbon pool. *Glob. Change Biol.* 17:798-818.

- Pospišil M, Pospišil A, Rastija M (2000). Effect of plant density and nitrogen rates upon the leaf area of sugar beet on seed yield and quality. *Eur. J. Agron.* 12:69-78.
- Saunders JM, Jones MB, Kansime F (2007). Carbon and water cycles in tropical papyrus wetlands. *Wetlands Ecol. Manage.* 15:489-498.
- Saunders JM, Kansime F, Jones MK (2012). Agricultural encroachment: implications for carbon sequestration in tropical African wetlands. *Glob. Change Biol.* 18:1321-1321.
- Schulze ED, Lange OL, Evenari M, Kappen L, Juschbom U (1975). The role of air humidity and temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert condition. *Oecologia* 19:303-314.
- Spigel RH, Coulter GW (1996). Comparison of hydrology and physical limnology of the East African Great Lakes: Tanganyika, Malawi, Victoria, Kivu and Turkana (with references to some North American Great Lakes). In: Johnson T. C., Odada E. O. (eds.), *The limnology, climatology, and paleoclimatology of the East African lakes*. Amsterdam, The Netherlands: Gordon and Breach Publishers.
- Thompson K, Shewry PR, Woolhouse HW (1979). Papyrus swamp development in Upemba: studies of population structure in *Cyperus papyrus* stands. *Bot. J. Linn. Soc.* 78:299-316.
- Turrall H, Burke J, Jean-Marc F (2011). Climate change, water and food security. Food and Agricultural Organization Water report. Viale delle Terme di Caracalla, 00153 Rome, Italy. ISBN 978-92-5-106795-6. <http://www.fao.org>. Accessed on 05/8/2013.
- Yin X, Nicholson SE, Mamoudou BB (2000). On diurnal cycle of cloudiness over Lake Victoria and its influence on evaporation from the Lake. *Hydrol. Sci.* 45(3):407-424.

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

Bokary Allaye Kelly^{1*} and Oumar Senou²¹Institut d'Economie Rurale (IER), Programme Ressources Forestières, CRRA-Sikasso, Mali.²Institut d'Economie Rurale (IER), Programme Ressources Forestières CRRA-Sotuba, Mali.

Received 24 February, 2017; Accepted 17 March, 2017

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

*Corresponding author. E-mail: bokarykelly@gmail.com. Tel. +223 66725407/75125022.

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.

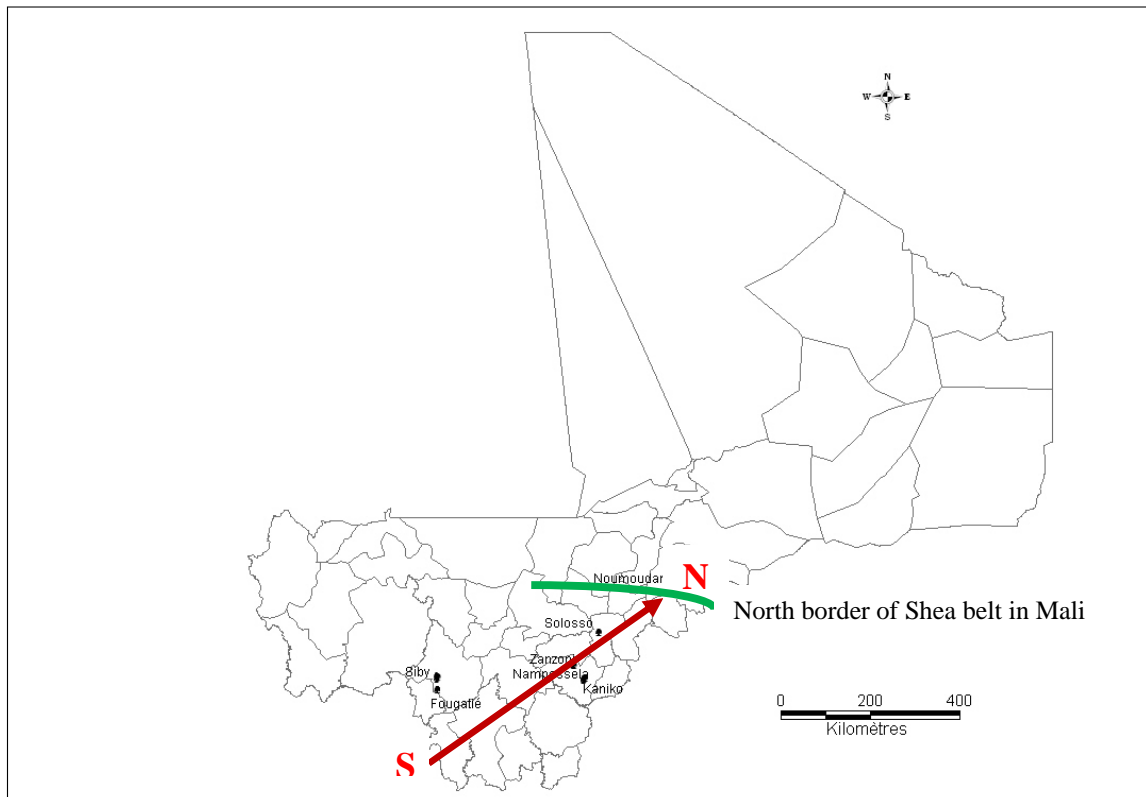
Experimental procedures

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 Fougatié 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



● = Study sites

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	Sudanian South (SS) (Isohyets 800-1100 mm)	12°22.686N; 008°20.258W
	Kaniko		12°19.115N; 005°22.007W
Sikasso	Nampossela	Sudanian North (SN) (Isohyets 500-800 mm)	12°19.879N; 005°20.456W
	Zanzoni		12°36.541N; 005°34.204W
Ségou	Solosso	Sahelian (Sa) (Isohyets 200-500 mm)	13°14.408N; 005°02.525W
Mopti	Noumoudama		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 $P \leq 0.001$, ** = highly significant at $P \leq 0.01$, * = significant at $P \leq 0.05$, 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 $P \leq 0.001$; 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 $P \leq 0.001$
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 $P \leq 0.001$, 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	9.99 ^{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 $P \leq 0.001$; 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	S	S	S	S
	P=0.000***	P=0.000***	P=0.000***	P=0.000***	P=0.000***

LSD means not significantly different at $P = 0.05$ were indicated by same character; *** = Very highly significant at $P \leq 0.001$; 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 (drier), 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. Ugeese 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.

ACKNOWLEDGEMENTS

The authors acknowledge the Projet d'Appui aux Filières Agricoles (PAFA) project funded by Agence Canadienne de Développement International (ACDI), for covering all financial expenses in conducting this study. They also acknowledge farmers (women and men) that participated efficiently in the field work and the villages' authorities and rural development services in the different study sites.

REFERENCES

- Aguzue OC, Akanji FT, Tafida MA, Kamal MJ (2013). Nutritional and some elemental composition of Shea (*Vitellaria paradoxa*) fruit pulp. Arch. Appl. Sci. Res. 5(3):63-65.
- Divine NB, Mohagir AM, Kapseu C, Mouloungui Z (2014). Production zones and systems, markets, benefits and constraints of Shea (*Vitellaria paradoxa* Gaertn) butter processing. OCL 21(2):D206.
- Djekota C, Diouf D, Sane S, Mbaye MS, Noba K (2014). Morphological characterization of Shea tree (*Vitellaria paradoxa* subsp. *paradoxa*) populations in the region of Mandoul in Chad. Int. J. Biodivers. Conserv. 6(2):184-193.
- Elias M (2013). Influence of agroforestry practices on the structure and spatiality of Shea trees (*Vitellaria paradoxa* C.F. Gaertn.) in central-west Burkina Faso. Agroforest. Syst. 87(1):203-216.
- Enaberue L, Okolo EC, Yusuf OA, Uwadiae E (2012). Density and phenotypic variation of *Vitellaria paradoxa* C. F. Gaertn. in Nigerian Guinea savannah. Ife J. Agric. 25:39-47.
- Fernande G, Honfo NA, Anita RL, Soumanou M, Van Boekel MAJS (2014). Nutritional Composition of Shea Products and Chemical Properties of Shea Butter: A Review. Crit. Rev. Food Sci. Nutr. 54(5):673-686.
- Gwali S, Nakabonge G, Okullo JBL, Eilu G, Nyeko P, Vuzi P (2012). Morphological variation among Shea tree (*Vitellaria paradoxa* subsp. *nilotica*) 'ethnovarieties' in Uganda. Gen. Res. and Crop Evol. 59:1883-1898.
- Kelly BA, Yossi H, Senou O (2011). Enrichissement des parcs à karité (*Vitellaria paradoxa* Gaertn. F.) en zone soudanienne au Mali: Identification et caractérisation agromorphologique des karités performants. Rapport d'Etude. Convention IER-CRRA/Sikasso - Programme d'Appui aux Filières Agricoles (PAFA).
- Nyarko G, Mahunu GK, Chimsah FA, Yidana JA, Abubakari AH, Abagale FK, Quainoo A, Poudyal M (2012). Leaf and fruit characteristics of Shea (*Vitellaria paradoxa*) in Northern Ghana. Res. Plant Biol. 2(3):38-45.
- Ugese FD, Baiyeri PK, Mbah BN (2010). Agroecological variation in the fruits and nuts of Shea butter tree (*Vitellaria paradoxa* C. F. Gaertn.) in Nigeria. Agroforest. Syst. 79:201-211.

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

Antonio Mendes de Oliveira Neto^{1*}, Jamil Constantin², Rubem Silvério de Oliveira Júnior², Naiara Guerra³, Eder Blainski², Hugo de Almeida Dan² and Diego Gonçalves Alonso²

¹Federal Institute Catarinense Campus of Rio do Sul, Rio do Sul, SC, Brazil.

²State University of Maringá, Maringá, PR, Brazil.

³Federal University of Santa Catarina Curitibanos Center, Curitibanos, SC, Brazil.

Received 24 February, 2017; Accepted 21 March, 2017

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 *Conyza* 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

*Corresponding author. E-mail: am.oliveiraneto@gmail.com. Tel: +55 (47) 3531 3733.

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 Oxisol eutrophic (Embrapa, 2013), clay texture. In Campina da Lagoa, main soil properties included 60.0 % clay, 27.0 % sand, pH H₂O = 6.0, and 28.58 g dm⁻³ of organic carbon (OC), while in Floresta, 61.0 % clay, 29.0 % sand, pH H₂O = 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

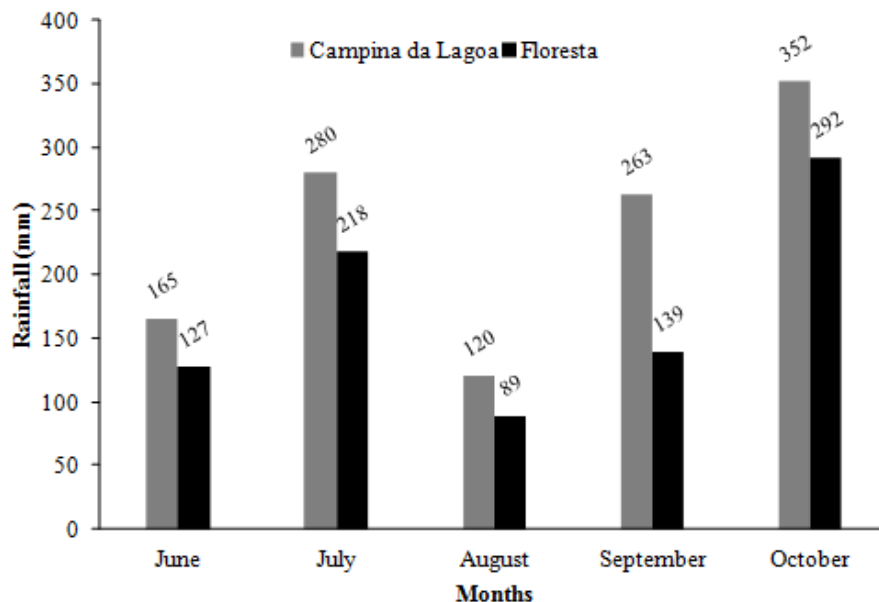


Figure 1. Monthly rainfall during the time of conducting experiments in two locations in Paraná.

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
<i>Conyza</i> spp. density (plants m ⁻²)	22	12
<i>Conyza</i> spp. average height (cm)	2	2
<i>Conyza</i> 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 CO₂ sprayer calibrated to deliver 200 L ha⁻¹ using 207 kPa as CO₂ 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 m² 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 $\geq 82\%$ 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 $\leq 5.3 \text{ plants m}^{-2}$.

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 $>15 \text{ cm}$. 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 $>16 \text{ cm}$ 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 letter 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 letter 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 diclosulam. 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.

ACKNOWLEDGEMENTS

The authors express his appreciation to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the grant awarded.

REFERENCES

- Armel GR, Richardson RJ, Wilson HP, Hines TE (2009). Strategies for control of horseweed (*Conyza canadensis*) and other winter annual weeds in no-till corn. *Weed Technol.* 23(3):379-383.
- Blainski E (2011). Herbicidas alternativos para o controle de *Conyza* spp. em diferentes estádios de desenvolvimento e monitoramento de fluxos de emergência em campo. 2011. Dissertation, University of Maringá, Maringá, Brazil, 71p.
- Constantin J, Oliveira Jr RS, Oliveira Neto AM, Blainski E (2012). Buva: fundamentos e recomendações para o manejo. Omnipax, Curitiba, 104p.
- Dan HA, Dan LGM, Barroso ALL, Procópio SO, Oliveira Jr, RS, Assis RL, Silva AG, Feldkircher C (2011). Atividade residual de pré-emergentes aplicados na cultura da soja sobre o milho cultivado em sucessão. *Planta Daninha* 29 (2):437-445.
- Embrapa. Centro Nacional de Pesquisa de Solos (2013). Sistema brasileiro de classificação de solos. Rio de Janeiro, Brazil. EMBRAPA. CNPS. 342p.
- Eubank TW, Poston DH, Nandula VK, Koger CH, Shaw DR, Reynolds DB (2008). Glyphosate-resistant horseweed (*Conyza canadensis*)

- control using glyphosate-, paraquat-, and glufosinate-based herbicide programs. *Weed Technol.* 22 (1):16-21.
- Kruger GR, Davis VM, Weller SC, Johnson WG (2008). Response and survival of rosette-stage horseweed (*Conyza canadensis*) after exposure to 2,4-D. *Weed Sci.* 56(5):748-752.
- Moreira MS, Melo MSC, Carvalho SJP, Nicolai M, Christoffoleti PJ (2010). Herbicidas alternativos para o controle de biótipos de *Conyza bonariensis* e *C. canadensis* resistentes ao herbicida glyphosate. *Planta Daninha* 28(1):167-175.
- Moreira MS, Nicolai M, Carvalho SJP, Christoffoleti PJ (2007). Resistência de *Conyza canadensis* e *C. bonariensis* ao herbicida glyphosate. *Planta Daninha* 25(1):157-164.
- Norsworthy JK, Mc Clelland M, Griffith GM (2009). *Conyza canadensis* (L.) Cronquist response to pre-plant application of residual herbicides in cotton (*Gossypium hirsutum* L.). *Crop Prot.* 28(1):62-67.
- Oliveira Neto AM, Constantin J, Oliveira Jr RS, Guerra N, Dan HA, Alonso DG, Blainski E, Santos G (2010). Estratégias de manejo de inverno e verão visando ao controle de *Conyza bonariensis* e *Bidens pilosa*. *Planta Daninha* 28(esp):1106-1117.
- Owen LN, Steckel LE, Koger CH, Main CL, Mueller TC (2009). Evaluation of spring and fall burndown application timings on control of glyphosate-resistant horseweed (*Conyza canadensis*) in no-till cotton. *Weed Technol.* 23(3):335-339.
- Santos G, Oliveira Jr RS, Constantin J, Francischini AC, Machado, MFPS, Mangolin CA, Nakalima JN (2014). *Conyza sumatrensis*: A new species resistant to glyphosate in the Americas. *Weed Biol. Manage.* 14: 106-114.
- Steckel LE, Craig CC, Hayes RM (2006). Glyphosate-resistant horseweed (*Conyza canadensis*) control with glufosinate prior to planting no-till cotton (*Gossypium hirsutum*). *Weed Technol.* 20(4):1047-1051.
- Trainer GD, Loux MM, Harrison SK, Regnier E (2005). Response of horseweed biotypes to foliar applications of cloransulam-methyl and glyphosate. *Weed Technol.* 19(2):231-236.
- Trezzi MM, Vidal RA, Patel F, Miotto Jr E, Debastiani F, Balbinot Jr AA, Moschen R (2015). Impact of *Conyza bonariensis* density and establishment period on soybean grain yield, yield components and economics threshold. *Weed Res.* 55:34-41.
- Vangessel MJ (2001). Glyphosate-resistant horseweed from Delaware. *Weed Sci.* 49:703-705.
- Vangessel MJ, Scott BA, Johnson QR, White-Hansen SE (2009). Influence of glyphosate-resistant horseweed (*Conyza canadensis*) growth stage on response to glyphosate applications. *Weed Technol.* 23(1):49-53.
- Vargas L, Bianchi MA, Rizzardi MA, Agostinetto D, Dal Magro T (2007). Buva (*Conyza bonariensis*) resistente ao glyphosate na região sul do Brasil. *Planta Daninha* 25(3):573-578.

Full Length Research Paper

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

Suzanne Sandrine Beack Bayengue^{1,2}, Mathieu Ndomou¹, Luther Martin Koanga Mogtomo¹, Rosalie Annie Ngono Ngane^{1*} and Clergé Tchiegang³

¹Laboratory of Biochemistry, Department of Biochemistry, University of Douala, P. O. Box 24157, Douala, Cameroon.

²Laboratory of Pharmacology and Toxicology, Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plant Studies, Ministry of Scientific Research and Innovation, P.O. Box. 13033 Yaoundé, Cameroon.

³Laboratory of Biochemistry and Food Technology, Department of Food and Nutrition Science, National School of Agro-Industrial Sciences (ENSAI), University of Ngaoundéré, P. O. Box 455, Ngaoundéré, Cameroon.

Received 31 January, 2017; Accepted 23 March, 2017

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 *Eremomastax 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

*Corresponding author. E-mail: angono@yahoo.com. Tel: (00237) 677 811 635.

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).

Distribution of traditional healers and mothers

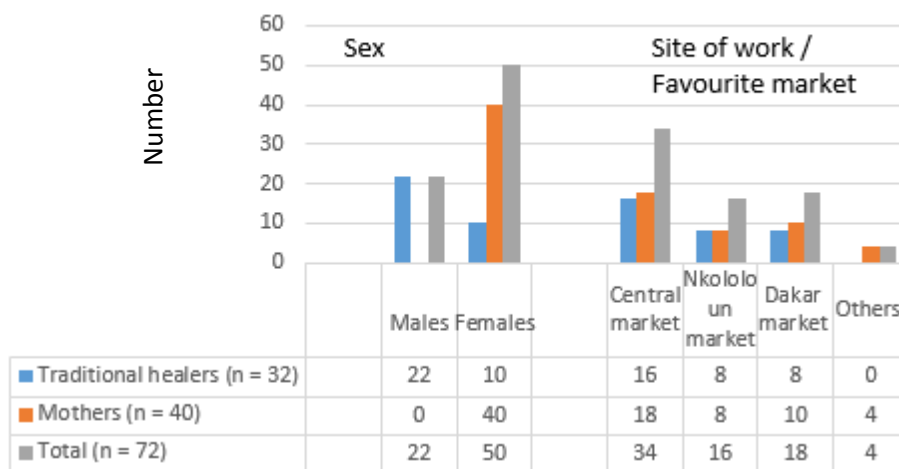


Figure 2. Distribution of traditional healers and mothers.

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 and 40 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 cordifolia* (*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. <i>Eremomastax speciosa</i> (Hochst.) Cufod Two sides, one side red	Acanthaceae	Shrub	Fresh leaves
2. <i>Alchornea cordifolia</i> (Schum. & Thonn.) Müll. Arg. Dry leaves Bobondji (Bassa)	Euphorbiaceae	Tree	Dry leaves
3. <i>Manihot esculenta</i> Crantz Kpwem (Ewondo)	Euphorbiaceae	Shrub	Fresh leaves
4. <i>Solanum lycopersicum</i> Linn. Tomatoe	Solanaceae	Herbs	Fresh fruit or box
5. <i>Tectona grandis</i> Linn. Ken-teck (Bassa)	Lamiaceae	Tree	Fresh bark
6. <i>Beta vulgaris</i> Linn. Beet	Amaranthaceae	Herbs	Root/whole plant/ Fresh leaves
7. <i>Dicliptera laxata</i> C.B. Clarke Green blood	Acanthaceae	Shrub	Fresh leaves
8. <i>Mimosa pudica</i> Linn. Mouko iyo (Duala)	Mimosaceae	Herbs	Whole plant
9. <i>Sacoglottis gabonensis</i> (Bail.) Urb Bidou (Beti)	Humiriaceae	Tree	Fresh bark
10. <i>Hibiscus sabdarifa</i> Linn. Foléré (Foufouldé)	Malvaceae	Herbs	Dry flowers
11. <i>Gnetum africanum</i> Welw. Eru, Okok	Gnetaceae	Creepers	Fresh leaves
12. <i>Ricinodendron heudelotii</i> (Bail.) Pierre ex Pax Djanssang (Bassa)	Euphorbiaceae	Tree	Fresh bark
13. <i>Eucalyptus sailgna</i> Smith	Myrtaceae	Tree	Fresh leaves
14. <i>Justicia ladanooides</i> Lam.	Acanthaceae	Shrub	Fresh leaves
15. <i>Anthocleista vogelli</i> Planch. Bopolopolo (Duala)	Loganiaceae	Tree	Fresh bark
16. <i>Entandrophragma candollei</i> Harms Koh-jock (Bassa)	Meliaceae	Tree	Fresh bark
17. <i>Bidens pilosa</i> Linn. Ward of poor	Asteraceae	Herb	Fresh leaves
18. <i>Cassia alata</i> Linn. Lonkana (Bassa)	Caesalpiniaceae	Shrub	Fresh leaves
19. <i>Moringa oleifera</i> Lam.	Moringaceae	Tree	Fresh/dry leaves or seed
20. <i>Amaranthus cruentus</i> Linn.	Amaranthaceae	Herb	Fresh leaves
21. <i>Ageratum conyzoides</i> Linn. Roi des herbes	Asteraceae	Herb	Fresh leaves
22. <i>Vernonia stellulifera</i> (Benth.) C. Jeffrey Ndolè sucré	Asteraceae	Herb	Fresh leaves
23. <i>Platostoma africanum</i> P. Beauv. Ewuda bie (Duala)	Lamiaceae	Herb	Fresh leaves and stern
24. <i>Alstonia boonei</i> De Wild. Kokmot (quinine)	Apocynaceae	Tree	Fresh bark
25. <i>Dacryodes edulis</i> (G. Don.) H. J. Lam. Prunier Sa'a (Beti)	Burseraceae	Tree	Fresh bark/leaves
26. <i>Ipomea</i> 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
<i>Alchornea cordifolia</i> (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	
<i>Anthocleista vogelli</i> Planch.	-	500 g	Decoction	30	01 full teaspoon, 3 times daily for 5 Days	-
<i>Beta vulgaris</i> 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.	
<i>Bidens pilosa</i> 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)
<i>Dicliptera laxata</i> C.B. Clarke	Two sides	300 g	Decoction	20-30	¼ or ½ Glass, 2 times daily for 5 to 7 Days	Antiviral (Meales)
	-	300 g	Infusion	15	¼ Glass, 3 times daily for 14 Days	
<i>Entandrophragma candolei</i> Harms	Tol (Bassa) and bark djanssang	01 kg	Decoction	20	Morning purge (150 ml), 01/Week for 02 Week	Anti-typhoid Anti-inflammatory Antibiotic
<i>Eremomastax speciosa</i> (Hochst.) Cufod	Bobondji	250 g	Decoction	10-15	¼ or ½ Glass*, 3 times daily for 7-14 Days	Antibiotic (diaper rash)
	Foléré					
	Beet					
	Fresh leaves grapefruit tree					
	-	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	
<i>Eucalyptus sailgna</i> Smith	-	300 g	Decoction	20-30	01 Full teaspoon I unlimited, 6 to 12 Days	Anti-typhoid Anti-jaundice
<i>Gnetum africanum</i> Welw.	Foléré	100 g	Infusion	10	½ glass, 3 times daily for 01 Days	Antibiotic
	01 small can of condensed milk	250 g	Crush			
<i>Hibiscus sabdariffa</i> Linn.	01 pineapple	150 g	Décoction	30	02 Full teaspoon, 2 times daily for 7 Days	-
<i>Justicia ladanooides</i> Lam.	-	300 g	Decoction	30	¼ Glass, 2 times daily for 7 Days	-
<i>Manihot esculenta</i> 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.

<i>Mimosa pudica</i> 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
<i>Ricinodendron heudelotii</i> (Bail.) Pierre ex Pax	-	01 kg	Infusion	10-15	¼ Glass, 3 times daily for 7 Days	Anti-splenomegaly
<i>Sacoglottis gabunensis</i> Bail.) Urb	Dry leaves of Bidou	01 kg	Decoction	15	Morning purge (150ml), 01/Week for 03 Week	Anti-malaria Anti-splenomegaly
<i>Solanum lycopersium</i> 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	-
<i>Tectona grandis</i> 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.

REFERENCES

- Abdullah K, Zlotking S, Parkin P, Grenier D (2011). L'anémie ferriprive des enfants. Programme canadien de surveillance pédiatrique. 6p.
- Adeleye A, Ezekiel AO, Smith S, Odusola O, Sobande J (2008). Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Mull.Arg., *Boerhavia diffusa* (L) and *Bridellia micranthal* (Hoscht) Baill. used in traditional medicine in Nigeria on *Helicobacter pylori* and four diarrhoeagenic bacterial pathogens. Afr. J. Biotechnol. 7(20):3761-3764.
- Agbor AM, Naidoo S (2015). Ethnomedicinal plants used by traditional healers to treat oral health problems in Cameroon. Evid. Based Complement. Alternat. Med. 2015:649832.
- Ami B, Yehuda S, Uri R, Edo S, Ron P, Shlomo A, Maya V, Yair A, Mona B (2012). Anaemia associated with acute infection in children. Isr. Med. Assoc. J. 14:484-487.
- Bhushan P (2005). WHO-CIPIH study nine on traditional medicine, Draft report. pp. 5-12.
- Bilanda DC, Dimo T, Mbatcham FW, Evéhé MS, Muluh J, Njifutié N (2004). Effet in vitro de de l'extrait au méthanol *Bidens pilosa* sur la chloroquino-résistance de *Plasmodium falciparum*. Pharm. Méd. Trad. Afr. 13:21-28.
- Dibong SD, Mpondo ME, Ngoye A, Kwin MF (2011). Plantes médicinales utilisées par les populations Bassa de la région de Douala au Cameroun. Int. J. Biol. Chem. Sci. 5(3):1105-1117.
- EDS-MICS (2011). Note de présentation des résultats préliminaires, Institut national de la statistique, Cameroun. 8 p.
- El Hioui M, Aboussaleh Y, Ahami AOT, Farsi M (2009). Contribution à l'étude de la prévalence de l'anémie chez les enfants préscolaires de la région de Kénitra, Maroc. Antropo 19:1-5.
- English M, Ahmed M, Ngando C, Berkley J, Ross A (2002). Blood transfusion for severe anaemia in children in a Kenyan hospital. Lancet 359:494-495.
- Kobto GK (2012). Conséquences de l'anémie maternelle sur le jeune enfant de la naissance à 18 mois de vie. Thèse en Santé publique et épidémiologie. Université Pierre et Marie Curie - Paris VI. pp. 26-30.
- Nayeem N, Karvekar MD (2011). Anti-microbial and anti-oxidant properties of the isolated compound from the methanolic extract from the leaves of *Tectona grandis*. J. Basic Clin. Pharm. 2(4):163-165.
- Oben EJ, Assi ES, Agbor AG, Musoro FD (2006). Effect of *Eremomastax speciosa* on experimental diarrhoea. Afr. J. Tradit. Complement. Altern. Med. 3(1):95-100.
- Okokon JE, Antia BS, Udoh AE, Akpan MM (2007). Antianaemic and Antimicrobial Activity of *Eremomastax speciosa*. J. Pharm. Toxicol. 2:196-199.
- OMS/UNICEF (2005). Focalisé sur l'anémie vers une approche intégrée pour un contrôle efficace de l'anémie. Déclaration conjointe. 2 p.

- UNICEF (2014). Les enfants en Afrique : statistiques clés sur la survie, la protection et le développement de l'enfance. Brochure UNICEF, Division politique et stratégie. 8 p.
- UNICEF (2015). Levels & Trends in Child Mortality. Report of a scientific group of UNICEF. 6 p.
- WHO (2009). WHO monographs on selected medicinal plants. Vol. 4. World Health Organization. 444 p.
- WHO (2015). The global prevalence of anemia in 2011. Report of a scientific group of WHO, Geneva. 43 p.
- World Meteorological Organization (2016). World Weather Information Service - Douala.



African Journal of Plant Science

Related Journals Published by Academic Journals

- *International Journal of Plant Physiology and Biochemistry*
- *Journal of Botany and Plant Pathology*
- *African Journal of Food Science*
- *International Journal of Biodiversity and Conservation*
- *Journal of Yeast and Fungal Research*

academicJournals