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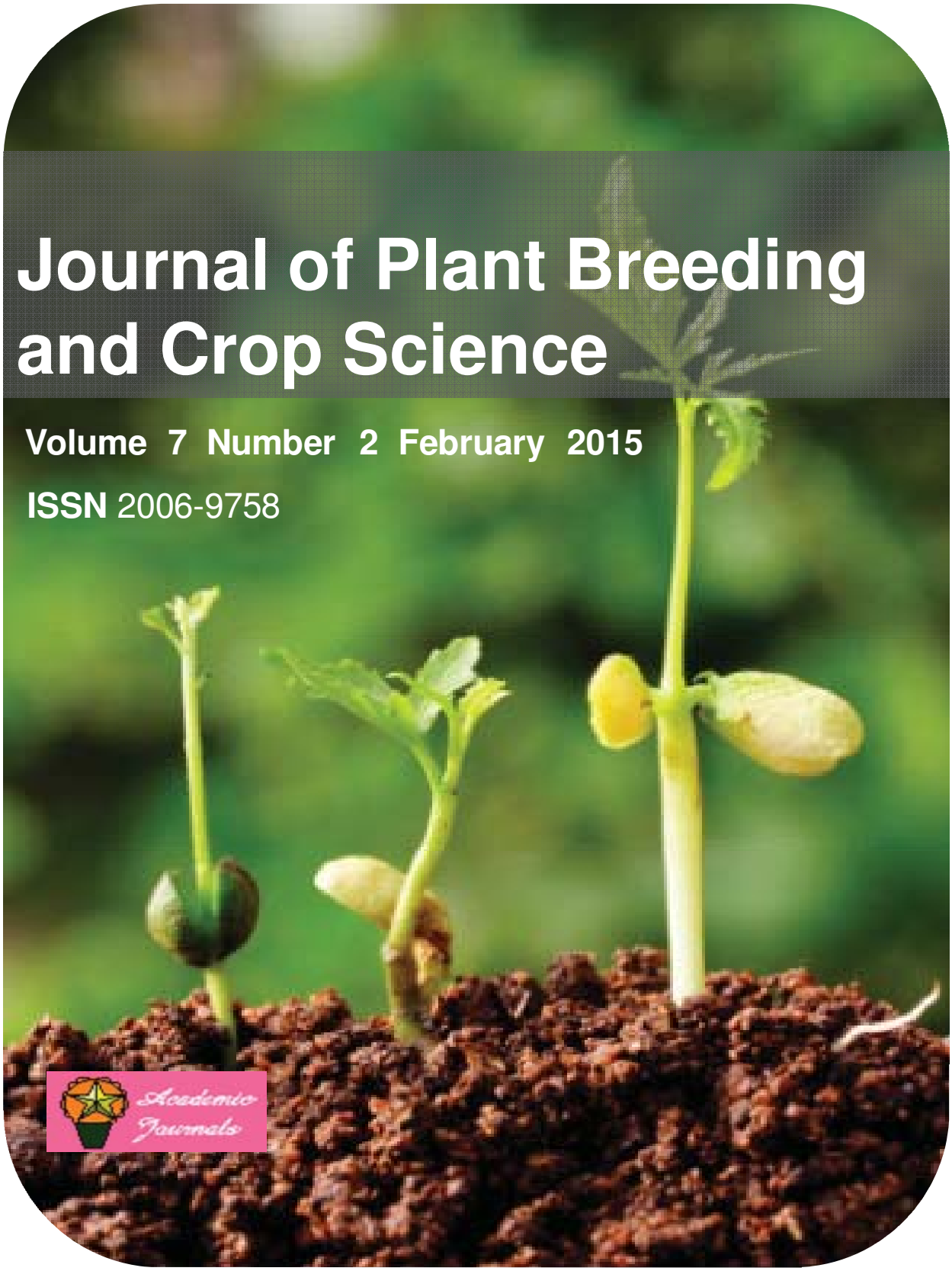

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Journal of Plant Breeding and Crop Science

Full Length Research Paper

Agro-morphological variability of shea populations (*Vitellaria paradoxa* CF Gaertn) in the Township of Bassila, Benin Republic

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Shea (*Vitellaria paradoxa* CF Gaertn) is a multipurpose forest tree species. This is one of the most integrated species in the cropping systems in the central and northern regions of Benin. It is also an important source of income for the population. Observations were made on some shea trees randomly selected in three vegetation types namely forests, fallows and farms. Data collection on quantitative and qualitative parameters such as length and width of leaves and fruits, tree diameter, fruit shape, crown shape, shape of leaf apex were made on 90 shea trees. The results show that the average density of shea trees per hectare varies (not significantly different) according to the three vegetation types (farms, fallow, and forests). The average diameter of tree trunk at man chest level was 37.35 ± 7.78 cm with a coefficient of variation (CV) within population was 21.09%. Variations between Shea populations in the study area were quite important and show the diversity of natural populations of the species. Leaves were predominantly oblong shape with an average length of 18.33 ± 3.21 cm and an average width of 6.92 cm ± 1.28; the leaf apex was in "pointed" shape. The fruits were dominantly oblong in the three vegetation types. The fruits had an average length of 4.49 ± 0.77 cm and a mean diameter of 3.56 ± 0.48 cm. The crown in shape of broom was observed so frequently in the different vegetation types. The longest and widest leaves and the longest and largest fruits were found in fields and fallows, while the smallest leaves and fruits were found in the forests.

Key words: Vegetation types, *Vitellaria paradoxa*, morphological diversity, Benin.

INTRODUCTION

Shea (*Vitellaria paradoxa* CF Gaertn) (Sapotaceae) is a tropical tree with multi-usage playing a socio- economic role in sub- Saharan Africa. In Africa, the area of distribution of shea tally with the area of Sudano-Sahelian climate. The species covers a geographical band from eastern Senegal to northwestern Uganda on a stretch of

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5000 km long, 500 to 700 km wide between 600 and 1500 mm isohyets (Hemsley, 1968; Boukoungou, 1987; Salle et al., 1991; Hall et al., 1996). Two subspecies have been identified presently (Djekota et al., 2014), *V. paradoxa* subsp. paradoxa is found in West and Central Africa (Salle et al., 1991; Fontaine et al., 2004; Sanou et al., 2005; Allal et al., 2008; Nyarko et al., 2012 while V. paradoxa subsp. nilotica is common in East Africa (Okullo et al., 2004; Byakagaba et al., 2011; Okiror et al., 2012; Gwali et al., 2011; Djekota et al., 2014). In the collection area in Benin, the species while enjoying the full protection of the forestry legislation is also saved by farmers during agricultural clearings. It is found in the form of natural population, and its predilection area goes from the region of Zou River (Atchérigbé latitude) to Malanville (Gbédji, 2003; Gnanglè, 2005), and is between 07°06' and 12°03' of north latitude. Its fruit plays socio-economic role of vital importance for the people of northern and central Benin. Almond obtained from the seed is transformed into shea butter and widely used in culinary cooking and strongly marketed in the sub-region and in the world. This oil is also used in the manufacture of cosmetics and pharmaceutical products. It is also used in traditional and social rituals such as marriages, funerals, coronations and rainmaking (Hall et al., 1996; Ferris et al., 2004; Moore, 2008; Gwali et al., 2012; Djekota et al., 2014). The wood of the shea butter tree is used for charcoal, mortar and pestle, furniture and construction, and the latex for glue making (Lovett and Haq, 2000a).

In term of agro-forestry importance in Benin, shea ranks second behind palm oils (Agbahungba et al., 2001). Shea is also the third Beninese largest export crop after cotton and cashew. Benin is the fourth shea almond producer in Africa after Mali, Burkina Faso and Nigeria (Dah-Dovonon and Gnanglè, 2006).

Despite the importance of this species, it is, however, subject to menace of all kinds especially related to high demography pressure, its low natural regeneration, the current practices of bushfires, these represent the leading cause of destruction of shea populations. The second cause of degradation of shea parks in Benin is their invasion by parasites, epiphytes and fungi. Promoting shea sector is a good lever to diversify agricultural production, fight against desertification and boost the development in the northern Benin. To this end, a better understanding of the variability within the gene pool of the species is necessary for its domestication, its conservation, continuation and improvement. Many studies have shown the existence of a high intra-specific variation (Chevalier 1943; Ruyssen 1957) among shea trees. Many authors have also shown a phenotypic variation and a correlation between its different physical properties Lovett and Haq (2004) in Ghana, Sanou et al. (2006) in Mali, Diarrassouba et al. (2007) and Djekota et al. (2014) in Chad. Therefore, a study on the shea diversity is necessary for a good conservation, good management and a selection of the best genetic resources of this tree species.

The objective of the present work was to study the agro- morphological variability among three vegetation types namely forests, fallows and farms for a better knowledge of individuals in their natural environments.

MATERIALS AND METHODS

Study area

The study was carried out in the northwest of Benin, in Bassila Township. The Township of Bassila is divided into four (4) districts and is covered on more than two fifths of its territory by forests. It extends over an area of 5,661 km^2 and is situated between 1º15' and 2º22' East longitude, 8º31' and 9º30' North latitude (Figure 1). There is a Sudano-Guinean climate and two (02) seasons in rotation. The rainy season starts from mid-April to mid-October and a dry season from mid-October to mid-April.

The average annual rainfall is between 1200 and 1300 mm and sometimes beyond 1500 mm in forest ecosystems (ASECNA, 2008).

The annual average temperature varies between 26 and 27ºC. Minimum temperatures of 17ºC was recorded in December-January and maximum of 40ºC in March-April (ASECNA, 2008).

Selection of villages

Six villages (Figure 1) were selected based on the following criteria: easy access to villages, the inhabitants of these villages should be part of one of the three major ethnic groups (Nago, Ani or Kotokoli), the inhabitants who participated in the workshop training organized by the Project for Conservation and Management of Natural Resources (ProCGRN) on improved techniques for collecting, processing and packaging of nuts and almond Shea and on butter manufacturing. It is about of village select by district: Bassila (Kikélé ; Adjiro); Manigri (Manigri-akanni); Pénéssoulou (Pénelan ; Nagayilé ; Kodowari) (Figure 1).

Experimental design

In each of the six selected villages, the same vegetation types were also selected (fallow, farms and forests). In each village and within each vegetation type, a plot of 1000 m^2 (50 m × 20 m) was delimited so a total of 18 plots. The geographical coordinates of the each plot was taken using a Geographical Positioning System apparatus (Garmin). Within each plot, five fruiting trees were randomly selected, then for the 18 plots a total of 90 Shea trees were selected. On each of the five trees, the length and width of 10 adult leaves were measured. Leaves and fruits were collected from the four (04) corners of the tree (North, South, East, and West). The same thing was done for the length and the diameter of 10 ripe fruits and the diameter of trunk up to a man chest, 1.30 m (DBH). Observations were noted on a morphological characterization of the tree and the describers analyzed.

Plants materials

The plant material consists of shea trees randomly selected in three vegetation types namely: farms, forests and fallows and in six villages. The selected trees were mature, at reproductive stage. Although, the sampling was randomly performed considering trees

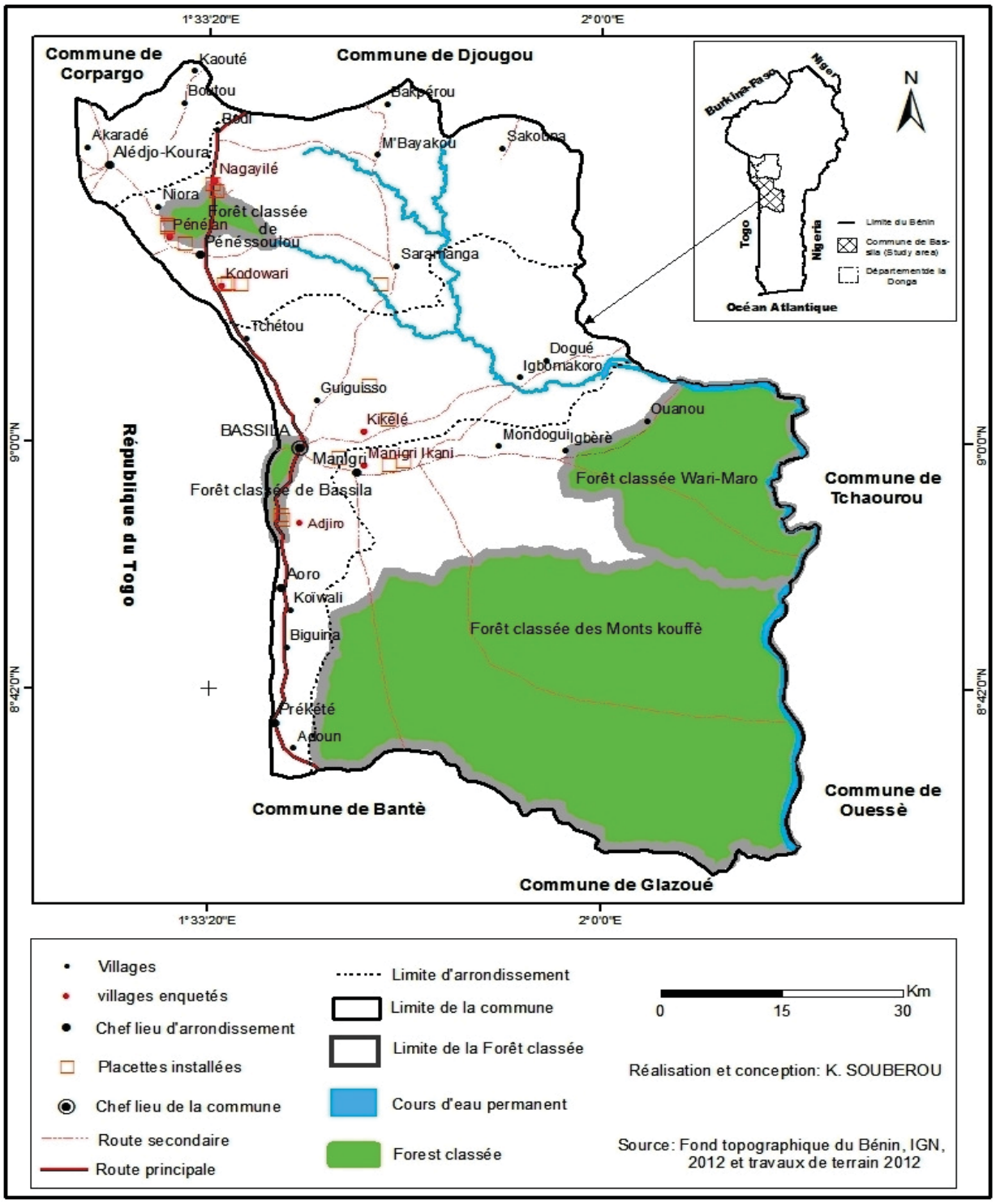

Figure 1. Geographical and Administrative Map of Bassila Township showing plots installed in the vegetation types.

that were spaced at least 10 m from each other to avoid the mixture of fallen fruits from two (02) different shea trees. For qualitative values, the following parameters were considered: the color of the bark, the shape of the crown, foliage density, the shape of the branches on the tree, the leaf shape, the shape of fruit, fruit type, and the appearance of the trunk.

Statistical analysis

The data collected at each site were encoded and saved in Microsoft Excel 2007 software. For quantitative data, the analysis of variance was carried out using SPSS (Statistical Package for the Social Sciences) for Window 16.0 and significant differences

Table 1. Average density of shea trees per hectare and per vegetation type.

Vegetation types	Farm	Forest	Fallow
Average density (shea tree/ha)	7.333	6.0	6.667

Table 2. Mean diameter of trees in different vegetation types.

Formations variables	Population			
	Farms	Forests	Fallows	Average inter-population
Average population (cm)	38.09[a]	38.19[a]	37.15[a]	37.35
Standard deviation	7.31	8.76	7.28	7.78
CV (%)	19.19	22.75	19.59	21.09

between means were detected using Newman-Keuls test. For leaf and fruit variables which conditions of normality and equality of variances were not satisfied, those variables are transformed and the test of Krsuskal-Wallis was used (a non-parametric alternative test analysis of variances) to separate averages at P= 0.05.

The classes of variations proposed and tested by Ouédraogo (1995) and Kouyaté (2005) in their study of West African populations of *P. biglobosa* composed of 1.663 individuals from five countries (Senegal, Mali, Burkina Faso, Niger and Chad) and on ethno-botanical aspects of the morphological, biochemical and phenological variability of *Detarium microcarpum,* were used to evaluate intra-and inter-population variation. The scale proposed by these authors is as follows:

1. Low variation (CV = 0 -10%)
2. Average variation (CV =10 -15%)
3. Moderate variation (CV=15 - 44%)
4. High variation (CV > 44%)

RESULTS

Inventory of Shea trees per hectare

Table 1 shows the results of the counting of the average density of shea trees per hectare and per vegetation type.

The analysis of Table 1 shows that the number of shea tree per vegetation type and per hectare was at least of 6 trees. These results show that the variability of density according to the three (03) vegetation types is not significantly. The density of shea trees in the farms, forests and fallows is almost the same. The highest (not significantly different) densities were found in the farms and forests while they were low in the fallows.

Morphological characterization of trees

Diameter of vegetation types

The average diameter of trees in different vegetation types is presented in Table 2. The analysis of Table 2 shows that the average diameter of shea trees in the farms and forests was almost identical that is these trees had almost the same size. The analysis of variance shows that there is no significant difference between the mean diameters of the three vegetation types. The trees of both vegetation types (farms and forests) show an average diameter greater than that of fallow 0.94 cm and 1.04 cm respectively.

The inter-population variation of trunk diameter was large enough for the CV (21.09) was between 15 and 44%.

Leaf size

All quantitative parameters measured on the leaf including: leaf length (LL) and leaf width (LW) were subjected for normality. The probability (P = 0.010) associated with this test was less than 0.05 therefore, the data were not normal. The main condition for using the test of variance analysis was not verified. The non-parametric test of Kruskal-Wallis was used in this case. The analysis of this test showed significant differences for the two quantitative parameters of the leaves (P = 0.001). The probability was less than 0.05, therefore, the median of length and width of leaf vary significantly between the three (03) vegetation types (Table 3). The leaves were longer in the farms than in the forests and fallows. The intra-and inter-populations for the length and width of leaves were large enough for the coefficients of variation were between 15 and 44%.

Fruits

Table 4 shows that the length and diameter of fruits vary according to vegetation types. Its value decreases from the farms to the forests through the fallows. The average length for inter-population of fruit observed was 4.49 cm. The longest fruits (4.69 cm) and the largest fruits (3.73 cm) were observed in the farms, while the smallest fruits were recorded in forests. The test of Kruskal-Wallis

Table 3. Average Length and width of leaves in different vegetation types.

Traits	Populations				
	Formations variables	**Farms**	**Forests**	**Fallows**	**Average inter population**
Leaf length	Average population (cm)	19.4[a]	18.35[b]	17.26[b]	18.33
	St. deviation	2.91	3.24	3.5	3.21
	CV (%)	15.02	17.64	20.31	17.65
Leaf width	Average population (cm)	7.2	6.63	6.95	6.92
	St. deviation	1.11	1.46	1.29	1.28
	CV (%)	15.45	22.11	18.65	18.73

Table 4. The average length and diameter of fruits from the different vegetation formation.

Traits	Formations population				
	Variability	**Farms**	**Forests**	**Fallows**	**Average inter population**
Fruit length	Average (cm)	4.69[a]	4.27[b]	4.52[b]	4.49
	St. deviation	0.96	0.61	0.74	0.77
	CV (%)	20.56	14.45	16.51	17.17
Fruit diameter	Average (cm)	3.73	3.4	3.57	3.56
	St. deviation	0.48	0.52	0.44	0.48
	CV (%)	12.99	15.42	12.37	13.59

showed a highly significant difference for all quantitative parameters of fruits (P = 0.001). The probability associated with the test was less than 0.05. Variations intra- and inter-populations for the length of the fruit were quite important because the coefficient of variation was between 15 and 44%. On the other hand, these intra-and inter population variations for diameter of the fruit were average because its coefficient of variation was between 10 and 15%.

Qualitative parameters

The color of the bark of trees sampled varies from black to light gray through the dark gray. The frequency of the black color of the bark was in increasing proportion from the farm (30%) to the forest (46.67%) and other colors (dark gray and light gray (ash) were in variable frequency within the three (03) vegetation types (Figure 2).

The trunk all the shea trees were rough in appearance. The shea trees studied had a crown in shape of a ball, broom, elliptical, or other (Figure 3). The broom shape (34.33%) was frequent in the three vegetation types. The ball shape was frequent in fallows and farms. The other forms of the crown were found in the forests.

The foliage density was average for almost the tree observed with opposite branches compared to the whorled branches in the forests and fallows.

The different shape of leaves observed (Figure 4) within the vegetation types were oblong (41 .66%), Elliptical (27.34%) and Oboval (31%). The leaves of the tree studied had almost oblong form with an apex in pointed shape (93%).

The regular fruit shape in the three (3) vegetation types (farms, forests and fallows) was oblong shape (68,33%) followed by spherical form and other forms (ovoid, elliptical (Figure 5) but in low percentage (31.67%). These different shapes vary from one village to another and from a vegetation type to another.

Correlation between leaf and fruit descriptors in the different vegetation types

The correlation values between quantitative parameters of fruits and leaves are presented in Table 5. The analysis of Table 5 shows that there was positive and significant correlation (r) between the length of fruits and fruit diameter in the farms (r = 0.579), forests (r = 0.145) and fallows (r = 0.503) as well as for the length and width of leaves (r = 0.157) in the farms. Similarly, there was negative and significant correlations between leaf length and fruit length in fallows (r = -0.189), leaf length and diameter of fruits in the farms (r = -0.176) as in the fallows (r = -0.186).

DISCUSSION

The study of agro- morphological characterization of

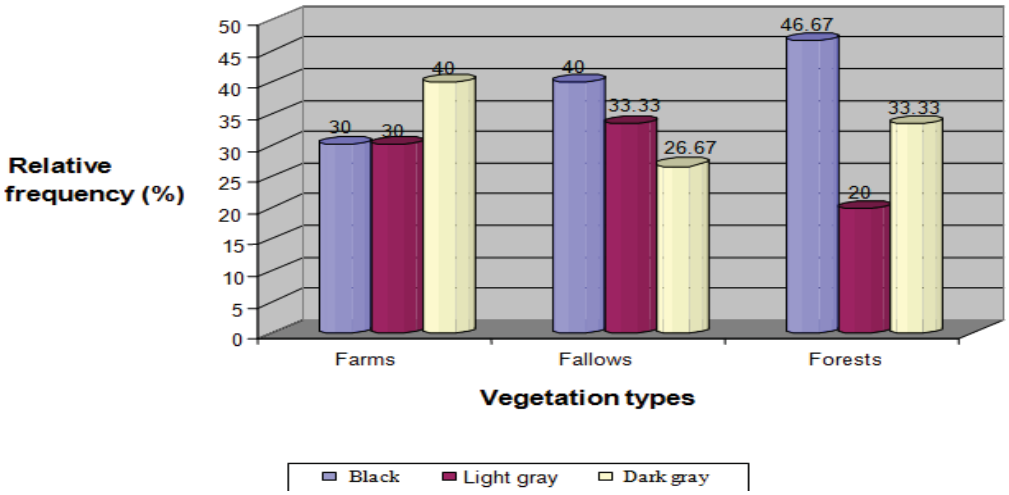

Figure 2. The color of the bark of trees per vegetation type.

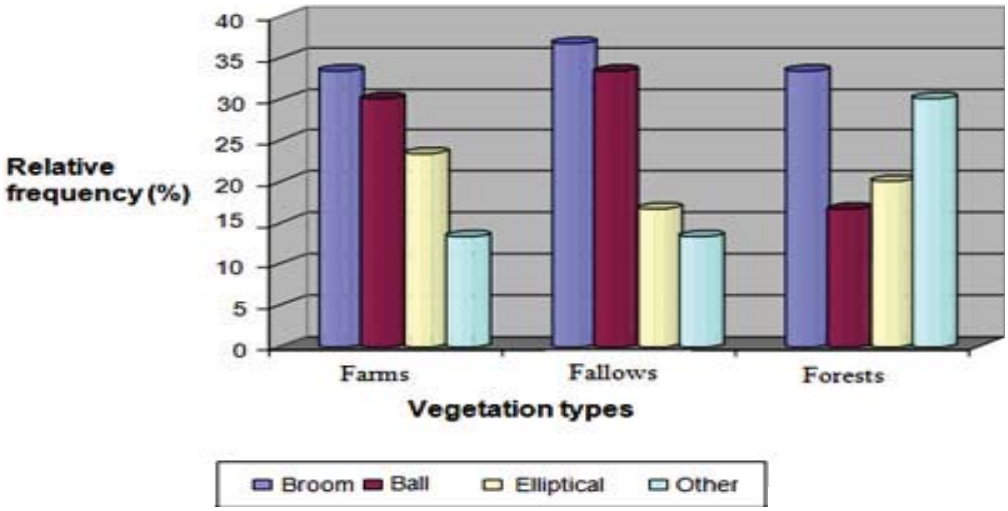

Figure 3. The color of the crown of shea trees per vegetation type.

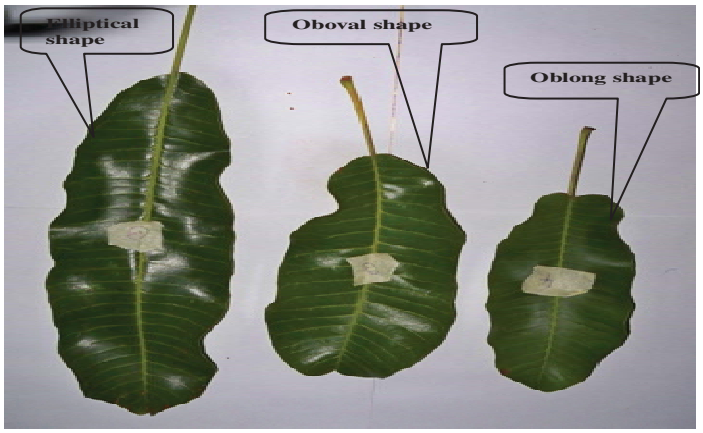

Figure 4. Leaf shape of *V. paradoxa* collected.

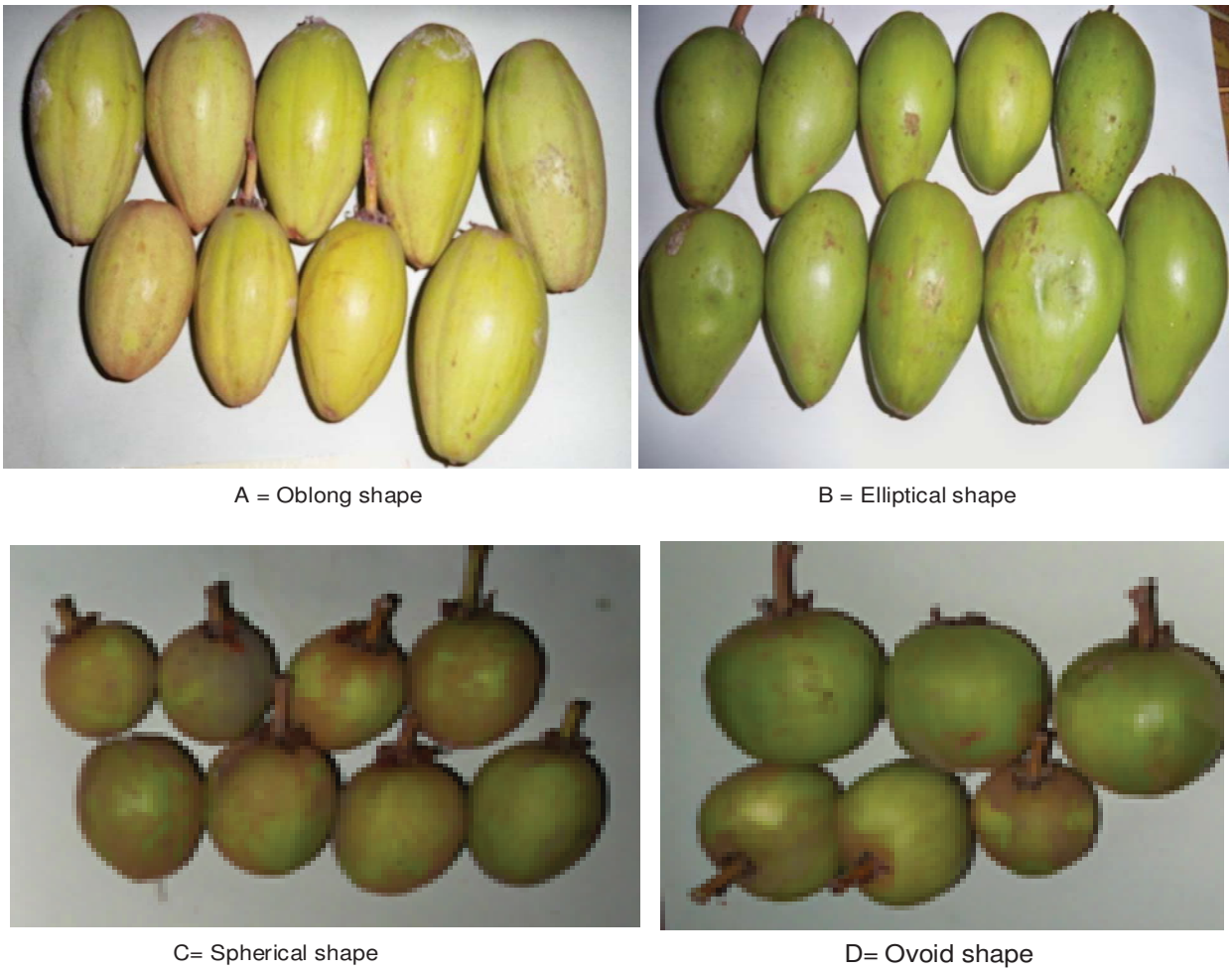

Figure 5. Different shapes of shea fruits.

Table 5. Correlations between quantitative parameters of leaves and fruits per vegetation type.

Parameters measured	Fruit length	Fruit diameter	Leaf width	Leaf length
Farm				
Fruit length	1	**0.579***	**0.213***	0.098
Fruit diameter	0.579*	1	0.238*	-0.176
Leaf width	0.213*	0.238*	1	0.157*
Leaf length	0.098	-0.176*	0.157*	1
Forest				
Fruit length	1	0.145*	0.023	0.179*
Fruit diameter	0.145*	1	0.038	0.055
Leaf width	0.023	0.038	1	0.006
Leaf length	0.179*	0.055	0.006	1
Fallow				
Fruit length	1	0.503	0.005	-0.189*
Fruit diameter	0.503*	1	0.122*	-0.186*
Leaf width	0.005	0.122*	1	-0.056
Leaf length	-0.189*	-0.186*	-0.056	1

*Values represent significant correlations.

populations of *V. paradoxa* in the northern Benin and more specifically in Bassila Township shows that the density of shea trees in the farms, forests and fallows is almost the same. However, the trees of *V. paradoxa* are more numerous in the farms within than the remaining two (02) habitats. The shea tree is threatened in forests and fallows because of the fires of vegetation, fraudulent cuts (industry use) and parasites (borers, fungi, epiphytes). The larger number of shea trees in the farms could be justified by the strong protection and maintenance of trees by farmers in this habitat because of the socio-economic importance of the species. In addition, of all the parameters measured, it appears that the highest values were observed in the farms. The values of parameters of trees recorded in forests are lower than those found in farms and fallows. Similar observations were reported by Sanou and Lamien (2011). Forest trees are smaller than those commonly found in farms and fallows because of the competition observed in forested areas. The trees are better distributed in the farms than in forests or fallows because of human intervention.

The average length and width observed in the populations of shea trees in Bassila Township were within the range of values defined by Thioulouze et al. (1997); length (minimum 5.4 cm and maximum 21.3 cm) and width (minimum 2.2 cm and maximum cm 6.8 cm). However in Chad zone, the lamina length is ranged from 15.5 to 26.3 cm, while the width of lamina varied from 3 to 5.4 cm (Djekota et al., 2014). Leaves of V. paradoxa found in Chad are not width than those found in other zones of West Africa. This shows that *V. paradoxa* is very diversified. Taking into account the agro-climatic zone, especially the Sudano-Guinean zone in which Bassila Township is, the average values measured were larger than those reported by Sanou et al. (2006) and Lovett and Haq (2000). The values obtained by these authors were respectively the Sudanian zone of 13.65 and 14.9 cm in length and 3.88 and 4.9 cm in width and for the Guinean area of 14.24 and 3.97 cm. The differences observed between the trees in Bassila Township and those of Ghana and Mali are caused by genotype or by the diversity of environmental conditions in each area of study and predetermine the behavior of a plant. The dimensions of the fruit of the Shea tree (length 3.6 cm diameter and 3.1) reported by Sanou et al. (2006) are low compared to the results of this study. Concerning the dimensions of the fruit, our results are low compared to those obtained by Djekota et al. (2014). These differences might be related to genotype or ecological conditions. In relation to the different coefficients of variation for most of the parameters measured, they were between 15 and 44% either within or between populations. This shows a fairly large variation in the populations of Shea tree in Bassila Township. In the present work, the coefficients of variation obtained between populations (farms, fallows and forests) are quite important compared to significant variations observed by Lovett and Haq in 2000 when studying the diversity of V. paradoxa in semi -arid areas of Ghana from 294 individuals distributed on twenty-four sites and 18 locations. This difference between the coefficients of variation could be explained by the fact that the study sites of Lovett and Haq were more numerous and varied on one hand and secondly the trees on which these researchers also worked were also many. The fairly large inter-population variation obtained shows the effect of the environment on the behavior of trees.

The values of correlation of leaf and fruit characteristics were low while they were high between the characteristics of the same organ, as shown by the results of Sanou et al. (2004) in Mali.

The results of qualitative morphological characteristics show that the color of the bark of Shea trees in Bassila Township was black contrary to the gray and light gray colors observed by Boukoungou (1987) and Chevalier (1943). The tree habit is quite variable. The crown of the tree was in ball, broom, elliptical shape. These shapes are similar to those obtained by Boukoungou (1987) and Diarrassouba et al. (2009). But the author also observed other shapes of the crown. According to Boukoungou (1987) the different shapes observed were not due to varietal differences but result from the action of bushfires during the formation of the structure of the tree by the disappearance of the lower branches and the destruction of small branches. The shape of the observed branches varies between opposite and whorled branches. The foliage density observed was similar to that reported by Desmarest (1958) except that observed in more densely manner by the author. The dominant shape of leaves and fruits of shea trees in the sampled population was oblong. This same observation was made by Chevalier (1943) with regard to leaf shape. In relation to fruit shape observed in Bassila Township, it was variable: oblong, spherical, ovoid and elliptical. In this study, four shapes of fruit were observed comparing to the results of Djekota et al. (2014) and Diarrassouba et al. (2009) who obtained respectively three in Chad region and five in Ivory Cost. Four shapes of fruit were noted in the farms contrary to Knight (1943) who observed two shapes (elliptical, spherical). This could be explained by the reduced number of Shea trees on which the researcher worked and also by phenotypic and genotypic differences (Lovett and Haq, 2000, Fontaine et al., 2004). The variations observed in different zones can be explained by some factors: natural and/or human selection, gene flow mediated from genetic drift, out crossing, environment (Yadina 1991; Irwin, 2000; Okullo et al., 2003; Vaughan et al., 2007; Tremblay et al., 2010; Abasse et al., 2011; (Djekota et al., 2014).

Conclusion

Agro-morphological characterization of populations of *V.*

paradoxa contributes to improve our better understanding of the species in Bassila Township. A high morphological variation was observed within shea populations from three different habitats. The variation intra and inter population is quite important for the length of the fruit while it was average as regards the diameter of the fruit. The study of qualitative parameters shows that the appearance of the trunk of all the Shea trees studied was rough. For the color of the trunk black was dominant with rectangular cracks. The shape of the crown was broom, with a relatively high frequency of into a ball and broom shapes in the farms and fallows. Foliage density was average for most of the observed trees with more opposite branches than whorled ones in the farms than in the forests and fallows. The leaves of the tree studied were mostly an oblong shape with an apex in pointed shape. The shape of leaves and fruits is discriminative. To ensure sustainable management of the shea sector, it would be desirable to continue this study by expanding to other ecological regions of Benin and integrating quality aspects of the pulp and amount of oil of almond of trees.

Conflict of Interest

The authors have not declared any conflict of interest.

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Optimization of micropropagation protocol for three cotton varieties regenerated from apical shoot

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The need for alternative strategies to obtain transgenic cotton via apical shoot was necessitated due to the recalcitrance of cotton regeneration from somatic embryogenesis, this has greatly slowed down the development of transgenic cottons. To this effect, an optimized regeneration system from apical shoot was developed for three varieties of cotton. Ninety-five percent seed surface sterility was observed in seed germination using a combination of hydrogen perioxide and Clorox as sterilizing medium. Highest shoot elongation rate was achieved on MS supplemented with 2.5 mg/L BAP + 0.1% (w/v) AC, rapid shoot growth occurred with kinetin supplemented media. Rooting efficiency of the three improved cultivars of cotton (*Gossypium hirsutum*), Samcot 9,11 and 13 were optimized using the optimum medium for rooting of difficult-to-root *in vitro* regenerated shoots of cotton which consist of MS basal salts and modified MS vitamins, supplemented with 3% sucrose, 0.2 mg/L IBA, without activated charcoal. In the end, an improved regeneration protocol with rooting efficiency up to 47% and regeneration rate up to 87% by combining rooting induction, indole acetic acid (IAA) shock and graft technique was developed.

Key words: *Allium sativum,* cotton tissue culture, transgenic plant, optimized regeneration of cotton.

INTRODUCTION

The focus of research in plant cell culture for many crop species was to be able to put species into tissue culture maintain or grow the plant cells, tissues or organs under sterile controlled laboratory conditions and ultimately regenerate a normal fertile plant. In comparison with other crops, successes in cotton tissue culture lag behind those of other crops. *In vitro* cultured cotton cells have been induced to undergo somatic embryogenesis in numerous laboratories using varied strategies (Shoemaker et al., 1986; Chen et al., 1987; Trolinder and Goodin, 1987; Kolganova et al., 1992; Zhang, 1994a; Zhang et al., 1996, 1999).

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Regenerated plants have been obtained from explants such as hypocotyls, cotyledon, root (Zhang, 1994a) and from various cotton species (Zhang, 1994b). Gould et al. (1991) reported a successful regeneration method of two cotton varieties; *G. barbadense* cultivars and *G. hirsutum* cultivars that was independent of genotype; however, rooting efficiency was low. Nasir et al. (1997), Morre et al. (1998) and Zapata et al. (1999) also reported the regeneration of cotton plants from shoot meristems. This method has also been successfully used in cotton transformation when combined with particle bombardment (McCabe and Martinell, 1993). Trolinder and Goodin (1987) reported regeneration of cotton plants from callus by somatic embryogenesis, and the efficiency of regeneration via somatic embryogenesis has been reported to improve significantly in recent years (Trolinder et al., 1989; Rajasekaran et al., 1996; Zhang et al., 2001), some difficulties still remain. Major limitations had been that only few cultivars can be induced to produce somatic embryos and regenerative plants. Most responsive lines are Coker varieties, which are no longer under cultivation (Feng et al., 1998). Aside from the genotype limitation, many of the plants regenerated from callus as somatic embryos are abnormal (Cousins et al., 1991; Trolinder and Goodin, 1987; Rajasekaran et al., 1996). Due to these shortcomings, cotton biotechnology has been a major task in cotton breeding and production. As an improved approach, Renfroe and Smith (1986) reported regeneration of cotton from isolated shoot meristem from seedlings of *G. hirsutum* L. cv. to obtain regenerated plants. Gould et al. (1991) extended this approach by using two *G. barbadense* cultivars and 19 *G. hirsutum* cultivars in his research, which showed that regeneration from shoot tips was genotype-independent. Saeed et al. (1997), Morre et al. (1998) and Zapata et al. (1999) also reported the regeneration of cotton plants from shoot meristems. However, rooting efficiencies were low in these reports (from 38 to 58%). In this report, an optimized regeneration protocol with improved rooting efficiency in shoot apex based cotton regeneration system is presented. Three factors that could affect the rooting efficiency of shoot apices were investigated in this research: 1) Effect of seed sterilization method, 2) Effect of shoot apex age, and 3) Effect of concentration of IAA shock. In the end, an improved regeneration protocol with rooting efficiency up to 87% was developed. The protocol uses cotton shoot apices as explants and combines basic rooting, IAA shock and grafting steps to increase rooting efficiency up to 47% and regeneration to 87%.

METHODOLOGY

Seed disinfection methods

Cotton seeds were de-linted in concentrated sulphuric acid (H_2SO_4) then washed in tap water. The de-linted seed were then wrapped in cheese cloth and soak in tap water for 1 h. Cotton seeds were disinfected via four methods:

Method 1: Cotton seeds were treated with 70% ethanol for 2 min prior to a 20 min exposure to 10% Clorox ® (5.25% sodium hypochlorite (NaOcCl)) solution with two drops of Tween 20 per 100 ml, and rinsed three times with sterile double-distilled water. The seeds were then placed on seed germination medium.
Method 2: Cotton seeds were treated with a 50% Clorox® (5.25% NaOcCl) solution with two drops of Tween 20 per 100 ml on a rotary shaker at 50 rpm for 20 min and rinsed at least three times with sterile double-distilled water. The seeds were then placed on seed germination medium.
Method 3: Cotton seeds were treated with 20% hydrogen peroxide for 2 h and rinsed three times with double-distilled water. The seeds were then placed overnight on a rotor shaker at 100 rpm. After removing the seed coat, the seeds were then placed on seed germination medium.
Method 4: Cotton seeds were treated as described in Afolabi-Balogun et al. (2011). After removing the seed coat, the seeds were placed on seed germination medium.

Seed germination

Three seeds were placed in each germination media (Afolabi-Balogun et al., 2011) and incubated in the dark at 28°C overnight and then in the light for 5 days. Upon removal from incubation, the number of elongated shoots was counted. Contamination was determined by visual inspection for fungal and/or bacterial growth.

Shoot apex isolation

The seedling apexes were isolated as described by Afolabi-Balogun et al. (2011). The epicotyl (shoot) was placed on MS+Kin medium. The plants were kept in growth room at 27± 2°C to 16 h light and 8 h dark at 70% humidity. The plantlets were grown for 10 days.

Shoot elongation and rooting development

Thirty shoots from each variety without root development were subjected to IAA shock at concentrations 0.1, 0.5, 1.0, 1.5 and 2.0 mg/ml for one minute. The treated shoots were rinsed and transferred to fresh MS medium for another three weeks. The number of rooted plants was recorded and the rooted plants were transferred to Magenta boxes containing MS medium and incubated in a culture chamber for four weeks before being transferred to the greenhouse.

Plantlets graft

Grating of un-rooted elongated shoots from MS medium after IAA shock onto the seedling stocks of the same variety was done by cutting the bottom of the scion into a wedge with a scalpel blade, then the upper part of the seedling stocks was cut under the first true leaf; and a slit (about 1.0 cm) on the stem was cut vertically. The decapitated end of the root stocks and matching cut ends of the scions were treated with 0.2 mg/L IAA + 0.1 mg/L GA. for 5 min. Then the treated scion was inserted into the slit and the cambiums were lined up. The grafted plant was then covered by a 1000 ml flask and kept in a humid chamber for a week. After which the flask was removed and the plants kept in the humid chamber for another week before being transferred to the greenhouse.

Data collection and analysis

Data were obtained at 25-30 days and at 42 days on the number

viable shoots and viable shoots with roots. Other observations made were on shoot health (on a rating scale 1 to 5 scale, with 1 being poorest chlorophyll development, and 5, best chlorophyll development), leaf abscission (on a 1 to 5 rating scale with a rating of 5 being complete leaf retention, and 1 complete leaf abscission), number of explants with callus, relative calli size, root length, and branching. Data was analyzed as a completely randomized design with three replications using ProcGLM of SAS program (SAS, 1987). Means of statistically significant (p=0.05) treatments were separated using LSD.

RESULTS

Seed disinfection methods

The extent of sterility was measured be physical examination of the culture bottle for contaminant such as mould. Maximum surface sterilization was observed with the seed disinfected with method 4 (number of contaminated seed is zero) (Figure 1). Methods 1 and 2 did not give perfect sterilization. Use of only 50% Clorox® gives the least sterilization. Combining Clorox® and hydrogen peroxide gave a better result, but this was still not as efficient as hydrogen peroxide.

Seed germination

All cotton seeds varieties germinated on MS though seedlings elongation on germination medium was very slow. For enhancement of growth, the tiny seedlings were transferred to different media supplemented with plant growth regulators (BAP, NAA, IBA) and activated charcoal (AC). The highest rate of elongation was achieved on MS supplemented with 2.5 mg/L BAP + 0.1% (w/v) AC.

Shoot apex isolation

Vigorous shoot growth was observed when kinetin is supplemented to the media.

Shoot elongation and rooting development

The rooting efficiency of the three varieties was significantly different in different concentrations of IAA (p=0.027) (Figure 2). The effect of different IAA shock concentrations varied from 6.7 to 47%. The highest efficiency (47%) was observed for a 1.5 mg/ml IAA and the lowest efficiency (12%) was observed for 0.1 mg/ml IAA. So the concentration of 1.5 mg/ml IAA was chosen for regeneration. The rate of rooting of elongated shoots cultured on various media is presented in Table 1. Optimum rooting was observed using ERM 4 which was about (47%) while the lowest rooting was observed with ERM 2 giving only (6.7%). Hence, a concentration of 1.5 mg/ml IAA was chosen in the regeneration system. The difference of rooting efficiency was not significantly different in all varieties (p=0.08). This result indicated that rooting efficiency is genotype independent.

Plantlet grafting

Rooting efficiency of plantlet as well as survival rate was improved to eighty five percent when plantlets were kept humid, pre-treating the scion and stock with 0.1 nmg/L IAA + 0.2 mg/L GA (Figure 3). Grafting is a very useful technique and is commonly used in horticultural crops.

DISCUSSION

Recently, several researchers have regenerated plants from shoot tip meristems (Zapata et al., 1999). Gould et al. (1991) reported that the yield of shoots *in vitro* from isolated apices depends on the incidence of contamination and rooting efficiency. In recent years, protocols involving proliferation of cotton shoots (Agrawal et al., 1997; Hemphill et al., 1998) have been published. The rooting efficiency ranged from 38 to 58% in their reports. Here we report an optimized regeneration protocol involving shoot tips regenerated directly without a callus phase, this method has the advantage of being genotype-independent; almost all cultivars can be regenerated from shoot tips. The use of shoot tips as explants in an *Agrobacterium*-mediated transformation system is a good way to overcome the obstacles in traditional *Agrobacterium*-mediated transformation. From the germination results, all seeds sterilized by hydrogen peroxide germinated in 5 days (Figure 1); seeds sterilized by both Clorox® methods had a lower germination rate (95 and 37%, respectively). The reason for those results may be that the residual of Clorox, specifically, chlorine, suppressed the germination of cotton seeds, while the residual of hydrogen peroxide is water and CO_2, did not affect the germination of cotton seeds.

The age of explants has a significant effect on shoot tip elongation (Table 2). The elongation rates of the three varieties were not significantly different from each other (p=0.1573). The elongation rate was also affected by the size of isolated tips. It was observed that if the starting size of the apex was less than 1 mm, the tips would not grow at all.

The efficiency of the rooting media was evaluated based on the increase in length and number of roots developed per seedling. The highest rate of elongation was achieved on MS supplemented with 2.5 mg/L BAP + 0.1% (w/v) AC however, MS + 3% (w/v) sucrose + 1.5 mg/IAA proved more effective for the development of better root system and the rooting of the plantlet was by grafting procedure.

The type and concentration of plant growth regulators

Figure 1. Effect of sterilization method on seed germination.

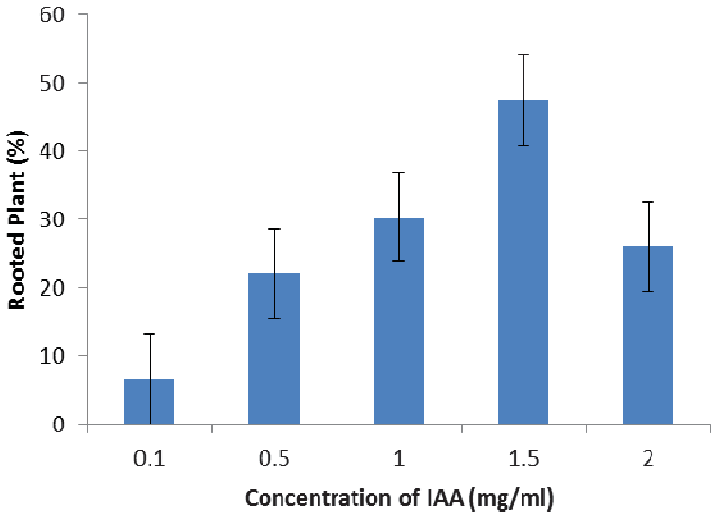

Figure 2. Effect of IAA shock on stimulating the rooting of previously unrooted Cotton shoot apices. Vertical bar represents the standard error of the 5 treatments of IAA.

Figure 3. 1-Rooting plant, 2-Effect of sterilization method 1 & 2 showing microbial growth,3-Germinating seed,4-Plantlet before grafting,5-Elongating apical shoot, 6-Grafted plantlet,7-Elongating apical shoot, 8- Cross section of work area, 9- Regenerated Plant in soil, 10-Plantlets in greenhouse.

Table 1. Elongation and rooting media composition used in optimization.

Medium	Media composition
ERM 1	½ MS + 1.5 % (w/v) sucrose
ERM 2	MS + 3 % (w/v) sucrose + 0.5 mg/L IAA
ERM 3	MS + 3 % (w/v) sucrose + 1.0 mg/L IAA + 0.1% (w/v) AC
ERM 4	MS + 3 % (w/v) sucrose + 1.5 mg/ IAA
ERM 5	0.1mg/l GA3 + 1.0 mg/L IAA

All media were solidified with 0.4% phytagel (Sigma).

Table 2. Mean number of explants elongated on elongation medium from 3 cotton varieties at 4 different ages.

Cotton variety	Age of Explant				
	5 days	7 days	9 days	11 days	Mean
Blec-Samcot 9	11.00±2.00++	25.33±2.08	28.67±0.57	30±0.0	23.75[a]
Blec-Samcot 11	13.33±3.06	26.70±0.57	28.00±1.029	33±0.57	24.33[a]
Blec-Samcot 13	14.67±3.21	26.67±2.08	28.67±0.57	30±0.0	25.00 [a]
Mean	12.75[c+]	25.75[b]	28.41[a]	29.75[a]	

+ Different letter label significant at p=0.05 level using LSD method, ++ Mean±Std.

Table 3. Regeneration response of apical shoot explant and split cotyledon node from cotton to the concentration of IAA.

Explant	Media composition	% Rooting (days)	
		14	27
	½ MS + 1.5% (w/v) sucrose	0[a]	0[b]
	MS + 3% (w/v) sucrose + 0.5 mg/l IAA	8[a]	54[a]
SA	MS + 3% (w/v) sucrose + 1.0 mg/l IAA + 0.1% (w/v) AC	4[a]	63[a]
	MS + 3% (w/v) sucrose + 1.5 mg/ IAA	18[a]	38[a]
	0.1 mg/l GA_3 + 1.0 mg/l IAA	17[a]	56[a]
Explant Mean		9.4[a]	42.2[b]
	½ MS + 1.5% (w/v) sucrose	8[a]	0[ab]
	MS + 3% (w/v) sucrose + 0.5 mg/l IAA	0[a]	4[a]
SCN	MS + 3% (w/v) sucrose + 1.0 mg/l IAA + 0.1% (w/v) AC	6[a]	23[a]
	MS + 3% (w/v) sucrose + 1.5 mg/ IAA	32[a]	67[a]
	0.1 mg/l GA_3 + 1.0 mg/l IAA	32[a]	29[a]
Explant Mean		25.6[a]	24.6[a]

Means followed by the same letter are not significantly different at p=0.05. Glu- Glucose, Suc- Sucrose, Ac. Char- Activated Charcoal. SA- Shoot apices SCN- Splited Cotyledon Node.

strongly influenced the organogenic potential of the apical shoot explant. The responding frequency of shoot seemed to depend more on concentration of indole-3-acetic acid (IAA). The regeneration response of apical shoot explant and split cotyledon node from cotton to the concentration of IAA is shown in Table 3. It is evident that without IAA regeneration of apical shoot is low and maximum shoot regeneration response has been observed with 1.5 mg/L IAA concentration along with MS. The further increment in IAA concentration to 2.0 mg/L along with MS showed decreased shoot regeneration response. We observed maximum number of shoots when GA was combined with IAA in all variety.

Conflict of Interest

The authors have not declared any conflict of interest.

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Morphological diversity and association of traits in ethiopian food barley (*Hordeum vulgare* l.) landraces in relation to regions of origin and altitudes

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One hundred and two barley accessions and five checks were evaluated using augmented design consisting of four complete blocks in 2012 main cropping season at holetta agricultural research center. Ten quantitative and six qualitative characters were recorded. Analysis of variance showed significant difference (p<0.01) among accessions for plant height, awn length, peduncle extrusion, thousand seed weight, number of seeds per spike, days to 50% flowering and days to maturity. Cluster analysis grouped accessions in to five distinct classes with maximum number of accessions (44) in cluster I and minimum (2) in cluster V. Principal component analysis showed that variances of 30, 17, 15 and 10% were extracted from the first four principal components, respectively, which contributed 72% of the total variation among accessions. Estimates of genetic diversity index based on qualitative characters showed high diversity index among characters at Arsi, Wellega and Wello, and diversity index increased in altitude between 2001 and 3000 m.a.s.l and decrease at altitude >3000. Phenotypic diversity was very high for kernel row number (H′=0.99), grain color (H′=0.90) and spike attitude (H′=0.85) and low for lemma color (H′=0.48). Days to flowering, days to maturity and numbers of seed per spike, from quantitative characters and kernel row number, grain color and spike attitude from qualitative characters were the most characters which contributed variances among accessions.

Key words: Cluster analysis, diversity index, principal component analysis, qualitative characters, quantitative characters.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an annual cereal crop which belongs to the genus *Hordeum* in the Tribe Triticeace of grass family Poaceae which contains about 350 wild species (Amanda, 2008). It is thought that barley has to be originated in the Fertile Crescent area of the Near East from the wild progenitor *Hordeum spontaneum* over 10,000 years ago (Badr et al., 2000; Blattner and Badani, 2001; Grando and Helena, 2005; Azhaguvel and

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Komatsuda, 2007; Dai et al., 2012). Barley is a progenitor *Hordeum spontaneum* over 10,000 years ago (Badr et al., 2000; Blattner and Badani, 2001; Grando and Helena, 2005; Azhaguvel and Komatsuda, 2007; Dai et al., 2012). Barley is a major crop, grown worldwide and in a wide range of climatic conditions; despite its importance as a crop species, little is known about the population genetics of barley and the effects of bottlenecks, adaptation, and gene flow on genetic diversity within and between landrace populations (Leino and Jenny, 2010; Tanto et al., 2010*).* The crop successfully grows in the arid climates of the Sahara, the Tibetan plateaus, the highlands of the Himalayas, and the Andean countries, the tropical plains of India and the mountains of Ethiopia (Grando and Helena, 2005).

Ethiopia is an important primary and secondary gene center for many field crop species, including barley, which were introduced centuries ago and since then adapted and developed wide genetic diversity (Abdi, 2011). Landraces represent over 90% of the barley cultivated in Ethiopia (Tanto et al., 2010*).* In Ethiopia barley is the fifth most important cereal crop both in area coverage and production, with around 1,013,623.72 ha and 18,155,830.29 qt respectively (CSA, 2012). It is grown both in *Meher* (June-September) and *Belg* (March-April) seasons. The diversity in soils, climate, altitude and topography, together with geographical isolation for long periods, are considered the main factors influencing the large diversity in Ethiopian barley (Harlan, 1976); social factors also play an important part in the diversification, thus, the morphological, biochemical and molecular groups in Ethiopian barley are the result of accumulated long-term mutations, hybridization, gene recombination and natural and human selection in heterogeneous environments (Lakew and Alemayehu, 2011*).*

In our country Ethiopia to conserve plant genetic resources including barley, the Plant Genetic Resources Centre of Ethiopia (PGRC/E), now the Institute of Biodiversity Conservation (IBC) was established in 1976. The primary mandates of IBC include the preservation of genetic diversity of crop plants, their wild relatives, and native species important to Ethiopian agriculture and biodiversity. Over 65 000 accessions from more than 120 plant species have been collected across the country and preserved *ex situ* at IBC. This germplasm collection includes a principal base collection of barley with >15,000 accessions (Abdi, 2011). However, most of collected and preserved landraces at the Gene Centre are not yet studied for their morphological diversity (Alemayehu and Parlevliet, 1997; Abdi, 2011). Therefore, this study is proposed with the following objectives:

1. To assess the extent of morphological variation in barley accessions in respect to regions of origin and altitudes of collection.
2. To cluster the accessions into relatively homogenous groups and to identify the major characters contributing to the overall diversity of the germplasm.

MATERIALS AND METHODS

Experimental materials

A total of 102 barley accessions were obtained from the Institute of Biodiversity Conservation, Addis Ababa, Ethiopia. The accessions were selected based on their region of origin and altitude (Table 1). Five standard checks (controls) (HB-42, Ardu, Shege, HB1307 and Balami), that were obtained from the Holetta Agricultural Research Center were included (a total of 107 genotypes were used in this study). The region of collection and altitude range is given in Table 1. Five gram of seed (100 kg ha^{-1}) was obtained from IBC for each accession.

Experimental site

The experiment was conducted at the Holetta Agricultural Research Center, Ethiopia, during the main cropping season of 2012 under rain fed condition. Holetta Agricultural Research Center is located at 9°3'N, 38°30'E with an altitude of 2400 m.a.s.l. It is 28 km west of Addis Ababa on Ambo road of and characterized with annual rainfall of 1044 mm, mean relative humidity of 60.6% and mean maximum and minimum temperature of 22.1 and 6.2°C, respectively (Figure 1).

Experimental procedures

The experiment was laid out in augmented randomized complete block design (Federer and Ragavarao, 1975) consisting of four blocks in which the 102 accessions were planted in un-replicated plots and the five checks were replicated four times (ones in each block) to estimate an error variance. The plot size used was one row with 2.5 m length, and 0.4 m between rows. Seeds were planted by hand with a seeding rate of 100 kg/ha. Plots were kept free from weeds**.**

Data collection

Based on the IPGRI descriptor list (IPGRI, 1994); ten quantitative and six qualitative characters were recorded (Tables 2 and 3). For each accession, 10 randomly selected individual plants were used for recording quantitative characters, except days to 50% flowering, days to maturity and thousand seed weight, which were recorded on plot basis.

ANALYSIS OF VARIANCE

Quantitative traits

All quantitative data were analyzed using SAS v 9.1.3 Software (SAS, 2004). A mixed model in which standard checks effect were considered as fixed, and accessions effect as random effect, was adopted as:

$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$

Where: Y_{ij} is response variable; μ is general mean, α_i is the fixed effect of i^{th} standard checks and random effect of accessions, β_j is the random effect of j^{th} block and e_{ij} is random errors. Mean squares

Table 1. Region of origin, altitude, and number of accessions used for this study

Region	Number of accessions by altitude groups(m.a.s.l)				
	Group I (1500-2000)	Group II (2001-2500)	Group III (2501-3000)	Group IV (3001-3500)	Total number of accessions
Arsi	3	3	3	3	12
Bale	3	3	2	3	11
Gojam	2	4	3	2	11
Gonder	2	0	7	2	11
Shewa	3	4	3	2	12
Sidamo	3	3	4	1	11
Tigray	3	4	4	1	12
Wellega	3	4	4	0	11
Welo	3	2	3	3	11
Total	25	27	33	17	102

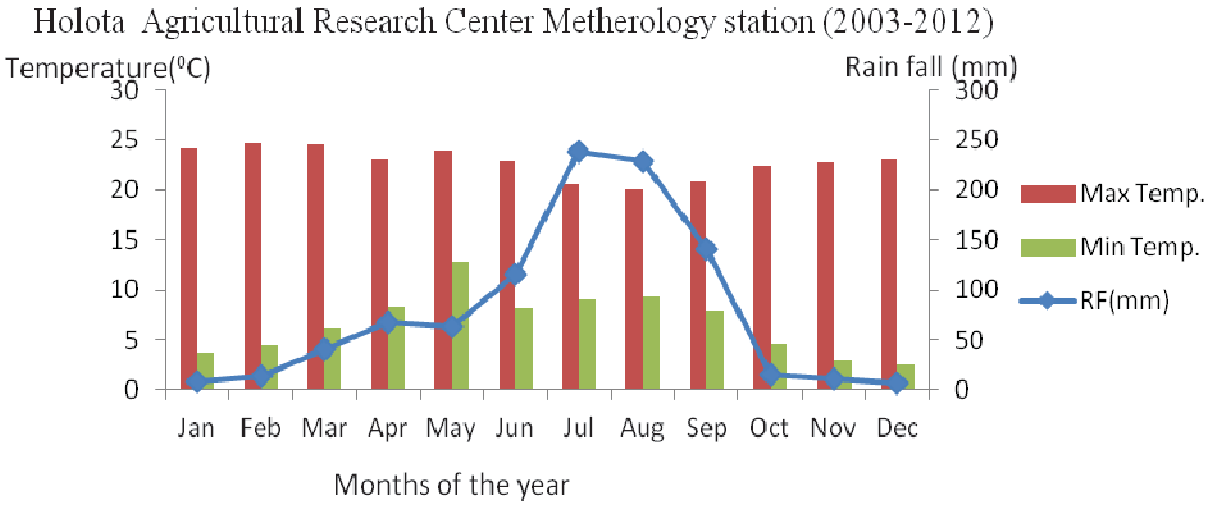

Figure 1. Climatic data of the experimental sites at Holetta agricultural research center minimum and maximum temperature and monthly rainfall.

were calculated as shown in Table 4. Estimates of σ_e^2, σ_g^2 and σ_b^2 were obtained by equating the obtained sum of squares to their expectancies, and solving the resulting system equations:

$$\sigma_g^2 = \frac{GenotypesMS - ErrorMS}{Blocks}$$

$$\sigma_c^2 = \frac{ControlsMS - ErrorMS}{Blocks}$$

$$\sigma_t^2 = \frac{test(Accessions)MS - ErrorMS}{Block}$$

Where genotypes = accessions + checks (controls).

Cluster analysis

Before undertaking multivariate analysis of variance in which two or more variables were analyzed at a time, the data was standardized to mean of zero(0) and a variance of one(1) to avoid differences in scales. One hundred two accessions and nine regions of origin were grouped into respective classes. The values of pseudo F statistic (PSF) and Hotellin's pseudo T^2 statistic were used for defining optimum number of clusters. Cluster analysis was made using the hierarchical cluster analysis. The PROC CLUSTER Procedure of SAS V9.1.3 (SAS, 2004) using Unweighted Pair Group Method using Arithmetic Average linkage (UWPGMA) was employed.

Principal component analysis

The principal component analysis (PCA) was computed to reduce the number of variables into a few correlated components that can explain much of the variability. It was performed using the

Table 2. List of quantitative characters recorded along with their code and definition.

Characters	Code	Character definition
Awn length (*cm*)	AWL	Distance from the tip of the spike to the end of the awn
Days to 50% flowering (count)	DFL	Number of days from planting to the day when 50% of the heads fully flower (heading) emerge from the boot of flag leaf in each row
Days to maturity (count)	DMA	Number of days starting from planting to the days when peduncles of the spikes in each row become complete yellow and mature
Number of fertile tillers per plant (count)	NFTPP	Number of fertile tillers (spike bearing) of randomly selected plants per plant, counted at maturity
Number of seeds per spike (count)	NSPS	Number of seed per spike on randomly selected plants counted at maturity
Peduncle extrusion length (*cm)*	PEDext	Distance from the auricle of flag leaf to the base of spike
Peduncle length (*cm*)	PDL	Distance from last node to base of the spike
Plant height (*cm*)	PLH	Length of randomly selected plants measured from the ground to the tip of the spike excluding awns at maturity
Spike length (*cm*)	SPL	length measured from base of spike to top of spikelets excluding the awns at maturity
Thousand seed weight (*g*)	TSW	The weight of 1000 seeds taken from each row in gram

Table 3. List of qualitative characters recorded along with their codes and descriptions.

Characters	Code	Character descriptions
Awn color	ACO	White (1), Brown (3), Black (5), Reddish (4)
Grain color	KCO	White (1), Red(2), Black(4), Purple (3)
Kernel row number	KRN	Two row (1), Six row(5), Irregular (3)
Lemma color	LMC	White (1), Black(4), Red(2), Purple (3)
Spike attitude	SPA	Erect (1), Horizontal(5), Semi-recurved (7)
Spike density	SPD	Lax (3), intermediate (5), dense (7)

correlation matrix to define the patterns of variation among landraces based on the mean of quantitative characters. And also helps to identify characters that load the most in explaining the observed variation. The PROC PRINCOMP Procedure of SAS V9.1.3 (SAS, 2004) was used for principal component analysis.

Qualitative traits

Estimate of diversity index

The Shannon-Weaver diversity index (H′) was used to compute the phenotypic frequencies to assess the phenotypic diversity for each character for all accessions, based on qualitative traits. It is used in genetic resource studies as a convenient measure of both richness and evenness using phenotypic data:

$$H = -\sum_{i-1}^{n} pi \ln(pi)$$

$H' = H/H_{max}$

$H_{max} = ln(n)$.

Where: H'= standardized relative diversity index; n = is the number of phenotypic classes per characters; Pi = is the proportion of the total number of entries in the i^{th} class; ln = natural logarithm.

RESULTS AND DISCUSSION

Analysis of variance

Analysis of variance indicated significant difference ($p<0.01$) among genotypes, accessions, controls and accessions vs. controls for all quantitative characters except awn length in controls, peduncle length in accessions vs. controls, spike length in genotypes, accessions and accessions vs. controls, number of fertile tiller per plant in controls and accessions vs. controls, and days to maturity in controls (Table 5). Hence, the result indicated the existence of high morphological variation in Ethiopian food barley landraces, in their regions of origin

Table 4. ANOVA table for sum of squares and their expectancies for the statistical genotypic model (Federer and Ragavarao, 1975)

Source of variation	Degree freedom	Mean square	Expected mean square
Blocks(b)	b-1	MSb	-
Genotypes(g)	g-1	MSg	$\sigma_e^2 + \sigma_g^2$
tests (accessions) (t)	t-1	MSt	$\sigma_e^2 + \sigma_t^2$
controls (c)	c-1	MSc	$\sigma_e^2 + \sigma_c^2$
tests vs. controls (t vs.c)	1	MSt vs.c	$\sigma_e^2 + \sigma_t^2 + \sigma_c^2$
Error	(b-1)(c-1)	MSe	σ_e^2
Total	n-1	-	$\sigma_e^2 + \sigma_g^2 + \sigma_b^2$

MSg = mean square of genotypes, MSb = mean square of blocks, MSt = mean square of test (accessions), MSc = mean square of controls, MSt vs.c = mean square of tests vs. controls, MSe = mean square of error, σ_e^2=expected error variance (MSe), σ_g^2 = Genotypic variance component, σ_t^2 = accessions variance component.

Table 5. Analysis of variance for ten quantitative characters.

Source of variation	Mean square										
	DF	PLH	AWL	PDER	PDL	SPL	TSW	NSPS	NFTPP	DFL	DMA
Block	3	470.87	0.16	4.10	43.48	1.57	70.17	79.13	0.53	50.61	19.51
MSg	106	107.45**	0.86**	5.83**	46.31*	1.05^{ns}	28.09**	185.81**	0.39*	86.20**	130.72**
MSt	101	98.66**	0.87**	5.47**	45.87*	0.88^{ns}	26.60**	162.52**	0.40*	69.52**	101.43**
MSc	4	302.99**	0.56^{ns}	11.54**	58.81*	5.29**	63.63**	555.81**	0.25^{ns}	14.84*	1.12^{ns}
MSt$_{vs.c}$	1	214.27**	1.09*	19.37**	42.07^{ns}	1.44^{ns}	36.79**	1056.57**	0.59^{ns}	2047.01**	2240.60**
MSE	12	19.24	0.22	1.24	18.41	0.62	1.52	15.39	0.16	3.32	1.60
Cort'd. total	121	13033.45	95.24	645.75	5260.68	124.13	3206.77	20118.48	45.83	9329.60	13934.57
Mean		104.78	11.45	13.89	37.69	8.09	45.23	37.63	3.71	78.82	125.80
SE		4.70	0.51	1.19	4.60	0.93	1.32	4.20	0.44	1.95	1.35
CV (%)		4.18	4.17	8.02	11.38	9.79	2.73	10.42	11.04	2.31	1.01

*, **, ns indicates significance at P=0.05 level, P=0.01 and non-significant, respectively. MSg = mean square of genotypes (controls + accessions),MSt = mean square accessions, MSc = mean square of controsl, MSt$_{vs.c}$ = mean square of accessions vs. control MSE = mean square of error(error variance), Cort'd total = corrected total, SE = standard error, CV(%) = coefficient of variation, PLH = plant height, AWL = awn length, PDER=peduncle extrusion, PDL = peduncle length, SPL = spike length, TSW = thousand seed weight, NSPS = number of seed per spike, NFTPP = number of fertile tiller per plant, DFL = days to 50% flowering, DMA = days to maturity.

and altitude groups. The same results were reported on morphological diversity of Ethiopia barley landraces by different authors (Tanto et al., 2009; Abay et al., 2009; Eticha et al., 2010; Dejene et al., 2010; Tanto et al., 2010; Muhe and Alemayehu, 2011; Jalata et al., 2011).

Morphological variation within regions

Estimate of genotypic variance for regions of origin among accessions showed highly significant difference ($p<0.01$) for plant height, peduncle extrusion, spike length, thousand seed weight, number of seeds per spike, days to 50% flowering and days to maturity in all regions. Similarly, awn length from Arsi, Sidamo, and Wellega; peduncle length from Bale, Shewa, Sidamo, Wellega and Wello; number of fertile tillering per plant from Arsi and Tigray significantly varied (Table 6). Analyses of diversity pattern, among accessions from different regions for quantitative characters revealed existence of morphological diversity within regions indicating differences in agro-ecological conditions across regions contributing for the observed morphological diversity. Similar results were also reported in several studies (Negassa, 1985; Demissie and Bjornstad, 1996; Dejene et al., 2010).

Morphological variation within altitudinal gradients

Most of the morphological characters showed significant variation among altitude groups except peduncle length in altitude group I (1500-2000) and IV (3001-3500), spike length in altitude group I and number of fertile tiller per plant in altitude group II (2001-2500) and IV (Table 6). The altitude group III (2501-3000) showed significant genotypic variation for all characters measured. In general, high genotypic variation was observed in an

Table 6. Estimate of genotypic variances for nine regions of origin and four altitude groups based on ten quantitative characters

Region	PLH	AWL	PDER	PDL	SPL	TSW	NSPS	NFTPP	DFL	DMA
Arsi	19.06**	0.09*	0.87**	4.58^{ns}	0.27*	6.77**	54.59**	0.13**	19.18**	33.52**
Bale	24.51**	0.02^{ns}	1.22**	8.33*	0.26*	6.66**	58.59**	0.01^{ns}	32.20**	43.46**
Gojam	35.38**	0.02^{ns}	1.31**	5.51^{ns}	0.24*	6.36**	63.94**	0.03$^{n\ s}$	34.63**	28.93**
Gonder	40.41**	0.02^{ns}	0.88**	1.74^{ns}	0.34*	9.64**	45.06**	0.01^{ns}	11.23**	25.56**
Shewa	33.41**	0.07^{ns}	1.34**	9.96*	0.47**	8.11**	63.52**	0.03^{ns}	15.39**	36.98**
Sidamo	25.89**	0.31**	1.30**	8.79*	0.26*	11.46**	58.05**	0.00	30.05**	54.18**
Tigray	53.59**	0.04^{ns}	0.81*	5.84^{ns}	0.25*	7.66**	91.18**	0.12**	35.47**	44.66**
Wellega	29.10**	0.54**	4.02**	9.32*	0.46**	9.29**	75.45**	0.06^{ns}	28.03**	36.29**
Wello	29.10**	0.05^{ns}	1.24**	10.44*	0.55**	7.77**	50.95**	0.00	18.85**	39.11**
Altitude group (m.a.s.l)										
Group I	31.18**	0.30**	1.50**	4.10^{ns}	0.13^{ns}	5.37**	54.89**	0.07*	33.32**	46.53**
Group II	19.63**	0.08*	1.56**	6.85*	0.27*	8.83**	59.28**	0.05^{ns}	31.08**	40.49**
Group III	26.52**	0.08*	1.17**	7.48**	0.35*	5.96**	48.93**	0.09*	15.69**	35.39**
Group IV	31.67**	0.10*	0.78**	5.73^{ns}	0.28*	11.31**	37.72**	0.03^{ns}	10.88**	22.58**

*, **, and ns indicates significance at P = 0.05, P = 0.01 and non-significant, respectively. PLH = plant height, AWL = awn length, PDER = peduncle extrusion, PDL = peduncle length, SPL = spike length, TSW = thousand seed weight, NSPS = number of seed per spike, NFTPP = number of fertile tiller per plant, DFL = days to 50% flowering, DMA = days to maturity; M.a.s.l. = meter above sea level; Group I (1500-2000), Group II (2001-2500), Group III (2501-3000) and Group IV (3001-3500).

altitude groups II and III, which comprised the major barley growing areas in the country. Similar result was reported by Demissie and Bjornstad (1996) and Dejene et al. (2010) where they found high variation concentration in areas between 2000-3000 and 2400-3000 m.a.s.l. respectively. This high variation attributed to mixed farming system, which is typically found in areas of higher elevation usually above 2000 m.a.s.l. Tanto et al. (2009) also reported the reduction of area of cultivation for barley as altitude decreased which indicated that barley is cool climate crop.

Cluster analysis

Cluster analysis for accessions

Cluster analysis grouped the 102 accessions in to five distinct groups (Table 7). Numbers of accessions per cluster varied from 44 accessions in cluster I to 4 accessions in cluster V. Cluster means and percent of populations (accessions) in each cluster are presented in Tables 7 and 8. Forty four accessions were found in cluster I, which was 43.1% of the total experimental materials. This cluster has been characterized by intermediate plant height, relatively the heaviest thousand seed weight, relatively higher number of fertile tillers per plant, early flowering and early maturity. Accessions grouped under cluster I were scattered along all regions and more at altitude group I (1500-2000) and II(2501-3000). Cluster II accounts 22.6% of the population and included 23 accessions and had shorter peduncle extrusion, longer days to 50% flowering and longer days to maturity. Majority of these accessions were collected at altitude group III (2501-3000) from all regions except Shewa and Tigray. Relatively accessions with shorter plant height, earlier days to 50% flowering, earlier maturity, and smaller thousand seed weight were grouped under cluster III which contribute 17.7% to the population (18 accessions).

Cluster IV consisted of thirteen accessions, 12.8% of the population, characterized by high number of seeds per spike and moderate in days to 50% flowering and days to maturity; which includes more accessions collected from Shewa and from all altitude groups. This cluster, cluster IV, contains accessions which have high number of seeds per spike and early mature, especially accession number 4879, 243571, 235068 and 242093. Cluster V included four accessions (3.9% of the population) and characterized by taller plant height, longer awn length, peduncle extrusion, peduncle length, spike length, and heavier thousand seed weight, fewer number of seeds per spike, lower number of fertile tillers per plant, relatively late days to 50% flowering and days to maturity, in which accessions were collected from Arsi, Bale and Wellega from altitude groups II (2001-2500), III (2501-3000) and IV (3001-3500).

Although the cluster analysis grouped barley accessions with greater morphological similarity, the cluster did not necessarily included all accessions from the same or adjacent sites. This result is in agreement with the work of Dejene et al. (2010) who reported that, clustering of accessions based on the agronomic characters revealed no distinct regional grouping patterns in which accessions from same or adjacent regions appeared in different clusters.

Table 7. Distribution of 102 barley accessions over five clusters by nine regions of origin and four altitude groups based on 10 quantitative characters

Regions	Clusters					No. of accessions
	I	II	III	IV	V	
Arsi	4	4	3	-	1	12
Bale	5	3	2	-	1	11
Gojam	5	6	-	-	-	11
Gonder	2	7	2	-	-	11
Shewa	2	-	4	6	-	12
Sidamo	6	1	3	1	-	11
Tigray	9	-	-	3	-	12
Wellega	7	1	1	-	2	11
Wello	4	1	3	3	-	11
Total	44	23	18	13	4	102
Altitude groups						
Group I	20	4	-	1	-	25
Group II	13	1	8	4	1	27
Group III	9	11	7	5	1	33
Group IV	2	7	3	3	2	17
Total	44	23	18	13	4	102

Table 8. The summary of cluster mean of 102 barley accessions for 10 quantitative characters

Characters	Cluster means				
	I	II	III	IV	V
Plant height	99.8	108.4	100.8	113.1	115.5
Awn length	11.5	11.0	11.8	11.8	11.7
Peduncle extrusion	14.9	12.5	13.1	14.4	17.1
Peduncle length	40.0	32.7	37.9	37.6	46.7
Spike length	8.2	8.3	7.6	7.5	8.6
Thousand seed weight	47.4	45.6	39.1	42.2	50.3
Number of seed per spike	25.1	40.7	44.6	58.5	25.5
Number of fertile tiller per plant	4.1	3.7	3.4	3.4	3.3
Days to 50% flowering	70.3	88.9	75.9	78.5	83.0
Days to maturity	116.0	138.2	117.6	125.1	135.5
Number of accessions	44	23	18	13	4

Clustering indicated that environment had an impact on the performance of barley and specifically altitude had great contribution for the variability of the characters.

Cluster analysis for regions

Regional cluster analysis grouped the nine regions of barley accessions in to four groups based on 10 quantitative characters (Table 9). Arsi, Bale, Gojam and Wellega grouped in to cluster I characterized with the longest spike length and earlier flowering. Cluster II was characterized with the longest plant height, awn length, peduncle extrusion, peduncle length, and number of seeds per spike, in which Shewa, Wello and Sidamo were grouped in this cluster. The shortest plant height, awn length, peduncle length, spike length, the heaviest thousand seed weight, the lowest number of seeds per spike and the highest Number of fertile tillers per plant were clustered under cluster III, in which Tigray is the source of collection for this cluster. Cluster IV comprised one region (Gonder) which was characterized with shorter peduncle extrusion; longer spike length, smaller number of fertile tiller per plant, delayed flowering and maturity. The same results were reported by Dejene et al. (2010) and Demissie and Bjornstad (1996).

Table 9. The summary of cluster means of nine regions of barley accessions for their 10 quantitative characters.

Characters	Cluster means			
	I	II	III	IV
Plant height	105.11	106.53	96.00	102.06
Awn length	11.35	11.91	11.08	11.25
Peduncle extrusion	14.77	14.52	12.36	11.74
Peduncle length	39.01	39.26	34.15	34.28
Spike length	8.24	7.97	7.77	7.82
Thousand seed weight	46.62	43.06	46.96	42.30
Number of seed per spike	31.84	42.52	31.37	40.16
Number of fertile tiller per plant	3.81	3.55	4.09	3.41
Day to 50% flowering	59.45	76.14	72.90	84.12
Day to maturity	124.83	119.97	119.76	130.34
Regions	**Arsi, Bale, Gojam Wellega**	**Shewa, Wello Sidamo**	**Tigray**	**Gonder**
Number of regions	4	3	1	1

Principal component analysis

Principal component analysis for accessions

The principal component analysis exhibited variances of 30, 17, 15 and 10%, were extracted for the first four principal components and accounts about 72% of total variation (Table 10). Days to 50% flowering, days to maturity, number of seeds per spike and peduncle extrusion showed greater loading for the variation in the first principal components. Similarly, thousand seed weight, days to maturity, spike length and number of seeds per spike contributed major variation in the second principal component. The variation in the third principal component were mainly due to number of fertile tiller per plant, peduncle extrusion, plant height, awn length and peduncle length, while the fourth principal component showed 10% of total variation with greater loading from awn length, plant height and spike length. In line with the present finding, Demissie and Bjornstad (1996) employed principal component analysis for detecting variation in 49 barley populations in which the first four PCs contributed 63% of total variation. Generally days to 50% flowering, days to maturity, and number of seeds per spike were the most loading characters for the variation among accessions.

Principal component analysis for regions

Principal component analysis showed that 83% of total variation among regions was extracted for the first three principal components having eigenvalue greater than one (Table 10). Peduncle extrusion, peduncle length, days to flowering, days to maturity and plant height gave the most loading contribution for the variation in first principal component which contributed 34% of the variation. The second principal component contributed 31% of the variation in which thousand seed weight, number of seed per spike, awn length and number of fertile tillers per plant contributed greater variation. Similarly, days to maturity, days to flowering, spike length and plant height were the most loading contributors for the third principal component.

Principal component analysis for altitude groups

The first two principal components extracted 93% of total variation among altitude groups having eigenvalue greater than one (Table 10). Number of seed per spike, peduncle extrusion, number of fertile tiller per plant and days to 50% flowering were the most loading contributors in the first principal component. Similarly, spike length, awn length and peduncle length were showed greater loading in the second principal component.

Diversity index

Estimates of Shannon Weaver diversity index over regions of origin and altitude groups showed high diversity index for the six qualitative characters studied. Phenotypic diversity was very high for kernel row number (H′=0.99), grain color (H′=0.90) and spike attitude (H′=0.85) and low for lemma color (H′=0.48) (Table 11). This is due to high ecological heterogeneity of the country, which is favorable condition for barley landrace cultivation. Except lemma color, all characters were high in phenotypic diversity over all regions of origin and

Table 10. Eigenvectors, total variance, cumulative variance, and eigenvalues for ten quantitative characters of 102 barley landrace in Ethiopia for first four, three and two principal components for accessions, regions and altitude groups respectively

Characters	Eigen vectors for accessions				Eigen vectors for regions			Eigen vectors for altitude groups	
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC1	PC2
PLH	-0.18	0.16	0.39	-0.51	0.30	0.25	0.32	0.32	0.23
AWL	0.14	-0.23	0.38	0.62	0.29	0.43	-0.01	0.24	-0.54
PDER	0.36	0.01	0.42	-0.11	0.49	0.05	0.10	-0.34	-0.24
PDL	0.29	-0.24	0.35	-0.28	0.42	0.09	0.21	-0.32	-0.27
SPL	0.21	0.35	0.31	0.42	0.27	-0.23	0.45	-0.22	0.56
TSW	0.24	0.57	0.12	-0.08	0.22	-0.51	0.01	-0.31	0.26
NSPS	-0.40	-0.34	0.19	0.16	-0.20	0.47	-0.02	0.35	-0.16
NFTPP	0.25	0.25	-0.45	0.17	0.08	-0.38	-0.23	-0.33	-0.03
DFL	-0.47	0.27	0.14	0.18	-0.35	0.06	0.52	0.33	0.24
DMA	-0.41	0.37	0.18	0.04	-0.31	-0.19	0.54	0.32	0.18
Eigen value	2.96	1.70	1.52	1.02	3.4	3.08	1.8	7.47	1.86
% of total variance	30	17	15	10	34.0	31.0	18.0	75.0	19.0
% cumulative variance	30	47	62	72	34.0	65.0	83.0	75.0	93.0

PLH = plant height, AWL = awn length, PDER = peduncle extrusion, PDL = peduncle length, SPL = spike length, TSW = thousand seed weight, NSPS = number of seed per spike, NFTPP = number of fertile tiller per plant, DFL50% = days to 50% flowering, DMA = days to maturity, PC = principal component.

altitude groups for this study. The same results were reported by different authors (Demissie and Bjornstad, 1996; Abdi, 2011; Lakew and Alemayehu, 2011).

Regional diversity index

Estimate of diversity index (H') pooled over regions of origin showed high phenotypic diversity among six qualitative characters (Table 11). The mean H' varied from 0.66 for Tigray to 0.83 for Arsi. Arsi, Wellega and Wello showed greater diversity index followed by Bale, Gojam, Gonder and Sidamo. Tigray and Shewa showed lower genetic diversity index. Among all characters, kernel row number from Gonder, grain color from Gojam, Shewa, and Wellega, spike density from Arsi and Tigray showed high genetic diversity index. Lemma color and awn color showed lower genotypic diversity index in most regions.

Altitudinal diversity index

Altitude groups showed high phenotypic diversity among six qualitative characters. The mean H' pooled over characters for four altitude groups varied from 0.65 for altitude group I (1500-2000 m.a.s.l) to 0.84 for altitudes group III (2501-3000 m.a.s.l) with mean value of 0.77 ± 0.07 (Table 11). Kernel row number from altitude group II (2001-2500), and III, grain color from altitude group I and III and spike density from altitude group III and IV (3001-3500) showed the highest diversity index. Altitude group II indicated lower genetic diversity index for lemma color. Difference in altitude gradient and agro-ecological setting gave high diversity variation in cereal crops especially barley landraces. The result indicated high H' in Ethiopia barley landrace in altitude group III (2501-3000 m.a.s.l). Diversity index decreased at an altitude above 3000 m.a.s.l. This result is in agreement with the work of Demissie and Bjornstad (1996) and Abdi (2011). Similarly, mean diversity index for characters increases with altitude reaching a maximum between 2400 and 2800 m.a.s.l and decreasing beyond that altitude (Engels, 1991). This indicates high genotypic diversity in barley is related to high rainfall and lower temperature at high altitudes, which shows barley that is a cool season crop.

Conclusions

One hundred and two barley accessions were evaluated for ten quantitative and six qualitative characters to assess morphological diversity and association of traits in Ethiopian food barley (*H. vulgare* L.) landraces in relation to regions of origin and altitudes. Analysis of variance and genetic diversity index indicated the existence of morphological diversity and association of traits in Ethiopian food barley (*H. vulgare* L.) landraces in relation to regions of origin and altitudes. Cluster analysis grouped one hundred two accessions in to five distinct groups. Number of accessions per cluster varied from 44 accessions in cluster I to 4 accessions in cluster V. Shannon-Weaver diversity index showed high and comparable levels of phenotypic diversity among the accessions. Phenotypic diversity was very high for kernel row number (H′=0.99), grain color (H′=0.90) and spike

Table 11. Estimate of Shannon-Weaver diversity index (H') of 102 Ethiopia barley landraces for nine region of origins and four altitude groups by six qualitative characters.

Region	Qualitative characters						Mean H'±SE
	SPA	KRN	ANC	LMC	GRC	SPD	
Arsi	0.84	0.82	0.95	0.57	0.85	0.98	0.83 ± 0.05
Bale	0.93	0.85	0.47	0.42	0.74	0.85	0.71 ± 0.08
Gojam	0.90	0.90	0.62	0.46	0.96	0.82	0.77 ± 0.07
Gonder	0.71	0.99	0.57	0.39	0.86	0.81	0.72 ± 0.08
Shewa	0.58	0.92	0.44	0.42	0.96	0.87	0.69 ± 0.10
Sidamo	0.87	0.91	0.52	0.38	0.90	0.86	0.74 ± 0.09
Tigray	0.69	0.76	0.43	0.34	0.75	0.99	0.66 ± 0.09
Wellega	0.90	0.69	0.91	0.61	0.96	0.89	0.82 ± 0.05
Wello	0.73	0.94	0.93	0.58	0.87	0.92	0.82 ± 0.05
Altitude group (m.a.s.l)							
Group I	0.57	0.69	0.52	0.53	0.97	0.66	0.65 ± 0.06
Group II	0.90	0.95	0.61	0.47	0.93	0.82	0.78 ± 0.08
Group III	0.70	0.98	0.84	0.59	0.97	0.96	0.84 ± 0.06
Group IV	0.93	0.89	0.49	0.28	0.49	0.93	0.66 ± 0.11
Total Mean	0.85	0.99	0.70	0.48	0.90	0.72	0.77 ± 0.07

SPA = Spike attitude; KRN = Kernel row number; ANC = Awn color; LMC = Lemma color; GRC = Grain color; SPD = Spike density, m.a.s.l = meter above sea level; Group I (1500-2000), Group II (2001-2500), Group III (2501-3000) and Group IV (3001-3500).

attitude (H′=0.85) and low for lemma color (H′=0.48). The mean H' pooled over characters for four altitude groups, were varied from H'=0.65 for altitude group I (1500-2000) to H'= 0.84 for altitude group III (2501-3000). Greater genotypic diversity index was observed in Arsi, Wellega and Wello and also high genotypic diversity was observed in altitude groups II (2001-2500), and III (2501-3000), which comprised the major barley growing areas in the country. Days to flowering, days to maturity and numbers of seeds per spike, from quantitative characters and kernel row number, grain color and spike attitude from qualitative characters contributed much of the variances among accessions. Based on the observed variation both for quantitative and qualitative characters, it could be concluded that studying the phenotypic diversity among barley accessions is important to identify the genetic potential of parental lines and increase the efficiency of the barley breeding programmers.

Conflict of Interest

The authors have not declared any conflict of interest.

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Correlation, path coefficient analysis and heritability of grain yield components in pearl millet (*Pennisetum glaucum* (L.) R. Br.) parental lines

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Twenty four parental lines of pearl millet A/B pairs developed jointly by Lake Chad Research Institute (LCRI), Maiduguri and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Kano during 1997 to 1999 were evaluated along with a seed parent (ZATIB) across five locations to determine yield and yield component relationships, heritability estimates as well as genetic advance. Correlation coefficient analysis showed that stand count (r=0.249), plant height (r=0.435) and head weight (r=0.958) significantly (p<0.05) and positively correlated with grain yield while days to 50% flowering significantly but negatively correlated (r=-0.539) with grain yield. There were negative but none significant correlation between grain yield with downy mildew score (r=-0.100) and *Striga* count (r=-0.095) while downy mildew score and *Striga* count negatively correlated with stand count (r=-0.155 and r=-0.065 respectively). Head weight has high positive and significant environmental, genotypic and phenotypic correlation coefficient with grain yield (r_e=0.920; r_g=0.900 and r_p=0.980). Positive and significant genotypic and phenotypic correlation coefficient exists between plant height and grain yield (r_g=0.593 and r_p=0.417). Path coefficient analysis indicated that stand count had strong positive direct effect (0.970) on grain yield followed by plant height (0.953). Head weight expressed high negative direct effect (-0.846) on grain yield. The parental lines under study showed high to moderate broad-sense heritability; with panicle length expressing the highest heritability (78.95%), followed by grain yield (75.43%) and head weight (73.30%). The rest characters expressed moderate heritability values. Panicle length and head weight displayed high heritability and high genetic advance indicating that the two traits might be controlled by additive gene effects. This suggests that selection in the segregating generation may be effective. Phenotypic correlation approximates genotypic correlation coefficient in this study, indicating that the influence of environment may be probably minimal and traits with high predictive values can be selected early in the breeding program as against traits with low predictive values.

Key words: Pearl millet, correlation, path analysis, heritability, genetic advance.

INTRODUCTION

Pearl millet is an important staple food security crop in Nigeria grown in 5.2 million hectares with a production of 4.62 million tones grain per year. It occupies about 32% of total area planted under cereals, and account for about

26% of total cereals production in Nigeria (Ndjeunga et al., 2010). Low productivity of pearl millet across all the millet growing belts in Nigeria is due to the cultivation of open pollinated varieties (OPVs) by farmers coupled with adverse biotic and abiotic stresses. It has been observed that single hybrid generally gives 20 to 30% more grain yield than OPVs (Rai et al., 2006). With the increasing population and rapid deployment of pearl millet into feed and instant value added products, significant increase in per hectare yield of the crop is required to meet the ever increasing demand, which can be made possible with the use of hybrids. Based on the availability of a commercially exploitable cytoplasmic-nuclear male-sterility system LCRI, Maiduguri along with ICRISAT embarked on pioneer research of developing commercial pearl millet hybrids using indigenous germplasm and converted breeding lines (Ezeaku and Angarawai, 2005). Out of the large pool of parental lines developed, 30 male sterile lines (A-lines) and their maintainer (B-lines) were selected based on uniformity, stable sterility and other characters such as seed set, exertion and vigor. These traits were evaluated based only on visual observation. As seed parents required for the production of millet hybrids, studies on the character association and heritability of the A/B lines are the first most significant step in embarking on single cross hybrid program. Estimation of correlation, path coefficient analysis, heritability and genetic advance would be useful in developing appropriate breeding and selection strategies. Therefore, understanding the yield and yield components relationship as well as heritability estimate of hybrid parental lines is essential in determining traits that contributes significantly to yield, facilitate their selection and utilization in hybrid development. Grain yield is a complex quantitative trait and is polygenetically controlled. Therefore, selection on the basis of grain yield alone is usually not effective. However, selection based on its components and secondary characters could be more efficient and reliable (Govindaraji et al., 2011). The purpose of this study was to gain sufficient knowledge of the interrelationship, path coefficient between yield and its components, heritability and genetic advance among pearl millet parental lines to determine criteria for selection that could be effectively used to identify the desirable lines with potential for high yield in single cross hybrid development program.

MATERIALS AND METHODS

A set of twelve A-lines and their maintainers (B-lines) developed by LCRI, Maiduguri and ICRISAT, Kano were evaluated in five locations namely, Minjibir, Bagauda, Zaria, Panda and Babura in 2000 during wet season in a randomized complete block design with four replications. The experimental unit was a four-row plot of 5 m long, spaced at 0.75 m apart and intra row spacing of 0.5 m. Inorganic fertilizer (NPK 15:15:15) was applied as a basal dose at the rate of 300 kg per hectare. Crops were thinned out to two plants per stand count two weeks after crop emergence. The crop was top dressed with 100 kg urea per hectare after three weeks of post crop emergence.

Data was taken from two middle rows for stand count, days to 50% flowering, downy mildew score (recorded following a 1-6 damage rating scale, where 1 = no symptom, 2 = 1-5% infected plants, 3 = 6-10% infected plants, 4 = 11-20% infected plants, 5 = 21-40% infected plants and 6 = > 40% infected plants), *Striga* count, plant height (cm), panicle length (cm), head weight (kg ha^{-1}) and grain yield (kg ha^{-1}) following the recommendation of International Board for Plant Genetic Resources (IBPGR) and ICRISAT descriptor list for pearl millet (Anonymous, 1993). Correlation coefficient was computed from variance and covariance components as suggested by Burton (1952), Wright (1960 and 1968) and Narasimharao and Rachie (1964). The correlation coefficient was partitioned into direct and indirect effects according to Dewey and LU (1959) and Turner and Stevens (1959). The genotypic, phenotypic, environmental correlation between yield and its components among themselves and genetic advance were worked out as per the methods suggested by Johnson et al. (1955) while heritability in broad sense was calculated according to the procedure described by Singh and Chaudhary (1977). All the data were analyzed using GENSTAT, 2009 edition.

RESULTS AND DISCUSSION

The correlation between pairs of variables sampled combined over five environments are presented in Table 1. Result showed that stand count (r=0.249), plant height (r=0.435) and head weight (r=0.958) significantly and positively correlated with grain yield at the 0.05 and 0.01 levels of probability while days to 50% flowering significantly but negatively correlated (r= -0.539) with grain yield. Several previous workers (Atif et al., 2012; Singh and Govila, 1989; Bidinger et al., 1993; Jindla and Gill, 1984) also found similar results in pearl millet. The negative but significant correlation of days to 50% flowering with grain yield shows that parental lines with shorter days to flowering tend to produce more grain yield and vice-versa. This negative correlation indicates that it is not possible to improve both traits simultaneously depending on the intensity of linkage or the degree of tradeoff between the two traits. Similarly, Tables 3 and 4 equally shows that the two traits exhibited negative and significant genotypic correlation coefficient (rg= -0.532) and phenotypic correlation coefficient (rp= -0.359). Although, some of the traits that exercise negative correlation with one another will be difficult to select for in characterization of desirable traits, those with negative association but none significant correlation will be disregarded in selection for crop or variety improvement (Ariyo et al., 1987; Henry and Krishna, 1990; Newall and Eberhart, 1961). There were negative but non significant

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Table 1. Combined correlation coefficients between yield and yield components in pearl millet parental lines grown across five environments.

Yield components	Grain yield	Stand count	Days to 50 % flowering	Downy mildew score	*Striga* count	Plant height	Panicle length	Head weight
Grain yield	**1.00**							
Stand count	0.249**	**1.00**						
Days to 50 % flowering	-0.539**	-0.157*	**1.00**					
Downy mildew score	-0.100	-0.155*	-0.033	**1.00**				
Striga count	-0.095	-0.065	-0.174*	-0.122	**1.00**			
Plant height	0.435**	0.054	-0.154	-0.002	-0.025	**1.00**		
Panicle length	0.154	0.012	-0.051	0.069	-0.030	0.236**	**1.00**	
Head weight	0.958**	0.258**	-0.545**	-0.032	0.063	0.432**	0.162*	**1.00**

*, ** Significant at 5% and 1% probability level respectively.

Table 2. Environmental correlation coefficients of grain yield on other parameters.

Trait	Days to 50% flowering	Downy mildew score	*Striga* count	Plant height	Panicle length	Head weight	Grain yield
Stand count	0.095	0.147	0.184	-0.135	0.191	0.117	0.048
Days to 50 % flowering		-0.009	0.107	-0.338	0.084	0.131	0.070
Downy mildew score			0.050	-0.021	0.040	-0.003	-0.040
Striga count				-0.048	-0.018	-0.184	-0.174
Plant height					-0.203	0.087	0.070
Panicle length						0.227	0.257
Head weight							0.920**

**Significant at 1% levels of significant.

Table 3. Genotypic correlation coefficients of grain yield on other parameters.

Trait	Days to 50% flowering	Downy mildew score	*Striga* count	Plant height	Panicle length	Head weight	Grain yield
Stand count	-0.549*	-0.688**	0.346	0.430*	0.543*	0.906**	0.904**
Days to 50 % flowering		0.005	-0.581*	0.288	-0.034	-0.545*	-0.532*
Downy mildew Score			-0.761**	-0.699**	0.051	-0.770**	-0.738**
Striga count				-0.100	-0.352	-0.842**	-0.793**
Plant height					0.367	0.573**	0.593**
Panicle length						0.385*	0.362*
Head weight							0.900**

*, **Significant at 5% and 1% levels of significant respectively.

correlation between grain yield with downy mildew score (r= -0.100) and *Striga* count (r= -0.095) while downy mildew score and *Striga* count negatively correlated with stand count (r= -0.155 and r= -0.065, respectively). This result indicates that these biotic stresses reduced grain yield and this could be due probably to the reduction in plant population.

The environmental, genotypic and phenotypic correlation coefficients of grain yield on other parameters are presented in Tables 2, 3 and 4. The correlation coefficient for most of the pairs of characters revealed the presence of strong positive and negative genotypic association between grain yield and other parameters assessed. The result further showed that genotypic correlation coefficients were higher than both the environmental and phenotypic correlation coefficients for

Table 4. Phenotypic correlation coefficients of grain yield on other parameters.

Trait	Days to 50% flowering	Downy mildew score	*Striga* count	Plant height	Panicle length	Head weight	Grain yield
Stand count	-0.305	-0.108	0.184	0.193	0.422*	0.626**	0.609**
Days to 50 % flowering		-0.003	-0.047	0.057	-0.002	-0.344	-0.359
Downy mildew score			-0.019	-0.191	0.034	-0.251	-0.262
Striga count				-0.049	-0.081	0.069	-0.071
Plant height					0.190	0.405*	0.417*
Panicle length						0.346	0.338
Head weight							0.980**

*, **Significant at 5% and 1% levels of significant respectively.

most of the parameters studied. Similar results were reported by Atif and Awadalla (2012) in pearl millet. Since environmental correlation coefficients approximate phenotypic correlation coefficients in this study, characters with strong genotypic association with grain yield will demonstrate consistent performance across wide range of environment. Head weight has high positive and significant environmental, genotypic and phenotypic correlation coefficient with grain yield (r_e=0.920; r_g=0.900 and r_p=0.980, respectively). Similarly, positive but significant genotypic and phenotypic correlation coefficient exists between plant height and grain yield (r_g=0.593 and r_p=0.417, respectively). The result suggests that these two traits, are less influenced by the environment and they could be improved in diverse environments. This finding is in agreement with Ezeaku and Mohammed (2006) and Kumari et al. (2013). The positive but highly significant correlation coefficient between stand count and grain yield for genotypic (rg=0.904) and phenotypic (rp=0.609) correlation coefficient indicates that optimum plant population generally promotes higher grain yield despite other environmental variables.

Downy mildew reduced plant height, head weight and grain yield since it correlated negatively with these traits for environmental, genotypic and phenotypic correlation coefficient. Ezeaku and Angarawai (2005) found downy mildew to adversely affect these traits. Negative environmental, genotypic and phenotypic correlation coefficient between *Striga* count with plant height, grain yield, panicle length, and head weight revealed that *Striga* attacks as expected would reduced plant height, grain yield, panicle length and head weight. The positive but significant genotypic correlation coefficient between head weight and plant height (rg=0.573) and their very low environmental correlation coefficient (re=0.018) indicates that selection for grain yield based upon the phenotypic performance of these characters alone may not be effective.

The significant genotypic correlation coefficient between head weight and plant height and between head weight and panicle length indicates that these two characters are independent of one another and they could be selected separately as they are components of grain yield. Both traits also influenced grain yield significantly and positively in this study. This shows that taller plants and longer panicles possess heavier head weight and greater grain yield to some extent than shorter plants, probably due to greater mobilization of assimilates to the panicle in taller plants. This result is in agreement with Gupta and Sidhy (1972) and Ezeaku and Mohammed (2006).

When large numbers of variables are included in a correlation study the association among themselves will be very complex. Thus path analysis is necessary to elucidate the true direct and indirect relationship among such characters. In this study path analysis was used to examine the relationship between grain yield and its components. Path coefficient analysis showing direct and indirect effects of yield and yield components are presented in Table 5. Stand count had strong positive direct effect (0.970) on grain yield followed by plant height (0.953). The high positive direct effect of stand count and plant height on grain yield is indicative of their important role in influencing grain yield. However, the negative indirect effects of panicle length and head weight on grain yield through stand count and plant height suggests the effect of downy mildew and *Striga* on these traits which is equally corroborated by the negative environmental genotypic and phenotypic correlation coefficient between downy mildew and *Striga* count on panicle length and head weight. Panicle length had negative direct effect (-0.214) on grain yield. Also, head weight expressed high negative direct effect (-0.846) on grain yield. This shows that increasing panicle length through selection may not necessarily lead to proportionate increase in grain yield. The disparity between Tables 1 to 4 which consistently indicated high positive correlation between head weight and grain yield and Table 5 which revealed high negative direct effect of head weight on grain yield justifies the need to clarify the nature of relationship between yield and yield components

Table 5. Path coefficient analysis of the direct and indirect effects of the yield components and their genotypic correlation coefficients with grain yield.

Trait	Direct effect on grain yield	Stand count	Days to 50 % flowering	Plant height	Panicle length	Head weight	Genotypic correlation coefficient
Stand count	0.970	0.00	-0.532	0.417	0.025	0.546	1.426**
Days to 50% flowering	-0.742	0.407	0.00	-0.214	0.527	0.404	0.382*
Plant height	0.953	0.410	0.274	0.00	0.350	-0.082	1.905**
Panicle length	-0.214	-0.116	0.007	-0.078	0.00	0.879	0.478*
Head weight	-0.846	-0.767	0.461	-0.485	-0.325	0.00	-1.962**

Significant at 5% and 1% levels of significant, respectively; residual effects = 0.125.

Table 6. Combined correlation coefficient (r), heritability (broad sense, h^2bs) and genetic advance (GA) (as per cent of mean) of the yield component characters in pearl millet parental lines

Traits	R	h^2bs	GA
Stand count	0.904**	57.15	7.05
Days to 50% flowering	-0.532	67.24	10.05
Plant height	0.593**	58.80	14.87
Panicle length	0.362	78.95	20.29
Head weight	0.900**	73.30	20.77
Grain yield	1.00	75.43	14.41

** Significant at 1% levels of significant.

using path coefficient analysis. This process has assisted in elucidating the true relationship between head weight and grain yield hence its direct selection will only be effective in improving grain yield in the absence of some biotic factors that affects head weight such as downy mildew and *Striga* infestation. Residual effect is low (0.125) indicating most of the yield component characters were considered in the present study.

Combined correlation coefficient, estimates of heritability and genetic advance as percent of mean are presented in Table 6. Estimates of heritability and their roles in predicting gains in crop species have been reported by Kang et al. (1983), Kole and Saha (2013) and Suthamathi and Dorairaj (1995). The parental lines under study showed high to moderate heritability with panicle length expressing the highest heritability (78.95%), followed by grain yield (75.43%) and head weight (73.30%). The rest characters expressed moderate heritability values. High heritability with positive but highly significant correlation coefficient was observed for head weight (73.30 and 0.900 respectively). Similarly, stand count and plant height expressed moderate heritability with positive and significant correlation coefficient. This finding is in agreement with Govindaraj et al. (2011). The high heritability in broad sense recorded for panicle length, head weight and grain yield indicates that genotype plays a most prominent role than the environment in determining the phenotype suggesting the preponderance of additive gene effects in the inheritance of the traits (Panse, 1957). This showed that phenotypic selection for these traits may likely be effective in hybrid development program. Similar results were also reported by Ghorpade and Metta (1993), Lakshmana and Guggari (2001) and Govindaraj et al. (2011). Phenotypic correlation approximates genotypic correlation coefficient in this study, suggesting that the influence of environment may probably be minimized and traits with high predictive values can be selected early in the breeding program as against traits with low predictive values. In this study panicle length, head weight, grain yield, stand count, days to 50% flowering and plant height exhibited high to moderate heritability estimates suggesting that these traits may be improved upon significantly.

The high genetic advance as percent of mean (> 20%) were recorded for panicle length (20.29) and head weight (20.77). The medium genetic advance as percent of mean (10 to 20%) were recorded for traits such as days to 50% flowering (10.05), plant height (14.87), and grain yield (14.41) while the low genetic advance as percent of mean (<10%) was recorded for number of plant stand (7.05). High heritability and high genetic advance was observed respectively for panicle length (78.95 and 20.29%), and head weight (73.30 and 20.77%). The progress that can be made in advancing mean value of population through selection program will depend on the heritability of the traits under consideration, the

phenotypic variation as well as the selection intensity. Therefore, result based only on heritability might not help in identifying traits that are needed to advance selection. Johnson et al. (1955) had also suggested that heritability estimates along with genetic gain is usually more helpful than the heritability alone in predicting the resultant effect from selecting the best individuals. The heritability gives information on the magnitude of the inheritance of traits, while genetic advance aid in formulating suitable selection criteria. Hence, traits that displayed high heritability and high genetic advance such as panicle length and head weight might be controlled by additive gene effects. This indicates that selection in the segregating generation may be effective.

Conclusion

Twenty four parental lines of pearl millet A/B pairs were evaluated along with ZATIB, a seed parent across five locations in northern Nigeria to determine yield and yield component relationships, heritability and genetic advance. The study is useful in developing appropriate hybrid breeding and selection strategies aimed at enhancing the performance of the resulting hybrids as traits that contributes significantly to yield will be found, selected and utilized.

The results revealed that stand count, plant height and head weight expressed positive and significant correlation with grain yield. Head weight had high positive and significant environmental, genotypic and phenotypic correlation coefficient with grain yield. Stand count also had strong positive direct effects with grain yield. The lines showed high to moderate broad-sense heritability; with panicle length expressing the highest heritability (78.95%), followed by grain yield (75.43%) and head weight (73.30%). With panicle length and head weight displaying both high heritability and high genetic advance, selection in the segregating generation may be effective.

Conflict of Interest

The authors have not declared any conflict of interest.

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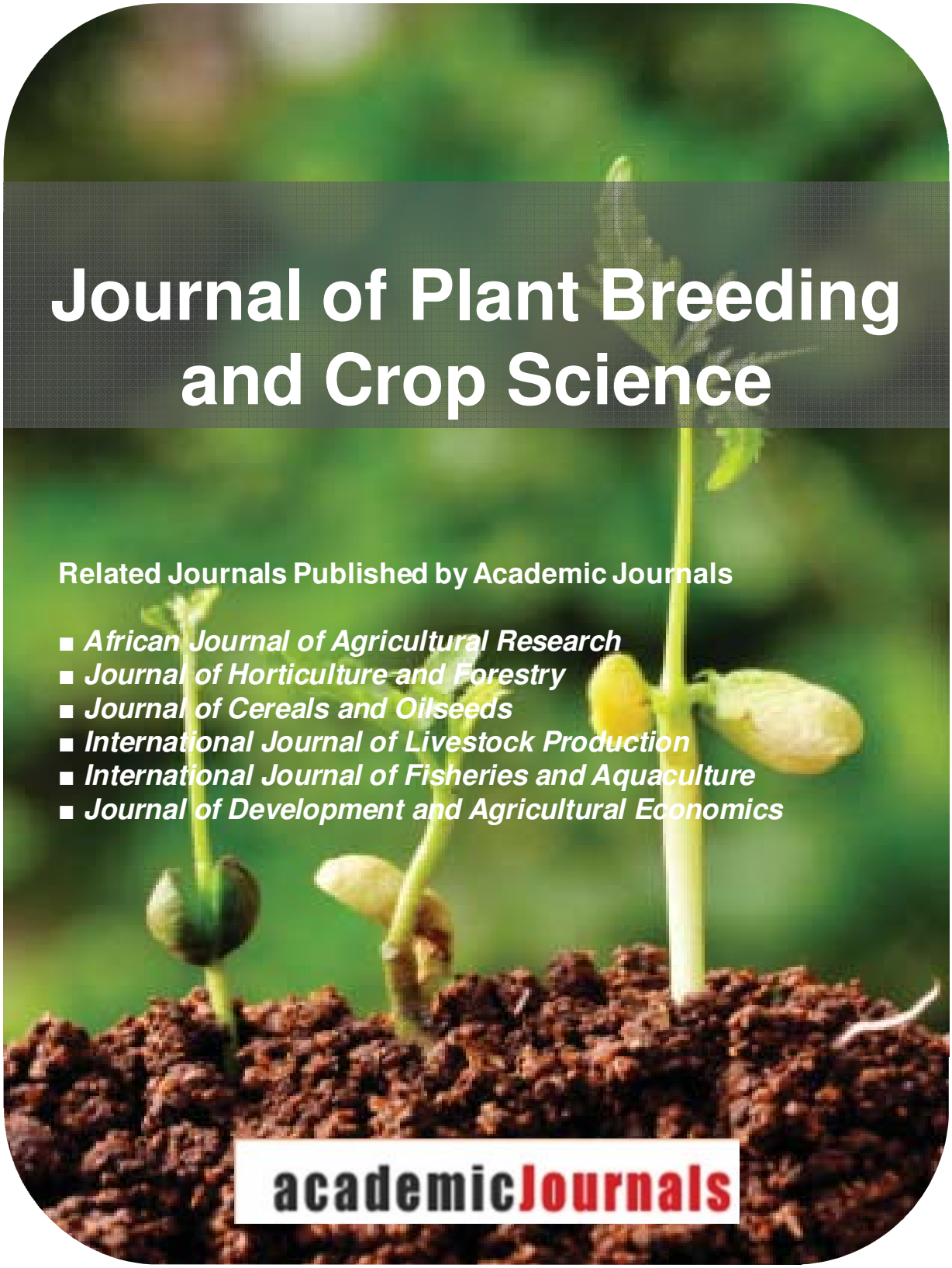

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