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Full Length Research Paper

Association and variation on boll and seed morphology among hybrids between linseed (*Linum usitatissimum* L.) and *Linum bienne* Mill. and their parents

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Linseed is the only cultivated species from the genus *Linum* and selection is the most frequently used method to develop varieties from the crop resulting in the reduction of the genetic diversity. *Linum bienne* Mill. is genetically more diverse than linseed and produces fertile hybrids with linseed. The author aimed for the development of hybrids with new combinations of genes useful for variety development programme. Morphological characters of parental, F1 and F2 hybrid plants were studied in field and cluster analyses, coefficient of variations (CV) and Nested analysis of variance (NANOVA) were used for the analyses. Cluster analyses from combined quantitative and qualitative characteristics were more powerful in grouping genotype. Selfed F2 hybrids scored the highest CV for all characteristics and seed-weight (20.36%). The degree of boll shattering was different among hybrids. F2 hybrids scored more phenotypic classes from seed coat colour. The differences in seed length and 1000-SW among the groups were significant (*P* = 0.017 and 0.033, respectively). Except for the differences in seed length, all the mean value differences in quantitative characteristics among sub-groups within the group were significant (P < 0.01). The result showed that the hybrids would be important populations to develop varieties for different traits. There was dragging of unwanted parental characters to hybrids due to a linkage. Assisting the process of crossing with markers associated with a trait would help to minimize the dragging of unwanted characters into hybrids.

Key words: *Linum bienne*, *Linum usitatissimum*, crop wild relative, segregation, crossing.

INTRODUCTION

Linseed/Flax (*Linum usitatissimum* L.) is one of the species from genus *Linum*, the largest genus of the Linaceae family containing 100 up to 230 species (Seetharam, 1972; Seegeler, 1983; Friis, 2000; Jhala et al., 2008). Of the c. 200 species of the genus, *Linum, L. usitatissimum* is the only cultivated species for oil in its seed and fibre in its stem (Zohary, 1999). From the beginning, linseed domestication involved the selection of some characters and more efficient self-fertilization (Durrant, 1976). *L. usitatissimum* is a self-pollinated species with less than 1% (Seegeler, 1983; McGregor, 1976) out-crossing but Mansby et al. (2000) reported a

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higher value of out-crossing; most of the linseed varieties have been developed by crossing within the gene pool of *L. usitatissimum* (Kurt and Evans, 1998). Ethiopia is one of the centres of origin of domestication for *L. usitatissimum* as a grain crop (Vavilov, 1951). Breeding using pedigree selection in linseed is the most common approach for crop improvement and is a straight forward process leading to homogeneous breeding lines (Salas and Friedt, 1995; Friedt, 1993) but this would lead to a higher rate of loss in genetic variation (Diederichsen, 2001). The observation that linseed cultivars in Canada showed a considerably lower rate of genetic variation than a world collection (Diederichsen, 2001) is an example where breeding programs dependent on selected varieties can result in a loss in genetic variations. After domestication and selection for variety development, linseed experienced bottlenecks in genetic diversity (Jaradat, 2015).

Heslop-Harrison (2002) reported a very small portion (0.1%) of the world plant species are grown as crops but still only a small proportion of the total genetic variability contained by this percentage of plants is used in commercial varieties. The wild relatives have vast genetic potential for the production of adapted commercial hybrids (Jaradat, 2015). *Linum bienne* Mill., the wild progenitor of cultivated linseed, is a potential donor of new alleles for *L. usitatissimum* genetic improvement (Soto-Cerda et al., 2011). Although *L. usitatissimum* and *L. bienne* as two different species have differences for many of their agronomic characteristics, *L. bienne* crosses and produces fertile offspring with cultivated linseed (Tammes, 1928). The two species have similar chromosome numbers (2n = 30) and the absence of differences in their parental ploidy levels and 'effective ploidy' as parental dosage between them may help the two species to develop a fertile hybrid (Lafon-Placette et al., 2018). Hybrids for cereal crops are the source of new combinations of genes and are vigours (Heslop-Harrison, 1990). Inter-specific crosses contributed for yield, drought and disease resistance and nutritional quality improvement of many crops (Desphande and Jeswani, 1951). Hybridizations of *L. usitatissimum* with other *Linum* species can improve some of linseed agronomic characteristics as suitable for industrial or nutritional quality (Nichterlein et al., 1986). Salt and Henderson (2017) also reported close relatives and progenitor species of many of our staple crops as having great potential significance in agriculture.

L. bienne is not growing in Ethiopian natural ecosystems (Friis, 2000) and in the present study the hybridization was between an American origin *L. bienne* (PI522290) and Ethiopian linseed cultivars. Therefore, the hybridization between *L. bienne* and Ethiopian linseed cultivar is not only hybridization between two different species but also between two geographically isolated species. Hybridization between linseed cultivars and *L. bienne* has been undertaken and in this study we aimed to determine the associations and variations among different hybrids and parental species for some agronomic characteristics of the two species and to present hybrid genotypes for future development of better linseed varieties for selected agronomic characteristics as well as for restoring the genetic diversity of linseed.

MATERIALS AND METHODS

Plant

Wild *Linum* spp., *Linum beinne* Mill. (PI 522290) acquired from the North Central Regional Plant Introduction Station - USDA, and six cultivated *L. usitatissimum* L.: MacBeth, a line from the Crop Development Centre at the University of Sasaktchewan, Canada; PI-523353 (in this paper named as HARC-15) from Holetta Agricultural Research Centre/Ethiopia; and accessions 13510, 237001, 235177 and 243817 from Ethiopian Biodiversity Institute (EBI) holdings were the germ-plasm used as parental plants for hybridization. Field characters of parental and hybrid plants were studied from June 2014 to November 2015 in three generations using rain-fed and irrigated fields. Seeds from the cultivated species were planted each week in five batches to match with the flowering time of *L. bienne,* it was the flowering time of the fourth batch of plants that matched with that of *L. bienne.* Parental genotypes were grown parallel with F_1 and F_2 hybrid genotypes for backcrossing and to check for environmental influence on the development of subsequent generations. Flowers from some plants were emasculated before their anthers released pollens. The emasculated flowers for control and crossing were protected by cellophane plastic paper after emasculation and after crossing for about 6 hours, sometimes less depending on the season and daytime temperature. The data from F_2 hybrid plants were scored from both selfed and backcrossed plants. Selfed F_2 hybrid plants were from seeds of selected F_1 plants and plants sampled randomly from volunteer plants grown in mixed stand.

Population grouping

The 76 sampled genotypes were grouped into different population groups to analyse the degree of variation among different population groups using Nested Analyses of Variances (NANOVA) technique. The first way of grouping was into three (F_2 hybrids, F_1 hybrids and parental plants); the second way was into four (selfed F_2 hybrids, backcrossed F_2 hybrids, F_1 hybrids, and parental plants, or F_2 hybrids, F_1 hybrids, wild parent and cultivated parental plants); and the third way was into five population groups (F_1 hybrids, selfed F_2 hybrids, backcrossed F_2 hybrids, cultivated parental plants, and wild parental plants). For cluster analysis F_2 hybrids were split into F_2 from HARC 15 × *L. bienne* (SF_2Ha), from accession 243817 and *L. bienne* (SF_2Hb) and F2 from volunteer and mixed stand hybrids (VSF_2H). That is the 76 sampled plants were grouped into seven sub-groups (Table 3 or Figure 4 for sub-groups' code).

Data collection

Boll size and shattering degree, 1000-seed-weight, seed size and seed colour were the characters used to analyze the associations and variations. Matured bolls, collected from both selfed and backcrossed F_2 hybrid and cultivated parental genotypes, were uniformly heated from 22 to 80°C for 40 min and then kept at 24°C for 15 min after which they were compared for degree of shattering with three scales (dehiscent = 1; semi-dehiscent = 2; and non-

Figure 1. Parental, F_1 and F_2 hybrid genotypes' seed coat colours: 1- 35 from volunteer selfed F_2 hybrid plants growing in a mixed stand; 36 - 51 from HARC-15 × *L. bienne*. selfed F_2 hybrids; 52 - 54 from accession243817 × *L. bienne* selfed F_2 hybrids; 55 - 63 from HARC-15 × *L. bienne* backcrossed F_2hybrids

dehiscent = 3). Thousand-seed-weight, from a bulk of 300 air-dried seeds with five replicas determined by using a balance with 0.001 g sensitivity. Seeds of each sample genotype were scanned using coloured Lexmark 2600 Series TWAIN Scanner and Adobe Photoshop CS with Image Ready Software to determine their length and width in mm. Five seeds positioned vertically or horizontally on the plane of the scanner were selected randomly and their length and width measured. Some seeds from the seed bulk of each sampled plant were drawn and displayed on a sheet of paper with specific codes and serial numbers (Figure 1).

The range and possible names of seed-coat colours from Figure 1 were put beside the displayed seeds. Then ten persons were independently assigned to name the colour of each of the displayed seeds.

Fatty acid compositions from some cultivars, *L. bienne* L. and their hybrids' intact seed samples were analyzed by using NIRSystem model 5000 (Foss NIRsystem Inc., MD, USA) in the reflectance mode at 1108 to 2492 nm with an 8 nm step. Each sample was scanned five times and the composition of each fatty acid in a sample seed determined from the mean of the five recodes.

Combining quantitative and qualitative data

The following major steps (Laghetti et al., 2008) were used to combine qualitative and quantitative characters data to generate the dissimilarity matrix (Table 4) useful for cluster analysis.

The first step of the method

For quantitative characteristics, minimum and maximum mean values as outer limits for each trait from the whole studied populations and then the difference between the maximum and minimum was determined. Then the distance between every two population groups was determined. The difference for 1000-seed-weight is 4.22 determined from Max (1000-SW) - Min (1000-SW) = 5.47 - 1.25 = 4.22, this value will be used to divide the difference between each two population groups to determine the distance between them for a trait. The 1000-seed-weight dissimilarity between two populations can be determined from the square of the difference between their 1000-seed-weight score. For example, 1000-seed-weight score for BCF_2H = [1000-SW_{BCF2H} - Min1000-SW]/dif (1000-SW) = [4.08 -1.25]/4.22 = 0.67, and for F_1H = [1000-SW_{F1H} - Min1000-SW]/dif(1000-SW) = [2.27 - 1.25]/4.22 = 0.24. Now the 1000-SW dissimilarity between BCF_2H and F_1H is (0.67 – 0.24)2 = 0.18. The same calculation was done for other quantitative traits between every two population groups and then added up.

The second step of the method

For qualitative characteristics, the scored value for a sub-trait, that is, zero or one, is divided by the square root of the total number of sub-traits $\left(\frac{0}{\sqrt{6}} = 0 \; and \; \frac{1}{\sqrt{6}} = 0.41\right)$ scored in the study to

Table 1. Mean±SD, CV and range values of BD, SL, SW and 1000-seed-weight of the seven plant population groups.

Trait	Parameter	Population							
		VSF_2H(35)	SF_2H^a(16)	SF_2H^b(3)	BCF_2H(9)	CP(6)	WP(1)	F_1H(6)	Total (76)
BD	Mean±SD	5.85±0.46	5.72±0.39	6.13±0.22	6.23±0.40	6.36±0.28	5.08±0.08	5.54±0.16	5.88±0.47
	CV	7.91	6.86	3.62	6.39	4.42	1.65	1.99	7.70
	Range	4.80-7.20	4.90-6.70	5.60-6.40	5.40-7.20	5.90-6.80	5.00-5.20	5.70-6.30	4.80-7.20
SL	Mean±SD	3.48±0.29	3.46±0.24	3.61±0.11	3.96±0.21	4.34±0.18	2.40±0.00	3.33±0.13	3.58±0.39
	CV	8.31	6.94	3.11	5.27	4.18	0.00	3.95	10.99
	Range	2.80-4.10	3.00-4.00	3.30-3.80	3.60-4.40	4.10-4.80	2.40-2.40	3.00-3.60	2.40-4.80
SW	Mean±SD	1.99±0.16	1.98±0.16	2.16±0.10	2.18±0.17	2.21±0.12	1.72±0.04	1.92±0.12	2.03±0.18
	CV^2	8.01	8.31	4.56	7.59	5.49	2.60	6.44	8.93
	Range	1.60-2.30	1.70-2.60	2.00-2.40	1.80-2.60	1.90-2.50	1.70-1.80	1.70-2.20	1.60-2.60
TSW	Mean±SD	2.93±0.60	2.65±0.38	3.02±0.01	4.08±0.45	5.47±0.81	1.25±0.01	2.27±0.27	3.14±0.99
	CV	20.36	14.29	0.36	11.10	14.79	0.80	11.73	31.68
	Range	1.95-4.36	1.99-3.70	3.00-3.03	3.33-4.69	4.02-6.50	1.24-1.26	1.96-2.82	1.24-6.50

VSF_2H = Volunteer selfed F_2 hybrids- from mixture of six crosses; SF_2H^a = Selfed F_2 hybrids between HARC-15 and *L. bienne*; SF_2H^b = Selfed F_2 hybrids between accession 243817 and *L. bienne*; BCF_2H= Back crossed F_2 from F_1 hybrids between HARC-15 and *L. bienne*; CP = Cultivated parents; WP = Wild parent; and F_1H = F_1 hybrids. Numbers in parenthesis such as (35) represent the number of sampled plant genotypes.

determine the sub-trait value for each population. For example, the values of the six sub-traits for VSF_2H and CP populations are 0.41, 0.41, 0.00, 0.00, 0.00, 0.00 and 0.41, 0.00, 0.00, 0.00, 0.00, 0.00, respectively. The dissimilarity for seed-coat colour between each two e.g. the populations VSF_2H and CP is given by: $(0.41\text{-}0.41)^2$ + $(0.41\text{-}0.00)^2$ + $(0.41\text{-}0.00)^2$ + $(0.41\text{-}0.00)^2$ + $(0.00\text{-}0.00)^2$ + $(0.00\text{-}0.00)^2$ = 0.17. The dissimilarity values for other populations were determined using the same calculation.

The third step of the method

After calculating total dissimilarity values between every two population groups for all measured traits, the calculated quantitative and qualitative trait values were combined. Total dissimilarity value between BCF_2H and F_1H = 0.18 + 0.17 = 0.35. If the two population groups were completely dissimilar with the five traits, this value could be 5 or if they were similar the calculated dissimilar value could be zero.

The final step of the method

The matrix of dissimilarities was generated from the earlier-calculated values useful for cluster analysis. From the dissimilarities matrix generated from the combination of quantitative and qualitative traits the second type of cluster analysis was performed.

Analysis

Descriptive statistics, cluster analysis and one-way nested analysis of variances were conducted for the associations and variations analyses using SPSS V-23 software and excel spreadsheet. Means with standard deviations of boll diameter (BD), seed length (SL), seed width (SW), 1000-seed-weight (TSW), and seed-coat colour (SC) frequency were determined for each of the 76 studied plants (Table S1). Two types of dendrograms were constructed: one from quantitative characters and the other from the combination of quantitative and qualitative characters. During the analyses, sample genotypes were grouped into different sub-groups to examine the nature of associations and variations among and within groups under different methods of analyses and population structures. Nested analyses of variances, an extension of one way ANOVA was used to determine the variations existing between every two population groups under different ways of grouping and the variance contribution (VC) of each population group to the total variance.

RESULTS

The maximum boll diameter, seed size and seed weights were scored from cultivated parental genotypes, whereas the least values for these traits were scored from the wild parental genotype. Six sub-classes of seed-coat colour, ranging from dark brown to yellow, scored from the study. Among the six sub-classes of seed-coat colour, brown, light brown and dark brown took the first (28, 36.8%), second (23, 30.3%), and third (15, 19.7%) highest frequencies from all the sampled plants, respectively. F_1 hybrids from different parents with different seed-coat colour developed only one type of seed-coat colour, light brown. Selfed F_2 hybrids (SF_2H) expressed all, except yellow, seed-coat colours scored in the study. Mean±SD, coefficient of variations (*CV*) and range of values for BD, SL, SW and TSW from seven population groups: VSF_2H, SF_2H^a, SF_2H^b, BCF_2H, F_1H, CP and WP were also determined and described in Table 1.

The highest variations among populations for boll size

Figure 2. Pictures showing degrees of boll shattering from cultivated and wild parental, F_1 hybrid and F_2 hybrid plants.

(7.91%), seed length (8.31%) and 1000-seed- weight (20.36%) were from selfed F_2 hybrids, grown voluntarily with mixed stand from different crosses for F_1 hybrid plants (Table 1). Seed width in the population was also with the second highest (8.01%) variation. The highest *CV* for the studied characters of a population and total sampled plants were 20.36 and 31.68%, respectively and both from 1000-seed-weight scores. For all studied characteristics, the highest mean values were scored by the cultivated parental plants and the least scored by the wild parental plant. Among hybrids, backcrossed F_2 hybrids scored the highest mean values for all characteristics.

The results in Table 1 revealed all types of hybrids were intermediate for all studied characteristics. The degree of bolls shattering was measured qualitatively by observing their relative size of the opening (Figure 2). Bolls from all selfed F_2 hybrids and from one group of backcrossed F_2 hybrids were the first to start opening their boll tips at 22°C and bolls from the second group of backcrossed F_2 hybrids started opening their tips at about a temperature of 50°C. Third group bolls collected from cultivated parents remained closed up to a temperature of 65°C but from 65 to 80°C c.50% of them developed little openings, heating them beyond 80°C did not bring change. Bolls from *L. bienne*, wild parent and F_1 hybrids were similar in shattering nature and showed the maximum degree of shattering without applying heat (Figure 2).

Backcrossed F_2 hybrids' bolls made two groups: one less open but larger boll, which are major features of cultivated linseed and the second group has well-opened bolls but small in size - a salient feature of wild relatives.

Most F_1, selfed F_2 and backcrossed F_2 hybrids had intermediate characters for most traits. Some showed wild parent characters for some traits and cultivated parent characters for other traits. One common characteristic for all hybrids was their bolls were

shattered, although the degree of boll shattering was minimal from some backcrossed F_2 hybrids.

There was very limited seed sample from the wild parent and fatty acid composition from this parental genotype was not determined.

Cluster analysis

Quantitative characters data based cluster analysis for the 76 hybrids and parental genotypes both as individuals and groups of populations (F_1 hybrids, selfed F_2 hybrids, backcrossed F_2 hybrids, wild parents and cultivated parents) consistently classified into four clusters: cluster I (F_1 and selfed F_2 hybrids), cluster II (backcrossed F_2 hybrids), cluster III (wild parent and cluster IV (cultivated parents). There was no overlapping among cluster mean values for the studied characteristics and all the characters were equally important to group the populations into four clusters. In the cluster analysis, the 76 genotypes initially split into cultivated parents and other groups (Figure 3).

Accession 237001 (#66) from cultivated parental plants and some selfed F_2 hybrids joined backcrossed F_2 hybrid group; one backcrossed F_2 hybrid (#63) joined F_1 and selfed F_2 hybrids group. Although some sampled plants joined a group of other plants, there was consistency between the two types of cluster analyses (Figures 3 and 4). There was no overlapping for 1000-seed-weight mean values among clusters and seed weight was the most important characteristic used to group the genotypes into the four clusters. That is why accession 237001(#66) and backcrossed F_2 hybrid (#63) with seed weight outside the range of their respective groups' genotypes seed weight were joined with other groups with lower seed weigh genotypes.

The seed-coat colours as qualitative data were combined with quantitative data for cluster analysis to see the effect of the combination in the clustering of the different groups of genotypes. Mean values of each trait for each population group were determined and tabulated in Table 3 for further calculation steps to generate the matrix of dissimilarities between every two population groups from combining both quantitative and qualitative characters.

From the total dissimilarity values or matrix (Table 4) the highest dissimilarity was between CP and WP and the next highest between BCF_2H and WP, whereas the least dissimilarity was between VSF_2H^{a} and SF_2H^{b}. Supported by Agglomeration Schedule Coefficients dendrogram (Figure 4) information suggested the different groups of plants to be classified into three clusters: cluster I (hybrid groups), cluster II (cultivated parents) and cluster III (wild parent). The different systems of clustering the genotypes indicated the existence of a large amount of diversity among the group and individual genotypes.

Nested analyses of variances (NANOVA)

From the nested analyses of variance, an extension of one way ANOVA (Table 5) showed splitting parental genotypes into wild and cultivated instead of splitting F_2H into SF_2 and BCF_2 hybrids to form four groups showed a relatively higher variation among groups in boll diameter and seed length but lower variation in seed width and seed weight. However, only the observed mean value differences for 1000-seed-weight among subgroups within groups were not significant. Only from seed weight and seed length, the observed mean values differences showed significant ($P< 0.05$) when the population is grouped into four groups. Except for seed length, all the characters showed significant variation in mean values among subgroups within the three, four and five groups (Table 5).

By comparing with the value of critical difference (CD) using Singh and Chaudhary method (1977; cited in Adugna et al., 2004), the observed differences between mean values of any two subgroups of genotypes were evaluated and only the differences between VSF_2H and SSF_2H HARC-*15* × *L. bienne* seed length and seed width mean values were insignificant observed differences. This result is supporting the conclusion that seed weight was the most important factor in grouping the 76 genotypes into four clusters.

DISCUSSION

Descriptive statistics and observational analysis

F_1 hybrids from MacBeth × *L. bienne*, HARC-15 × *L. bienne* and 15310 Early × *L. bienne* were with positive heterosis in palmitic and oleic but with negative heterosis in linolenic fatty acid compositions referring to their cultivated parental genotypes and the 262 genotypes. These heteroses were also reflected in the saturated to unsaturated ratio differences. F_1 hybrids (MacBeth × *L. bienne*) scored the highest palmitic fatty acid composition from the palmitic fatty acid composition determined from 262 genotypes. F_2 hybrids from reciprocal backcrosses scored the least stearic fatty acid compositions: female gamete from cultivated parent and male gamete from F_1 hybrid had 2.91%, and female gamete from F_1 hybrid and male gamete from cultivated parent had 4.42% stearic fatty acid composition. These compositions were reduced to 49.48 and 23.26%, respectively from the composition (5.76%) scored by HARC-15 as negative and significant heterosis. The report from Tulu et al. (2018) showed maize (*Zea mays* L) hybrids developed with positive and significant heterosis in yield but negative and significant heterosis in days to anthesis (DA) and days to silking (DS) from different maize lines as desired traits. Alleles from seed-coat colour controlling genes were blending in the F_1 hybrids: all the F_1 hybrids from brown, olive and

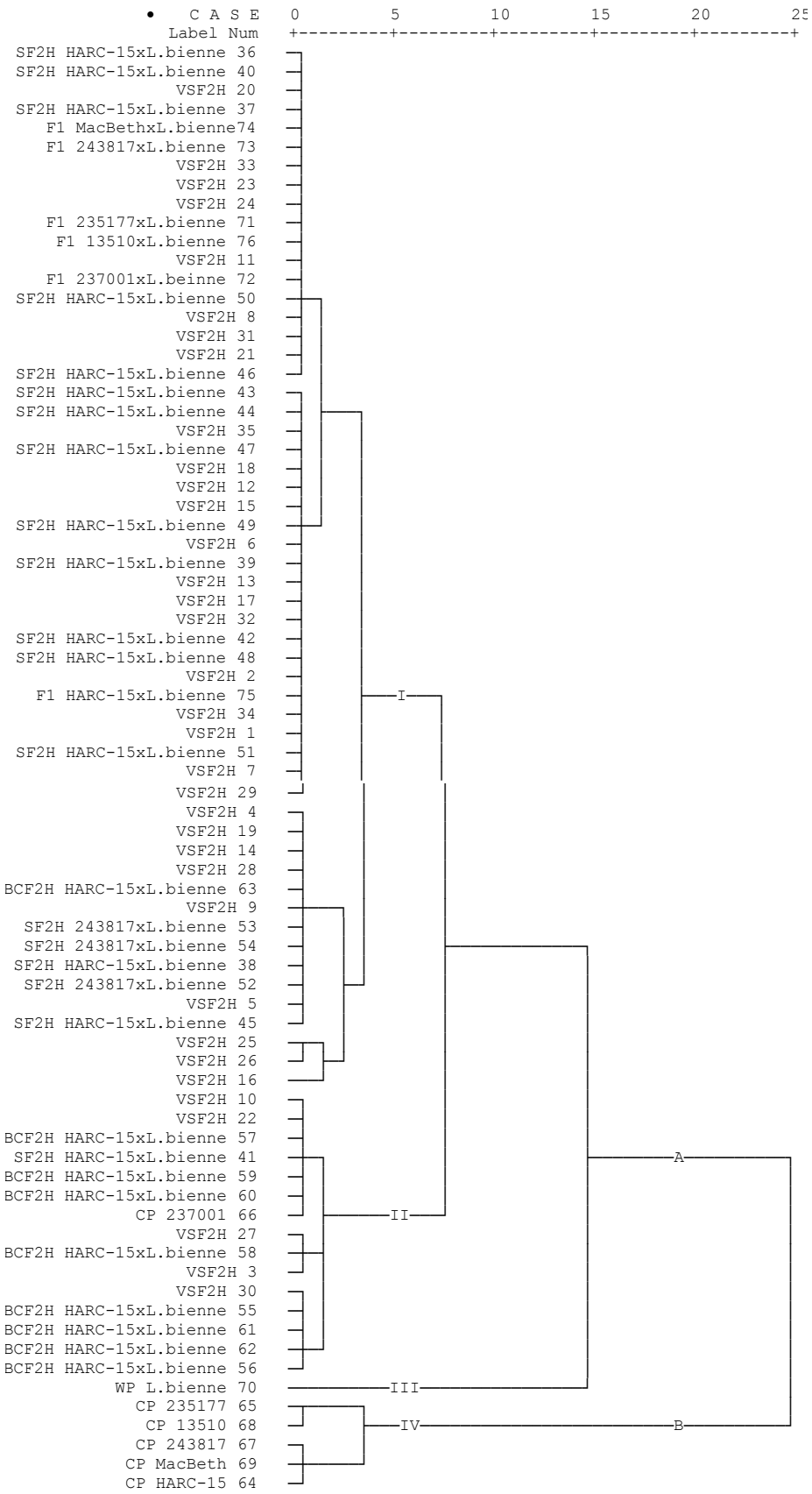

Figure 3. A dendrogram from quantitative characters cluster analysis of the entire sampled plants.

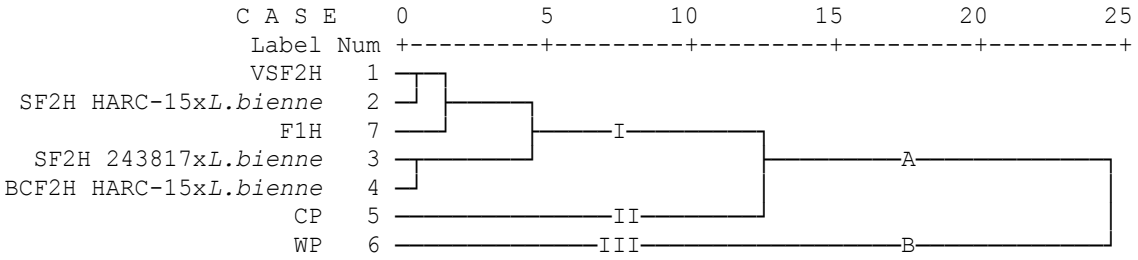

Figure 4. A dendrogram from combined quantitative and qualitative characters for cluster analysing average linkage between population groups.

yellow seeded genotype parents uniformly developed only light brown seeds. However, in the F2 hybrids, these alleles segregated into different classes of seed-coat colour. Worku and Heslop-Harrison (2018) reported the presence of segregation of genotypes for some important agronomic traits. The yellow seed-coat colour was reappearing after the development of the F_3 hybrid generation. Three genes (one as a basic and the other two as modifier genes) determined the development of linseed seed-coat colour (Rajan and Sengupta, 1970; Tammes, 1922). Yellow seed-coat colour can result when the basic gene and either of the two or both modifier genes are recessive. There was variation in the degree of yellowness among seeds from linseed cultivars. Accession 237001 genotype was relatively light yellow whereas other groups of yellow-seeded genotypes were deep yellow relatively. This variation has been reflected in F_1 plants from the crosses between each of these two groups of yellow and brown seeded linseed genotypes. F1 plants developed from the crossing between accession 237001 and other brown-seeded accessions were only brown-seeded, whereas those between other yellow-seeded and brown-seeded accessions developed only brown-seeded. Worku et al. (2015) reported Ethiopian linseed germplasm has a diversified genetic structure regarding genes controlling floral and seed coat colours.

Selfed F_2 hybrids grown voluntarily with mixed stand from different crosses for F_1 hybrid plants scored the highest variability for almost all characters considered in the study. Diederichsen and Raney (2008) reported there is more genetic variation in this group which influences their phenotype. On seed weight variability, there are different reports: 20.5% as the highest CV from all studied characteristics of 2934 accessions (Diederichsen and Raney, 2006) and 20.8% for 3,089 accessions (Diederichsen, 2007) were for seed weight. However, without specifying the variability value, Akbar et al. (2003) reported seed weight was with low variances and this indicates non-additive genes control the trait and there is a high difference between phenotypic and genotypic coefficients of variances as an indication of the presence of more environmental influence. Another research result reported seed weight is influenced by dominant gene action (Kurt and Evans, 1998; Kumar and Chauhan, 1980). Diederichsen and Raney's (2006) report showed the accessions grown in different years showed almost constant CV and in this research, there was no significant variation in parental seed weight in the three growing seasons. Therefore, targeting this trait to improve yield and oil content would be effective since the relationship between mean seed weight and subsequent grain yield is positive (Tyson, 1989).

This study showed generally, the hybrids were intermediate for most and vigour for some agronomic characters. F_1 hybrids between MacBeth and L*. bienne*, HARC-15 and *L. bienne*, and between accession 15310 and *L. bienne* scored higher palmitic fatty acid composition: 6.74%, 7.04 and 7.08 than the fatty acid composition 5.79, 5.69 and 6.56% from MacBeth, HARC-15 and accession 13510, respectively. However, F_2 hybrids with reciprocal backcrosses between HARC-15 and F_1 between HARC-15 and *L. bienne* had a similar amount of fatty acid compositions with HARC-15. Bayahi and Rezgui (2018) reported that F_1 and F_2 hybrids derived from crosses between two Chickpea (*Cicer arietinum* L.) varieties (Desi and Kabuli) were superior in yield to the best and mean parent. Similarly, Mohammed et al. (2019) reported that sugarcane genotypes the source of resistance against smut exists among genotypes and can be used to develop new high yielding sugarcane varieties superior to the parental genotypes. One of the seed characters of the wild species with the least mean value is 1000-seed weight, 1.25 g, and a similar result, 1.1 to 2.7 g has been reported (Diederichsen and Hammer, 1995). Seetharam (1972) reported that 1000-seed-weight and oil content from different hybrids were intermediate between their parents. The segregation of backcrossed F_2 hybrids into only two classes: (1) small boll size and highly shattering; and (2) large boll size and less dehiscent, may indicate alleles from genes controlling boll size and shattering are linked-coupled linkage. This linkage would be important for breeders to separate important agronomic characters from unimportant ones. In linseed non-dehiscent capsules, branching habit and variability in the fatty acid profile are some of the examples of the breeding efforts and results of interaction of many inherited factors (Hall

Table 2. Fatty acids composition (%) of some parental linseed germplasm and their hybrids with *L. bienne*.

Genotype	Fatty acids composition percentages					
	Palmitic (C16 : 0)	Stearic (C18 : 0)	Oleic (C18 : 1)	Linoleic (C18 : 2)	Linolenic (C18 : 3)	Sat/unsta ratio (Cn: 0/Cn:n)
MacBeth	5.79	5.08	15.16	14.08	59.38	0.12 (10.87%)
MacBeth × *L. bienne*	6.74*	5.90	20.60	14.09	52.94	0.14 (12.64%)*
HARC-15	5.69	5.76	16.81	14.14	56.94	0.13 (11.45%)
HARC-15 × L. bienne	7.04*	5.67	22.17	14.05	50.58	0.15 (12.71%)*
13510 Early	6.56	5.83	22.07	14.63	51.25	0.14 (12.39%)
15310 Early × *L. bienne*	7.08*	5.45	22.67	14.31	50.27	0.14 (12.53%)*
HARC-15 × (HARC-15 × *L. bienne*)	5.56	2.91†	16.60	15.78	58.57	0.09 (8.47%)
(HARC-15 × *L. bienne*) v HARC-15	5.39	4.42†	17.12	15.24	58.36	0.11 (9.81%)
Average (n = 262)	6.21	5.12	18.70	14.69	55.04	0.13 (11.33%)
Range (n = 262)	5.03-7.08	2.91-6.55	13.97-23.84	13.69-15.78	49.63-60.40	0.02-0.15

Hybrids between wild relative and cultivated germplasm had a lower percentage of linolenic acid but a higher percentage of palmitic acid compared with the composition of the fatty acids of their cultivated parental germplasm (Table 2). In general, their saturated to unsaturated fatty acids ratios were higher as indicated by * than the ratios from their cultivated parents and the average from total samples (n = 262).

Table 3. Mean values of quantitative characters and scores from qualitative traits used to combine quantitative and qualitative characters.

Population group	Quantitative traits				Qualitative trait (seed colours)*					
	BD	SL	SW	1000-SW	1	2	3	4	5	6
VSF_2H (35)	5.85	3.48	1.99	2.93	1	1	1	1	0	0
SF_2H^a (16)	5.72	3.46	1.98	2.65	1	1	1	1	1	0
SF_2H^b (3)	6.13	3.61	2.16	3.02	1	1	0	1	0	0
BCF_2H (9)	6.23	3.96	2.18	4.08	1	1	0	0	0	0
CP (6)	6.36	4.34	2.21	5.47	0	1	0	0	0	1
WP (1)	5.08	2.40	1.72	1.25	0	0	0	1	0	0
F_1H (6)	5.54	3.33	1.92	2.27	1	0	0	0	0	0

VSF_2H = Volunteer selfed F_2 hybrids- from mixture of six crosses; SF_2H^a = Selfed F_2 hybrids between HARC-15 and *L. bienne*; SF_2H^b= Selfed F_2 hybrids between accession 243817 and *L. bienne*; BCF_2H=Back crossed $F_2$from F_1hybrids between HARC-15 and *L. bienne*; CP = Cultivated parents; WP = Wild parent; and F_1H = F_1 hybrids. BD=Boll diameter; SL=seed length; SW=seed width; 1000-SW- 1000-seed-weight. *****Qualitative trait (seed coat colour) described as follows: light brown (1), brown (2), dark brown (3), olive (4), light brown to yellowish (5), and yellow (6); and 0 stands for absence whereas 1 for presence of a subtract in a population.

et al., 2016).

Cluster analysis

Cluster analysis is useful to evaluate genetic diversity of groups of genotypes (Begum et al., 2007) under the assumption that populations within the same cluster have smaller differences among themselves than between those belonging to different clusters. As the number of characters used for cluster analysis is increased, especially including both qualitative and quantitative characters, classification among sampled genotypes was strong. That is, using both quantitative and qualitative characteristics in classification had more power to classify genotypes into clear clusters (Figure 4) than using quantitative characteristics alone (Figure 3). One genotype (#63) from (HARC-15 × *L. bienne*) × HARC-15BCF_2H group shifted to SF_2H genotypes' group although they were not considered as an independent cluster group, whereas one genotype (#41) from HARC-15 × *L. bienne* selfed F_2H group shifted to BCF_2H group (Figure 3). These genotypes had the least (3.33±0.00 g) and the highest (3.66±0.03 g) seed weight from their respective groups (Table S1). This shows that seed weight is an important factor when discriminating genotypes. Fuet al. (2002) reported that samples obtained from crosses between two cultivars could cluster with samples related in pedigree but not with their expected group. The formation of independent groups (Figure 4) by the three populations: hybrids, wild and cultivated parents in cluster analysis and the occurrence of considerable differences between backcrossed and selfed F_2 hybrids in fatty acid composition pattern (Table 2) which has high heritability (Rai et al., 1989) are valuable indicators that F_2 hybrids would contribute

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SF_2H^b (3)	6.13	3.61	2.16	3.02	1	1	0	1	0	0
BCF_2H (9)	6.23	3.96	2.18	4.08	1	1	0	0	0	0
CP (6)	6.36	4.34	2.21	5.47	0	1	0	0	0	1
WP (1)	5.08	2.40	1.72	1.25	0	0	0	1	0	0
F_1H (6)	5.54	3.33	1.92	2.27	1	0	0	0	0	0

VSF_2H = Volunteer selfed F_2 hybrids- from mixture of six crosses; SF_2H^a = Selfed F_2 hybrids between HARC-15 and *L. bienne*; SF_2H^b= Selfed F_2 hybrids between accession 243817 and *L. bienne*; BCF_2H=Back crossed $F_2$from F_1hybrids between HARC-15 and *L. bienne*; CP = Cultivated parents; WP = Wild parent; and F_1H = F_1 hybrids. BD=Boll diameter; SL=seed length; SW=seed width; 1000-SW-1000-seed-weight. *****Qualitative trait (seed coat colour) described as follows: light brown (1), brown (2), dark brown (3), olive (4), light brown to yellowish (5), and yellow (6); and 0 stands for absence whereas 1 for presence of a subtract in a population.

Table 4. Matrix of total dissimilarity values generated from combination of quantitative and qualitative characteristics.

Correlation	VSF_2H	SF_2H^a	SF_2H^b	BCF_2H	CP	WP	F_1H
VSF_2H	0						
SF_2H^a	0.184	0					
SF_2H^b	0.335	0.593	0				
BCF_2H	0.710	1.024	0.272	0			
CP	1.447	1.969	1.023	0.493	0		
WP	1.531	1.593	2.375	3.300	4.500	0	
F_1H	0.611	0.713	0.844	1.043	2.117	0.912	0

See Table 3 for population codes.

functional mapping populations important for a linseed genetic map.

Stearoyl-ACP-Desaturase (SAD) gene, which is responsible for the production of the fatty acid desaturase enzyme that converts oleic acid (C18:1) to linoleic acid (C18:2), has relatively more genetic diversity in *L. bienne* than in cultivated linseed (Allaby et al*.,* 2005). Therefore, hybrids between *L. bienne* and *L. usitatissimum* would be a useful genetic resource to develop a variety useful for specific purposes by using diverse germplasm from *L. bienne*. Unfortunately, the amount of seeds from *L. bienne* was not enough to determine fatty acid composition and the researchers could not make a comparison between hybrids and wild parent on this character.

Nested analysis of variance (NANOVA)

Type of grouping, number of groups and the variability of the characteristic considered in the analysis (Table 5) were some of the factors for the observed mean values differences among groups and subgroups within groups to be significant or non-significant. The contribution of variations among groups to total variations and level of significances increased as the group split further or the number of groups increased. Seed weight mean values showed relatively more variations with P-values between 0.033 and 0.265 among groups of all forms of grouping the genotypes.

Conclusion

It is practically easy to get fertile hybrids between *L. usitatissimum* and *L. bienne* and high diversity in seed coat colour and 1000-seed-weight which could associate with other traits like oil content and productivity. Hybrids would be a potential genetic resource for the development of a linseed variety useful for specific end-uses such as fatty acids. As the proportion of cultivated germplasm genetic composition in F_1 hybrids changed from 50 to 75% in the F_2 hybrids through backcrossing with cultivated parental genotype, the lower percentage linolenic and higher palmitic changed to the cultivated parental content. Therefore, hybrids with higher genetic

Table 5. Mean squares for BD, SL, SW and 1000-SW among groups, subgroups within group and within subgroups.

Trait	No. of groups	Source of variation	SS	Df	MS	*F-ratio*	*P-value*	VC (%)
Boll diameter	Three	AG	2.658	2	1.329	0.338	0.732	0.00
		ASGwG	15.735	4	3.934	24.374	0.000**	31.98
		WSG	60.199	373	0.161	-	-	68.02
	Four PG →CPG &WPG (PG = parental genotypes)	AG	9.643	3	3.214	1.102	0.469	19.38
		ASGwG	8.750	3	2.917	18.072	0.000**	17.09
		WSG	60.199	373	0.161	-	-	63.52
	Four F_2H →SF_2H & BCF_2H	AG	8.9326	3	2.9775	1.0080	0.498	0.18
		ASGwG	9.4604	3	2.9540	19.5393	0.000**	28.29
		WSG	60.1992	373	0.1614	-	-	71.53
	Five	AG	15.9178	4	3.9794	5.4426	0.161	29.11
		ASGwG	2.4752	2	0.7312	7.6684	0.001**	6.50
		WSG	60.1992	373	0.1614	-	-	64.38
Seed length	Three	AG	10.443	2	5.2214	0.8169	0.504	4.63
		ASGwG	25.566	4	6.3914	105.3791	0.000**	64.60
		WSG	22.623	373	0.0607	-	-	30.77
	Four PG →CPG &WPG (PG = parental genotypes)	AG	26.628	3	8.876	2.839	0.207	64.42
		ASGwG	9.380	3	3.127	51.554	0.000**	15.78
		WSG	22.623	373	0.0607	-	-	19.80
	Four F_2H →SF_2H & BCF_2H	AG	19.523	3	6.508	1.184	0.446	11.64
		ASGwG	16.486	3	5.495	90.604	0.000**	58.01
		WSG	22.623	373	0.061	-	-	30.35
	Five	AG	35.708	4	8.927	59.399	0.017*	76.16
		ASGwG	0.301	2	0.150	2.478	0.085	0.52
		WSG	22.623	373	0.061	-	-	23.31
Seed width	Three	AG	0.816	2	0.408	0.582	0.600	0
		ASGwG	2.804	4	0.701	29.788	0.000**	36.67
		WSG	8.777	373	0.024	-	-	63.33
	Four PG →CPG &WPG (PG = parental genotypes)	AG	1.845	3	0.615	1.040	0.488	21.99
		ASGwG	1.775	3	0.592	25.142	0.000**	21.50
		WSG	8.777	373	0.024	-	-	56.51
	Four F_2H →SF_2H & BCF_2H	AG	2.150	3	0.717	1.462	0.381	11.52
		ASGwG	1.470	3	0.490	20.830	0.000**	26.31
		WSG	8.777	373	0.024	-	-	62.18
	Five	AG	3.179	4	0.795	3.600	0.229	36.10
		ASGwG	0.441	2	0.221	9.381	0.000**	7.20
		WSG	8.777	373	0.024	-	-	56.70
1000-seed-weight	Three	AG	131.477	2	65.738	1.883	0.265	42.08
		ASGwG	139.656	4	34.914	126.201	0.000**	41.46

Table 5. Contd.

	WSG	103.192	373	0.277	-	-	16.46
Four PG →CPG &WPG (PG = parental genotypes)	AG	190.138	3	63.379	2.513	0.235	43.27
	ASGwG	80.995	3	25.217	97.588	0.000**	38.19
	WSG	103.192	373	0.277	-	-	18.53
Four F_2H →SF_2H & BCF_2H	AG	207.9555	3	69.3185	3.2916	0.177	72.36
	ASGwG	63.1770	3	21.0590	76.1204	0.000**	14.99
	WSG	103.1919	373	0.2767	-	-	12.66
Five	AG	266.616	4	66.654	29.518	0.033*	82.72
	ASGwG	4.516	2	2.258	8.162	0.000**	1.69
	WSG	103.192	373	0.277	-	-	15.59

BD = Boll diameter; SL = seed length; SW = seed width; 1000-SW = 1000-seed-weight; SS = sum of squares; df = degree of freedom; MS = mean squares; VC = variation component; AG = among groups; SGwG= subgroups within group; WSG = within subgroups; CPG = cultivated parental plants; WPG = wild parental genotype; F_2H = F_2 hybrids; SF_2H = selfed F_2 hybrids;and BCF_2H hxb = backcrossed F_2 hybrids from HARC-15×*L.bienne*.* = significant at α < 0.05 level, and ** = significant at α < 0.01 level.

composition from the wild parent would be important lines for lower linolenic and higher palmitic fatty acids content. Hybridization between *L. usitatissimum* and *L. bienne* can result in the introgression of several alleles from wild to cultivated linseed which would help future linseed breeding programmes by providing combinations of new alleles. The introgression of alleles from wild to cultivars would help cultivars restore and maintain their genetic diversity. The hybrids also could provide useful mapping populations to forward the development of a linseed genetic map. Considering more characteristics, especially from the combination of qualitative and quantitative traits, for cluster analysis is a more powerful method to utilize the genetic variation in genotypes and to classify them into well discriminated groups. Assisting the process of hybridization with markers associated trait would help to minimize the dragging of unwanted characters into hybrids. Marker assisted hybridization would also reduce the time required to get genotypes for specific purposes.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Table S1. Table S1.Mean±SD values of five repeated measurements for each studied traits of 76 genotypes

S.N^{o}	Sampled plant	BD	SL	SW	TSW	SC
1	VSF_2H1	5.84±0.21	3.16±0.15	1.86±0.09	2.67±0.02	1
2	VSF_2H2	5.50±0.28	3.48±0.15	2.10±0.10	2.66±0.04	1
3	VSF_2H3	6.80±0.19	3.86±0.05	2.14±0.11	3.83±0.03	2
4	VSF_2H4	6.60±0.22	3.72±0.04	2.16±0.09	3.38±0.05	2
5	VSF_2H5	5.94±0.11	3.84±0.11	2.20±0.07	2.74±0.06	1
6	VSF_2H6	6.06±0.17	3.48±0.04	1.92±0.04	2.32±0.01	1
7	VSF_2H7	5.76±0.17	3.26±0.19	2.00±0.20	2.87±0.12	2
8	VSF_2H8	5.22±0.19	3.28±0.11	1.88±0.24	2.41±0.05	2
9	VSF_2H9	6.40±0.14	3.50±0.20	2.06±0.15	3.28±0.06	3
10	VSF_2H10	6.10±0.14	3.60±0.07	2.14±0.05	3.75±0.17	4
11	VSF_2H11	5.40±0.20	3.32±0.16	1.74±0.09	2.02±0.03	3
12	VSF_2H12	5.68±0.23	3.82±0.13	1.92±0.15	3.00±0.09	4
13	VSF_2H13	5.68±0.13	3.46±0.15	1.96±0.11	2.67±0.01	1
14	VSF_2H14	6.16±0.05	3.70±0.07	2.12±0.13	3.35±0.04	2
15	VSF_2H15	5.88±0.39	3.52±0.16	2.02±0.04	2.44±0.04	3
16	VSF_2H16	6.28±0.22	3.04±0.15	1.88±0.11	3.55±0.02	3
17	VSF_2H17	5.68±0.26	3.50±0.12	1.88±0.15	2.68±0.01	3
18	VSF_2H18	5.60±0.20	3.62±0.13	1.96±0.13	3.00±0.01	4
19	VSF_2H19	6.76±0.25	3.86±0.05	2.12±0.08	3.39±0.02	3
20	VSF_2H20	5.56±0.26	3.14±0.09	1.84±0.05	2.14±0.02	3
21	VSF_2H21	5.20±0.27	3.14±0.11	1.94±0.11	2.33±0.01	1
22	VSF_2H22	6.12±0.11	3.70±0.23	2.14±0.05	3.80±0.03	2
23	VSF_2H23	5.46±0.05	3.04±0.05	1.84±0.09	2.01±0.01	3
24	VSF_2H24	5.32±0.33	3.02±0.13	1.82±0.08	1.97±0.02	2
25	VSF_2H25	5.54±0.30	3.14±0.05	1.82±0.11	3.43±0.03	2
26	VSF_2H26	5.62±0.23	3.42±0.13	1.92±0.11	3.32±0.03	3
27	VSF_2H27	6.44±0.19	3.90±0.10	2.14±0.11	3.80±0.04	1
28	VSF_2H28	6.24±0.11	3.60±0.16	2.12±0.11	3.33±0.01	3
29	VSF_2H29	5.92±0.19	3.38±0.08	1.94±0.05	2.99±0.02	1
30	VSF_2H30	6.28±0.16	4.00±0.10	2.14±0.05	4.32±0.03	2
31	VSF_2H31	5.28±0.29	3.24±0.17	1.88±0.11	2.32±0.03	2
32	VSF_2H32	5.76±0.05	3.46±0.15	1.98±0.13	2.78±0.02	2
33	VSF_2H33	5.58±0.11	3.36±0.09	2.16±0.11	2.33±0.02	1
34	VSF_2H34	5.52±0.15	3.48±0.04	1.94±0.09	2.55±0.02	4
35	VSF_2H35	5.70±0.16	3.64±0.09	2.04±0.05	3.01±0.01	3
36	Selfed (HARC-15 x *L. bienne*)	5.60±0.23	3.18±0.18	1.90±0.23	2.34±0.01	1
37	Selfed (HARC-15 x *L. bienne*)	5.48±0.40	3.42±0.08	1.84±0.05	2.31±0.02	3
38	Selfed (HARC-15 x *L. bienne*)	6.14±0.38	3.68±0.04	2.02±0.11	2.83±0.03	3
39	Selfed (HARC-15 x *L. bienne*)	5.76±0.19	3.56±0.11	2.18±0.04	2.50±0.04	5
40	Selfed (HARC-15 x *L. bienne*)	5.64±0.18	3.14±0.05	1.84±0.09	2.34±0.01	1
41	Selfed (HARC-15 x *L. bienne*)	5.92±0.13	3.80±0.12	1.94±0.05	3.66±0.03	4
42	Selfed (HARC-15 x *L. bienne*)	5.70±0.34	3.36±0.09	2.00±0.10	2.65±0.02	1
43	Selfed (HARC-15 x *L. bienne*)	5.26±0.25	3.42±0.16	2.00±0.07	2.99±0.02	4
44	Selfed (HARC-15 x *L. bienne*)	5.30±0.16	3.52±0.25	2.00±0.07	2.99±0.03	2
45	Selfed (HARC-15 x *L. bienne*)	6.48±0.15	3.58±0.11	2.18±0.26	2.68±0.01	3
46	Selfed (HARC-15 x *L. bienne*)	5.30±0.16	3.64±0.11	2.02±0.22	2.34±0.01	1
47	Selfed (HARC-15 x *L. bienne*)	5.80±0.27	3.64±0.11	2.08±0.08	2.97±0.03	1
48	Selfed (HARC-15 x *L. bienne*)	5.70±0.30	3.36±0.13	1.80±0.07	2.67±0.01	3
49	Selfed (HARC-15 x *L. bienne*)	5.94±0.30	3.58±0.15	1.96±0.11	2.49±0.01	2
50	Selfed (HARC-15 x *L. bienne*)	5.50±0.23	3.48±0.18	1.94±0.24	2.00±0.01	2
51	Selfed (HARC-15 x *L. bienne*)	5.92±0.22	3.00±0.00	1.90±0.07	2.71±0.04	4

Table S1. Contd.

52	Selfed 243817 x *L. bienne*	6.00±0.29	3.66±0.09	2.06±0.05	3.03±0.01	1
53	Selfed 243817 x *L. bienne*	6.24±0.15	3.54±0.15	2.24±0.09	3.02±0.01	1
54	Selfed 243817 x *L. bienne*	6.14±0.17	3.64±0.05	2.18±0.04	3.01±0.00	2
55	HARC-15 x (HARC-15 x *L. bienne*)	6.36±0.28	4.18±0.04	2.34±0.18	4.45±0.02	2
56	HARC-15 x (HARC-15 x *L. bienne*)	6.12±0.29	4.26±0.11	2.30±0.14	4.65±0.02	2
57	HARC-15 x (HARC-15 x *L. bienne*)	6.16±0.27	3.72±0.16	2.10±0.19	3.66±0.02	2
58	(HARC-15 x *L. bienne*) x HARC-15	6.60±0.12	4.10±0.12	2.24±0.05	3.99±0.02	2
59	(HARC-15 x *L. bienne*) x HARC-15	5.78±0.13	3.76±0.15	2.02±0.15	3.67±0.02	2
60	(HARC-15 x *L. bienne*) x HARC-15	5.68±0.19	3.96±0.11	2.18±0.08	3.98±0.03	2
61	(HARC-15 x *L. bienne*) x HARC-15	6.42±0.36	3.94±0.11	2.24±0.11	4.67±0.02	2
62	(HARC-15 x *L. bienne*) x HARC-15	6.66±0.34	3.94±0.05	2.22±0.13	4.34±0.01	2
63	(HARC-15 x *L. bienne*) x HARC-15	6.30±0.26	3.82±0.16	2.00±0.10	3.33±0.00	1
64	HARC-15 (Parental genotype)	6.68±0.08	4.42±0.08	2.20±0.00	6.47±0.02	2
65	235177 (Parental genotype)	6.40±0.07	4.18±0.08	2.16±0.05	5.05±0.03	2
66	237001 (Parental genotype)	5.94±0.05	4.18±0.08	2.06±0.11	4.03±0.01	6
67	243817 (Parental genotype)	6.36±0.05	4.46±0.05	2.26±0.11	5.97±0.01	2
68	13510 (Parental genotype)	6.10±0.07	4.22±0.08	2.28±0.08	5.32±0.01	2
69	MacBeth (Parental genotype)	6.66±0.05	4.60±0.12	2.30±0.14	6.00±0.00	2
70	*L. bienne* (Parental genotype)	5.08±0.08	2.40±0.00	1.72±0.04	1.25±0.01	4
71	235177 x *L.bienne* (F_1)	5.46±0.05	3.26±0.09	1.84±0.09	2.08±0.02	1
72	237001x *L.bienne*(F_1)	5.36±0.05	3.24±0.15	1.88±0.13	1.99±0.02	1
73	243817x*L.bienne* (F_1)	5.80±0.07	3.40±0.16	1.96±0.15	2.19±0.03	1
74	MacBeth x *L.bienne* (F_1)	5.54±0.05	3.34±0.11	1.94±0.09	2.38±0.02	1
75	HARC-15 x *L. bienne* (F_1)	5.64±0.05	3.42±0.11	2.04±0.09	2.79±0.02	1
76	13510 x *L. bienne* (F_1)	5.44±0.05	3.32±0.11	1.84±0.09	2.20±0.02	1
	Total	5.88±0.47	3.58±0.39	2.03±0.18	3.14±0.99	

VSF_2H = volunteer selfed F_2 hybrids from six crosses; Seed coat description: 1 = light brown; 2 = brown; 3 = dark brown; 4 = olive; 5 = light brown to yellowish; 6 = yellow

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Adaptability and yield stability of bread wheat (*Triticum aestivum*) varieties studied using GGE-biplot analysis in the highland environments of South-western Ethiopia

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The objectives of this study were to evaluate released Ethiopian bread wheat varieties for yield stability using the GGE biplot method and identify well adapted and high-yielding genotypes for the highland environments of South-western Ethiopia. Twenty five varieties were evaluated in a randomized complete block design with three replications at Dedo and Gomma during the main cropping season of 2016 and at Dedo, Bedelle, Gomma and Manna during the main cropping season of 2017, generating a total of six environments in location-by-year combinations. Combined analyses of variance for grain yield indicated highly significant (p<0.001) mean squares due to environments, genotypes and genotype-by-environment interaction. Yield data were also analyzed using the GGE (that is, G, genotype + GEI, genotype-by-environment interaction) biplot method. Environment explained 73.2% of the total sum of squares, and genotype and genotype X environment interaction explained 7.16 and 15.8%, correspondingly. The first 2 principal components (PC1 and PC2) were used to create a 2-dimensional GGE biplot and explained 63.88 and 15.71% of GGE sum of squares, respectively. The GGE biplot identified two wheat growing mega-environments. The first mega environment consisted of environments E1 (Gomma-2016), E2 (Dedo-2016), E3 (Bedele-2017), E4 (Manna-2017) and E5 (Gomma-2017) with G6 (Ogolcho) as a vertex genotype. The second mega environment consisted of E6 (Dedo-2017) with G8(Hulluka) as its vertex genotype. Genotypes (G10) Mekelle-4, (G7) Hoggana, (G16) Danda'a and (G14) Ga'ambo did not fit in any of the mega-environments. Genotypes (G5) Hidasse, (G15) Kakaba, (G21) Sofumar, (G11) Shorima, (G20) Tay, (G14) Ga'ambo, (G17) Gassay and (G4) Millan were found to be the most stable genotypes with mean grain yield exceeding the grand mean. Genotypes (G14) Ga'ambo and (G20) Tay were found to be benchmarks/ideal genotypes and could be used as checks to evaluate the performance of other genotypes and also can be recommended for wider cultivation in the highland environments of South-western Ethiopia. However, bread wheat breeding research should be started to identify higher yielding genotypes for these environments with testing sites established at Bedelle and Dedo to address the two mega environments.

Key words: GGE biplot, GXE interaction, Ideal genotypes/environments, mega-environments.

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the major cereals grown for use as food and industrial raw materials in Ethiopia. It is an important staple food in the diets of many Ethiopians, providing an estimated 12% of the daily

per capita caloric intake for the country's over 90 million population (FAO, 2017). It is annually grown in 1.7 million hectares of land which is 13.38% of the total area of land used for cereal production (CSA, 2018). It ranks second after maize contributing 15.17% of the total annual cereal production. Among the nine National Regional States of the country, Oromia and Amhara, respectively, account for 898,455.57 ha (52.9%) and 554,284.49 ha (32.7%) of the total national wheat production area, while the remaining 14.4% is accounted for by the Southern Nations Nationalities and Peoples Regional State (SNNPR) and other regional states (CSA, 2018). When production is considered, 58.7% (26,699,177.73 quintals(Qts)) and 29.1% (14,047,074 Qts) of the total national wheat production are, respectively, contributed by Oromia and Amhara regions with an additional 12.2% coming from SNNPR and other regional states (CSA, 2018).

In the highlands of South-western Ethiopia, including Jimma and Illubabore zones, wheat is grown in 3% of the national and 5% of the regional total wheat production area (CSA, 2018). Tef, high land pulses, maize, wheat and barley are the major crops grown in both zones. Wheat is, however, becoming an important crop because of its higher yield potential and higher market price compared to other crops. In 2017, meher season annual production of wheat in Jimma and Illubabore zones was 701,047.43 and 170,327.59 Qts with productivity of 20.56 and 25.70 Qts/ha, respectively. Though the average productivity in both zones is less than both the national (26.75 Qts/ha) and the regional (29.65 Qts/ha) average productivity (CSA, 2018). It shows potential of the zones in wheat production, which can be improved further if improved varieties and management practices are applied.

Even though research on wheat has been going on for a long time in the country, the highland environments of Jimma and Illubabore zones have not been considered among the target agroecologies. This was mainly due to the fact that priorities were given to the central highlands and varieties which are currently in production were bred and selected specifically for their adaptation to the central highlands where combined use of those improved varieties and their improved production packages have played an immense role in significantly improving wheat productivity. Contrary to this, in parts of South-western highland, wheat is still grown following traditional practices using low yielding and low quality mixed seed obtained from local market owing to lack of well adapted and high yielding varieties. Therefore there is an urgent need to identify well adapted and high yielding improved varieties and avail to the farming communities to promote production and productivity of wheat in these areas within the possible short time. Evaluating adaptation of the already existing nationally released varieties is the best cost effective and time efficient approach to identify those varieties before starting breeding program from the grass root level.

Looking at the diversity of the highland environments of the South-western Ethiopia, it is not obvious whether to make varietal recommendation for the whole region from variety performance evaluation conducted in a single environment or test at specific environment and make site specific varietal recommendation. Furthermore, no information is available regarding how many wheat mega-environments are available in the regions. Multi-location performance evaluation trial (MLPET) of the nationally released bread wheat varieties was proposed to identify varieties for specific and broad adaptation and also to characterize the environments and group homogenous environments into a single and more representative one in terms of discrimination ability. In order to identify best performing adapted genotypes for specific or wider adaptation, genotype-by-environment (GXE) interaction and stability analysis are the major methodologies employed in plant breeding.

A number of statistical packages are available for effective analyses of yield data obtained from MLPET and identifying genotypes for specific and wider adaptation by generating information on the degree of GXE interaction. The Wricke (1962) ecovalence, Finlay and Wilkinson (1963) regression coefficient, Eberhart and Russell (1966) regression coefficient and deviation from regression, Shukla (1972) stability variance parameter, Pinthus (1973) coefficient of determination, Lin et al. (1986) Cultivar superiority measure (Pi), GGE biplot (Yan et al., 2000), AMMI Stability Value (ASV) (Purchase et al., 2000), Yield stability index (YSI) (Farshadfar et al., 2011), Multivariate analysis methods (principal component analysis, principal coordinate analysis, factor analysis, cluster analysis and additive main effects and multiplicative interaction (AMMI) are some of the packages available to date.

Yan et al. (2000) proposed the methodology known as genotype and genotype-by-environment (GGE) biplot for graphical display of GXE interaction pattern of MLPET data with many advantages. GGE biplot analysis considers both genotype (G) and genotype-by-environment interaction effects and graphically displays GXE interaction in a two way table (Yan et al., 2007). GGE biplot is an effective method based on principal component analysis (PCA) to fully explore MLPET data. It allows visual examination of the relationships among the test environments, genotypes and the GXE interactions. GGE Biplot is an effective tool for; environmental evaluation (the power to discriminate among genotypes

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Table 1. Background information of bread wheat varieties used in the study.

No	Entry code	Varieties	Year of release	Area of adaptation(*masl)	Source
1	G1	ETBW 5879	2011	2200-2600	KARC
2	G2	ETBW 6095	2011	1800-2400	KARC
3	G3	Worrakatta	2014	-	KARC
4	G4	Millan	2015	-	KARC
5	G5	Hidasse	2012	2200-2600	KARC
6	G6	Ogolcho	2012	1600-2100	KARC
7	G7	Hoggana	-	-	KARC
8	G8	Hulluka	2012	2200-2600	KARC
9	G9	Mekelle-3	-	-	KARC
10	G10	Mekelle-4	-	-	KARC
11	G11	Shorima	2011	2100-2700	KARC
12	G12	Mekelle-1	-	-	KARC
13	G13	Mekelle-2	-	-	KARC
14	G14	Ga'ambo	2011	750	KARC
15	G15	Kakaba	2010	1500-2200	KARC
16	G16	Danda'a	2010	2000-2600	KARC
17	G17	Gassay	2007	1890-2800	KARC
18	G18	Alidoro	2007	2800-3100	KARC
19	G19	Digelu	2005	2000-2600	KARC
20	G20	Tay	2005	1900-2800	KARC
21	G21	Sofumar	2000	2300-2800	KARC
22	G22	Mada-Wolabu	2000	2300-2800	KARC
23	G23	Pavon-76	1982	750-2500	KARC
24	G24	Geferson	-	-	KARC
25	G25	King bird	-	-	KARC

KARC: Kulumsa Agricultural Research Center; *masl: Meters above the mean sea level

in target environment), genotype evaluation, mega environment analysis (e.g., "which- won- where" pattern), where by specific genotype can be recommended to specific mega environment and ranking of genotypes (based on their mean performance and stability). The objectives of this study were, therefore, to evaluate released Ethiopian bread wheat varieties for yield stability using the GGE biplot method, and identify well adapted and high-yielding genotypes for the highland environments of South-western Ethiopia.

MATERIALS AND METHODS

Experimental materials and test environments

Twenty five nationally released bread wheat varieties (Table 1) were obtained from the National Bread Wheat Research Coordinating Center (NBWRCC) based at Kulumsa Agricultural Research Center (KARC) for use in this study. The genotypes were evaluated in six environments, over two growing seasons, in the highlands of South-western Ethiopia. The experiments were conducted at Dedo and Gomma during the main cropping season of 2016 and at Dedo, Bedelle, Gomma and Manna during the main cropping season of 2017 generating a total of six environments in location-by-year combinations. Hence the six environments were E_1, E_2, E_3, E_4, E_5 and E_6 representing Gomma-2016, Dedo-2016, Bedele-2017, Manna-2017, Gomma-2017 and Dedo-2017, respectively.

Experimental design and field management

The experiments were laid out in a randomized complete block design with 3 replications at all environments. Each plot had six rows in a plot size of 3 m × 1.2 m (3.6 m^2) with spacing of 20 cm between rows and 5 cm between plants. Fertilizer was applied at the rate of 150 kg Diammonium phosphate (DAP) and 200 kg urea/ha. Both urea and DAP were given through split application, half dose at planting and the remaining half at full tillering stage. At planting the portions of both DAP and urea were mixed and drilled into the rows and mixed with soil before planting. Seeds were drilled into the rows at the rate of 150 kg/ha. The remaining half doses of both fertilizers were applied at full tillering through top dressing. Weeds were controlled by 3 to 4 times hand weeding. Data were recorded on all agronomic characters and grain yield. However, only grain yield was considered for stability analysis. The central four rows were hand harvested and threshed separately to determine grain yield. The moisture content of the grain was adjusted at 12.5% and grain yield was converted to quintals/ha.

Statistical analysis

Analyses of variance (ANOVA) were conducted separately for individual environments according to Gomez and Gomez (1984). Bartlett's test was used to assess the homogeneity of error

Table 2. Combined analyses of variance for grain yield and the percentage sum of square of the 25 bread wheat genotypes evaluated in six environments in the highlands of South-western Ethiopia.

Sources of variation	Df	SS	SS%	MS	F-val	Pr>F
Environment (E)	5	75837.8	73.2	15167.6	1151.5	<.0001
Replication within E	2	25.91	0.02	12.95	0.98	0.3751
Genotype (G)	24	7415.58	7.15	308.983	23.46	<.0001
G X E	120	16382.7	15.8	136.52	10.37	<.0001
Error	298	3925	3.78	13.17	-	-
Total	449	103587	100	-	-	-
Grand Mean = 29.22			CV(%)= 12.4			

variances between environments to determine the validity of the combined analysis of variance across environments. Combined analyses of variance were performed with the PROC GLM procedure in SAS (2014) versions 9.3 software. Comparison of treatment means was done using Fischer's least significant difference (LSD) test at 5% probability levels. In performing the combined analyses of variance genotypes were assumed to be fixed while environments were assumed random. The following statistical model was used for combined analysis of variance over environments:

$$Yijk = \mu + Gi + Ej + GEij + Bk(j) + \epsilon ijk$$

where, Yijk, Observed value of genotype i in block k of environment (location) j; μ, grand mean; Gi, effect of genotype I; Ej, environment or location effect; GEij, the interaction effect of genotype i with environment j; Bk(j), The effect of block k in location (environment) j and ϵijk, error (residual) effect of genotype i in block k of environment j

The combined analysis of variance was carried out to estimate effects of environment (E), genotype (G) and GXE interaction. Levels of significance of these variables were determined by using F-test.

Genotype main effect and genotype by environment interaction effect (GGE) biplot analysis

The GGE biplot analysis was conducted by using Genstat version 18th software. GGE biplot methodology which is composed of two concepts, the biplot concept (Gabriel, 1971) and the GGE concept (Yan et al., 2000) were used to visually analyze the wheat varieties. This methodology uses a biplot to show the factors (G and G X E) that are important in genotype evaluation and that are also the sources of variation in G X E interaction analysis of MLPET data (Yan, 2001). The general model for GGE Biplot is as follow:

$$Yij - \mu - \beta j = \lambda 1 \text{Ɛ} i1 \eta j1 + \lambda 2 \text{Ɛ} i2 \eta j2 + \text{Ɛ} ij$$

where, Yij, the performance of the i^{th} genotype in the j^{th} environment; μ, The grand mean; Bj, the main effect of the environment j; λl and λ2, singular value for IPCA1 and IPCA2, respectively; Ɛi1 and Ɛi2, eigen vectors of genotype i IPCA1 and IPCA2, respectively; ηj1 and ηj2, eigen vectors of environment j for IPCA1 and IPCA2, respectively and Ɛij = Residual associated with genotype i and environment j.

RESULTS AND DISCUSSION

Combined analyses of variance for grain yield revealed highly significant (P<0.0001) mean squares due to genotypes, environments and GXE interaction. Environment, genotype and GXE interaction explained 73.2, 7.15 and 15.8% of the total sum of squares, respectively (Table 2). This agrees very well with a previous study which reported that environment accounted for 80% of the total variation while genotype and G XE interaction accounted for the remaining 20% of the total variation in MLPET of bread wheat (Kaya et al., 2006).

High percentage of sum of squares attached to environment indicated that environment played a dominant role in influencing yield performance of the bread wheat genotypes. The GXE interaction was highly significant (p<0.001) and accounted for 15.80% of the sum of squares implying the need for investigating the nature of variable responses of the genotypes to environments. Presence of the GXE interaction indicates that the phenotypic expression of one genotype might be superior to another genotype in one environment but inferior in a different environment. In other words, when significant GXE interactions are present, the effects of genotypes and environments are statistically non-additive (or the differences between genotypes depend on the environment). The presence of a significant GXE interaction complicates interpretation of the results. That means, it is difficult to identify superior genotypes across environments when GXE interaction is highly significant.

In general, from the combined analyses of variance (Table 2) superiority of genotypes across environments could not be identified by considering their mean grain yield performance because GXE interaction was highly significant. It was earlier suggested that in situations where GXE interactions minimize the usefulness of genotypes, yield levels, adaptation and stability are taken into account in MLPETs (Kang and Pham, 1991). Crossa et al., (1990) elaborated the relevance of qualitative or crossover interactions in agriculture and appropriate statistical analyses are required for quantifying them. Furthermore, the traditional analysis of variance determines the values of each variance source and the significance of the contribution of each component, but it does not partition the interaction into several components and thus other types of analyses should be performed.

Hence, such multi-environment trial data along with a highly significant GXE interaction requires measures of stability analysis techniques that will help to get more information on the GXE interaction as well as to assess the adaptation regions of the genotypes according to their favorable interaction. However, the findings of these study are in accordance with other researchers (Fentaw, 2011; Mehari et al., 2015; Misganaw and Fisseha, 2016) who reported that variety of environmental factors are important in selecting wheat genotypes under Ethiopian conditions.

The lowest and the highest mean grain yields were 6.43 Qt/ha obtained in G8 (Hulluka) at E5 (Gomma-2017) and 68.78 Qt/ha obtained in G6 (Ogolcha) at E3 (Bedelle-2017), respectively. E3 (Bedelle-2017) was the highest yielding environment with mean grain yield of 48.2 Qt/ha and E4 (Manna-2017) was the least yielding environment with mean grain yield of 12.6 Qt/ha, which was far below the grand mean (29.2 Qt/ha) (Table 3). Better soil condition and distribution of rainfall at E3 (Bedele-2017) helped better yield performance while poor fertility status of the soil and terminal moisture stress caused low yield performance at E4 (Manna-2017). The genotypes ranked differently from one environment to another environment in their grain yield performance showing deferential responses to environments and possibly a cross-over type of genotype X environment interaction. Among the genotypes G20 (Tay) (38.03 Qt/ha), G14 (Ga`ambo) (34.34 Qt/ha) and G15 (Kakaba) (33.94 Qt/ha) were the first three best yielders in terms of grain yield data pooled across environments.

Genotype main effect and genotype-by-environment interaction (GGE) biplot analysis

The GGE (genotype main effect (G) and genotype-by-environment interaction (GE)) concept is based on the understanding that genotype main effect (G) and genotype-by-environment interaction (GEI) are the two sources of variation that are relevant to genotype evaluation and that they must be considered simultaneously for appropriate genotype evaluation (Yan, 2001). The graphical method was employed to investigate environmental variation and interpret GXE interaction. The partitioning of GXE interaction through GGE biplot analysis showed that IPCA 1 and IPCA 2 accounted for 63.88% and 15.71% of sum of squares, respectively, with a total of 79.59% variation for grain yield.

The Polygon View of the GGE Biplot (The "which-won-where" patterns)

The polygon view of the GGE biplot points out the best genotype in each environment. It graphically addresses important concepts such as crossover interaction, mega environment differentiation, particular adaptation, etc. (Yan and Tinker, 2005). The term mega environment analysis defines the partitioning of a crop growing region into different target zones (Gauch and Zobel, 1997). Polygon views of the GGE biplot based on symmetrical scaling for the which-won-where pattern of genotypes and environments is given below in Figure 1.

The GGE bi-plot showed six vertex genotypes, G8 (Hulluka), G7 (Hoggana), G14 (Ga'ambo), G10 (Mekelle-4), G6 (Ogolcho) and G16 (Danda'a). There were six rays, which divided the biplot into six sections. The environments fell into only two sections but the genotypes were distributed throughout all the six sections. The vertex genotype of each sector is the one that gave the highest grain yield in the environments which fell within that sector (Figure 1).

The GGE biplot identified two wheat growing mega-environments. The first mega environment consisted of environments E1 (Gomma-2016), E2 (Dedo-2016), E3 (Bedele-2017), E4 (Manna-2017) and E5 (Gomma-2017) with a vertex genotype G6 (Ogolcho). Hence, G6 (Ogolcho) was the winning genotype in most of the environments. E6 (Dedo-2017) was the only environment that was found in the second mega environment with G8 (Hulluka), as its vertex genotype. It was also noted that no mega-environments fell into sectors where genotype G10 (Mekelle-4), G7 (Hoggana), G16 (Danda`a) and G14 (Ga`ambo) were the vertex genotypes, indicating that these genotypes were not suitable to any of the test environments.

Ranking of varieties based on mean grain yield and stability performance

In GGE biplot methodology, the estimation of grain yield and stability of genotypes was done using the average environment (tester) coordinate (AEC) methods (Yan and Hunt, 2002). The line passing through the biplot origin is called the average environment (tester) coordinate (AEC), which is defined by the average PC1 and PC2 scores for all environments. The AEC ordinate separates genotypes with below average means from those with above average means. So genotypes with mean grain yield exceeding grand mean grain yield were G15 (Kakaba), G5 (Hidasse), G25 (King bird), G1 (ETBW 5879), G16 (Danda'a), G14 (Ga'ambo), G17 (Gassay), G20 (Tay), G11 (Shorima), G18 (Alidoro) and G4 (Millan) (Figure 2). The line, which passes through the origin and is perpendicular to the AEC, represents the stability of genotypes. Either direction away from the biplot origin, on the axis, indicates greater GXE interaction and reduced stability. For selection, the ideal genotypes are those with both high mean grain yield and high stability. In the biplot, they are close to the origin and have the shortest vector from the AEC. A longer projection to the AEC, regardless of direction, represents a greater tendency of the GXE

Table 3. Mean grain yield (Qt/ha) of 25 bread wheat varieties, evaluated in the highland environments of South-western Ethiopia.

No.	Entry Code	Genotypes	Test environments (Location X year combinations)						Mean	Overall rank
			Gomma-2016 (E1)	Dedo-2016 (E2)	Bedele-2017(E3)	Manna-2017(E4)	Gomma-2017(E5)	Dedo-2017(E6)		
1	G1	ETBW 5879	33.7	18.7	58.4	10.4	15.86	47.67	30.79	10
2	G2	ETBW 6095	38	16.8	39.7	10.8	11.38	30.94	24.60	21
3	G3	Worrakatta	27.2	12.4	47.9	11.97	21.26	44.49	27.54	19
4	G4	Millan	39.1	20.6	60.26	10.7	13.16	44.55	31.40	9
5	G5	Hidasse	35.1	19.8	56.2	9.3	10.31	38.85	28.26	16
6	G6	Ogolcho	41.1	27.1	68.78	13.1	21.51	27.13	33.12	5
7	G7	Hoggana	18.2	22	17.58	10.1	19.22	35.31	20.40	25
8	G8	Hulluka	24.1	23.5	37.9	12.2	6.43	51.6	25.96	20
9	G9	Mekelle-3	36.6	15.5	48.9	13.3	13.16	39.31	27.80	18
10	G10	Mekelle-4	25.4	35.1	17.59	16.1	20.21	27.37	23.63	22
11	G11	Shorima	35.4	26.8	57.58	14.7	17.07	41.1	32.11	7
12	G12	Mekelle-1	28.7	23.52	40.77	13.5	18.69	43.4	28.10	17
13	G13	Mekelle-2	29.9	25.99	36.47	14.7	12.93	51.3	28.55	14
14	G14	Ga'ambo	38.3	24.23	65.1	13.5	17.55	47.33	34.34	2
15	G15	Kakaba	35.9	24.8	52.95	15.8	26.7	47.51	33.94	3
16	G16	Danda'a	32.9	15.5	57.89	11.5	13.39	46.78	29.66	11
17	G17	Gassay	38.5	26.4	60.5	13.8	15.52	47.49	33.70	4
18	G18	Alidoro	36.3	29.3	56.73	14.3	21.66	33.18	31.91	8
19	G19	Digelu	25.7	15.9	32.7	14.1	12.79	40.42	23.60	23
20	G20	Tay	41.5	28.5	60.69	20.6	27.16	49.75	38.03	1
21	G21	Sofumar	33.2	15.4	55.86	10.5	19.72	41.6	29.38	13
22	G22	MadaWolabu	30.7	22.1	43.75	10.2	18.22	45.16	28.36	15
23	G23	Pavon-76	31	24	46.2	10.7	17.64	47.2	29.46	12
24	G24	Geferson	23.8	16.9	36.4	8.1	16.11	38.38	23.28	24
25	G25	King Bird	31.7	31.4	48.9	11.3	21.69	51.01	32.67	6
Mean			32.5	22.49	48.2	12.6	17.17	42.3	29.21	-
CV(%)			8.14	10.82	6.47	7.48	8.5	17.3	-	-
F-test			**	**	**	**	**	*	-	-

*and ** represent statistically significant differences at 0.05 and 0.01 probability levels, respectively.

interaction of a genotype that means less stability across environments. Thus, G15 (Kakaba), G21 (Sofumar), G11 (Shorima), G20 (Tay), G14 (Ga'ambo), G17 (Gassay) and G4 (Millan) were the most stable genotypes with mean grain yield exceeding grand mean grain yield. On the other hand, G10 (Mekelle-4) and G6 (Ogolcho) were far from AEC (long vector) indicating their least stability.

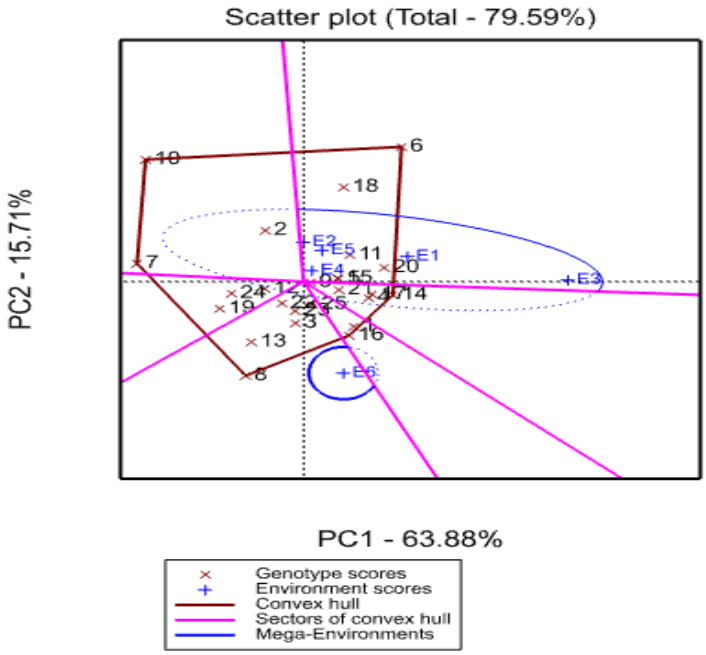

Figure 1. Polygon view of the GGE biplot using symmetrical scaling for the which-won-where pattern of the genotypes environments. Details of environment are given in Table 2. Numbers 1 to 25 represent genotypes as indicated in Table 2.

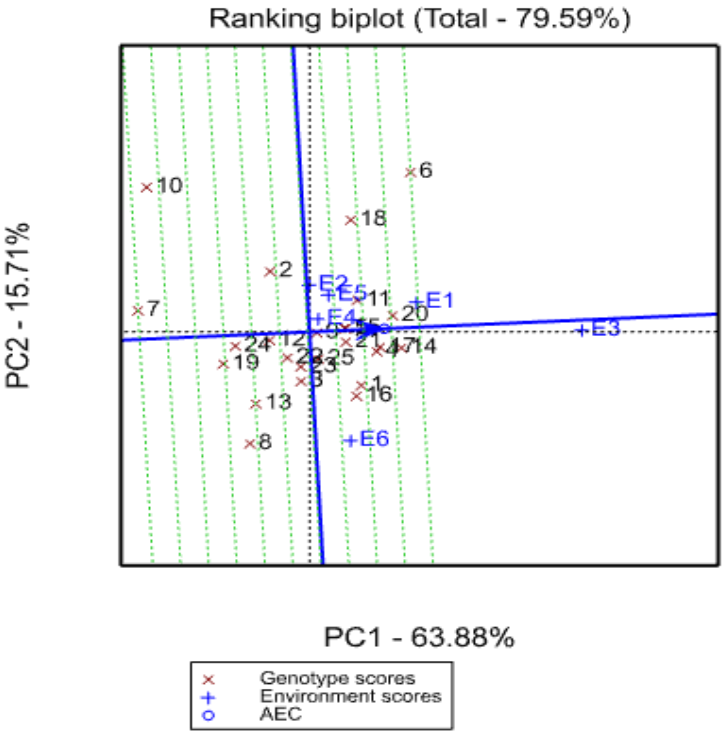

Figure 2. Average environment coordination (AEC) views of the based on environment-focused scaling for the mean grain yield performance and stability of 25 bread wheat genotypes tested across six environments. Details of environment are given in Table 2. Numbers 1 to 25 represent genotypes as indicated in Table 2.

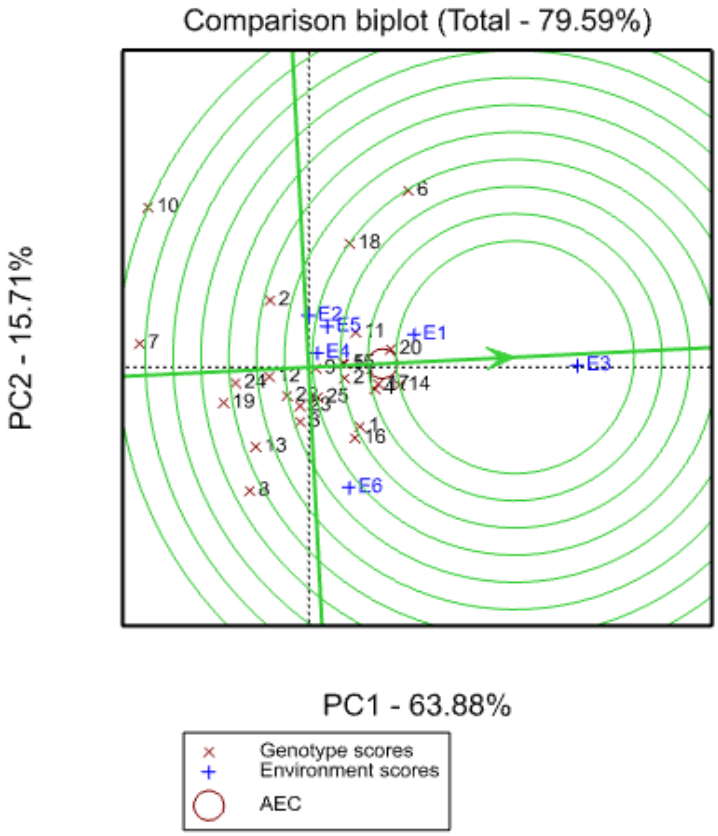

Figure 3. GGE biplot with scaling focused on genotypes, for the evaluation based on the ideal genotype of 25 bread wheat genotypes across six environments. Details of environment are given in Table 2. Numbers 1 to 25 represent genotypes as indicated in Table 2.

However it should be noted that the former genotype represent low yielding compared to grand mean and instable genotypes while the later exemplifies higher yielding but instable genotypes.

Evaluation of varieties based on the ideal genotype

An ideal genotype is expected to have the highest mean grain yield performance and stability in performance across environments (Farshadfar et al., 2012). Though such an ideal genotype may not exist in reality, it can be regarded as a reference for genotype evaluation (Kaya et al., 2006). The ideal genotype is located in the first concentric circle in the biplot. Genotypes found closer to the ideal genotypes are desirable genotypes and those found far from the ideal genotype are considered as undesirable genotypes.

Thus, the ideal genotype can be used as a benchmark for selection. Genotypes that are far away from the ideal genotype can be rejected in early breeding cycles while genotypes that are close to it can be considered in further tests (Yan and Kang, 2003). Mean grain yield performance and stability of 25 bread wheat genotypes tested across six environments. Details of environment are given in Table 2. Numbers 1 to 25 represent genotypes as indicated in Table 2. Accordingly, genotypes placed near to the first concentric circle, G14 (Ga'ambo) and G20 (Tay) were found to be benchmarks for evaluation of bread wheat genotypes (Figure 3). G4 (Millan), G17 (Gassay), G11 (Shorima), G16 (Danda'a) and G1 (ETBW 5879) were located near the ideal genotype, thus were desirable genotypes. Undesirable genotypes were those distantly located from the first concentric circle, namely, G10 (Mekelle-4), G7 (Hoggana), G2 (ETBW 6095), G12 (Mekelle-1), G19 (Digelu), G24 (Gefferson), G8 (Hulluka) and G13 (Mekelle-2).

Evaluation of environments relative to ideal environments

Discriminating ability and representativeness are important properties of a test environment. An ideal environment should be differentiating the tested

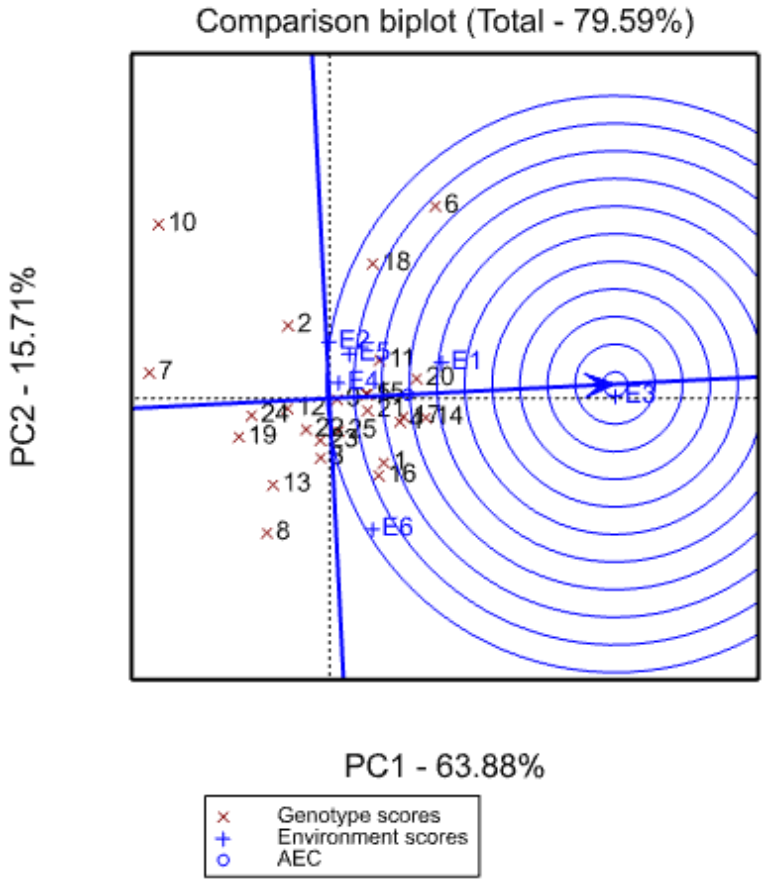

Figure 4. GGE biplot with scaling focused on environment, for the comparison of environments with ideal environment. Details of environment are given in Table 2. Numbers 1 to 25 represent genotypes as indicated in Table 2.

genotypes and at the same time be a representative of the target agro-ecology (Yan, 2001; Yan and Kang, 2003). Similar to ideal genotype, an ideal environment is defined and shown by the small circle. Meaning that the environment is more desirable and discriminating when located closer to the center of a circle or to an ideal environment. Yan et al. (2001) suggested that favorable test environments should have large PC1 scores (more discriminating of the genotypes) and near zero PC2 scores (more representative of an average environment). Accordingly, E3 (Bedele-2017), which had the longest vector which fell into the center of concentric circles, was considered as an ideal environment in terms of being the most representative of the overall environments and the most powerful to discriminate genotypes. Thus, E3 (Bedele-2017) was an ideal environment which could be used as a benchmark to evaluate the remaining environments. E1 (Gomma-2016) was closer to the ideal environment, thus, it was regarded as the most desirable environment to select widely adapted genotypes (Figure 4). Conversely environments E6 (Dedo-2017), E2 (Dedo-2016), E4 (Manna-2017) and E5 (Gomma-2017) were located far from the ideal environment, thus were considered as less powerful to discriminate the genotypes.

Conclusion

The results from this study indicated that bread wheat genotypes responded deferentially to environments with significant genotype X environment interaction. Genotypes G15 (Kakaba), G21 (Sofumar), G11 (Shorima), G20 (Tay), G14 (Ga'ambo), G17 (Gassay) and G4 (Millan) were the most stable. Genotypes G14 (Ga'ambo) and (G20) Tay were benchmarks/ideal genotypes that could be used as checks when evaluating the performance of other genotypes and also can be recommended for wider cultivation in the highland environments of South-western Ethiopia. The study also identified two bread wheat mega environments. Therefore, bread wheat breeding research should be started to identify higher yielding genotypes for the highland environments of South-western Ethiopia with

testing sites established at Bedelle and Dedo to address the two mega environments.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Determination of concentration of heavy metals in ginger using flame atomic absorption spectroscopy

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Concentrations of four heavy metals (Ni, Zn, Cd and Pb) in two ginger varieties were determined using flame atomic absorption spectroscopy (FAAS) with wet acidic digestion methods. Results showed that the concentration of zinc is 0.86 to 1.17 mg/kg in Hargama and 0.63 to 0.87 mg/kg in Bolbo varieties. The concentration of nickel for Hargama and Bolbo are 0.15 to 0.18 and 0.17 to 0.21 mg/kg, respectively. Zinc concentration in Hargema variety is statistically significantly different from Bolbo variety. However, no statistically significant differences were observed in nickel concentrations. Concentration of zinc is relatively greater than concentration of nickel in the samples. Concentrations of both metals are below permissible limits set by WHO/FAO and could not cause health problems. In addition to this, the concentration level of both metals is lower than toxicity levels. But concentrations of cadmium and lead metals were below the method detection limit.

Key words: Concentration, ginger, heavy metal, permissible limit, toxicity.

INTRODUCTION

Ginger is a medicinal herb and belongs to Zingiberaceae family, genus *Zingiber* and species *officinale* (Gupta and Sharma, 2014). It is widely used as a spice and medical treatment for certain diseases. Ginger contains several compounds and its major components are 6-gingerol, 6-shogaol, and 6-paradol that possess strong antioxidant activity (Prasad and Tyagi, 2015) and it possesses health benefits. Ginger also contains different nutrients such as protein, fats, insoluble fibers, soluble fibers, carbohydrates and vitamins (Shirin and Jamush, 2010; Ajayi et al., 2013).

Spices contain essential elements like Na, K, Cu, Zn, Ca, Mg, Fe and Mn as well as non-essential or toxic elements such as Hg, Cd, Pb and Cr metals (Longhurst, 2010; Belay and Tadesse, 2014). Low intake of essential metals produces deficiencies, while higher consumption may cause toxicity. However, non-essential metals are lethal and toxic to human even at low concentrations.

Non-essential metals are ranked among the most hazardous toxic substances owing to their persistence in the environment and absorption in food chain (Khan et al., 2013; Muhammad et al., 2013). Toxic effects of metals include vomiting, diarrhea, headache, irritability, hypertension, heart, lung, kidney, liver and intellectual

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problems and cancer (Shah and Ara, 2012). Toxic metals are extremely persistent in the environment even at low concentrations and have been reported to produce damaging effects on human and animals because there is no good mechanism for their elimination from the body (Loannidou et al., 2005; Adah et al., 2013).

There is evidence that lead pollution can induce aggressive behavior in animals which can also occur in humans (Nkansah and Amoako, 2010). Krejpcio et al. (2007) reported that concentration of zinc in spices from Polish markets is found to be 5.96 to 16.95 mg/kg while Nkansah and Amoako (2010) researched that the concentration of zinc in spices from Ghana is found to be 73 g/kg. Wagesho and Chandravanshi (2015) indicated that the concentration of zinc in some parts of Ethiopian ginger is 38.5 to 55.2 mg/kg. Agrawal et al. (2011) reported that the concentration of zinc is 0.46 to 2.74 mg/kg while Devi et al. (2008) showed that the concentration of zinc is 44.93 mg/kg in Indian spices.

The contents of trace metals in herbal medicinal plants from Turkey are found in the ranges: 0.2 to 2.7 µg/g for cadmium, 0.1 to 2.8 µg/g for lead, 1.4 to 11.3 µg/g for nickel and 5.2 to 83.7 µg/g (Soylak et al., 2006). According to result of Komy (2005), concentration of lead and cadmium in cumin spice is 0.33 and 0.22 µg/g, respectively. Gaya and Ikechukwu (2016) studied that the concentration of heavy metal in Nigeria for ginger spice are (in mg/kg) 7.45±0.02, 3.42±0.01, 2.70±0.01 and 10.13±0.02 for cadmium, nickel, lead and zinc, respectively. Ozkutlu et al. (2006) reported that the concentration of cadmium is 0.07 mg/kg and that of zinc is 5.00 mg/kg in ginger spice. The permissible limit of nickel, zinc, lead and cadmium are 0.05, 0.1, and 0.1 mg/kg (WHO/FAO, 2011) and 0.2 mg/kg (Sharma, 2014), respectively.

For people in the areas covered in this research, ginger is common spice in food per day and a known medicinal remedy. As people directly consume ginger as spice and medicine, some heavy as well as trace elements that could cause health damage in the long run may be taken indirectly. Thus, study of heavy metals in ginger is of paramount importance. This research aimed to determine the concentration of heavy metals in ginger variety in some areas of Southern part of Ethiopia.

MATERIALS AND METHODS

Description of study area

This research was conducted in Kembatta Tembaro Zone in the Southern part of Ethiopia. This region is one of main ginger producing regions in Ethiopia. Three ginger producing woredas: Kachebira, Tambaro and Hadero were considered to collect samples. Figure 1 displays the administrative map of the study area.

Sample collection protocol

Fresh rhizomes of two ginger varieties namely hybrid (Bolbo) and Hargema samples were collected from ginger producing model farmers in the three selected woredas. Total of six samples were collected from three selected Woredas (two varieties from each woreda). Figure 2 presents ginger varieties considered in this work.

Sample preparation

The collected samples were washed thoroughly with tap water to remove absorbed particulates from the soil and then rinsed by de-ionized water. Its thin outer cover skin was removed with plastic knife and then chopped into pieces of approximately same size in order to facilitate drying uniformity. Samples were exposed to sunlight for two days to reduce moisture content. The samples were dried in the oven (carbolated fusion furnace) at a temperature of 105°C for 24 h to have dry mass basis (Wagesho and Chandravanshi, 2015). The dried samples were powdered in high speed universal disintegrator (Model F100) in a stainless steel mill till obtaining fine particles that pass through a 0.5 mm mesh and kept dry in a cleaned polyethylene bag.

Acid digestion method

A mass of 0.5 g of sieved powder of the samples was weighed out (Model ABS 220-4M) into acid washed glass beaker. Then the powder was digested with addition of 4 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%) in wet digestion system (Wagesho and Chandravanshi, 2015). After digestion, the solution was diluted with 10 mL de-ionized water. The same digestion procedure was followed for blank solution that was used for calibration curve determination (with minimum of correlation coefficient $R^2 = 0.9977$).

Experimental setup

Flame atomic absorption spectrophotometer (FAAS) (Model 210 VGP) was used to measure absorbance of each metal from which concentration of heavy metals was deduced. Hallow cathode lamps of specific wavelength were used as an exciting energy. Working conditions of experimental setup are shown in Table 1.

Statistical analysis

Data entry management and preliminary summaries were done on Microsoft Office Excel spread sheet. Means of data collected were determined. All analyses were carried out in triplicates and data presented as means. One-way analysis of variance (ANOVA) at $p < 0.05$ was used to determine statistically significant differences in the mean concentrations of metals among varieties as well as within a given variety in study areas. For comparison of the mean of the treatments, the Fisher's least significant difference (LSD) test were used to check the significance level. Data were further manipulated with ASA and SPSS 20.

RESULTS AND DISCUSSION

Concentrations of four heavy metallic elements (Zn, Ni, Cd, and Pb) in the digested samples of ginger were analyzed by FAAS. Results are shown in Table 2. Among the analyzed metals lead and cadmium were below the method detection limit. Mean concentrations of zinc range from 0.63 to 1.17 mg/kg while that of nickel are in the range of 0.15 to 0.21 mg/kg.

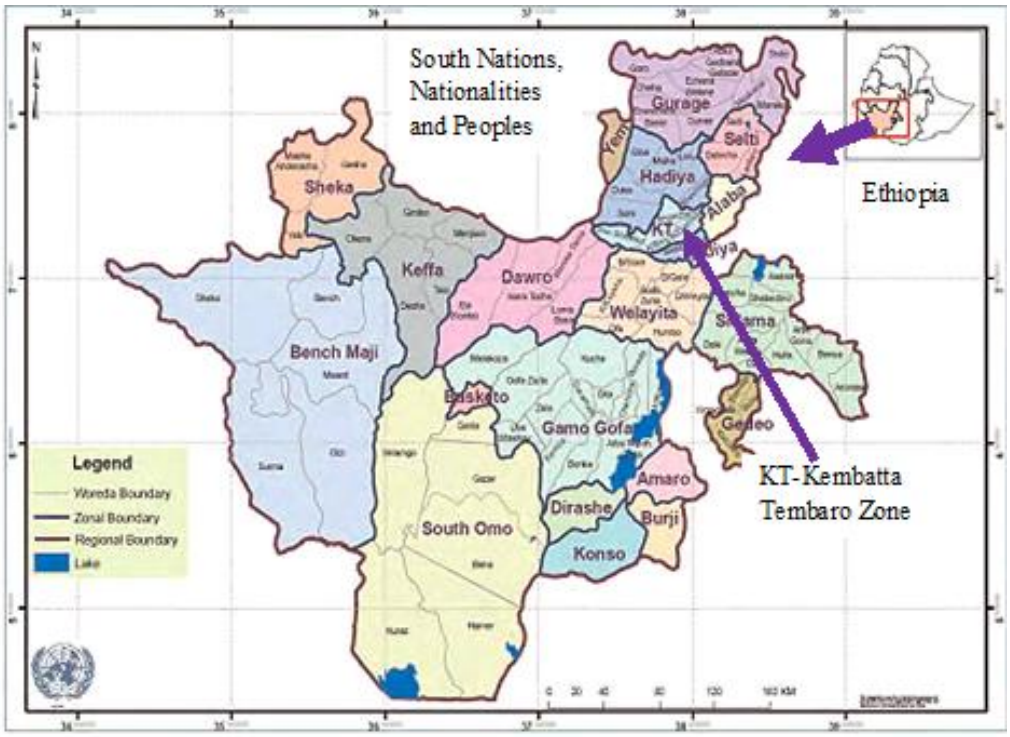

Figure 1. Map of study area as indicated by an arrow (retrieved at: www.rippleethiopia.org/page/snnpr).

Figure 2. Ginger varieties considered in this work (a) Hargema and (b) Bolbo (Hybrid).

Zinc (Zn)

Minimum and maximum zinc concentration of ginger in the studied area is 0.63 and 1.17 mg/kg, respectively. As shown from Table 2, varieties had differences in concentration in the three places considered in this work. One-way analysis of variance showed that the mean concentration of zinc of Hadero is statistically significantly different among other two sites, at $p < 0.05$. Variety wise, Fisher's combined probability test using the LSD criterion for significance determination indicated that the mean concentration of zinc is statistically significantly different from each other with $p < 0.05$ within the study area. As shown in Table 2, the variety Hargam possessed high zinc concentration as compared to Bolbo variety.

According to the study conducted in some parts of

Table 1. Working condition of the experimental setup.

Metal	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Energy (erg)	IDL (mg/l)	MDL (mg/g)	MQL (mg/g)	Recovery (%)
Cd	228.9	0.7	2.0	3.07	0.005	0.0002	0.0003	105
Ni	232	0.2	7.0	2.928	0.001	0.002	0.01	104
Pb	217	0.7	3.0	3.16	0.1	0.002	0.007	-
Zn	213.9	0.7	2.0	3.047	0.005	0.0006	0.002	93.2

IDL-Instrument detection limit, MDL-method detection limit, MQL-method quantification limit.

Table 2. Concentration of heavy metals in this work.

Study area	Variety	Heavy metal concentration (mg/kg)			
		Ni	Zn	Cd	Pb
Kachebira	Bolbo	0.20^{ba}	0.63^{c}	ND	ND
	Hargama	0.18^{bc}	0.95^{b}	ND	ND
Tambaro	Bolbo	0.17^{bc}	0.68^{c}	ND	ND
	Hargama	0.15^{c}	0.86^{b}	ND	ND
Hadero	Bolbo	0.21^{a}	0.87^{b}	ND	ND
	Hargama	0.18^{ba}	1.17^{a}	ND	ND
CV	-	8.79	8.61	-	-
LSD	-	0.029	0.132	-	-

Means with the same letter in a given column are not significantly different, ND-below method detection limit.

Ethiopia with dry weight digestion method, concentration of zinc in ginger is 38.5 to 55.2 mg/kg (Wagesho and Chandravanshi, 2015). Agrawal et al. (2011) reported that concentration of zinc is 0.46 to 2.74 mg/kg in India. Current result is in good agreement with results found in India but far less than that obtained in Ethiopia in previous study. Moreover, the present work has reported very low concentration of zinc as compared to Nkansah and Amoako, (2010) which is in Ghana (73 g/kg). Krejpcio et al. (2007) reported that the content of zinc concentration of spices in Polish markets is found to be 5.96 to 16.95 mg/kg which is higher than results obtained in this work. The mean concentration of zinc determined in this study is lower than the value determined in India (Devi et al., 2008) but greater than the value obtained (0.03-0.04 mg/kg) in Nigeria (Ajayi et al., 2013). The content of zinc in ginger sample of the current study (Ethiopia) is less than the permissible limit set by WHO/FAO (2011) in edible plants (50 mg/kg).

Nickel (Ni)

Minimum and maximum value of concentration obtained in this work for nickel is 0.15 and 0.21 mg/kg, respectively. Bolbo variety had relatively higher concentration of nickel than Hargama variety, but not statistically significant. Geographically, there is statistically significant difference in nickel concentration in Hadero, however, other two areas had statistically insignificant differences. In contrast to zinc, Bolbo variety possesses more nickel concentration than Hargama variety. However, the difference did not show statistical significance.

Nickel concentration of the present study is lower than the nickel content determined in Ethiopia in previously conducted research (5.46-8.40 mg/kg) (Wagesho Chandravanshi, 2015). The current work is also lower than nickel content obtained (43 g/kg) in Ghana (Nkansah and Amoako, 2010). Nickel content determined in the present study (Ethiopia) is higher than the permissible limit set by WHO/FAO (2011) in edible plants (1.63 mg/kg). However, nickel toxicity in human is not a very common occurrence because its absorption by the body is very low (Jabeen et al., 2010).

Lead (Pb) and cadmium (Cd)

In this experiment, both metals were below detection limits of the experimental technique employed. However, lead and cadmium were observed in some previous

studies. Reports of Agrawal et al. (2011) showed that lead and cadmium concentrations in India were 0.5 to 12.60 mg/kg and 0.92 to 2.27 mg/kg, respectively. A research conducted on heavy metals in spices collected from Polish markets showed that the concentration of lead is 0.21 to 0.78 mg/kg and that of cadmium is 0.02 to 0.04 mg/kg. Moreover, it was determined to be 0.30 mg/kg for cadmium, in Nigeria (Oladoye and Jegede, 2016).

Conclusion

Flame atomic absorption spectroscopy was used to determine concentration of heavy metals (Ni, Zn, Pb and Cd) in ginger varieties with wet digestion method. Statistically significant difference of zinc concentration was observed within varieties as well as between values within the given study area. Nickel concentration showed a non-statistically significant difference among varieties but value from one study area (Hadero) showed statistically significant difference. Both zinc and nickel were found to be below WHO/FAO permissible limits and could not cause health problems. Lead and cadmium were not detected.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Genetic diversity, variability and characterization of the agro-morphological traits of Northern Ghana Roselle (*Hibiscus sabdariffa* var. *altissima*) accessions

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Roselle (*Hibiscus sabdariffa* var. *altissima*), a bast fibre crop adapted to the warm climate of Northern Ghana, offers a great economic potential not yet explored for lack of information on its distribution, collection, and genetic diversity. Little variability is reported in exotic genotypes to merit trait improvement. The objective of this study is to investigate distribution and diversity in roselle of Northern Ghana. Twenty-five accessions collected from seven districts were field evaluated in a 5×5 lattice square design in three replications at twelve qualitative and five quantitative morphological traits. Data were analysed for within- and between-population variability and multivariate analysis. Large within-population variability of SDI 0.72 to 0.87 was identified in accessions of Kassena-Nankana East district. The most variable traits, plant height and branch number, varied from 184 cm to 284 cm with six accessions HA-44, HA-47, HA-43, HA-38, HA-52, and HA-42 having the tallest plants and least basal branching of four. Mean flowering time was between 96 and 104 days. Mean Euclidean distance of 3.03 ± 0.90 ranged from 0.41 to 5.17. Based on means across pairwise distances of 2.22 and 3.94, three accessions were divergent, namely, HA-61 (3.94), HA-57 (3.66) and HA-59 (3.63). Clustering and principal components analyses delineated three distinct groups. The first three PCs explained 100% of the variance. The ample diversity in roselle awaits exploitation for genetic improvement, particularly for fibre yield.

Key words: Bast fibre crop, cluster analysis, discriminant analysis, genetic diversity, morphology, PCA, roselle.

INTRODUCTION

Hibiscus sabdariffa var. *altissima* Wester, hereinafter referred to as roselle, is the cultivated fibre type with inedible calyx. Roselle is less known than its vegetable type, *H. sabdariffa* var. *sabdariffa*, and is underutilized for its bast fibre in Sub-Saharan Africa (SSA), although it is an important fibre commodity in Asia. Roselle fibre is ideal for making cordage due to its salt-resistant trait (Crane, 1949; Cook, 1960), for packaging sacs, paper products, upholstery, and a fabric for shoes and bag (Managooli, 2009). New found uses include a bio-composite for automobile parts and building materials such as fibre board (Alves et al., 2010; Junkasem et al., 2006). Roselle accounts for about 20% of bast fibre crops. From 1961 to 2016, bast fibre crop acreage in

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Table 1. Accessions of roselle (var. *altissima*) evaluated by morphological traits in Ghana in 2017

Accession	Collection site	District	Accession	Collection site	District
HA-37	Sumbrungu	Bolgatanga Municipality	HA-50	Yorogo	Bolgatanga Municipality
HA-38	Sirigu	Kassena-Nankana West	HA-51	Nawasa	Gonja North
HA-39	Chuchuliga	Builsa North	HA-52	Korania	Kassena-Nankana East
HA-40	Bolgatanga	Bolgatanga Municipality	HA-53	Nawasa	Gonja North
HA-41	Yua	Kassena-Nankana West	HA-54	Wiasa	West Mamprusi
HA-42	Pungu	Kassena-Nankana East	HA-55	Yua	Kassena-Nankana West
HA-43	Sirigu	Kassena-Nankana West	HA-56	Navrongo	Kassena-Nankana East
HA-44	Manyoro	Kassena-Nankana East	HA-57	Dua	Bongo
HA-45	Korania	Kassena-Nankana East	HA-58	Korania	Kassena-Nankana East
HA-46	Chuchuliga	Builsa North	HA-59	Navrongo	Kassena-Nankana East
HA-47	Manyoro	Kassena-Nankana East	HA-60	Gowrie	Bongo
HA-48	Saboro	Kassena-Nankana East	HA-61	Zaare	Bolgatanga Municipality
HA-49	Bolgatanga	Bolgatanga Municipality			

Sub-Saharan Africa (SSA) increased from 15,000 to 25,000 ha, equivalent to 67% growth, whereas bast fibre yield dropped from 1.15 to 0.67 t/ha, corresponding to 42% reduction. Nine countries, namely, Angola, Central African Republic, Democratic Republic of Congo, Ethiopia, Madagascar, Mali, Mozambique, Nigeria, and South Africa were engaged in bast fibre production. Current bast fibre production in SSA amounts to 16,000 metric tons/year. Research and development in roselle as a potential for bast fibre production in Ghana is lacking, despite a wide range of morphotypes found in the northern sector of the country (Ankrah et al., 2018).

A collection of roselle will control loss of this biodiversity. Assessment of genetic variability and diversity in roselle will provide information for development of improved cultivars and for conservation management. In a preliminary survey of knowledge of roselle in Northern Ghana, the indigenous folk asserted that roselle is threatened, as previous morphotypes are no longer common (personal communication). Moreso, the widely reported lack of variability in exotic roselle germplasm which is hampering efforts for genetic improvement is a legitimate concern (Omalsaad et al., 2014; Yusof and Saud, 2009; Hanboonsong et al., 2000).

Genetic diversity is a dynamic property of germplasm and its estimation may be based on morphological evaluation, biochemical, or molecular assessment (Bhandari et al., 2017). Among the three approaches, morphological characterization offers less costly and readily assessable measurement making them attractive to breeders for a genetic improvement program. Morphological evaluation is labour intensive, requires large plant population size, exhibits low rate of polymorphism and is constrained by environmental sensitivity and higher risks of biased estimates (Botha and Venter, 2000). Despite these drawbacks, morphological evaluation provides sufficient information on crop characteristics and reveals sources of useful genotypes for trait improvement (Camussi et al., 1985). Genetic diversity studies on roselle (var. *altissima*) are rather scanty and limited to work reported by Ankrah et al. (2018) who assessed thirty-six wild roselle accessions in Ghana; a study on a roselle bast fibre characterization study in Kenya (Mwasiagi et al., 2014), and some comparative variability study between kenaf and roselle (Sie et al., 2009; Cheng et al., 2004; Siepe et al., 1997). Further collection of 25 roselle accessions in northern Ghana is hereby evaluated for genetic variability and diversity information. The objective of this research is to estimate genetic diversity in a further collection of twenty-five accessions of roselle in northern Ghana based on agro-morphology evaluation.

MATERIALS AND METHODS

Plant material, experimental design and crop management

Seeds of twenty-five accessions of roselle (Table 1) were supplied by farmers located in seven districts in Northern Ghana covering a geographical area of latitude 9° 39′ to 10° 59′ N and longitude 0° 47’ to 1° 23’ W with an elevation of 119 to 238 masl (Figure 1). Field trial was carried out from June 26, 2017 to November 30, 2017 on the research fields of the Department of Horticulture, Kwame Nkrumah University of Science and Technology, Kumasi. This site is located at latitude 6^{o} 40′39 N and longitude 1° 33′58 W at an elevation of 258 masl in the semi-deciduous forest zone of Ghana. Average monthly rainfall within this period was 4.6 mm. The soil type was sandy loam Auroso Orchrosols with a pH of 5.9.

Seeds were planted in 5×5 lattice square design with three replications on 0.5 m × 2.0 m plot with an alley of 1.0 m to give 20 plants/plot. Irrigation was carried out as and when required. The pre-emergence weeds, nut grass (*Cyperus rotundus*) and *Panicum maximum* were controlled with WeedKill (glyphosate, 400 g/L) at a rate of 3.0 L/ha and post-emergence weeds by hand weeding with a hoe. The predominant insect pests, cotton stainer (*Dysdercus* sp.) and thrips (*Thysanoptera)* were controlled with Sumitex (dimethoate

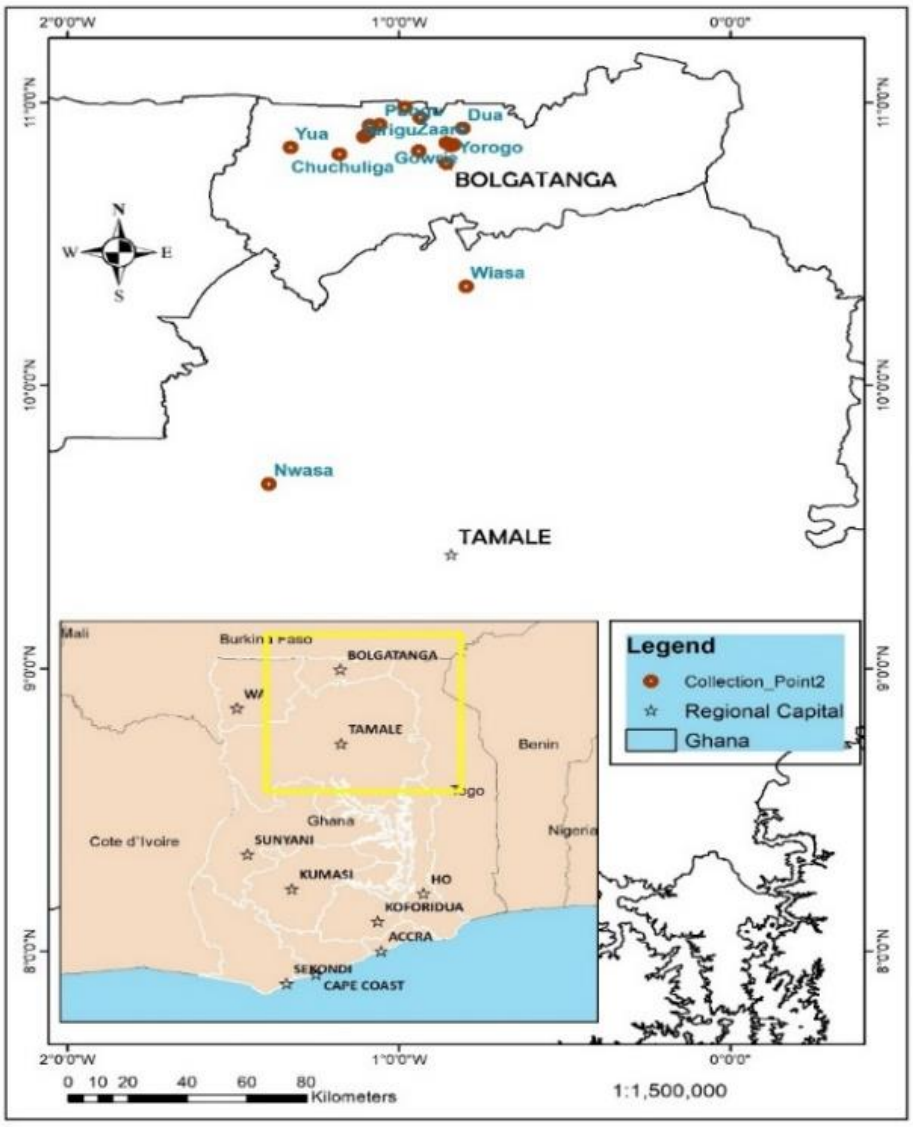

Figure 1. A schematic of Ghana map showing roselle (var. *altissima*) seed collection sites in the Northern and Upper East regions.

400 g/L) at a rate of 1 L/ha.

Data collection

Days to 50 % flowering (DTF) were recorded beginning at 90 days after planting (DAP). At 150 DAP, 12 qualitative and five quantitative traits on 10 competitive plants per plot were collected. The descriptors of roselle (var. *altissima*) were adapted from El-Naim et al. (2012) and Coffie (2016). The qualitative traits included plant type, (PT: predominant colour of the plant; green (1), pigmented (3), red (5)); branching habit (BH: extent of branching; few (1), intermediate (2), extensive (3)); growth habit, (GH: form of growth; non-bushy (1), bushy (2)); stem pubescence (SPB: feel of the stem; smooth (1), hairy (2), rough (3), spiny (4)).

Other traits were leaf form (LF: shape of leaf; entire (1), trilobed (3), pentalobed (5)); size of leaf (LS: shape of the leaf blade; slender (1), broad (2)); leaf pubescence (LPB: presence or absence of hair; smooth (1), hairy (2)). Calyx pigmentation (CPG: predominant colour of the calyx; green (1), pigmented (2), red (3), calyx pubescence (CPB: presence or absence of hair; smooth (1), hairy (2); and capsule shape (CSH: predominant shape of the capsule; ovoid (1), round (2)) were also evaluated. The remaining traits were petal colour (PC: predominant colour of the petals; yellow (1), purple (2)), and throat colour (TC: colour of the flower throat; yellow (1), crimson (3)).

Days to 50% flowering was measured as number of days from planting to 50 % of plants in a plot having at least one open flower. Plant height (PH, cm) was estimated as height from ground level to growing tip; height at first branching (HFB, cm) as distance from ground level to first primary branch, and basal diameter (BD, mm) as diameter of the stem at 5 cm above ground. Finally, branching number (BN) was determined by counting the number of primary reproductive branches along the stem. Micrometre screw gauge and meter rule were used to measure diameter and heights, respectively.

Statistical analysis

Frequencies of occurrence of the twelve qualitative traits and their percentages were computed to reveal morphological variabilities. Principal Components Analysis (PCA) was performed on the qualitative data with PROC PRINQUAL of SAS to reveal the discriminatory power of the traits identifying groups on the basis of their similarities. The quantitative traits were analyzed by computing means, standard deviation, minimum and maximum values and coefficient of variation (CV). Entry means (X_i) and standard deviation (σ) were used to divide accession scores into five phenotypic classes (x_i) of equal width of 1.0σ, for the entire data spanning (xi-2σ)≥ X_i ≥(xi+2σ). The frequency of genotypes in the ith class (P_i) was used to deduce the standardized Shannon-Wiener Diversity Index (SDI) for within-population variation, (Shannon, 1948), where:

$$H' = -\Sigma \frac{(Pi * lnPi)}{\ln n} \quad (1)$$

P_i was computed as n_i/N, where n_i is the number of individuals of the ith class, and N is the total number of individuals; n is the number of classes. The between-population variation was assessed by analysis of variance of the lattice square design based on the random effects model presented as,

$$Y_{ijk} = \mu + R_j + B(R) + G_i + \varepsilon_{ijk} \quad (2)$$

In this model, Y_{ijk} is genotype response, G_i, in replication R_j, in block B_k and ε_{ijk} as the error associated with the genotype i = 1…, t, replication j =1…, r, and block nested within replication k =1…, s. The expected mean squares (EMS) were derived from analysis of variance. Pairwise genetic similarity between accessions was based on Euclidean distance computed as:

$$d(x,y) = \sqrt{\sum_i^n (Px_i - Py_i)^2} \quad (3)$$

where d = the Euclidean distance; i = trait; n =total number of traits; x_i = value for trait x; and y_i = value for trait y. Mean Euclidean distance for each accession was calculated to estimate dissimilarity between the population. Cluster analysis was performed on the distance matrix using Ward's minimum variance method (Ward, 1963). A stepwise discriminant analysis identified traits that contributed most to the variance by minimizing Wilk's lambda (Wilk, 2006). Principal components analysis (PCA) was performed on the distance matrix to depict relationships among the genotypes and determine the loadings that were effective in discriminating between accessions. A scatterplot of the first and second principal components was constructed to reveal relationships between traits and between accessions. The SAS 9.3 program (SAS Institute Inc, 2011) was employed for all statistical computations.

RESULTS

Variability in qualitative traits

The roselle collection was represented by nine accessions (36%) in Kassena-Nankana East, five accessions (20%) in Bolgatanga Municipal, and four (16%) in Kassena-Nankana West. The others were two accessions (8%) each in Bongo, Builsa-North, and Gonja-North districts and one (4%) accession in West Mamprusi. A total of 750 plants were evaluated. Roselle exhibited large variability in all qualitative traits except growth habit, calyx pubescence and capsule shape. All plants exhibited non-bushy growth, with hairy calyx and round capsules. Leaf size and petal colour were somewhat variable with 80.1% slender leaves, 19.9% having broad leaves, and 72.4% yellow and 27.6% purple petals, respectively.

The highly variable traits were plant type (40.7% uniform green, 31.7% pigmented, 27.6% red), branching habit (25.1% few, 60.1% intermediate, 14.8% extensive), and stem pubescence (44.5% smooth, 26.1% hairy, 27.9% rough, 1.5% spiny). The others were, leaf form (6.0% entire, 30.3% tri-lobed, 63.7% penta-lobed), leaf pubescence (62.1% smooth, 37.9% hairy), calyx pigmentation (40.7% uniform green, 31.7% pigmented, 27.6% red), and throat colour (40.7% yellow, 59.3% crimson) (Table 2). Plate 1 shows variation in calyx, flower, stem and leaf morphology.

Principal component analysis of qualitative data

A scatter plot of the qualitative traits revealed that the first three principal components (PC) with eigenvalues greater than 1.0 had large contribution to the variance. The first two PCs accounted for 65% of the total variance, with PC1 43.64% and PC2 21.71%. Based on length of the vectors, plant type and calyx pigmentation exerted greatest contribution to the variance, followed by petal colour and throat colour, while branching habit contributed least (Figure 2).

Four major groups of roselle (var. *altissima*) were identified, namely, Group I, consisting of three genotypes HA-42, HA-44, HA-49 with predominantly penta-lobed and hairy leaves, and few branched rough and spiny stems; Group II with five genotypes HA-38, HA-51, HA-54, HA-57, HA 59 distinguished by extensive branching; group III having three genotypes, HA-39, HA-41, HA-60 were clustered entirely on their broad leaf trait, and finally, group IV with five genotypes, HA-48, HA-53, HA-55, HA-56, HA-58 were green plant type with few branching, smooth stem, slender and entire smooth leaves, green calyx, yellow petals and yellow throat. Genotypes HA-40, HA-46, HA-47 and HA-50 were not clustered with other genotypes as were HA-37, HA-43, HA-45, HA-52, and HA-61. Growth habit, calyx pubescence, and capsule shape were not discriminatory as these were not represented on the biplot.

Within-population variation in quantitative traits

The Shannon-Weiner Diversity Index (SDI) ranged from 0.00 to 1.00 with a mean of 0.82 ± 0.19. All traits exhibited high mean SDI values of 0.74 to 0.85 (Table 3). Plants of all accessions exhibited variation in number of

Table 2. Distribution of qualitative morphological traits in roselle (var. *altissima*) collected from Northern Ghana and evaluated in 2017.

Trait	Description	Score	No. of plants	Percentage
Plant type (PT)	Uniformly green	1	305	40.7
	Pigmented	3	238	31.7
	Uniformly red	5	207	27.6
Branching habit (BH)	Few	1	188	25.1
	Intermediate	2	441	60.1
	Extensive	3	111	14.8
Growth habit (GH)	Non-bushy	1	750	100
	Bushy	2	0	0
Stem pubescence (SPB)	Smooth	1	334	44.5
	Hairy	2	196	26.1
	Rough	3	209	27.9
	Spiny	4	11	1.5
Leaf form (LF)	Entire	1	45	6.0
	3-lobed	3	227	30.3
	5-lobed	5	478	63.7
Leaf size (LS)	Slender	1	601	80.1
	Broad	2	149	19.9
Leaf pubescence (LPB)	Smooth	1	466	62.1
	Hairy	2	284	37.9
Calyx pigmentation (CPG)	Green	1	305	40.7
	Pigmented	2	238	31.7
	Red	3	207	27.6
Calyx pubescence (CPB)	Smooth	1	0	0
	Hairy	2	750	100
Capsule shape (CSH)	Ovoid	1	0	0
	Round	2	750	100
Petal colour (PC)	Yellow	1	543	72.4
	Purple	2	207	27.6
Throat colour (TC)	Yellow	1	305	40.7
	Crimson	3	445	59.3

days to flowering with SDI values as high as 0.92 to 1.00 except HA-43, HA-46, HA-51, HA-55, and HA-60 whose individual plants consistently flowered on the same day, hence their SDI values were 0.00. Based on accession means across traits, HA-38, HA-42, HA-47, HA-57 and HA-58 were the most variable with SDI values of 0.90 ± 0.08 to 0.93 ± 0.05. The most variable accessions in the individual traits were, for plant height, HA-45, HA-51 and HA-59 (SDI: 0.97 to 1.00); height after first branching, HA-43 and HA-58 (SDI: 0.97); branch number, HA-37, HA-47, HA-50, HA-55, HA-57, HA-58, and HA-60 (SDI: 0.97 to 1.00), and for basal diameter, HA-42 and HA-57 (SDI: 0.95 to 0.96). The district having highest roselle fibre diversity by rank were Kassena-Nankana East (0.87 ± 0.09), Bolgatanga Municipal (0.83 ± 0.10) and West-Mamprusi (0.82 ± 0.09) (Table 3).

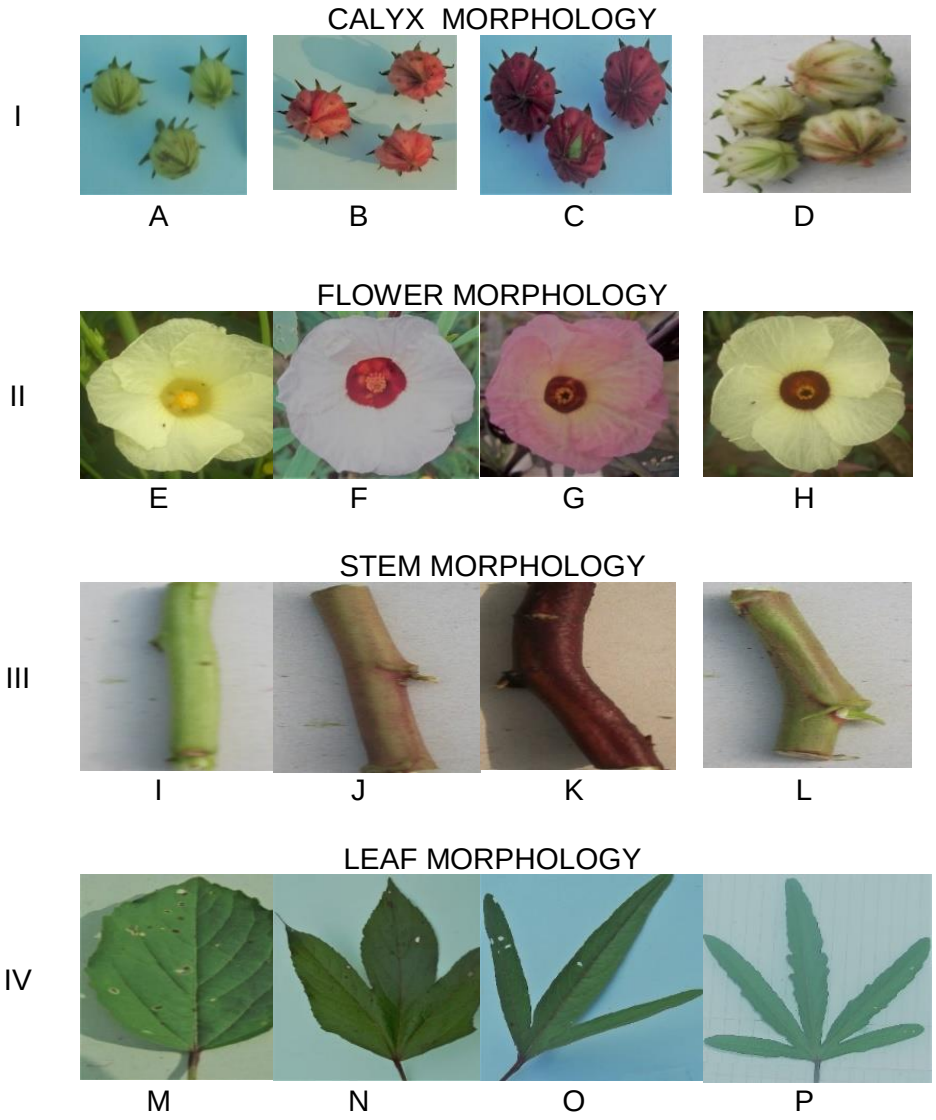

Plate 1. Image of plant parts of mature roselle (var. *altissima*). Panel I: round fruit of a (A) full green plant enclosed in fibrous green calyx; (B) pigmented plant enclosed in fibrous pink calyx; (C) full red plant enclosed in inedible fibrous red calyx; (D) green-pigmented plant enclosed in fibrous green-pigmented calyx. Panel II: (E) Bright yellow flower with deep-yellow throat of a full green plant; (F) pale purple flower with crimson throat on a pigmented plant; (G) purple flower with crimson throat on a red plant; (H) pale yellow flower with crimson throat on a green-pigmented plant. Panel III: (I) full green stem; (J) pigmented stem; (K) full red stem; (L) green-pigmented stem. Panel IV: (M) broad entire leaf; (N) broad trilobed leaf; (O) slender trilobed leaf; and (P) slender pentalobed leaf. Sub-classes: A, E, I are from a full green genotype; B, F, J are from a pigmented (brownish-green with patches of red pigments) genotype; C, G, K are from a full red genotype; D, H, L are from a pigmented (bright green with patches of red pigments) genotype. Variants of leaf morphology are characteristic of all the genotyes in the study.

Between-population variation

Analysis of variance revealed a strong replication effect ($P \leq 0.05$) for all traits except height at first branching. Genotype effect was important ($P \leq 0.05$) except for basal diameter and height at first branching. Block nested within replication was also not important (Table 4). Mean plant height of the collection was 244.85 ± 37.49 cm and ranged from 154 to 342 cm. Mean height at first branching was at 8.05 ± 2.39 beginning at a minimum height of 2.40 cm to 19.50 cm. Branch number ranged from 4 to 21 with mean of 8.71 ± 3.22. Basal diameter

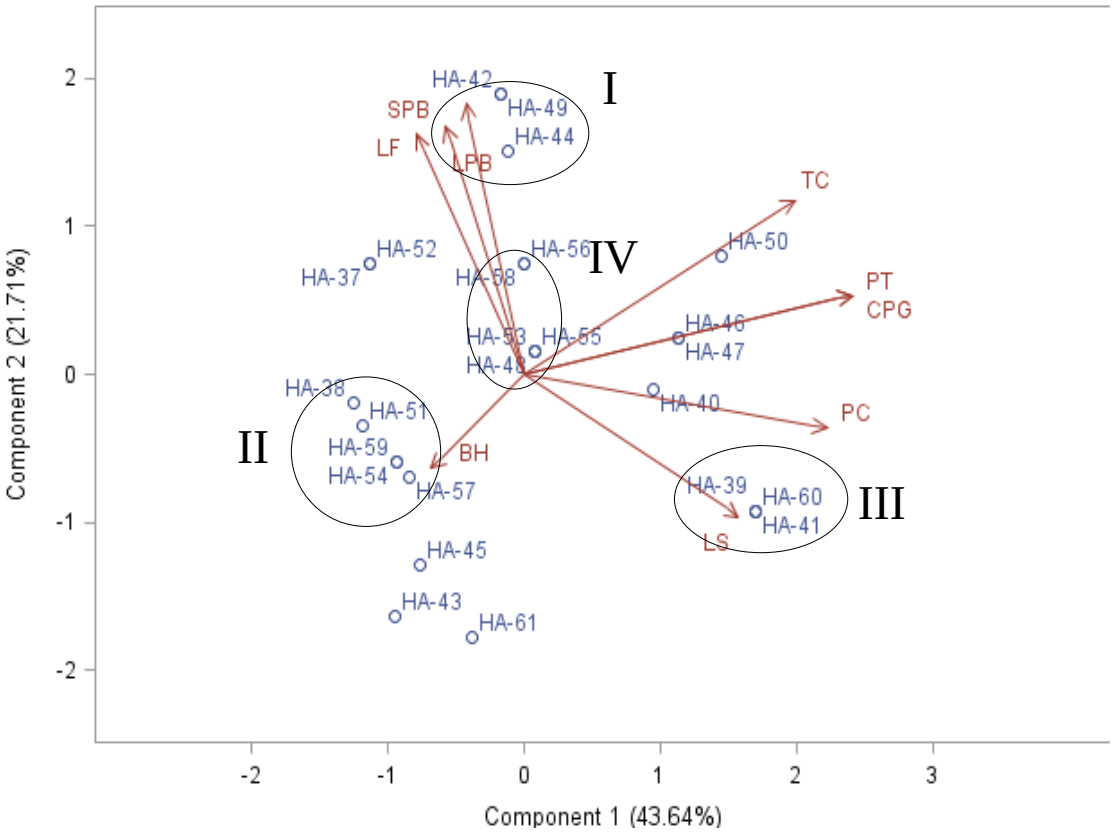

Figure 2. Principal components biplot of PC1 and PC2 of 25 northern Ghana roselle (var. *altissima*) accessions evaluated in 2017 on 12 qualitative traits.

varied within wide limits in a range of 12.19 to 32.59 mm with a mean of 21.04 ± 3.50 mm. Accessions flowered from 96 to 104 days after planting. On average, flowering occurred at 99.40 ± 1.34 days (Table 5).

Based on district means, the maximum plant height of 262.03 ± 17.04 cm was recorded in West Mamprusi populations and the least plant height of 214.82 ± 42.97 cm was recorded in Builsa-North populations. District mean for height at first branching varied from 7.74 ± 0.03 cm in Builsa-North to 8.32 ± 0.31 cm Bolgatanga Municipal. On district mean basis, branch number was least 6.75 ± 0.03 in Builsa-North and highest 10.73 ± 2.59 in Gonja-North. For basal diameter, the district mean ranged from 22.63 ± 3.17 mm in West Mamprusi to 19.25 ± 0.00 in Builsa-North, while days to flowering period varied from 99.84 ± 0.44 in Kassena Nankana-West to 97.67 ± 0.96 in West Mamprusi (Table 6). Ranking based on tallest plants, highest branching points, fewer branch numbers, and largest basal diameter revealed West Mamprusi, Kassena-Nankana West and Bongo districts to be the top three districts having genotypes of high fibre yield potential (Table 6).

Based on accession means, plant height varied from 184.83 ± 14.81 cm to 283.50 ± 27.00 cm. Short genotypes were HA-46 (184.43 ± 14.81 cm), HA-59 (187.30 ± 15.82 cm), HA-61 (202.87 ± 12.37 cm), HA-41 (209.27 ± 21.88 cm), and HA-51 (216.97 ± 28.43 cm). The tall plant genotypes were HA-44 (275.37 ± 30.87 cm), HA-47 (277.40 ± 26.30 cm), HA-43 (277.57 ± 21.88), HA- 38 (279.00 ± 22.92), HA- 52 (280.77 ± 22.54), and HA-42 (283.50 ± 27.00 cm). All accessions exhibited some branching, but to varying extents and somewhat at the same height above ground. On accession mean basis, height at first branching ranged from 6.86 ± 1.62 cm in HA-60 to 9.60 ± 3.69 cm in HA-57 with branches numbering between 6.40 ± 1.45 in HA-49 to 12.97 ± 3.59 in HA-51 (Table 7). On individual plant basis, as few as four and as many as 21 branches were present. Accessions with few branches were HA-49 (6.40 ± 1.45), HA-45 (6.53 ± 1.61), HA-58 (6.73 ± 1.66), HA-46 (6.73 ± 1.91), and HA-39 (6.77 ± 1.36). Accessions with extensive branching were HA-56 (10.40 ± 3.57), HA-48 (10.50 ± 2.87), HA-61 (10.70 ± 2.60), HA-43 (11.63 ± 3.80), HA-42 (11.83 ± 3.31), HA-38 (12.87 ± 3.40), and HA-51 (12.97 ± 3.59).

Mean basal diameter ranged from 18.96 ± 1.89 mm in HA-56 to 24.55 ± 2.99 mm in HA-61. Accessions with large mean basal diameter were HA-57 (22.11 ± 2.86 mm), HA-58 (22.31 ± 3.34 mm), HA-42 (22.38 ± 2.94 mm), HA-54 (22.63 ± 3.17 mm), and HA-55 (23.45 ± 3.45

Table 3. Shannon Weiner Diversity Index of 25 roselle (var. *altissima*) accessions in Ghana in 2017 based on morphological evaluation.

Acc[1]	District	Plant height (cm)	Height at first branch (cm)	Branch number	Basal diameter (mm)	Days to 50% flowering	Accession mean	District mean	Rank
HA-37	Bolgatanga Municipal	0.74	0.87	0.99	0.75	0.92	0.85±0.11	0.83±0.10	2
HA-40		0.69	0.88	0.76	0.84	0.92	0.82±0.09		
HA-49		0.89	0.84	0.81	0.88	0.94	0.87±0.05		
HA-50		0.86	0.81	0.97	0.77	0.92	0.87±0.08		
HA-61		0.57	0.71	0.68	0.82	0.92	0.74±0.14		
HA-57	Bongo	0.89	0.88	1.00	0.95	0.92	0.93±0.05	0.80±0.29	4
HA-60		0.75	0.91	0.97	0.73	0.00	0.67±0.39		
HA-39	Builsa-North	0.84	0.8	0.88	0.61	0.92	0.81±0.12	0.77±0.29	6
HA-46		0.92	0.95	0.92	0.89	0.00	0.74±0.41		
HA-51	Gonja-North	0.98	0.69	0.89	0.80	0.00	0.67±0.39	0.72±0.27	7
HA-53		0.67	0.74	0.74	0.78	0.92	0.77±0.09		
HA-42	Kassena-Nankana East	0.95	0.91	0.75	0.96	0.92	0.90±0.08	0.87±0.09	1
HA-44		0.90	0.82	0.68	0.71	1.00	0.82±0.13		
HA-45		0.97	0.86	0.88	0.82	0.92	0.89±0.06		
HA-47		0.83	0.94	1.00	0.86	0.94	0.91±0.07		
HA-48		0.92	0.78	0.76	0.92	0.92	0.86±0.08		
HA-52		0.87	0.86	0.75	0.72	0.92	0.82±0.08		
HA-56		0.89	0.68	0.96	0.62	0.92	0.81±0.15		
HA-58		0.86	0.97	0.97	0.87	0.92	0.92±0.05		
HA-59		1.00	0.79	0.76	0.88	0.92	0.87±0.10		
HA-38	Kassena-Nankana West	0.94	0.99	0.83	0.88	0.92	0.91±0.06	0.79±0.28	5
HA-41		0.86	0.69	0.81	0.93	0.92	0.84±0.10		
HA-43		0.84	0.97	0.84	0.91	0.00	0.71±0.40		
HA-55		0.83	0.78	0.99	0.91	0.00	0.70±0.40		
HA-54	West Mamprusi	0.72	0.80	0.74	0.90	0.92	0.82±0.09	0.82±0.09	3
Mean		**0.85±0.10**	**0.84±0.09**	**0.85±0.11**	**0.83±0.10**	**0.74±0.38**			

[1]Acc: Accession.

mm) (Table 7). Flowering occurred about the same time in all genotypes with accession means DTF ranging from 97.67 ± 0.96 to 101.33 ± 1.92 DAP. Three accessions HA47, HA-54, and HA-59 flowered earlier than 100 DAP, at 97.67± 0.96 days while only HA-37 flowered at 101.33 ± 1.92 days (Table 7). Based on fibre yield, plant height, branching points, branch number, and largest basal diameter, genotypes HA-42, HA-52, HA-38, HA-43 and HA-47 were considered to be the top five with economic value in terms of fibre yield (Table 7).

Correlation of quantitative traits

The Pearson correlation coefficients, *r*, were low, -0.01 to 0.13. Basal diameter showed weak positive significant correlation with plant height (r = 0.11; R^2 = 0.012) and branch number (r = 0.13; R^2 = 0.017) but a negative significant correlation with height at first branching (r = -0.08; R^2 = 0.006). Height at first branching showed a low positive significant relationship with days to 50 % flowering (r = 0.12; R^2 = 0.014) accounting for 1.40% of the variation. The remaining traits showed non-significant correlations be it positive or negative (Table 8).

Genetic distances among accessions

The overall mean genetic distance based on Euclidean estimates was 3.03 ± 0.90 covering a range of 0.14 to

Table 4. Mean squares of traits of northern Ghana roselle (var. *altissima*) accessions evaluated in a lattice square design in 2017 in Ghana.

Source	df	Plant height	Height at first branching	Branch number	Basal diameter	Days to 50% flowering
Replication	2	2844.30**	1.00	14.41**	64.35**	4.51*
Block (Replication)	8	191.39	0.70	2.05	4.41	0.81
Genotype	20	2606.79**	1.11	14.48**	6.41	3.39**
Error	40	449.40	1.80	1.76	6.74	1.19

**(P<0.01); * (P<0.05).

Table 5. Means, standard deviations, range, and coefficient of variation of morphological traits evaluated on 25 roselle (var. *altissima*) accessions collected from northern Ghana in 2017.

Trait	Mean	SD	Min - Max	CV (%)
Plant height (cm)	244.85	37.49	154.00 - 342.00	15.31
Height at first branching (cm)	8.05	2.39	2.40 - 19.50	29.67
Branch number	8.71	3.22	4.00 - 21.00	37.01
Basal diameter (mm)	21.04	3.50	12.19 - 32.59	16.65
Days to 50% flowering	99.40	1.34	96.00 - 104.00	1.35

SD: standard deviation; CV: coefficient of variation

Table 6. Mean, standard deviation, and range of phenotypic traits evaluated in 25 roselle (var. *altissima*) accessions from 7 districts in northern Ghana in 2017.

District	Plant height (cm)	Height at first branch (cm)	Branch number	Basal diameter (mm)	Days to 50% flower	Rank
Bolgatanga Municipal	234.38±19.42 (172-305)	8.32±0.31 (4.10-17.50)	7.92±1.72 (4.00-17.00)	21.68±1.70 (14.32-31.09)	99.80±0.87 (99-104)	**5**
Bongo	255.74±15.08 (198-331)	8.23±1.94 (2.40-17.00)	7.12±0.02 (4.00-10.00)	20.97±1.62 (15.20-27.65)	99.84±0.23 (99-100)	**3**
Builsa-North	214.82±42.97 (160-314)	7.74±0.03 (3.50-17.20)	6.75±0.03 (4.00-11.00)	19.25±0.00 (12.47-29.18)	99.00±1.41 (96-100)	**7**
Gonja-North	224.91±10.45 (163-277)	8.32±0.03 (4.10-15.40)	10.73±2.59 (5.00-21.00)	20.88±0.83 (16.65-29.66)	99.34±0.47 (99-100)	**6**
Kassena-Nankana East	251.28±32.02 (154-342)	7.93±0.44 (4.10-19.50)	8.87±1.81 (4.00-21.00)	20.80±1.40 (12.19-30.41)	99.20±1.23 (97-104)	**4**
Kassena-Nankana West	258.17±33.05 (160-331)	7.82±0.53 (3.40-14.30)	10.29±2.55 (5.00-20.00)	21.38±1.39 (12.57-32.59)	99.84±0.44 (99-101)	**2**
West Mamprusi	262.03±17.04 (220-293)	7.82±2.23 (4.10-13.10)	7.47±1.94 (4.00-13.00)	22.63±3.17 (18.13-30.12)	97.67±0.96 (97-99)	**1**

5.17 (Table 9). Very low distances were recorded between accessions HA-40 and HA-53 (0.41), HA-37 and HA-58 (0.84), and HA-38 and HA-43 (0.91), whereas large distances were recorded between accessions HA-55 and HA-59 (5.02), HA-57 and HA-59 (5.11), and HA-60 and HA-61 (5.17). Based on means across pairwise distances, which varied between 2.22 and 3.94, three accessions were divergent, namely HA-61(3.94),

Table 7. Means, standard deviations, and range of phenotypic traits of 25 roselle (var. *altissima*) accessions evaluated in Ghana in 2017

Accession	Plant height (cm)	Height at first branch (cm)	Branch number	Basal diameter (mm)	Days to 50% flower	Rank
HA-37	250.40±19.40 (219-297)	8.05±2.85 (4.40-17.50)	7.07±1.74 (4.00-11.00)	21.83±3.02 (15.67-28.71)	101.33±1.92 (100-104)	**12**
HA-38	279.00±22.92 (231-328)	7.42±2.10 (3.40-10.50)	12.87±3.40 (7.00-20.00)	20.59±2.25 (17.42-25.20)	99.67±0.96 (99-101)	**3**
HA-39	245.20±34.91 (200-314)	7.72±3.19 (3.50-17.20)	6.77±1.36 (5.00-10.00)	19.25±2.76 (15.32-29.18)	98.00±1.44 (96-99)	**14**
HA-40	233.23±26.18 (179-289)	8.19±1.97 (4.60-12.70)	8.43±2.80 (4.00-16.00)	20.62±3.15 (15.67-27.38)	99.67±0.48 (99-100)	**17**
HA-41	209.27±21.88 (160-248)	7.52±1.48 (4.50-11.50)	9.60±3.04 (5.00-18.00)	20.61±5.85 (12.57-31.23)	99.33±0.48 (99-100)	**22**
HA-42	283.50±27.00 (239-341)	7.60±1.75 (4.20-10.30)	11.83±3.31 (8.00-21.00)	22.38±2.94 (17.89-28.18)	99.33±0.48 (99-100)	**1**
HA-43	277.57±21.88 (240-316)	7.75±2.00 (4.20-12.00)	11.63±3.80 (7.00-20.00)	20.87±4.47 (14.80-31.41)	100.00±0.00 (100-100)	**4**
HA-44	275.37±30.87 (229-332)	8.15±2.37 (4.30-15.20)	8.73±2.03 (5.00-14.00)	19.58±1.56 (15.24-22.13)	100.33±1.27 (99-102)	**6**
HA-45	233.03±14.68 (206-261)	7.46±2.07 (4.70-12.50)	6.53±1.61 (4.00-10.00)	20.87±3.09 (15.63-27.80)	98.33±0.96 (97-99)	**18**
HA-46	184.43±14.81 (160-210)	7.76±2.00 (4.10-11.20)	6.73±1.91 (4.00-11.00)	19.25±3.86 (12.47-26.07)	100.00±0.00 (100-100)	**25**
HA-47	277.40±26.30 (212-323)	7.25±1.82 (4.20-11.30)	7.37±1.50 (5.00-10.00)	21.99±3.45 (15.81-29.30)	97.67±0.96 (97-99)	**5**
HA-48	225.37±12.12 (208-251)	8.60±3.2 (4.30-19.50)	10.50±2.87 (7.00-18.00)	19.62±1.96 (16.36-23.81)	100.67±0.96 (100-102)	**20**
HA-49	238.73±32.03 (202-300)	8.69±2.29 (4.50-14.00)	6.39±1.45 (4.00-9.00)	20.34±4.00 (14.32-28.80)	99.33±0.48 (99-100)	**16**
HA-50	250.43±25.84 (213-305)	8.04±3.11 (4.10-16.30)	7.03±1.50 (4.00-10.00)	21.07±3.42 (16.15-29.61)	99.33±0.48 (99.00-100)	**11**
HA-51	216.97±28.43 (163-261)	8.69±2.37 (5.20-15.40)	12.97±3.59 (8.00-21.00)	21.63±3.42 (16.61-29.66)	99.00±0.00 (99-99)	**21**
HA-52	280.77±22.54 (238-317)	8.26±2.90 (4.40-18.30)	8.77±3.02 (5.00-19.00)	21.94±2.58 (18.93-30.00)	98.33±0.96 (97-99)	**2**
HA-53	232.30±20.87 (190-277)	8.34±2.16 (4.10-12.80)	8.90±2.63 (5.00-15.00)	20.29±2.37 (16.55-27.28)	99.67±0.48 (99-100)	**19**
HA-54	262.03±17.04 (220-293)	7.82±2.23 (4.10-13.10)	7.47±1.94 (4.00-13.00)	22.63±3.17 (18.13-30.12)	97.67±0.96 (97-99)	**9**
HA-55	266.82±29.30 (219-331)	8.58±2.14 (4.50-14.30)	7.04±1.35 (5.00-10.00)	23.45±3.45 (18.40-32.59)	100.36±0.49 (100-101)	**7**
HA-56	248.87±30.80 (203-321)	8.16±1.33 (4.70-10.50)	10.40±3.57 (5.00-17.00)	18.96±1.89 (13.15-22.70)	99.67±0.48 (99-100)	**13**
HA-57	266.40±33.10 (208-331)	9.60±3.69 (2.40-17.00)	7.13±1.81 (4.00-10.00)	22.11±2.86 (16.36-27.65)	99.67±0.48 (99-100)	**8**
HA-58	251.40±32.83 (209-342)	8.25±2.34 (4.10-12.40)	6.73±1.66 (4.00-9.00)	22.31±3.34 (17.41-29.24)	100.67±2.40 (99-104)	**10**
HA-59	187.30±15.82 (154-210)	7.71±1.68 (4.10-11.30)	8.97±3.90 (4.00-20.00)	19.52±4.59 (12.19-30.41)	97.67±0.96 (97-99)	24
HA-60	245.07±29.10 (198-319)	6.86±1.62 (4.30-9.70)	7.10±1.54 (4.00-10.00)	19.82±2.07 (15.20-23.77)	100.00±0.00 (100-100)	15
HA-61	202.87±12.37 (172-223)	8.70±2.20 (4.50-17.00)	10.70±2.60 (7.00-17.00)	24.55±2.99 (19.78-31.09)	99.30±0.47 (99-100)	23

Table 8. Pearson correlation coefficients of five quantitative traits of *altissima* accessions.

Variable	Plant height (cm)	Height at first branching (cm)	Branch number	Basal diameter (mm)
Height at first branching	-0.02			
Branch number	0.03	0.01		
Basal diameter	**0.11****	**-0.08***	**0.13****	
Days to 50% flowering	-0.03	**0.12****	-0.02	-0.01

**(P<0.01); * (P<0.05).

Table 9. Euclidean distances of 25 northern Ghana roselle (var. *altissima*) accessions evaluated by morphological characterization in 2017.

Accession	Mean ± SD	Min - Max	Accession	Mean ± SD	Min - Max
HA-37	3.05 ± 0.86	0.84 – 4.73	HA-50	2.30 ± 0.67	1.05 – 3.64
HA-38	3.35 ± 0.93	0.91 – 4.77	HA-51	3.41 ± 0.63	2.41 – 4.63
HA-39	3.04 ± 0.88	1.35 – 4.94	HA-52	2.82 ± 0.69	1.44 – 4.40
HA-40	2.22 ± 0.71	0.41 – 3.31	HA-53	2.30 ± 0.74	0.41 – 3.64
HA-41	2.71 ± 0.66	1.56 – 4.38	HA-54	3.12 ± 0.82	1.21 – 4.39
HA-42	3.19 ± 0.81	1.31 – 4.83	HA-55	3.19 ± 0.89	1.17 – 5.02
HA-43	2.90 ± 0.86	0.91 – 4.24	HA-56	2.73 ± 0.80	1.35 – 4.39
HA-44	2.76 ± 0.80	1.46 – 4.65	HA-57	3.66 ± 0.87	1.95 – 5.11
HA-45	2.82 ± 0.83	1.35 – 4.17	HA-58	2.82 ± 0.86	0.84 – 4.48
HA-46	3.47 ± 0.91	2.06 – 4.83	HA-59	3.63 ± 0.87	2.05 – 5.11
HA-47	3.40 ± 0.92	1.21 – 4.79	HA-60	3.23 ± 0.82	2.16 – 5.17
HA-48	3.04 ± 0.85	1.45 – 4.79	HA-61	3.94 ± 0.64	2.41 – 5.17
HA-49	2.70 ± 0.76	1.33 – 4.06	**Overall mean**	**3.03 ± 0.90**	**0.14 – 5.17**

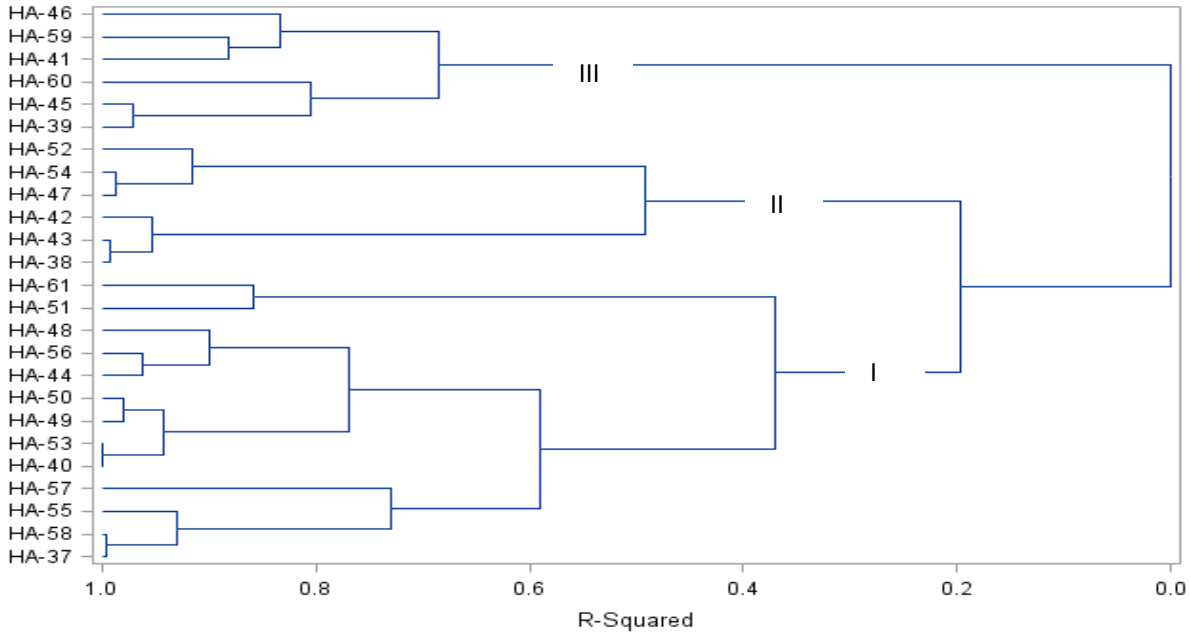

Figure 3. A dendrogram based on Ward's minimum variance of 25 northern Ghana roselle (var. *altissima*) accessions evaluated by morphological traits in field trials in Ghana in 2017.

HA-57 (3.66) and HA-59 (3.63). Accessions HA-40 (2.22), HA-50 (2.30) and HA-53 (2.30) were the least divergent genotypes (Table 9).

Cluster analysis

Accessions were clustered into three distinct groups (Figure 3). Cluster I comprised 13 accessions, HA-37,HA-40, HA-44, HA-48, HA-49, HA-50, HA-51, HA-53, HA-55, HA-56, HA-57, HA-58, and HA-61, with mean genetic distance of 2.58 ± 0.89. Cluster I accessions were grouped based on highest branching points and late flowering (Table 10). Accessions HA-57, HA-61, HA-49, HA-48, and HA-55 exhibited branching points at heights exceeding 8.50 cm above ground. Similarly, accessions HA-37, HA-48 and HA-58, flowered beyond 100 DAP. Mean branching point and flowering were 8.47 ± 2.54 cm and 99.92 ± 1.21 DAP, respectively.

Cluster II was made up of six accessions, HA-38, HA-

Table 10. Means, standard deviations, and differences of clusters of 25 northern Ghana *altissima* accessions evaluated in 2017.

Trait	Overall means	Cluster I	Diff	Cluster II	Diff	Cluster III	Diff
PH	244.85±37.49	242.83±32.92	-2.02	276.71±23.87	31.86	217.38±34.16	-27.47
HFB	8.05±2.39	8.47±2.54	0.42	7.68±2.16	-0.37	7.51±2.08	-0.54
BN	8.71±3.22	8.62±3.05	-0.09	9.98±3.65	1.27	7.62±2.67	-1.09
BD	21.04±3.50	21.25±3.29	0.21	21.73±3.27	0.69	19.89±3.91	-1.15
DTF	99.40±1.34	99.92±1.21	0.52	98.78±1.23	-0.62	98.89±1.25	-0.51

PH = Plant height; HFB = Height at first branching; BN = Branch number; BD = Basal diameter; DTF = Days to 50% flowering; Diff = cluster means – overall means.

Table 11. Principal components analysis of 25 roselle (var. *altissima*) accessions studied based on five quantitative traits.

Trait	PC1	PC2	PC3
Plant height (cm)	0.38	0.69	0.17
Height at first branching (cm)	0.74	-0.42	-0.23
Branch number	0.18	0.12	0.86
Basal diameter (mm)	0.71	0.41	-0.30
Days to 50% flowering	0.48	0.56	0.34
Eigenvalues	1.45	1.16	1.01
Cumulative eigenvalues	1.45	2.61	3.62
Percentages	40.05	31.77	28.18
Cumulated percentages	40.05	71.82	100.00

42, HA-43, HA-47, HA-52 and HA-54 with a mean genetic distance of 2.36 ±0.92. The six accessions of cluster II were separated based on highest mean plant height of 276.71 ± 23.87 cm, high branching number (9.98 ± 3.65), and largest basal diameter (21.73 ± 3.27 mm) (Table 10). The very tall accessions of interest were HA-42 (283.50 ± 27.00 cm), HA-52 (280.77 ± 22.54 cm), HA-38 (279.00 ± 22.92 cm), HA-43(277.57 ± 21.88 cm), HA-47 (277.40 ± 26.30 cm), and HA-54 (262.03 ± 17.04 cm). Accessions with large basal diameter in excess of 22.30 mm were HA-61, HA-55, HA-54, HA-42, and HA-58.

Accessions HA-39, HA-41, HA-45, HA-46, HA-59, and HA-60 of cluster III had an overall mean genetic distance of 2.40 ± 0.48 and were segregated on the basis of short plant height (217.38 ± 34.16 cm), least number of branches (7.62 ± 2.67), smallest basal diameter (19.89 ± 3.91 mm) and lowest branching point (7.51± 2.08 cm) (Table 10). Accessions of interest having the least number of branches were HA-49 (6.39 ± 1.45), HA-45 (6.53 ± 1.61), HA-58 (6.73 ± 1.66), HA-46 (6.73 ± 1.91), and HA-39 (6.77 ± 1.36).

Stepwise discriminant analysis

Three of the five quantitative morphological traits produced adequate discrimination of the accessions based on minimization of Wilk's lambda. Branch number (Wilk's lambda 0.27**; F=5.54), contributed the most variance to the data, followed by plant height (Wilk's lambda 0.08**; F=4.84) and then days to 50% flowering (Wilk's lambda 0.04**; F=2.51). Height at first branching and basal diameter were not discriminatory.

Principal components analysis

The first three principal components (PCs) with eigenvalues greater than 1.00 contributed 100% to the variance in the data. Contributions to the total variance were for PC1, 40.05%, with major loadings in height at first branching (0.74) and basal diameter (0.71). The PC2 contributed 31.77% of the variance with major loadings in plant height (0.69) and days to 50% flowering (0.56). Total contribution of PC1 and PC2 to the variance was 71.82%. The PC3 accounted for 28.18% of the total variance, with much contribution from branch number (0.86) (Table 11).

Biplot of PC1 and PC2 revealed four major uncorrelated groups (Figure 4A). Group I accessions (HA-38, HA-42, HA-43 HA-52, HA-54) had large values of plant height and basal diameter. Accessions of group II (HA-39, HA-45, HA-60) were assembled, based on least number of branches per plant. The eight accessions of group III (HA-37, HA-40, HA-44, HA-49, HA-50, HA-51, HA-53, HA-56, HA-58) had least values of plant height,

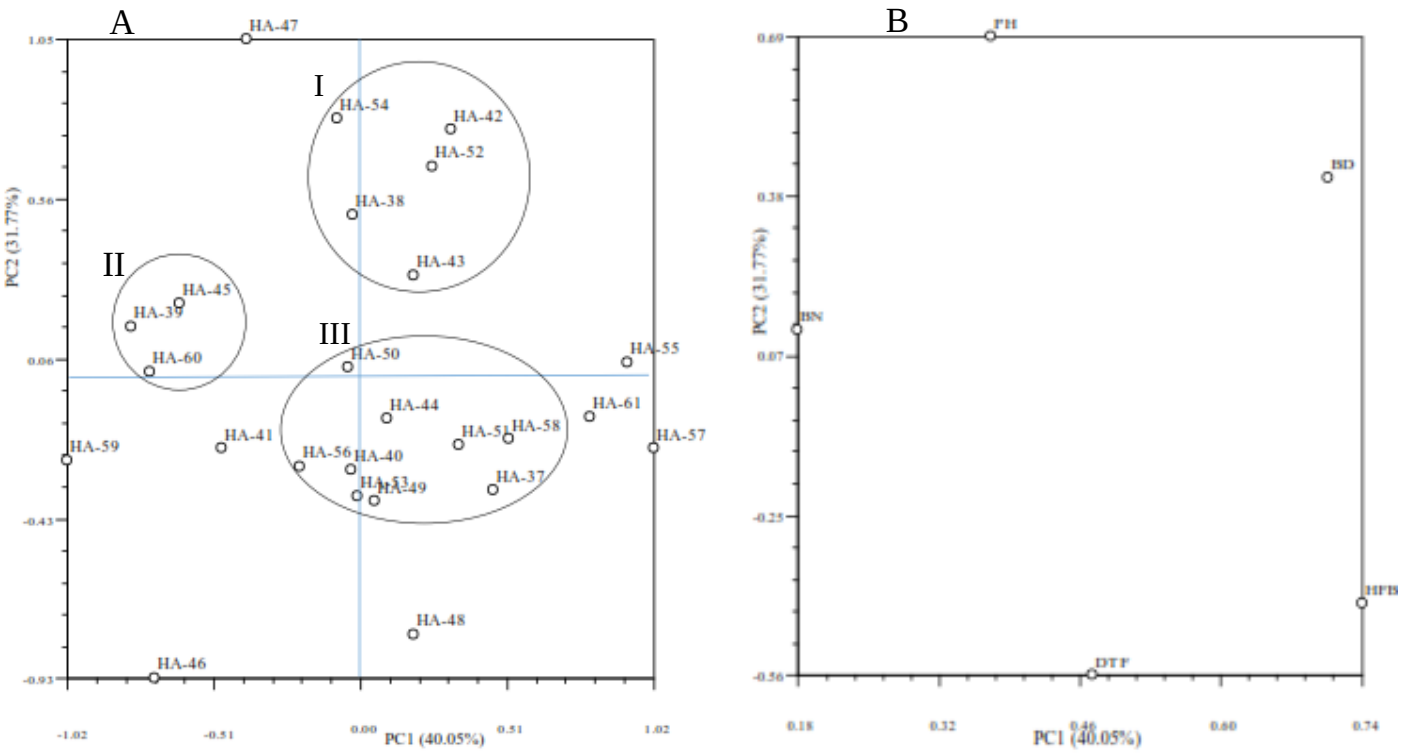

Figure 4. Principal components biplots of (A) 25 roselle (var. *altissima*) accessions and (B) five quantitative traits evaluated in a field trial in Ghana in 2017.

basal diameter, branch number and medium values of height at first branching and days to flowering. Accessions HA-41, HA-46, HA-47, HA-48, HA-55, HA-57, HA-59, and HA-61 were separated from the rest. All traits contributed positively to the total variance as they grouped to the right of the origin, 0.00, of the PC1 axis. Positive correlation was observed between plant height and basal diameter as well as plant height and branch number. Negative correlation was observed for height at first branching and days to flowering (Figure 4B).

DISCUSSION

Roselle is a crop well adapted to the hot climates of SSA and has thrived over several decades in limiting soil nutrients and marginal environments. In Ghana, indigenous communities that have fair knowledge of roselle (var. altissima) utilize them in homesteads for domestic fibre production for making ropes. In contrast, roselle is commercially cultivated in Asian countries where its bast fibre is exploited in many industries. Since the study of Coffie (2016), who reported that the center of diversity of roselle (H. sabdariffa var. sabdariffa) lies along a northern Ghana- Ouagadougou-Mali belt, no study has been carried out to investigate the distribution and diversity in roselle (var. altissima) in northern Ghana. Additionally, there is dearth of knowledge on the economic potential of roselle (var. altissima) in Ghana. The distribution and assessment of genetic diversity in roselle is herein reported. The roselle seed collection was obtained from seven districts in northern Ghana where Kassena-Nankana East was represented more than the other districts. This non-uniform collection could not be avoided owing to the widespread lack of knowledge on roselle. In addition, similarity in seed morphology with the vegetable type roselle posed challenges in obtaining adequate information on the fibre type. Despite these drawbacks, indigenous knowledge of the aged farmers provided sufficient guide to the locations of roselle (var. *altissima*) cultivation. Chivenge et al. (2015) stated that aged folk possessed sufficient indigenous knowledge about under-utilized crops in SSA, and that, this knowledge needs to be harnessed in a rapid manner for utilization, conservation, and cultivar development in the midst of climate change threats.

The large variability in qualitative traits which depicted diverse leaf forms, plant type, calyx and flower pigmentation were consistent with the morphology of the vegetable roselle. Coffie (2016), in her study of 35 roselle (var. *sabdariffa*) genotypes from across West Africa reported on substantial variability among the accessions. On plant type, roselle (var. *altissima*) had more green genotypes (40%) with substantially fewer branching

genotypes (25%) than the vegetable type (9% green; 1% with few branching). In current study, roselle (var. *altissima*) leaves were predominantly slender (80%) and penta-lobed with few tri-lobed (64% and 30%, respectively) forms, contrasting with the fundamentally broad (68%), tri- (49%) and pentalobed (36%) leaves of var. *sabdariffa*. Calyx pigmentation was similar to that in var. *sabdariffa*, but majority of the plants had yellow petals (72%) and somewhat equal distribution of yellow (41%) and crimson (59%) throats. In var. *sabdariffa*, however, 39% had yellow petals and 91% crimson throat.

The qualitative characteristics of roselle concur with an earlier report on 36 roselle fibre genotypes from northern Ghana (Ankrah et al., 2018), which exhibited ample variability in plant type, branching habit, stem pubescence and leaf form. The absence of variability in growth habit, calyx pubescence and capsule shape indicates that these traits are conserved in roselle (var. *altissima*). On the contrary, there are reports of variability in growth habit and capsule shape of roselle (var. *sabdariffa*) (El-Tahir and El-Gabri, 2013; Coffie, 2016). Because qualitative traits are not influenced by environment, the variations identified in roselle could be largely genetic. For the purpose of fibre production, roselle with tall green stem and few or no branches at high branching points are most desirable. Indigenous knowledge purports that green stems produce higher fibre yield of better quality. While selection methods based on the phenotypic expression would likely achieve the desired improvement in fibre yield, further work is needed to verify this claim.

Principal components analysis of the qualitative data revealed that the first two PCs cumulatively explained 65% of the total variance. Of the nine qualitative traits that were significant for the structuring of roselle genotypes the contribution of four traits, namely, plant type, calyx pigmentation, petal and throat colour were substantial (Figure 2). Further studies on structuring of roselle should concentrate on these four traits.

The high SDI values observed in the 25 roselle populations and within the seven districts is indicative of a large within-population variation. Notwithstanding, the high values could have been caused to some extent, by mixture of seeds. Further work is needed to clean up the seeds. To the best of our knowledge, this is the first report, which estimates SDI in roselle (var. *altissima*). Medagam et al. (2015) estimated SDI values of agro-economic traits of roselle (var. *sabdariffa*) to be 0.32 to 2.00.

On the basis of large values of plant height, high branching points, and large basal diameter, the most desired accessions with high fibre yield potential included HA-38, HA-42, HA-47, HA-57 and HA-58. The districts of largest diversity in a decreasing order were Kassena-Nankana East, Bolgatanga and West Mamprusi. The import of this finding is that, future collection of roselle in Ghana should focus on these districts. The other notable districts with large diversity in roselle were Bongo and Kassena-Nankana West.

Except for height at first branching and basal diameter, a strong genotype main effect for plant height, branch number and days to 50% flowering indicated a large between population variability. The large replication effect confirmed mixing up of seeds at the various collection points. Strong genotype effect in plant height and branch number was identified in some Sudan, Egypt, and Iran roselle (var. sabdariffa) collections, respectively (Javadzadeh and Saljooghianpour, 2017; Abou El-Nasr et al., 2014; Ibrahim et al., 2013). Similarly, a large genotype effect in number of days to flowering in roselle (var. *sabdariffa*) was reported by Ibrahim et al. (2013). The large variability was unexpected as roselle is cleistogamous, and selfing more often restricts variability. Phenotypic differences arise from genotypic and environmental components. Chief among the environmental factors in roselle development is the day length effect (Warner and Erwin, 2003; Mansour, 1975). At flowering, growth in height and stem diameter slow down and limit increase in plant height as occurs in kenaf (Dempsey, 1975). The wide differences in plant height could be attributed to the significant genotype effect for days to flowering. In contrast, no accession differences were observed for basal diameter as all stems were of almost similar girth. This characteristic of roselle warrants further study into the performance of roselle at various geographical areas in Ghana.

The large values of branch number at predominantly low branching points in roselle was unexpected. Although there is no defined planting distance for roselle, the planting distance of 20 × 50 cm within and between rows, respectively, may have contribued to the extensive branching, together with other environmental influences. The import of this finding suggest a much narrower planting distance to increase plant height and decrease number of branches. Sermsri et al. (1987) suggested planting distance of 5 to 15 cm for within row spacing and 20 to 40 cm for between row spacing as ideal to maximizing fibre yield potential of roselle (var. *altissima*) as well as its plant density. In addition, few reports have confirmed that wider plant spacing of 50 to 80 cm in roselle (var. *sabdariffa*) increased branching and reduced plant height (Okosun et al., 2006; Shalaby and Razin, 1989).

Basal diameter was found to have significant positive correlation with plant height and branch number, but negative correlation with height at first branching. This association appears to be beneficial since tall plants with large basal diameter would also have branching, if any, at high points. For high yield and good quality fibre, plants with high branching points are desired as the long fibre strands would have few or no interruptions. The low correlation coefficients indicate that only 1.21 and 1.70% of the variation in plant height is explained by variation in basal diameter and branch number, respectively. Similarly, 0.64% of the variation in basal diameter is

explained by height at first branching (Table 8). Coffie (2016) reported low to moderate positive correlation coefficient of r = 0.11 to 0.41 in plant height with number of internodes, branch number, leaf area and height at first branching in var. *sabdariffa* genotypes. Very low to moderate correlation coefficients were reported in basal diameter and plant height of 0.56 ($P < 0.01$), basal diameter and number of branches of 0.42 ($P < 0.05$), basal diameter and days to 50% flowering of 0.047 ($P>0.05$), in kenaf genotypes in Bangladesh (Mostofa et al., 2002). Knowledge regarding association of agronomic traits and their yield potential provides guidelines in crop improvement based on correlated traits, especially for characters that are difficult to evaluate or take long time to express. Roselle is ambiphotoperiodic as it can flower both in short days or long days (Mansour, 1975). The duration of the growing season and length of day are critical factors that have significant influence on the fibre yield characteristics of roselle (Dempsey, 1957).

Roselle (var. *altissima*) typically grows to a height exceeding 250 cm in height with very few branches at high branching points at optimal environmental conditions, which includes adequate irrigation, good soil nutrients, warm temperature and minimum day length of about 12 h 30 min. With a typical tropical day length that consisted of 12 h 30 min and average temperature of 25°C (World Weather and Climate Information, 2017) of the growing season, the genotypes studied were expected to have tall plant height. Plant height ranged from 154 to 342 cm and majority exhibited extensive branching at the lower stem. Low branching points and extensive branching are hindrance to fibre quality as fineness of fibre strands are reduced by the knotty branch points. Of the 25 roselle genotypes, only five accessions, HA-39, HA-45, HA-46, HA-49 and HA-58 exhibited few branches to merit selection for improvement. On the basis of tall plants exceeding 250 cm and large stem diameter greater than 20 mm, twelve accessions, HA-37, HA-38, HA-42, HA-43, HA-44, HA-47, HA-50, HA-52, HA-54, HA-55, HA-57, and HA-58 were selected for further studies on yield improvement.

The mean genetic distance of 3.03 ± 0.90 based on Euclidean estimates represents a substantial genetic diversity in the region. Coffie (2016) reported a mean genetic similarity of 0.27 ± 0.26 based on squared correlation coefficient among 35 var. *sabdariffa* landraces from West Africa. The fairly large genetic distances of the current roselle population suggest that the accessions were divergent. Because the genetic distance was based on morphological evaluation, influence of environment on the Euclidean estimates cannot be ruled out. Despite being a self-pollinating plant, the unexpected wide genetic variability may have arisen from forces such as gene or seed flow, climate and environmental variability, or mutation. An outcrossing rate of less than 1% in roselle (Young, 1995; Vaidya, 2000) over many generations could create ample variability.

Clustering of the accessions was independent of geographical origin, suggesting movement of seeds across the region. Each of the three clusters comprised at least one desirable trait of economic value. Hence, for any genetic improvement in bast fibre potential, there should be selection of parents across the three clusters. The findings of Bakasso et al. (2013) revealed two major clusters in 124 roselle (var. *sabdariffa*) collections from Niger on the basis of ten agro-morphological traits which included plant height, branch number and basal diameter. Coffie (2016) reported three main clusters in 35 roselle (var. *sabdariffa*) accessions based on six agro-morphological descriptors. Satyanarayana et al. (2015) reported of clustering of 60 roselle (var. *sabdariffa*) genotypes from India into seven clusters based on eleven agro-phenological traits. In their work, they showed that fibre yield per plant and dry stick weight were the most important while plant height and basal diameter were the least contributors to the variance.

Three of the five quantitative traits, namely, branch number, plant height, and days to 50% flowering achieved the largest minimization of Wilk's lambda and were the most efficient discriminatory traits. Further studies on structuring and genetic diversity in roselle should consider these three traits. In roselle (var. *sabdariffa*) discriminatory triats were found to be plant height, number of internodes, basal diameter, flowering time. and 100-seed weight (Coffie, 2016; Bakasso et al., 2013).

The first three PCs that explained 100% of the total variance revealed that all five traits were relevant in structuring of roselle.. However, from the PCs, height at first branching, number of branches and plant height were critical in providing a selection guide when considering fibre potential of roselle plants. On this basis, it is beneficial to select tall plants at high branching points rather than at low branching points. Delineating accessions into different groups have proved relevant for selecting desirable parents to maximize genetic variance in breeding programs (Chakravarty and Basu, 1972). Hybridization of genotypes from uncorrelated groups is therefore expected to result in beneficial improvement in agronomic character performances. In contrast, the tetraploid nature rather promotes breeding success with members in similar groups than in uncorrelated groups. The biplots of the PCA showed a good contribution of the accessions HA-46, HA-47, HA-57 and HA-59 to the variance. Overall, a large diversity residing in roselle (var. *altissima*) genotypes was revealed, hence, trait improvement in these accessions is possible.

Conclusion

The qualitative and quantitative descriptors revealed wide differences in morphology in roselle. Variability estimates were highest for Kassena-Nankana district. The predominant morphotype was non-bushy, uniform green, basal branching, and variable plant heights, with smooth

slender pentalobed leaves, yellow petals, crimson throats, hairy calyxes, and round capsule. The extensive branching was unexpected and represented a departure from roselle accessions previously described, highlighting the existence of other morphotypes in roselle gene pool awaiting collection and characterization. The variable plant height, number of branches, and days to 50% flowering with some accessions having heights as high as 300 cm, few branches and early flowering offer the possibility of selection for improvement in these traits. The substantial genetic distance highlights the existence of polymorphic alleles for the quantitative traits. The three distinct clusters represent diverse groups that can be hybridized to exploit heterotic effect. Sixteen accessions were considered useful for roselle breeding program on the basis of tall plant height and few branches, namely, HA-37, HA-49, HA-50, HA-55, HA-57, and HA-58 in Cluster I, HA-38, HA-42, HA-43, HA-47, HA52, and HA-54 in Cluster II, and finally, HA-39, HA-44, HA-45, HA-46 in Cluster III. The correlated accessions that were grouped on the biplot was in agreement with the clustering based on Euclidean distance. This observation indicate the power of cluster analysis and PCA in identifying relationships among genotypes. The most important discriminatory traits, branch number, plant height, and days to 50% flowering should be considered in future studies on roselle. Because roselle is day-length sensitive, evaluation in other geographical locations in Ghana would be necessary.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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