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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

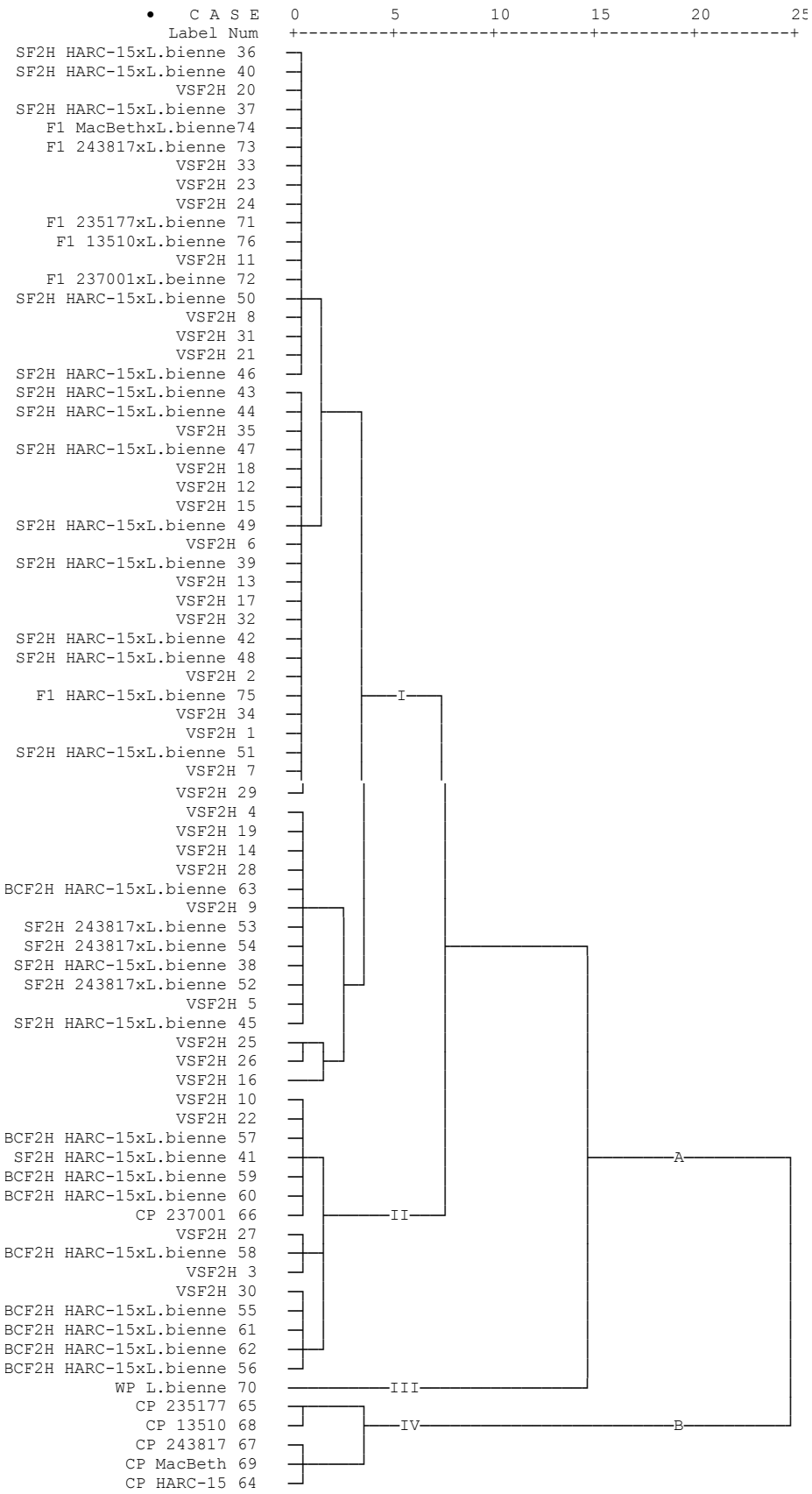

Figure 3. A dendrogram from quantitative characters cluster analysis of the entire sampled plants.

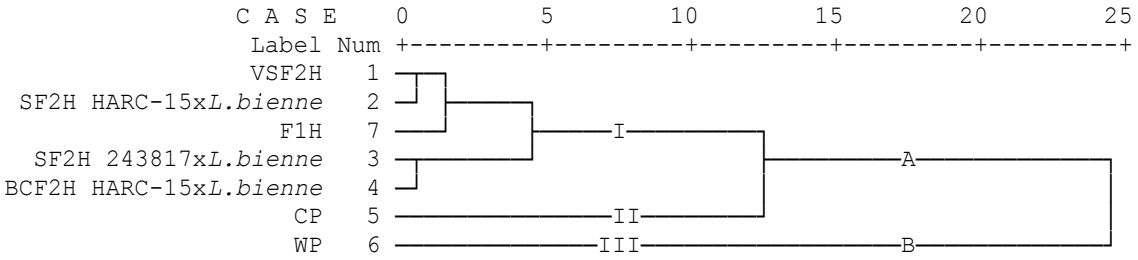

Figure 4. A dendrogram from combined quantitative and qualitative characters for cluster analysing average linkage between population groups.

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