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Research Paper

Phenotypic variability and characteristics of lentil (*Lens culinaris* Medik.) germplasm of Ethiopia by multivariate analysis

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Abstract. Multivariate statistical techniques, which simultaneously analyze multiple measurements on each individual under investigation, are widely used in the analysis of genetic diversity. The objective of this study were to classify groups of genotypes based on morphological diversity, identification of the major traits contributing to the diversity and identify superior genotypes for future use of the Ethiopian germplasm. Eleven variables were subjected to analysis by applying the method of Mahalanobis's generalized distances (D2), Principal Components Analysis (PCA), and a cluster analysis following the method of the Unweight Pair Group Method using Arithmetic Averages (UPGMA). The result of the analyses of variance and dendrogram revealed the existence of considerable genetic diversity among lentil genotypes, the source of origin and population groups for yield and yield contributing characters indicating the scope and guarantee for use in the breeding programmes. PCA showed that the most important traits that contributed to the genetic divergence were above ground biomass, seed yield, number of seeds per plant, days to maturity and number of pods per plant. UPGMA grouped into 6 major and 15 sub clusters characterized by distinct morphological feature irrespective of their origin.Considering yield and agronomic performance, cluster distance and cluster mean, accessions belonging to cluster B.1 and cluster F can be used as parents for the hybridization and back crossing programme for the development of high yielding lentil genotypes resistant to lentil rust disease.

Keywords: Cluster analysis, multivariate, PCA, UPGMA.

INTRODUCTION

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is a diploid (2n = 2x = 14 chromosomes) self-pollinating annual species with a haploid genome size of an estimated 4063 Mbp (Arumuganathan and Earle, 1991). The largest lentil producer country is India, followed by Canada and Turkey, accounting for 68% of the global production (Erskine, 2009). In Africa, Ethiopia, Morocco and Tunisia are the leading producer but 61% of the area and 68% of production is from Ethiopia (FAOSTAT, 2009). In Ethiopia, lentil is grown for human consumption as a rich source of protein (23 to 24%) (Senayet and Wondimu, 1994). Lentil is one of the high valued crops in the country compared with other cereals and pulse crops that fetch a high market price besides the crop is generally grown in rotation with cereals to break the cereal disease cycles and to fix atmospheric nitrogen, thus reducing the demand of other cereal crops for nitrogen fertilizers (Geletu et al., 1996). Global, African

Table 1. Geographical, climatic and soil features of the experimental sites.

Agro-ecological	Sirinka *	Sinana	Chefe Donsa*
Range of temp. (°C)	21-32	9.3- 20.9	8.9-28.3
Mean annual rainfall (mm)	876	808	851
Altitude (masl)	1850	2450	1900
Latitude	12°11′ N	7° 7′ N	08° 44′ N
Longitude	39.62′ E	40°10′ E	38° 95′ E
Soil texture	Clay soil	Clay soil	Light soil
Soil type	Eutric Vertisol black soil	Pellic vertisol slightly acidic	Afisols and vertisol black soil

and Ethiopia lentil productivity is about 887 kg/ha, 644 kg/ha (Erskine, 2009) and 1168 kg/ha, respectively (CSA, 2012). Numerous factors such as the low yield potential of the local cultivars and its susceptibility to biotic and abiotic stress limit the yield and the seed quality of lentil (Muehlbauer et al., 2006). To avoid the risk of genetic vulnerability and to widen the genetic background, it is crucial to increase the genetic resources available. In order to reach this objective the key is to study the genetic resources of the gene pool.

Quantitative traits provide an estimate of genetic diversity. Genetic diversity can be assessed at four levels of organization that is, among species and among populations, within populations and within individuals (Hunter, 1996). Morphological characterization is the first step in the classification and description of any crop germplasm (Smith and Smith, 1989). Various numerical quantitative techniques have been successfully used to classify and measure the pattern of phenotypic diversity in the relationship of germplasm collections of economically important traits of lentil germplasms (Erskine and Witcombe, 1984; Erskine et al., 1989; Ahmad et al., 1997; Abebe et al., 2001; Sarker and Erskine, 2006; Fratini et al., 2007; Abebe et al., 2008).

The use of established multivariate statistical methods is an important strategy for classifying germplasm, ordering variability for a large number of accessions or analyzing genetic relationships among breeding materials. Multivariate statistical techniques, which simultaneously analyze multiple measurements on each individual under investigation, are widely used in the analysis of genetic diversity irrespective of the data set (morphological, biochemical, or molecular marker data) (Mohammadi and Prasanna, 2003). In order to promote lentil germplasm use as well as guidelines for future collections, plant breeders must understand the patterns of variability and the grouping of the available germplasm. Three multivariate methods (generalized distance as, indicated by D^2 statistic, principal component analysis (PCA) and average linkage cluster analysis (UPGMA), were used to describe and group sets of individual lentil accessions, simultaneously taking into account several characteristics and the relationships between them (Rao, 1952; Hair et al., 1987, 1995; Franco et al., 1997). Multivariate analysis techniques were used for diversity analysis for lentil (Edossa et al., 2010; Tyagi and Khan, 2011; Abdul Latief et al., 2011; Roy et al., 2013). In order to properly utilize the lentil genotypes of Ethiopia and the diversity present in the field and gene bank, there must be a proper characterization and evaluation of the collected landraces and exotic lines using multivariate analyses. In light of this, the objective of the study was to classify groups of genotypes based on the morphological genetic diversity, to identify the major traits contributing to the diversity, and to find superior genotypes for future use of the Ethiopian germplasm.

MATERIALS AND METHODS

Description of the study site

The field experiments were conducted at Sirinka Agricultural Research Center (SRARC) northeastern part of Ethiopia, Chefe Donsa, sub-center of the DebreZeit Agricultural Research Center (DZARC) in the central highlands, and Sinana Agricultural Research Center (SARC), southeastern part of Ethiopia during the 2010/11-2011/12 cropping seasons (Table 1).

Plant materials

228 genotypes were used for this study, out of which 158 were planted for morphological evaluation at SRARC in the 2010/11 cropping season. Of these, 104 genotypes were from the Ethiopian lentil landrace collected from six major lentil production regions over 16 adminstrative zones (Figure 1) and kept by the Institute of Biodiversity Conservation (IBC) representing the total lentil germplasm holdings. As well as other six commercial released varieties and 48 parental and elite lines from different sources included. In the 2011/2012 cropping season, 228 genotypes plus RILs were included in the study across three locations Sirinka, Chefe Donsa, and Sinana. Each germplasm collection, even though considered a single population, represents a bulk of different individuals or populations which are accessions treated henceforward as genotypes only for experimental

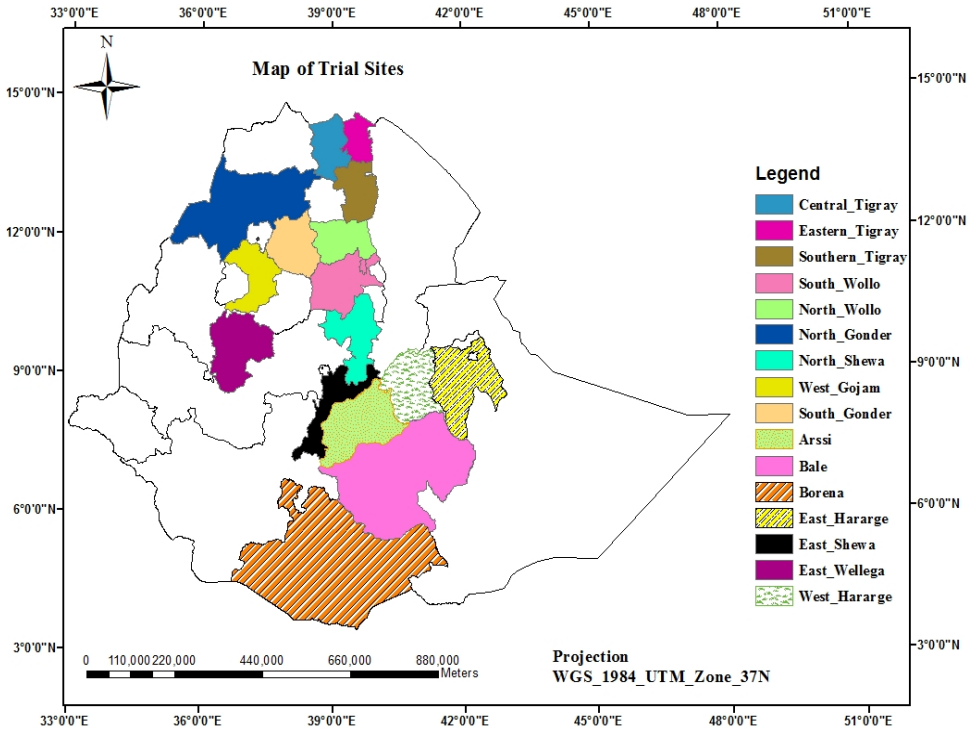

Figure 1. Map of Ethiopia showing areas of collection sites (shaded region) of the 104 landrace accessions.

purposes (Table 2).

Experimental layout and design

In the 2010/11 cropping season, the experiment was laid down in a randomized complete block design (RCBD) with three replications at SIARC. Spacing between plants and rows was 5 and 20 cm, respectively, and the row length was 2 m with a plot size of 0.8 m^2 at Sirinka. In 2011/12 cropping season, the genotypes were planted at SIARC in July, Chefe Donsa in August, and SARC in mid -September in an Augmented Design (Table 1).

Data collection

Data were recorded on 10 important phonological, yield and yield component characters. Besides rust disease severity reaction was score for each genotype. The observations were recorded on 10 randomly selected and tagged plants on each plot for number of pods/plant (NP), number of seeds/ pod (NS), seed weight per plant (SWPP), number of seeds per plant (NSPP), and plant height(cm) (PH) and for 100-seed weight (g) (SW), days to flowering (DF), days to maturity (DM), above ground biomass (g) (BI), seed yield per plant (g) (SY), rust disease severity score (RDS).

Data analysis

The data collected from RCBD were subjected to the analysis of variance (ANOVA) using the GenStat Release 13.3. software program (VSN International Ltd., 2010). Group analyses were carried out based on the mean value of all agro-morphological traits. Moreover, these mean values were used in the analysis of variance on the same software to test group differences with respect to their source of origin and population group. To compute variance components for each location and for the combined analysis across locations for data collected from the augmented designs, the analysis of variance was carried out using the formula outlined by Lin et al. (1986) and Yau and Hamblin (1994) using GenStat Release 15.1 [VSN International Ltd., 2012].

Cluster analysis

The data of those genotypes characterized during the two cropping seasons were analysed to perform cluster analysis by multivariate analysis applying the method of principal components analysis (PCA) (Eriksson et al., 1999), and a cluster analysis following the method of unweight pair group method using arithmetic averages (UPGMA). Sneath and Sokal (1973) were used to classify the 228 genotypes into groups (clusters) and determine

Table 2. List of 228 Ethiopian lentil genotypes, source of origin and population group used in the study.

Source of origin	No. of genotypes	Name	Population group
Tigray	8	Acc. no. 219957, 235383, 237503, 237504, 241785, 242604, 243447	Landrace
Amhara	54	Acc. no. 36003, 36025, 36028, 36039, 36041, 36061, 36071, 36085, 36088, 36089, 36097, 36103, 36104, 36105, 36137, 36139, 36150, 36162, 36165, 36168, 207258, 207274, 207287, 207309, 212745, 215248, 215249, 223221, 228242, 229179, 229182, 229183, 231247, 235013, 235015, 235016, 235017, 236484, 236486, 236487, 237502, 238978, 238979, 241784, 241786, 243433, 243436, 243440, 243443, 244606, 244610, 244615, 244619, 244623	Landrace
Oromya	29	Acc. no. 36001, 36007, 36009, 36013, 36015, 36019, 36023, 36029, 36033, 36042, 36048, 36058, 36110, 36120, 36131, 203141, 215806, 216877, 228809, 230521, 230833, 230834, 230837, 231248, 235698, 236438, 236892, 237027, 238971	Landrace
SNNP	2	Acc. no. 36147 and Acc. no.,228243	Landrace
Somali	1	Acc. no. 230832	Landrace
DZARC	6	/ILL4225 x ILL4605/ /ILL 6821/ *Alemaya*, /ILL 1 x ILL 1169//ILL 6027/ *ADAA*, /ILL 7978/ *Teshale*, / Alemaya x FLIP88-41L/ *Derash*, /ILL 7981/ *Aleme Tena* and P160/ILL 2704/ /*Chekol*/	Commercial varieties
Unknown	10	Acc. no. 36134, 207260, 211062,211078, 211110, 220120, 211131, 233349, 233973, 241782	Landrace
ICARDA	22	X2003S 222/ILL 213/, X2003S 238 /ILL 4605/, X2006S 128/ILL 5480/, L-9-12, X2002S 219 /ILL 6821/, X2006S 129 /F2/, X2005S 215 /ILL 6002/, X2006S 133/FLIP87-21L/ /ILL 4349 x ILL4605//ILL 6211/, X2006S 130/FLIP 93-46L/ /ILL 7547/, FLIP-2004-7L, X2003S 223, X2003S 195/ILL 7115/, X2006S 130/FLIP 96-46 L//ILL 7978/, X2002S 221/FLIP 96-47 L//7979/, X2002S 221 /7980/, X2003S 233 /ILL 8009/, X2006S 134/ILL8174/, 2006S 122 /FLIP 2003-43L/ /ILL 7010 x ILL 1939/ /ILL 9932/, /FLIP 2003-56L/ /ILL 9945/X2006S 127, /ILL 2573 x ILL 7537/ /FLIP 2003-62 L/ /ILL 9951/X2006S 122, X2002S 219 /shehor-74/ /ILL 7554/, 2003S 236	Parents
DZARC	11	EL-142 /ILL 5071/, EXOTIC #DZ/2008 AK, R-186XFLIP-86-38L-24, ILL-358 X ILL-2573-2-2000, 87S-93549XEL-1O3-4, 87s-93549XEL-03-5, Chekol X R-186-1, R-186X FLIP-86-38L, Chekol x R-186-2, EL-142 X R-186-2, EL-142 X R-186-3	Breeding line
ICARDA	15	ILL-590/NEL 590/, FLIP-2006-60L, FLIP-97-68L, FLIP-04-26L, ILL-28501, FLIP-2006-20L, FLIP-87-68L, /ILL 6037 x ILX 87062/ FLIP2005-24L/ ILL-10045, FLIP-97-16L/ILL 8078, ILL-10680, FLIP-2004-37L, FLIP-84-95L /ILL 5722, FLIP-97-61L, L-830, Precoze/ILL 4605/	Breeding line
ICARDA	85	RIL1- RIL85	RIL

distance among the source of origin and population groups using the GenStat Release 13.3 (VSN International Ltd. 2012) statistical soft ware. The patterns of distribution of morphological variation were analyzed using Mahalanobis' generalized distances (D^2) (Mahalanobis, 1936). The D^2 is applied to estimate the

Table 3. Mean square values for agro-morphological character of lentil (*Lens culinaris*) based on source of origin at Sirinka in the 2010/11 cropping season.

Source of variation	DF	DM	NPP	NSS	PWPP	SWPP	NSPP	PH	SW	BI	SY
Rep	576.89	1255.7	3076.8	1.31	1.73	2.06	8983.1	377.14	0.023	6357ns	46706.5
Origin group	131.53**	3516.53**	739**	0.64**	3.58**	1.53**	2155.8**	85.43**	6.13**	60728**	7790.4**
Origin group × geno.	66.14**	212.79**	336.6**	0.26**	2.17**	0.61**	793**	46.3**	0.67**	342**	4441.9**
Residual	13.92	45.61	127.1	0.054	0.0577	0.169	200.9	8.15	0.094	6161	997.2
s.e.	3.7	6.8	11.27	0.23	0.24	0.41	14.18	2.85	0.31	78.49	31.58
cv%	6.5	7	32.3	19.8	16.5	37.1	33.7	8.3	12.1	27.3	37.4

**, * = Significant at 1% and 5 % probability levels, respectively. DF = days to 50% flowering, DM = days to 90% maturity, NP=number of pods per plant, NS = number of seeds per pod, PWPP = Pod weight per plant, SWPP = Seed Weight per Plant, NSPP = Number of Seeds per Plant, PH = Plant height in cm, SW= 100-seed weight in gram, BI = Biomass, SY = seed yield.

Table 4. Mean comparison of agro-morphological character based on source of origin at Sirinka in the 2010/11 cropping season.

Source of origin	DF	DM	NPP	NSS	PWPP	SWPP	NSPP	PHH	SW	BI	SY
Tigray region	56.65^{abc}	91.42^{a}	36.56^{bc}	1.19^{abc}	1.42^{cd}	1.18^{abcd}	44.6^{bc}	36.78^{d}	2.474^{bd}	289^{abc}	96.8^{acd}
Improved varieties	56.31^{abc}	104.8^{bc}	38.33^{bc}	1.36^{cd}	1.974^{e}	1.64^{be}	50.8^{bc}	32.09^{a}	3.072^{fg}	324.7^{bcd}	113.18^{d}
Amhara region	56.76^{abc}	91.83^{a}	36.32^{bc}	1.15^{ab}	1.33^{cd}	1.05^{abc}	42.76^{b}	35.13^{cd}	2.34^{bcd}	263.5^{a}	79.9^{abc}
Oromaya region	56.13^{ab}	89.41^{a}	37.83^{c}	1.11^{ab}	1.42^{d}	1.06^{abc}	43.58^{b}	35.69^{cd}	2.23^{abc}	296^{abc}	80.8^{abc}
ICARDA	58.09^{ac}	100.82^{b}	27.8^{a}	1.06^{a}	1.964^{e}	0.954^{a}	29.22^{a}	33.11^{ab}	2.908^{ef}	257.1^{a}	68.45^{ab}
SNNP region	54.76^{a}	87.74^{a}	35.6^{abc}	1.4b^{cd}	1.4^{bcd}	0.93^{a}	44.1^{abc}	35.2^{abcd}	2.156^{ab}	239.6^{a}	65.01^{a}
Somalia region	59.4a^{bcd}	93^{ab}	43.3^{abc}	1.3^{abcd}	0.803^{a}	0.991^{ab}	53.73^{abc}	38.16^{bcd}	1.656^{a}	270.8^{ab}	81.8^{abcd}
DZARC	57.66^{abc}	106.63^{c}	38.92^{bc}	1.36^{cd}	1.166^{ab}	1.52^{bde}	53.59^{c}	33.8^{abc}	2.751^{e}	375.6^{bd}	111.4^{d}
Global core	61.97^{d}	116.6^{d}	30.26^{ab}	1.283^{cd}	1.26^{abc}	1.2^{abcd}	40.14^{b}	32.55^{ab}	3.215^{g}	337.8^{bc}d	93.62^{acd}
Unknown	56.56^{abc}	90.64^{a}	34.3^{abc}	1.432^{d}	1.32^{bcd}	1.15^{abc}	47.51^{bc}	34.32^{abcd}	2.26^{abcd}	277.7^{abc}	86.4^{abcd}

Means within columns with different letters are significantly different ($P < 0.05$).

distances within and between clusters.

RESULT

Analysis of variance based on the source of origin and type of population

The analysis of variance showed a highly significant ($p \leq 0.01$) difference among sources of origin for all traits except pod set score, justifying the appropriateness of further analysis (Table 3). The landrace collected from different regions of Ethiopia depicted early flowering and short maturity period. Genotypes originated from the ICARDA and elite breeding lines from the DebereZeit Agricultural Research Center (DZARC) recorded late flowering and long maturity period (Table 4). The maximum number of pods per plant was recorded in Somalia region followed by the breeding lines from DZARC and commercial improved varieties collected from DZARC. While, low number of pods per plant was recorded for genotypes introduced from ICARDA. The maximum 100 seed weight was noted for the elite breeding lines from ICARDA and commercial varieties obtained from DZARC; the lowest 100 seed weight was recorded for the landraces collected from the SNNP and Somalia regions. The highest seed yield and above ground biomass were recorded for the commercial varieties obtained from DZARC and elite breeding lines from DZARC. Low seed yield was depicted that landraces originated from the SNNP region (Table 4).

The analysis of variance based on population

Table 5. Mean square values for agro-morphological character of lentil (*lens culinaris*) based on type of population at Sirinka in the 2010/11 cropping season.

Source of variation	DF	DM	NPP	NSS	PWPP	SWPP	NSPP	PHH	SW	BI	SY
Rep	564.1	1255.36	3068	1.3	1.73	2.06	8983.3	377.14	0.02	6358	46707
Popn type	347.4**	13171.4**	1579.7**	0.32	15.34**	3.09	1990.7**	309.9**	24.6**	73378.0**	8908.9**
Popn type × geno.	66.8**	246.58**	342.6**	0.27	2.09**	0.63**	840.4**	45.16**	0.69**	35204.0**	4558.7**
Residual	13.94	45.46	126.7	0.05	0.06	0.17	200.9	8.15	0.09	6161	997.2
s.e.	3.7	6.7	11.26	0.23	0.24	0.41	14.18	2.85	0.31	78.5	31.6
cv%	6.5	7	32.1	19.6	15.6	36.8	33.5	8.3	12.1	27.3	37.4

**, * = Significant at 1 and 5 % probability levels, respectively. DF= days to 50% flowering, DM = days to 90% maturity, NP = number of pods per plant, NS = number of seeds per pod, PWPP = Pod weight per plant, SWPP = Seed Weight per Plant, NSPP= Number of Seeds per Plant, PH = Plant height in cm, SW = 100-seed weight in gram, BI = Biomass, SY = seed yield.

Table 6. Mean comparison of agro-morphological characters based on type of population at Sirinka in 2010/11 cropping season.

Popn type	DF	DM	NPP	NSS	PWPP	SWPP	NSPP	PHH	SW	BI	SY	PSS
Landrace	56.52^{a}	90.9^{a}	36.65^{b}	1.169^{a}	1.361^{a}	1.06^{a}	43.67^{b}	35.37^{b}	2.299^{a}	275.5^{a}	81.74^{a}	4.546^{a}
Breeding lines	59.15^{b}	106.8^{b}	31.16^{a}	1.201^{a}	1.888^{b}	1.172^{b}	38.47^{a}	33.1^{a}	2.966^{b}	310.7^{b}	86.61^{a}	5.124^{b}
Improved varieties	56.31^{a}	104.8^{b}	38.33^{b}	1.355^{b}	1.974^{b}	1.629^{c}	50.8^{b}	32.09^{a}	3.072^{b}	324.7^{b}	113.18^{b}	5.381^{b}

Means within columns with different letters are significantly different ($P < 0.05$).

group revealed a highly significant ($p \leq 0.01$) variation for all agronomic traits except for number of seeds per pods (Table 5). The Ethiopian landrace collection showed early flowering and short maturity period whereas exotic elite breeding lines tooke a long time to flower and mature (Table 6). Higher values for the yield component traits were associated with improved varieties, and lower values were associated with elite breeding lines and Ethiopian landrace collection (Table 6). The study depicted the top 20 high yielding genotypes obtained from ICARDA (9), DARC (7), GC (2), Amhara (1) and Tigray (1) (data not shown). From linear ANOVA we can infer that selection should be practices based on the phenotypic performance of individual genotypes rather than considering source of origin and type of material. It is explicit that there is no relationship between geographic distribution and genetic diversity of lentil in this study. Further multivariate analysis is required to have a better picture.

Genetic diversity and relatedness study by multivariate methods

The similarity matrix based on the euclidean test depicted the maximum identity was recorded between parent lines (X2003S 195/7115/) and (X2002S 219 /6821/) (100%) and the minimum similarity was recorded between breeding line (R-186XFLIP-86-38L-24) with landrace (Acc.no. 228242) (62%) Sirinka 2010/11. For combined analysis over locations, the maximum identity was recorded between landraces (Acc. no. 241785) and (Acc. no. 236487) (99.5%) from Tigray and Amhara regions (data not shown). The most distantly related genotypes recorded (77.7%) between RIL 191 and RIL 16 indicated that these two RIL accumulated and segregated the two extreme genes of parent types.

Cluster analysis

Lentil genotypes used in this study were grouped into 6 major and 15 sub clusters at 87 and 95% similarity coefficient cutting edge, respectively (Figure 2). The maximum genetic distance was noted between landrace, Acc.no. 36001 and elite breeding line (EL-142 X R-186-3) where they held the two extreme position of the dendrogram. The former originated from landrace collection in

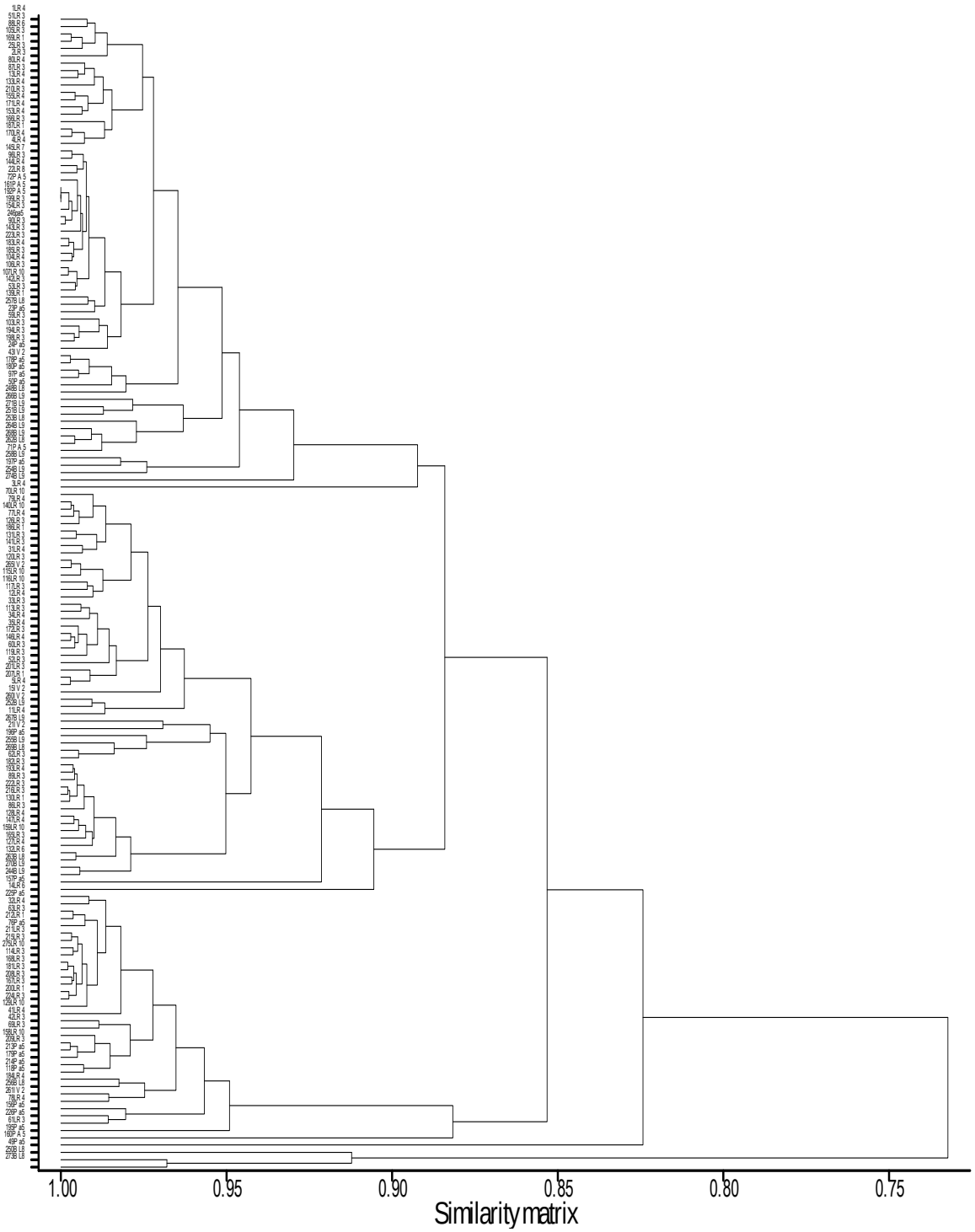

Figure 2. UPGMA dendrogram showing the genetic relationships among 228 genotypes of lentil analysis for 2011/12 cropping season. Numbers in the dengrogram shows the lentil genotype codes.

Oromya region and the latter, a putative wilt resistant elite breeding line, from DZARC. Cluster A was the largest of all clusters containing 65 genotypes. Of these 60% landraces originated from Ethiopia, 18% parent lines, 18% breeding lines, and one improved variety. This cluster had a morphological feature of a low number of seeds per pods and a long plant height. Cluster B comprised 55 genotypes, with 42 landraces, 4 improved varieties, 2 parents and 7 breeding lines.

Cluster C, the third largest group, comprised 33 genotypes of which 71% was composed of landrace collection from Ethiopia, 24% wilt resistant donor parents

Table 7. Contribution of quantitative variables into the PC1, PC2 and PC3 vector.

Variables	2011			2012	
	PC1	PC2	PC3	PC1	PC2
DF	0.02	0.05	0.02	0.02	-0.20
DM	0.03	0.13	-0.06	0.02	-0.21
NP	0.04	-0.14	0.48	0.09	0.38
NSS	0.001	-0.002	0.005	0.0003	0.001
SWPP	0.003	-0.005	0.02	0.003	0.005
NSPP	0.07	-0.20	0.83	0.06	0.19
PHH	0.02	0.02	0.04	0.02	0.00
SW	0.001	0.0001	-0.01	0.00	-0.01
BI	0.96	0.26	-0.01	0.92	-0.36
SY	0.25	-0.93	-0.26	0.38	0.78
Rust disease severity score				-0.01	0.01
Percentage of variation	90.89	5.99	1.86	74.98	12.92

and one improved variety and another elite breeding line resistant to wilt. Cluster D and E were represented by only a single distinct wilt resistant breeding line.

Cluster F had three lines composed of one distinct parent line (X2006S 128/5480/) with a different agronomic merit, and the other two were elite wilt resistant breeding lines originated from DARC (R-186 X FLIP-86-38L-24 and EL-142 X R-186-3). The dendrogram was also constructed for the 2011/12 combined data for 228 genotypes including lentil RIL mapping population for rust were evaluated and grouped into six major and twelve sub clusters (data not shown). Except the RIL and some out tailed accessions, the same trends were observed with that of Sirinka 2010/11.

Principal components analysis (PCA)

In the present study, the first three Principal Coordination axes (PCs) accounted for 98.7% of the total phenotypic variation of the eleven characters. The first PCA explained 90.9% of the total variations. However, the associated communalities showed that a higher proportion of the variance due to number of seeds per plant and number of pods per plant was not properly described by the first principal components. For this reason, the second and the third principal component that these two characters explained described 91.8% of the total variance were taken into consideration (Table 7).

Contribution of characters to the divergence of genotypes

Based on the PCA, the value of the vectors for each trait and result of variation accounted each axis in the two seasons were shown Table 7. The two dimensional charts (PCA 1 and PCA 2) of the genotype and best performing genotypess selected are presented in Figure 3. The clustering of the genotypes into six major groups was largely followed and affected by the value of the first PC which accounted 90.9 % of the total variation. In the first PC, the most important traits that contributed to the genetic divergence were above ground biomass and seed yield. All traits had positive loading vector that contribute variation among accessions. In the second PC, which explained 6% of the total variation, the predominant traits were above ground biomass and days to 90% maturity. The third PC, which accounted for 1.8% of the total variation, was dominated by two important yield component traits that is, number of seeds per plant and number of pods per plant. These two traits accounted 95% of the genetic divergence among the genotypes; the same trait contributed to the genetic divergence in the 2012 cropping season (Table 7).

Genetic diversity study between groups

Grouping based on source of origin

The Mahalanobisis inter-population genetic distance (D^2) among the source of origin ranged from 0.001 to 28.1 (Table 8). Samples introduced from the ICARDA were distantly related to samples collected from Somalia region (D^2 = 28.12), and the least genetic distance recorded between landrace originated from Amhara and Tigray region (D^2 = 0.001) (Table 8).

Genotypes from the different sources of origin were grouped into two major clusters of distinct genetic populations at 72% of similarity coeffecient cutting point (Figure 4). The first major cluster comprised the Ethiopian landrace collected from different regions. The second major cluster includes parents introduced from ICARDA, elite breeding lines from the ICARDA core set and commercial improved varieties released by DZARC and

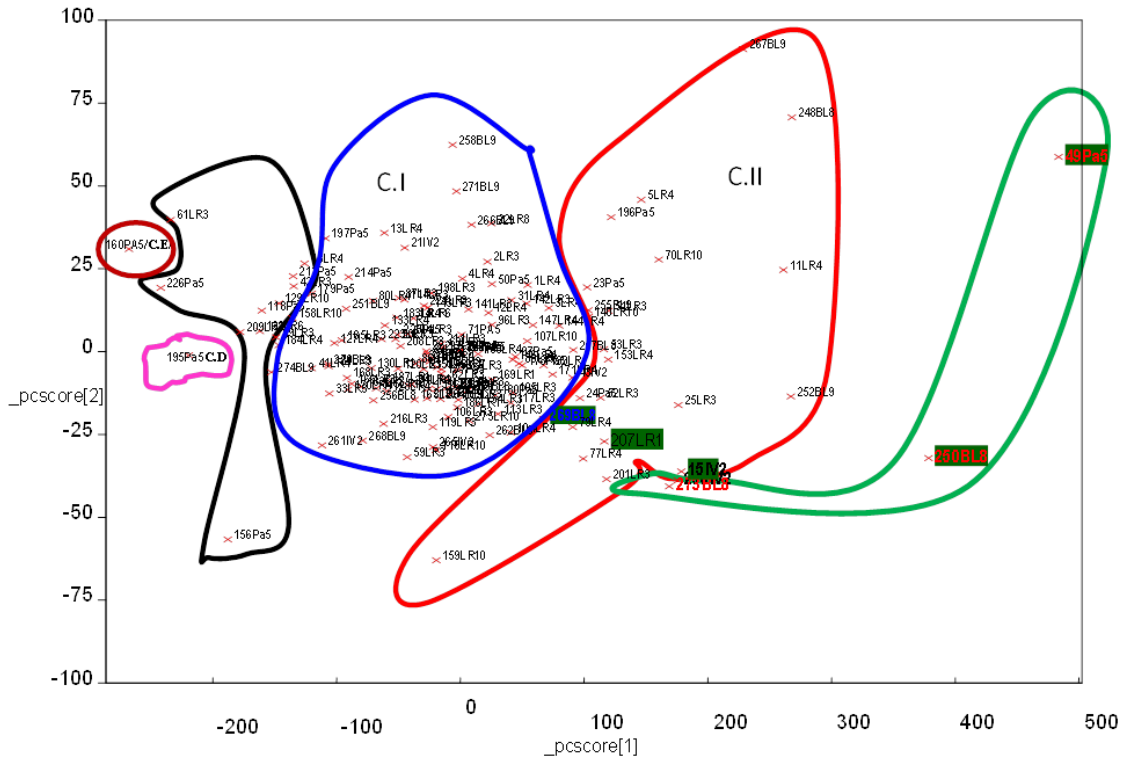

Figure 3. Scatter distribution and representation of 158 lentil accessions in the space defined by the first two principal components scores.

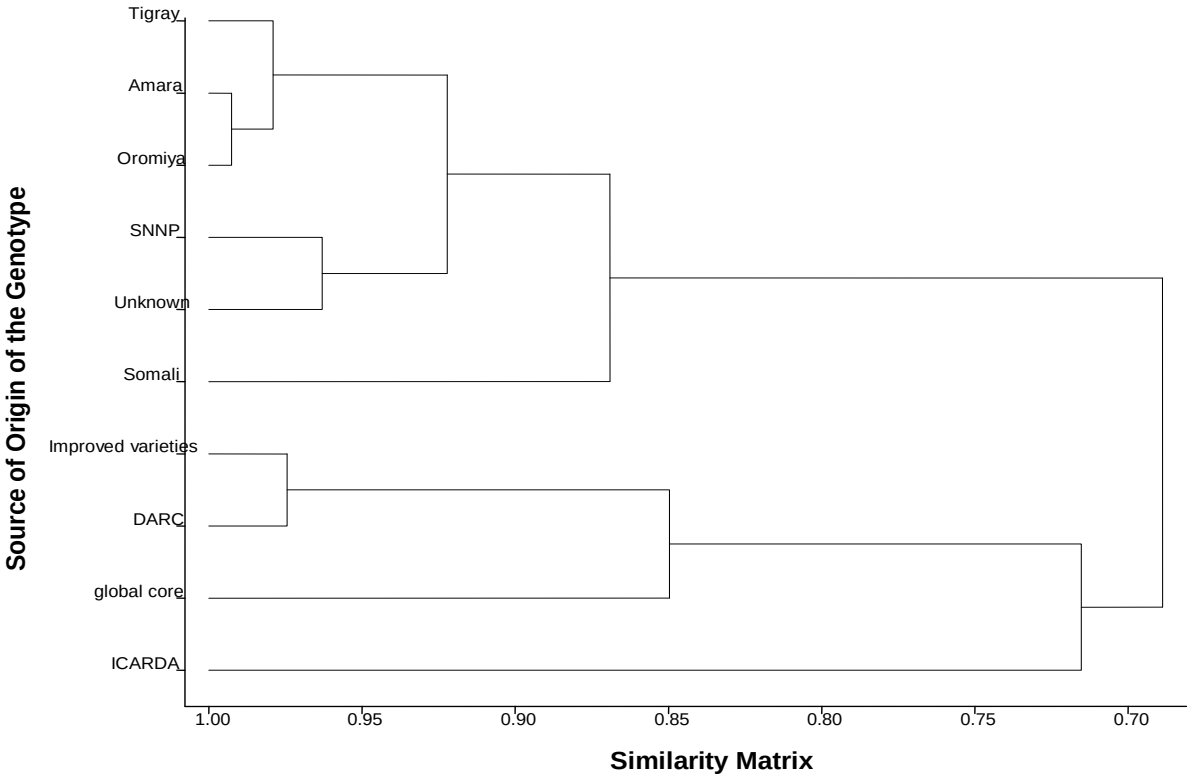

Figure 4. Dendrogram based on Euclidean using the method of average link (also UPGMA) based on source of origin at Sirinka 2012.

elite breeding lines for different agronomic merit originated from DZARC. The first main cluster had three sub-clusters at a 95% similarity matrix cutting point, whereas, the second major cluster comprised genotypes collected from different source was distinctly different (Figure 4). The study depicted that selection should be practiced based on the phenotypic performance of individual accessions rather than considering source of origin and types of material.

Visualizing relationship based on population group

A similarity matrix based on material types showed, high levels of dissimilarities observed among the different population group. The least similarity was recorded between landrace originated from Ethiopia with improved varieties (45.9 %) (Table 9).

DISCUSSION

The maximum phenotypic identity was recorded between parents with their recombinant inbreed line (93.5%) (Table 9). The range for genetic distance was 4.83 up to 22.45 with an average genetic distance (D2 = 10.52) (Table 9). Considering the different groups of analyses, the genetic distance depicted among the material type was by far higher compared with the genetic distance noted within genotypes and source of origin. Based on a cluster assignment at a probability of 0.68 (68%) as a cutting-point populations were assigned fully into three major clusters (Figure 5).

As per the analysis of variance a highly significant variations among the genotypes for the majority of the morphological traits is a sign of the presence of a high degree of genetic variation implying the great potential of the genotypes in future breeding programs through selection. In support of these findings, a high degree of genetic variation for days to flowering, days to maturity, plant height, pods per plant and seed yield per plant were reported for the Ethiopia lentil germplasm (Erskine and Witcombe, 1984; Seifu, 1988; Geletu and Yadeta 1994; Geletu et al., 1996; Asnake and Geletu, 2003; Tigist, 2003; Edossa et al., 2010).

In this study, we chose to follow the criterion used by Clifford and Stephenson (1975) and adopted by Guei et al. (2005), which suggested that the first three principal components (PC) are often the most important in reflecting the variation patterns among accessions, and the characters associated with these are more useful in differentiating accessions. In the present study, the first three Principal Coordination axes (PCs) accounted for 98.74% of the total phenotypic variation of eleven characters. The clustering of the genotypes into six major groups was largely followed and affected by the value of the first PC which accounted 90.9% of the total variation. The most important traits that contributed to the genetic divergence inorder of importance were seed yield, above ground biomass, days to 90% maturity, number of seeds per plant and number of pods per plant. Previous morphological analysis and lentil path coefficient analysis also indicated that number of seeds per plant, number of pods per plant, days to 90% maturity and plant height were the important traits contribute to yield difference among the genotypes (unpublished data). In agreement to these findings, similar associations were reported by Toklu et al. (2009) and Edossa et al. (2010) the first three and four PCs accounted 73.13 and 86.3% of the total phenotypic variation, respectively. They reported most important traits were above ground biomass, number of seeds per plant, number of pods per plant and seed yield; additionally they reported flowering period and days to maturity was the main contributor to the variation.

Lentil genotypes used in this study were grouped into 6 major clusters. The dendrogram revealed the existence of a considerable genetic diversity among lentil germplasm. Cluster A has a morphological feature of a low number of seeds per pods and a long plant height. Cluster B was characterized by high number of pods per plant, number of seeds per pods, number of seeds per plant, above ground biomass, and seed yield. Clusters C, D and E have a morphological feature associated with poor agro-morphological traits such as pod weight per plant, seed weight per plant, number of seeds per plant, plant height, above ground biomass and poor yielder. Cluster F had morphological characteristics of high above ground biomass, number of pods per plant, number of seeds per plant, seed weight per plant, seed yield and take a long time to flower and mature. The correlation between the cophenetic matrix and the Euclidian distance matrix (r = 0.88) showed a good fit to the data set, validating the observed clusters as interpreted according to Rohlf (2004).

As per the Mahalanobisis inter-population genetic distance (D^2) among the source of origin wide range of genetic distances were recorded. In agreement with this findings Roy et al. (2013) observed material originated from Ethiopian had showed both the most similarly and distantly related genotypes. However, Edossa et al. (2010) recorded a narrow range of 0.825 to 11.018 with an average value of 3.92 inter-population genetic distance (D^2) among landrace originated from different sources of administrative regions in Ethiopia. Genotypes from the different sources of origin were grouped into two major clusters of distinct genetic populations of the Ethiopian landrace collected from different regions and the second major cluster includes exotic genotypes elite breeding lines from DZARC. Dendrogram clearly showed that they had evolved from different lines of ancestry or derived from independent events of both natural and artificial selection that separated them into different population groups within the cultivated lentil (*Lenus culinaris*). Despite the wide genetic diversity of lentil

Table 8. Nei's original measures of Similarity matrix (below diagonal) and Intergroup Mahalanobis (D^2) genetic distance (above diagonal) based on source of origin.

Source of origin	1	2	3	4	5	6	7	8	9	10
Tigray (1)	-	11.72	0.001	0.046	3.49	0.238	2.616	11.2	18.08	0.416
Varieties (2)	78.9	-	11.79	13.00	7.22	14.40	18.28	0.23	1.30	10.53
Amara (3)	99.3	72.1	-	0.038	3.58	0.22	2.54	11.25	18.21	0.40
Oromiya (4)	98.2	72.2	98.6	-	4.30	0.08	2.11	12.33	19.83	0.41
ICARDA (5)	85.6	62.9	87.3	87.1	-	5.53	11.86	8.33	9.32	5.11
SNNP (6)	92.3	66	93.8	93.3	80.6	-	1.4	13.5	21.9	0.4
Somali (7)	87.2	57.1	87.7	86.4	58.4	85.1	-	16.2	28.1	1.5
DARC (8)	81.4	97.4	74	74.9	61.5	68.8	67.4	-	2.52	9.50
ICARDA core set (9)	77.4	84.1	74.9	72.9	80.9	65.5	59.2	85.9	-	17.59
Unknown (10)[1]	92.4	78.7	91.2	90.3	75.7	96.3	88.3	82.2	74.7	-

Table 9. Nei's original measures of genetic identity (below diagonal) and Intergroup Mahalanobisis (D2) genetic distance (above diagonal) based on type of populations on average mean of the combined data.

Population group	Breeding line	Global core	Improved variety	Landrace	Parent	RIL
Breeding line	-	10.15	18.63	10.15	10.15	10.15
ICARDA core set	78.9	-	18.63	8.44	8.44	8.44
Improved variety	80.5	68.6	-	18.63	18.63	18.63
Landrace	75.6	52.2	45.9	-	6.31	6.31
Parent	81.2	82.5	69.5	75.6	-	4.83
RIL	68.2	82.4	54.9	65.3	93.5	-

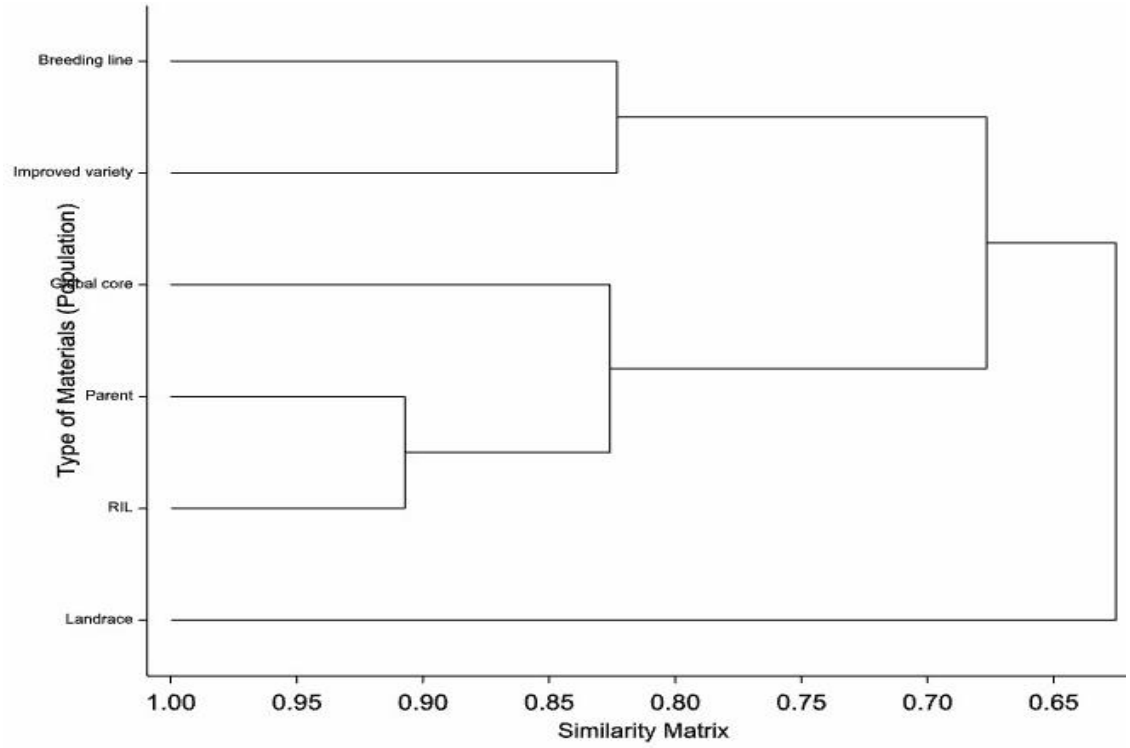

Figure 5. Cluster analysis using the method of average link distances between different type of population type.

[1] Unknown: Ethiopian landraces but their source of collection not mentioned in the passport data

germplasm of Ethiopia the grouping pattern of landraces and genotypes did not reflect any association with geographic origin. Some genotypes from ICARDA, Amhara and Oromya were dispersed throughout the dendrogram indicating that some genotypes of these region have a close resemblance with those of other regions for the variables under consideration.

Based on the mean phenotypic performance, improved varieties were characterized by high number of seeds per pods, seed weight per plant, number of seeds per plant, above ground biomass, resistant to rust disease and seed yield. Besides, elite breeding lines could be a potential source for number of pods per plant, plant height, 100 seed weight and resistant source for lentil rust disease. Landrace which originated from Ethiopia was associated with earliness, long plant height, and susceptiblity to lentil rust. Low seed yield and yield components were associated with some groups of landrace originating from SNNP region, Amhara region, RIL population and putative parent from ICARDA. Despite the wide genetic diversity of lentil germplasm of Ethiopia the grouping pattern of genotypes and landrace did not reflect any association with geographic origin. Accessions from ICARDA, Amhara and Oromya were dispersed all over the tree, indicating that some genotypes of these regions have a close resemblance with those of other regions for the variables under consideration. The study depicted that selection should be practiced based on the phenotypic performance of individual accessions rather than considering source of origin and types of material. Therefore, crosses between parents with maximum divergence would be more responsive to improvement since they are likely to produce higher heterosis and desirable genetic recombination. Thus, improved varieties of *Alemaya* and *Derash*, landrace (Acc.no. 242604) and breeding line (Chekol X R-186-2) from sub-cluster B-1 parent line X2006S 128/5480/, breeding lines R-186XFLIP-86-38L-24 and EL-142 X R-186-3 from cluster F performed consistently better in the four environments (Figure 3). These genotypes should be considered better parents for recombination breeding for yield and rust disease reaction.

Grouping based on the material nature showed high levels of inter-population genetic distance observed among the different population groups compared with the genetic distance noted within genotypes and source of origin. Dendrogram clustered into three major clusters. The first cluster constituted exclusively Ethiopian landraces with distinct class, the second cluster composed of elite breeding lines with different agronomic merits and improved varieties. The third class constituted putative resistant and susceptible parent lines and their recombinant inbreed lines (RIL).

CONCLUSION

The linear ANOVA showed a highly significant ($p < 0.01$) genetic variation of different yield and yield contributing characters within the genotypes, among source of origin and material type, justifying the appropriateness of further analysis. Lentil genotypes used in this study were grouped according to agro-morphological nature of the genotypes rather than source of origin and material type. The dendrogram revealed that the existence of considerable genetic diversity among lentil genotypes, the source of origin and population groups for different yield and yield contributing characters indicated the scope and their warranty to use in breeding programmes. The most important traits that contributed to the genetic divergence inorder of importance were seed yield, above ground biomass, days to 90% maturity, number of seeds per plant and number of pods per plant. These agro-morphological trait can be used as a basic character for developing a mini-core set of the Ethiopian germplasm. However, there is no relationship between geographic distribution and genetic diversity in terms of important traits of lentil in this study. Thus, we suggest that parental selection should be made on the basis of systematic assessment of genetic distance in each genotype rather than on geographic difference. Hence, considering yield and agronomic performance, cluster distance and cluster mean, accessions belonging to cluster B.1 and cluster F can be used as parents for the hybridization and back crossing programme for the development of high yielding lentil genotypes resistant to lentil rust disease.

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