Evaluation and characterization of ICARDA elite germplasm of lentil

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Abstract

Food legumes are a central part of the diet for many communities around the world and lentil could be an excellent choice to provide more nutritious foods. Lentil breeding program aims to develop adapted, high yielding, biotic and abiotic tolerant varieties. The success of any breeding program is dependent on the availability of genetic materials with sufficient diversity. In order to investigate the genetic diversity in elite germplasm of lentil, a total of 138 genotypes received from ICARDA were evaluated in research field of Seed and Plant Improvement Institute during 2015-2016 cropping season. These materials were studied in an observatory design by evaluating 28 quantitative and qualitative traits according to the Bioversity international descriptor. Results indicated that the highest Shannon index belonged to ground color of testa (1.33), testa pattern (0.85) and color of testa pattern (0.8). The traits seed weight per plant (CV=111.23%), grain yield (CV=81.58%) and pod weight per plant (CV=72.55%) had the highest coefficient of variation. The highest 100-seed weight and grain yield were recorded for genotypes 118 and 69, respectively. Genetic relatedness of genotypes was investigated by their pedigrees and values of genetic distance. Analysis of proximity values based on quantitative traits showed that genotypes 6 and 114 had the highest similarity and genotypes 42 and 130, had the highest genetic distance. The results of discriminant analysis of principal components indicated a successful classification based on quantitative traits in differentiating groups of genotypes. Totally, results indicate the presence of a valuable genetic diversity which could be used in advanced breeding programs.

Key words: Gene Bank, Genetic diversity, Gene pool, Genetic resources.

INTRODUCTION

Food legumes are a central part of the diet for many communities around the world. Pulse crops, especially lentil could be an excellent choice to provide more nutritious foods for millions of people who suffer from obesity, overweight, and micronutrient malnutrition (Thavarajah, 2018). Lentil provides carbohydrate, protein, fiber, macro-and micronutrients, and vitamins to support human health. In addition, through its ability in nitrogen fixation and reduction of use for chemical fertilizer, lentil contributes to sustainable farming system.

Currently, this crop is mainly cultivated in semi arid regions of South Asia, North America, Australia and Africa (Verma *et al.,* 2014). Archaeological evidence implies that lentil is one of the first cases of domestication in the Near East (Ahmad *et al*., 1997). The plant has been grown in the Fertile Crescent, the place where it was cultivated at least about seven centuries BC and its cultivation has spread around the Mediterranean, the Middle East, Ethiopia and India. Canada, contributing 38% of the world's production, the largest producers of lentil followed by India, Turkey, and Australia (FAO, 2015). The area under lentil cultivation in Iran was 131,000 ha with a production of 83,000 t, during 2015-16 cropping season (Anonymous, 2016). The major reasons for low production of lentil in developing countries are low research priorities and governmental

support compared to cereals and its limited cultivation to margins with low input conditions which are heavily influenced by biotic and abiotic stresses. To overcome these constraints and increase the production, lentil breeding program aims to develop adapted, high yielding, biotic and abiotic tolerant varieties (Sarker and Kumar, 2011).

Studies show that although lentil is one of the oldest domesticated plants, the most lentil cultivated areas are allocated to native populations for example farmers in the south east Anatolia region of Turkey commonly grow landraces (Bicer and Sakar, 2008). The same is true in many regions of Northern Spain (Cristobal *et al.,* 2014) as well as in Iran. This clearly indicates that it has received less attention in lentil breeding programs than other legumes and crops (Cristobal *et al.,* 2014). Research for lentil breeding has been increasing following the FAO/WHO report on poverty and protein deficiency societies in 1965. The efforts of plant breeders have since been focused on increasing the production of this plant. The conservation and sustainable use of plant genetic resources is a prerequisite for tackling the challenges ahead, ensuring food security and reducing poverty (FAO, 2012). Hence, gene banks were established throughout the world with the aim of maintaining a wide range of genetic resources and improving local cultivars with adaptation to adverse conditions and stressful environments which ensures sustainable production through establishing equilibrium in proportion of input to output by reduced need for chemical fertilizers and increased efficiency of water use and nutrient utilization as well as extensive production of alternative crops for farmers through the development of industrial and pharmaceutical plants (Haussmann *et al.,* 2004).

Various studies have been carried out on the evaluation of phenotypic and genotypic variation in different local, regional or international collections of lentil. Barulina (1930) for the first time studied the details of the morphological description of the local lentil species and landraces in Asia and accordingly, reported a wide range of diversity (Sultana *et al.,* 2005). Sultana *et al.* (2005) in studying the morphological diversity of lentil species reported two phenotypic groups for traits such as stem color, pubescence, tendril length, pod pigment, pod dehiscence and cotyledon color. Tahir and Omer (2017) used Agro-morphological and molecular characterization to evaluate the genetic diversity among nine lentil genotypes in Iraq and according to ascending hierarchical clustering and PCA concluded that, nine genotypes were discriminated into three main clusters. They also reported considerable variation (40%) between local and ICARDA populations. Ferguson and Robertson (1999) compared the phenological and agro-morphological traits of 310 accessions from some wild lentil species and observed that *L. culinaris* subs p*orientalis* had more leaves, peduncle, pod and seed per plant than cultivated species. Pouresmael and Ghanavati (2012) reported significant intra- and inter-species variation in lentil accessions of the National Plant Gene Bank of Iran through studying morphological traits diversity such as 100 seed weight, rachis length, stipule shape and pod pigmentation. Cristobal *et al.*(2014) characterized fifteen morpho-agronomical characters of 27 lentil landraces from the region of Castilla and Leon and reported five principal components which explained 83.7% of the cumulative variance. Toklu *et al.* (2009) studied diversity of 23 agro-morphological characteristics in 39 genotypes from the south east Anatolia region of Turkey and seven commercial varieties by using Principal component analysis to determine relationship among traits.

Knowing the genetic diversity of a crop collection on the one hand is useful in selecting suitable parents in a breeding program (Jaradat, 1991), and on the other hand, is valuable for the efficient management of gene banks (Rezaie and Frey, 1990). A broader variation increases the likelihood of finding genes or gene combinations desired by the breeder. Measuring the genetic variation in germplasm is important for determining the economic value and the efficiency of conservation and utilization of genetic reserves. In addition, the characterization and evaluation of accessions is one of the primary activities for the improvement of plants, which helps to identify the potential of stored germplasm and provides a source for future studies (Kameswara and Bramel, 2000).

A total of 990 accessions of Lentil collection of National Plant Gene Bank of Iran were investigated for trend and pattern of genetic diversity of fifteen morphological and phenological characteristic in relationship with geographical distribution in the country. The observed diversity in this study was about 58% of potential diversity. Accessions from different provinces were significantly different for most of the traits (Jafar Aghaei *et al.,* 2005). A total of 302 accessions of the lentil collection of the National Plant Gene Bank of Iran from 13 provinces were evaluated for seed and pod characteristics including number of seed per pod, pod dehiscence, cotyledon color, color pattern on seed testa, background color of testa and pod pigmentation. Pod dehiscence and number of pods per plant showed the highest diversity among the quantitative traits (Saman *et al.,* 2012).

ICARDA comprises the largest and most representative collection of lentil landraces (Ford *et al.,* 2007) so that many recent cultivar releases by national programs are selections from land races in the ICARDA germplasm collection. This research was performed to evaluate morphological variation in some lentil elite genetic resources received from ICARDA and discover the relationship among these populations for their use in conservation and breeding programs to widen the genetic base of germplasm in the country.

MATERIALS AND METHODS

A total of 138 elite genotypes of lentil (Table 1) received from International Center for Agricultural Research in the Dry Areas (ICARDA) were evaluated and characterized in research field of Seed and Plant Improvement Institute in Karaj (31°27′N, 54°53′E at altitude of 1220 m above sea level) during 2015-2016 cropping season. The genotypes were planted on-ridges of two-meter rows with a distance of 60 cm between the rows in an observatory design. Planting was done manually in the second half of March. Total rainfall, seasonal maximum and minimum temperature during cropping seasons (March.-July) are shown in Table 2. During the growing seasons hand weeding was performed and irrigation was done to maintain the soil water content close to 70% field capacity at the depth of 50 cm.

Twenty quantitative and eight quantitative traits (Table 3) including flower color (FC), tendril length (TL), pod pigmentation (PP), pod dehiscence (PD), ground color of testa (TC), testa pattern (TP), color of testa pattern (CPT), cotyledon color (CC), branch number (BN), days to flowering (DF), days to 50% flowering (DF50), days to podding (DP), days to 50% podding (DP50), days to pod filling (DPF), days to maturity (DM), plant height (PH), first pod height (FPH), plant weight (PWE), pod number per plant (PNP), pod weight per plant (PWP), pod length (PL), pod width (PWI), number of seed per pod (NSP), seed number per plant (SNP), seed weight per plant (SWP), 100-seed weight (SW), grain yield (GY) and biomass (BM) were evaluated according to Bioversity international descriptor (IPGRI, 1985). The scale used for scoring qualitative characteristics is shown in Table 3. Five random selected plants from each plot were used for determination of mean of quantitative traits.

Descriptive statistics including range, mean, standard deviation and coefficient of variation were estimated for quantitative traits and median, mode and Shannon index (Shannon and Weaver, 1949) for qualitative traits. The coefficients of correlation for quantitative and qualitative traits were estimated by Pearson and Spearman methods, respectively. To classify the studied germplasm based on quantitative traits, a similarity matrix was produced by calculating the proportion of shared traits in the set of genotypes by propShared function in adegenet package. The similarity values were then inverted and converted to distance values by logarithm function. The resulting distance matrix was used in hierarchical cluster analysis through complete linkage method. The germplasm was also classified by cluster analysis for quantitative traits through complete linkage approach based on Euclidean distances. Correlation between two proximity matrices of genotypes produced by qualitative and quantitative traits was estimated by mantel method and the significance of the coefficient of correlation was tested by using the Monte Carlo simulation based on 999 replications. The method of discriminant analysis of principal components (DAPC) (Jombart *et al.,* 2010) was performed to determine the amount of differentiation among the groups developed by cluster analysis. All statistical analyses were performed by using basic functions or package adegenent in R software v3.4.4.

RESULTS

Qualitative traits

The results of descriptive analysis showed that the violet flower color, prominent tendril, lack of pod pigmentation, pod dehiscence, green ground of testa, absence of testa pattern, absence of color for testa pattern and orange/red cotyledon color were the most frequent characteristics in the studied population based on mode statistics (Table 4, Figure 1). The highest Shannon index (1.33) belonged to ground color of testa and testa pattern (0.85) and color of testa pattern (0.8), respectively. Tendril length had the lowest value of Shannon index (0.31).

The traits color of testa pattern and testa pattern had the highest coefficient of correlation ($r=0.973$, $p<0.01$) (Table 5). Cotyledon color was significantly correlated with flower color ($r=0.197$, $p<0.05$) and ground color of testa ($r=0.603$, $p<0.01$).

Analysis of proximity values of the studied germplasm based on qualitative traits showed that genotypes 1 and 112, as well as 52 and 98 had the highest genetic distances (Figure 2). A large number of genotypes, including 189 pairs, had the least observed genetic distance.

Table 1. ICARDA elite lentil genotypes used in this study.

No.	Pedigree	No.	Pedigree	No.	Pedigree	No.	Pedigree	No.	Pedigree	No.	Pedigree
1	-	26	ILL7537XILL7711	51	ILL4605XILL7982	76	ILL5883XILL8142	101	ILL6434XILL6972	126	ILL5588xILL99
2	ILL7502XLL590	27	ILL7537XILL7711	52	ILL6434XILL8095	77	ILL5883XILL10956	102	ILL6434xILL8072	127	Sel.80S42188xILL223
3	ILL10956XILL5883	28	ILL7537XILL857	53	ILL7940XILL7981	78	ILL7617XILL5883	103	ILL6434XILL8072	128	-
4	ILL7981XLL7706	29	ILL10870XILL8176	54	ILL5562XILL4400	79	ILL7617XILL5883	104	ILL1005XILL6972	129	ILL6209xILL5671
5	ILL6024XLL8009	30	ILL8009XILL5480	55	ILL5562XILL4400	80	ILL7617XILL5883	105	ILL4605XILL1005	130	ILL5582xILL5845
6	ILL4605XLL10872	31	ILL6037xILL7012	56	ILL6199XILL6994	81	ILL7617XILL5883	106	ILL4605XILL1005	131	-
7	L-5125XLL8006	32	ILL6037xILL7012	57	ILL1005XILL9977	82	ILL5883xILL6458	107	ILL323XILL4605	132	ILL6243xILL1939
8	ILL7980XLL7666	33	-	58	ILL5883xILL6458	83	ILL7012xILL6994	108	ILL7938XILL1005	133	ILL7163xILL7554
9	ILL5883XLL7201	34	-	59	ILL7980xILL6994	84	ILL6994xILL9932	109	ILL7938XILL1005	134	ILL5883xILL6994
10	ILL7949XLL8072	35	ILL5582xILL6475	60	ILL323xILL1918	85	ILL8010xILL7983	110	ILL7938XILL1005	135	-
11	ILL6129XLL7980	36	ILL7989xAKM279	61	ILL6037xIILL7012	86	ILL7723xILL5883	111	ILL7938XILL1005	136	-
12	ILL7617xILL5883	37	-	62	ILL6994xILL5725	87	ILL7012xILL6994	112	ILL7938XILL1005	137	ILL7620xILL9836
13	ILL761xILL5883	38	ILL1005xILL6972	63	ILL6994xILL5725	88	ILL6994xILL5725	113	ILL6129XILL1005	138	ILL7620x91517
14	ILL4605XILL6024	39	ILL7502XILL590	64	ILL6994xILL5725	89	ILL5883xL4147	114	ILL8108xILL7938		
15	ILL4605XILL7537	40	ILL6024XILL7686	65	ILL7980xILL6994	90	ILL6447xL 4147	115	ILL10005xILL1005		
16	ILL4606XILL7537	41	ILL7981xILL7706	66	-	91	ILL6212xILL6994	116	ILL9977xILL1005		
17	ILL8008XILL7201	42	ILL4605XILL10872	67	ILL6783XILL98	92	-	117	ILL6434xILL7938		
18	ILL7199XILL7012	43	ILL4605XL-5152	68	ILL8008X 87062	93	ILL6209xILL5671	118	ILL9977xILL1005		
19	ILL7199XILL7012	44	ILL1005XILL9942	69	ILL7620X 91517	94	ILL7985XILL6037	119	ILL9977xILL1005		
20	ILL7199XILL7012	45	ILL1005XILL9942	70	ILL7620 X91517	95	ILL8066XILL6024	120	-		
21	ILL7616XILL7723	46	ILL590XILL6002	71	ILL7620X 91517	96	ILL4605XILL1005	121	UJL197x ILL4400		
22	ILL6002XLL5883	47	ILL6037XILL7981	72	ILL6434XILL6972	97	ILL4605XILL1005	122	ILL466xILL212		
23	ILL6434XLL5883	48	ILL8009XILL7979	73	ILL6434XILL8072	98	ILL323XILL4605	123	ILL4349xILL4605		
24	ILL7934XLL5883	49	ILL7537XILL7982	74	ILL590XILL6002	99	ILL6129XILL1005	124	ILL4349xILL4605		
25	ILL9835XILL7010	50	ILL7537XILL7982	75	ILL7204XILL6994	100	ILL6129XILL1005	125	ILL2129xILL13		

Table 2. Climate parameters during different months of growth period in Karaj in 2015-16.

	Mar.	Apr.	May	June	July
Maen Tem. (°C)	11	15	21	26	28
Min. Tem. (°C)	0.1	0.5	8.2	10	15
Min. Tem. (°C)	22	32	35	39	41
Precipitation (mm)	25	52	13	0	0

Table 3. Scale of scoring qualitative characteristics for the evaluation of ICARDA lentil elite germplasm.

Trait	Scale					
Flower Color (FC)	1 White	2 White with blue veins	3 Blue	4 Violet	5 Pink	6 Others
Tendril Length (TL)	0 Rudimentary	1 Prominent				
Pod Pigmentation (PP)	0 Absent	1 Present				
Pod Dehiscence (PD)	0<10% dehiscence	1>10% dehiscence				
Ground Color of Testa (TC)	1 Green	2 Grey	3 Brown	4 Black	5 Pink	6 Green-Gray
Testa Pattern (TP)	0 Absent	1 Dotted	2 Spotted	3 Marbled		
Color of Testa Pattern (CPT)	0 Absent	1 Olive	2 Grey	3 Brown		
Cotyledon Color(CC)	1 Yellow	2 Orange/ red	3 Olive/ green			

Table 4. Descriptive statistics for qualitative traits for the evaluation of ICARDA lentil elite germplasm.

Trait	Minimum	Maximum	Median	Mode	Shannon Index
FC	1	5	4	4	0.79
TL	0	1	1	1	0.31
PP	0	1	0	0	0.53
PD	0	1	1	1	0.58
TC	1	6	2	1	1.32
TP	0	3	0	0	0.85
CPT	0	4	0	0	0.80
CC	1	3	2	2	0.59

FC: Flower Color, TL: Tendril Length, PP: Pod Pigmentation, PD: Pod Dehiscence, TC: Ground Color of Testa, TP: Testa Pattern, CPT: Color of Testa Pattern, CC: Cotyledon Color.

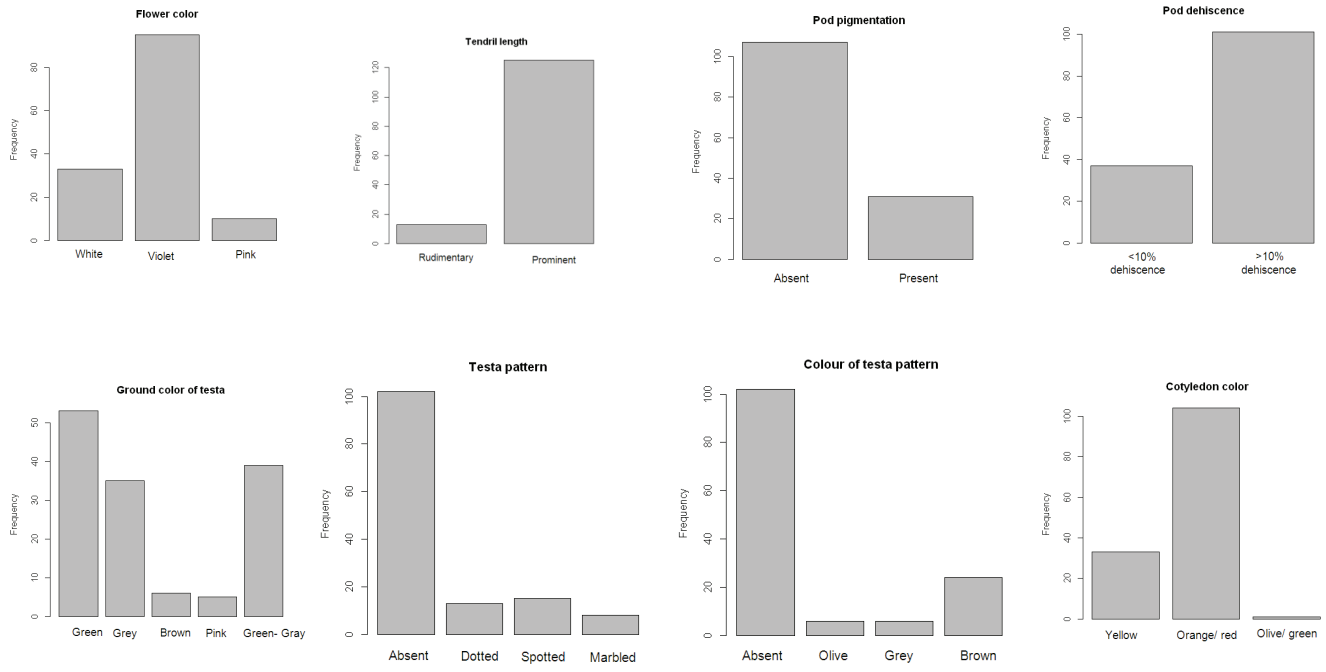

Figure 1. Frequency distribution of qualitative traits measured in lentil elite germplasm of ICARDA.

Table 5. Spearman coefficients of correlation among qualitative traits for the evaluation of ICARDA lentil elite germplasm.

Trait	TL	PP	PD	TC	TP	CPT	CC
FC	-0.048	0.064	0.055	0.138	0.054	0.152	0.197*
TL		-0.064	-0.083	0.01	0.008	0.024	0.112
PP			0.169*	0.06	-0.012	0.012	0.09
PD				0.157	-0.036	-0.003	0.089
TC					0.115	0.157	0.603**
TP						0.973**	0.104
CPT							0.158

*: Correlation is significant at the 0.05 level.
**: Correlation is significant at the 0.01 level.
FC: Flower Color, TL: Tendril Length, PP: Pod Pigmentation, PD: Pod Dehiscence, TC:Ground Color of Testa, TP:Testa Pattern, CPT: Color of Testa Pattern, CC: Cotyledon Color.

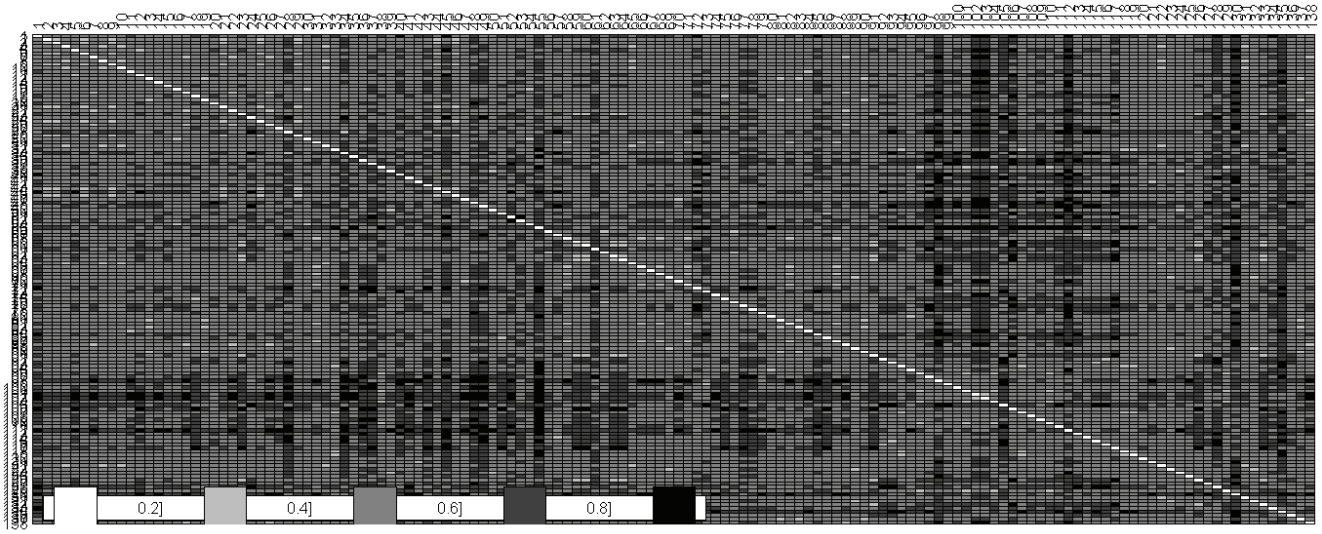

Figure 2. Heat map of distances among lentil elite germplasm of ICARDA based on qualitative traits.

The studied genotypes were located in nine groups by cluster analysis (Figure 3). Group 1 included the genotypes 1, 2, 4, 7, 18, 24, 30, 31, 45, 59, 77, 81, 85, 99, 100, 107, 112, 113, 127, 130 and 135. This group had the highest variation for the traits flower color, testa pattern and color of testa pattern. Genotypes 3, 27, 37, 47, 64, 83, 84, 87, 94, 96, 116, 117, 120, 123, 131, 134 and 136 were located in group 2. The trait pod dehiscence showed the highest variation in this group along with group 6. Group 3 comprised of genotypes 5, 6, 13, 14, 15, 20, 34, 35, 39, 40, 42, 43, 48, 49, 54, 56, 57, 63, 73, 80, 82, 86, 89, 93, 97, 101, 102 and 118. This group ranked second in terms of variation in the trait pod pigmentation after group 6 and in the trait color of testa pattern after group 1. Group 4, containing genotypes 8, 9, 12, 26, 50, 60, 67, 70, 78, 90, 103, 114, 115, 129 and 132 lacked a particular feature in terms of the highest or lowest variation of the measured traits, therefore it was intermediately situated among other groups. Genotypes 10, 19, 21, 22, 41, 51, 66, 69, 105, 108, 109, 111, 119, 124 and 126 were situated in group 5 which had the highest and lowest variation of the traits cotyledon color and pod pigmentation, respectively. Twenty genotypes including 11, 16, 25, 28, 32, 36, 38, 55, 61, 62, 71, 72, 74, 75, 79, 92, 106, 121, 128 and 137 were classified in group 6 which had the highest variation in the traits tendril length, pod pigmentation and pod dehiscence (along with group2). Group 7 consisting of genotypes 17, 23, 29, 33, 52, 53, 76, 95, 98 and 125 had the highest variation of the trait ground color of testa. Group 8 with genotypes 44, 58, 65, 88, 91 and 104 and Group 9 with genotypes 46, 68, 110, 122, 133 and 138 were the least variant groups for the traits flower color, tendril length, pod pigmentation, pod dehiscence, ground color of testa, testa pattern, color of testa pattern and cotyledon color. In fact these two groups lacked variation in the trait tendril length which could be considered as their particular feature.

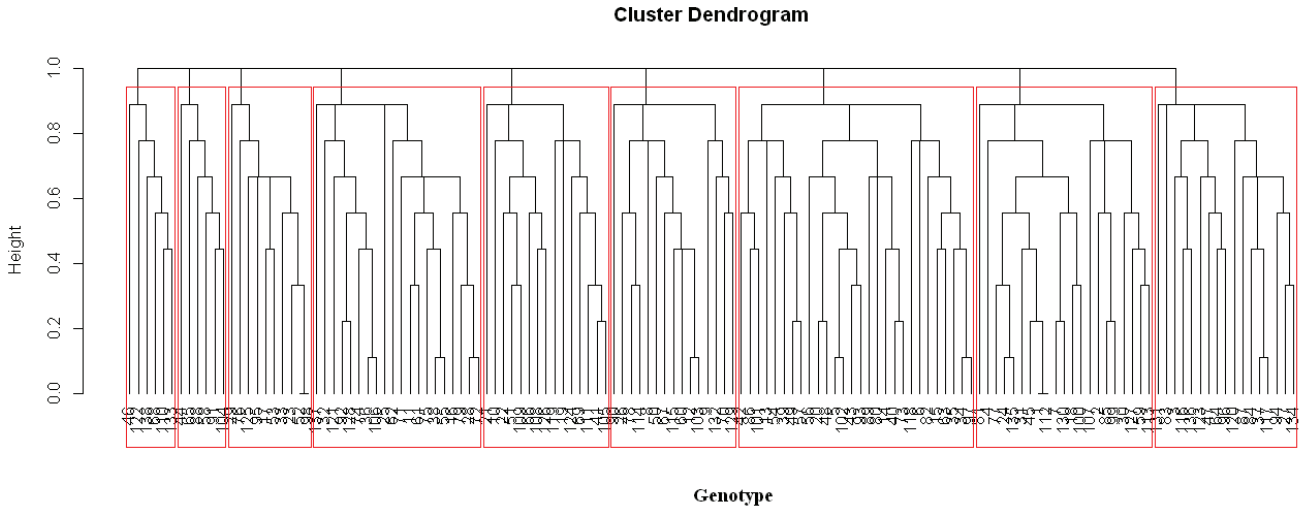

Figure 3. Dendrogarm of cluster analysis of ICARDA lentil elite germplasm based on qualitative traits.

Table 6. Distribution features of quantitative traits measured in ICARDA lentil elite germplasm.

Trait	Range	Minimum	Maximum	Mean	Coefficient of variation (%)
DF	14	52	66	58.25	5.87
DF50	17	52	69	62.84	4.75
DP	12	61	73	66.46	4.12
DP50	15	62	77	70.62	3.83
DPF	16	71	87	76.91	3.80
DM	22	81	103	90.43	4.83
PH (cm)	16.33	16.67	33	25.69	11.96
FPH (cm)	11	5	16	9.49	24.17
PWE (g)	5.10	0.50	5.6	1.79	46.67
PNP	13.36	1.42	14.78	7.23	37.11
PWP (g)	2.68	0.025	2.7	0.68	72.55
PL (mm)	8.33	7.33	15.67	10.94	12.17
PWI (mm)	4.0	5.0	9.0	6.53	11.41
NSP	3.33	1	4.33	1.28	29.89
SNP	41	0.67	41.67	13.48	66.20
SWP (g)	6.33	0	6.33	0.55	111.23
SW (g)	6.38	0.21	6.59	3.77	35.31
GY (gm^{-2})	49.76	0.65	50.41	12.44	81.58
BMS (gm^{-2})	160.71	14.29	175	73.32	35.95

BN: branch number, DF: days to flowering,DF50: days to 50% flowering, DP: days to podding, DP50: days to 50% podding, DPF: days to pod filling, DM: days to maturity, PH: plant height, FPH: first pod height, PWE: plant weight, PNP: pod number per plant, PWP: pod weight per plant, PL: pod length, PWI: pod width, NSP: number of seed per pod, SNP: seed number per plant, SWP: seed weight per plant, SW: 100- seed weight, GY: grain yield, BMS: biomass.

Quantitative traits

The level of diversity in the studied germplasm for the quantitative traits was measured by coefficient of variation (Table 6). The results indicated that the traits seed weight per plant (CV=111.23%), grain yield (CV=81.58%) and pod weight per plant (CV=72.55%) had the highest coefficient of variation. The lowest CV belonged to the traits day to pod filling (CV=3.80%) and days to 50% podding (CV=3.83%).

Genotypes 102, 92, 108, 112, 99, 23, 99, 94 and 100 had the highest number of traits branch number, days to pod filling, days to maturity, first pod height, pod width, pod number per plant, pod weight per plant, pod length and pod width, respectively. The lowest number of traits branch number, days to 50% flowering, days to maturity, plant height, pod width, pod number per plant,

pod length, seed number per plant, 100-seed weight and grain yield belonged to genotypes 133, 62, 69, 37, 123, 20, 65, 108, 41 and 122, respectively. Grain yield of at least 5% of the whole genetic materials under study was higher than 32.42 (gm^{-2}). Almost half of the total germplasm had a height higher than 25 cm. Genotypes with 100-seed weight higher than 6.15 g comprised of approximately 5% of the population.

The highest coefficient of correlation ($r=0.868$, $p<0.01$) was observed between the traits pod weight per plant and plant weight (Table 7). The traits plant weight and seed number per plant ($r=0.722$, $p<0.01$), biomass and grain yield ($r=0.718$, $p<0.01$), and days to podding and days to flowering ($r=0.714$, $p<0.01$) also showed high significant positive correlations. Grain yield showed significant positive correlations with branch number, plant weight, pod number per plant, pod weight per plant, pod length, pod width, seed number per plant, seed weight per plant and 100-seed weight.

Analysis of genetic distances based on quantitative traits showed that genotypes 6 and 114 had the highest similarity and genotypes 42 and 130 possessed the highest genetic distance (Figure 4).

Four different groups of genotypes were separated by the dendrogarm of cluster analysis (Figure 5). The average values of these groups for the measured traits are presented in Table 8. Group1 included genotypes 1, 2, 3, 4, 5, 6, 7, 10, 11, 13, 14, 15, 18, 21, 22, 24, 25, 26,

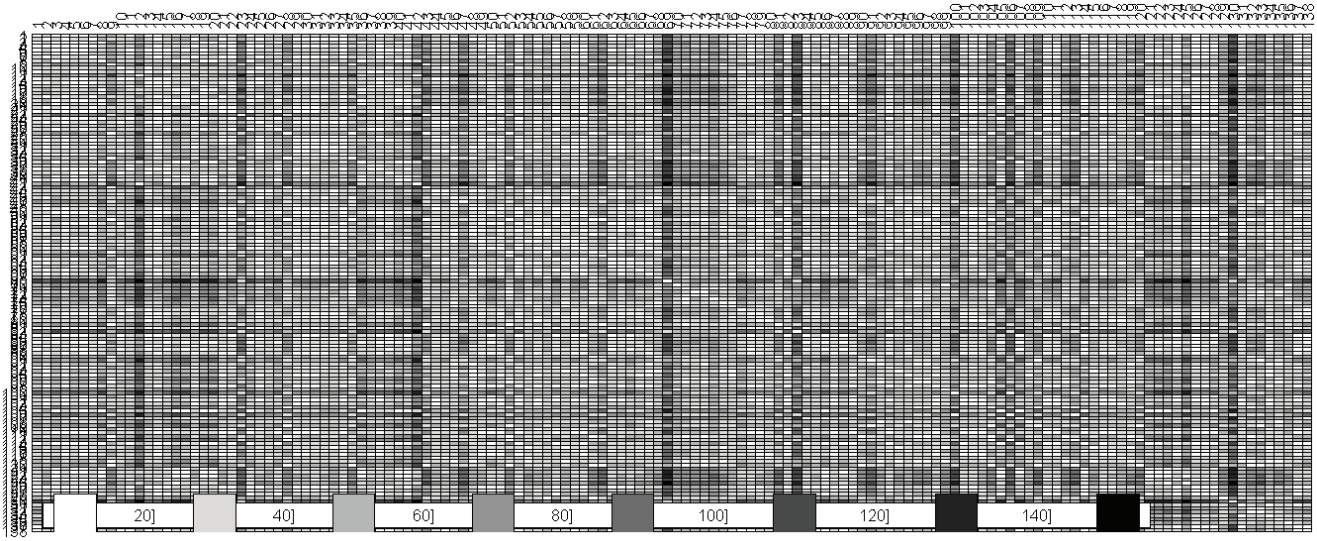

Figure 4. Heat map of Euclidean distances among lentil elite germplasm of ICARDA based on quantitative traits.

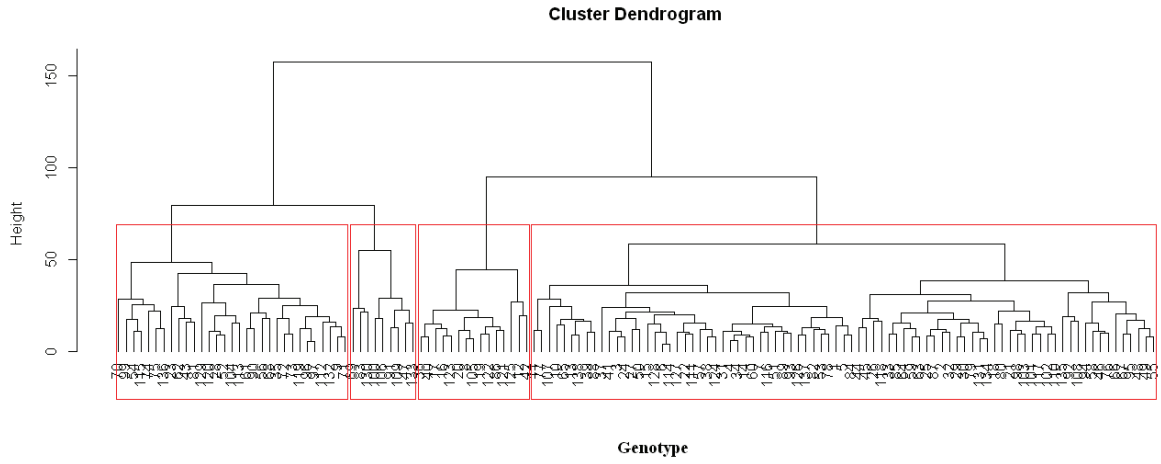

Figure 5. Dendrogram of cluster analysis of ICARDA lentil elite germplasm based on quantitative traits.

Table 7. Pearson coefficients of correlation among quantitative traits for the evaluation of ICARDA elite lentil germplasm.

Trait	DF	DF50	DP	DP50	DPF	DM	PH	FPH	PWE	PNP	PWP	PL	PWI	NSP	SNP	SWP	SW	GY	BMS
BN	.218*	.213*	.296**	.326**	.282**	.244**	0.016	.230**	.550**	-0.12	.340**	0.114	.223**	-0.078	.254**	.171*	.365**	.208*	.202*
DF		.635**	.714**	.496**	.390**	.496**	-0.095	.238**	0.147	-0.149	0.032	-0.059	0.119	-.239**	-0.116	-0.006	.245**	0.077	0.046
DF50			.659**	.478**	.364**	.447**	-0.065	.233**	0.063	-.248**	-0.137	-0.065	0.091	-.237**	-.184*	-0.095	.196*	-0.059	-0.021
DP				.590**	.460**	.552**	-0.123	.268**	0.136	-.201*	-0.036	-0.03	0.161	-.304**	-0.128	-0.02	.258**	0.052	0.049
DP50					.527**	.464**	-0.057	.218*	.199*	-0.079	0.068	-0.083	0.076	-0.112	-0.035	0.037	.290**	0.025	0.094
DPF						.592**	0.053	.252**	.256**	-.170*	0.093	0.007	.202*	-.244**	-0.057	0.042	.431**	0.048	0.099
DM							0.068	.288**	.203*	-.328**	-0.024	-0.031	0.143	-.279**	-.186*	0	.299**	-0.084	0.122
PH								.386**	.378**	-0.161	.269**	0.151	.282**	-0.065	0.139	.204*	.391**	0.131	.319**
FPH									.402**	-.292**	.203*	.249**	.324**	-.230**	0.031	.196*	.453**	0.113	.196*
PW1										0.087	.868**	.322**	.403**	-0.003	.722**	.421**	.537**	.407**	.407**
PNP											.409**	-0.008	-0.153	.217*	.625**	0.151	-0.167	.254**	.208*
PWP												.307**	.283**	0.101	.890**	.490**	.358**	.436**	.377**
PL													.594**	0.041	.235**	.401**	.347**	.212*	.225**
PW2														-0.141	0.155	.236**	.455**	.324**	.303**
NSP															.258**	0.007	-0.162	0.08	0.098
SNP																.443**	0.151	.472**	.392**
SWP																	.253**	.210*	.210*
SW																		.387**	.415**
GY																			.718**

*: Correlation is significant at the 0.05 level.
**: Correlation is significant at the 0.01 level.
BN: branch number, DF: days to flowering,DF50: days to 50% flowering, DP: days to podding, DP50: days to 50% podding, DPF: days to pod filling, DM: days to maturity, PH: plant height, FPH: first pod height, PWE: plant weight, PNP: pod number per plant, PWP: pod weight per plant, PL: pod length, PWI: pod width, NSP: number of seed per pod, SNP: seed number per plant, SWP: seed weight per plant, SW: 100- seed weight, GY: grain yield, BMS: biomass.

27, 29, 30, 31, 32, 33, 34, 37, 38, 39, 41, 44, 45, 46, 48, 49, 50, 51, 53, 55, 57, 58, 59, 60, 63, 64, 65, 66, 67, 76, 77, 78, 79, 80, 82, 84, 85, 87, 88, 89, 92, 93, 94, 95, 98, 101, 102, 103, 107, 108, 110, 111, 114, 116, 117, 118, 124, 126, 127, 128, 131, 134, 135, 137 and 138 and had the highest mean of days to flowering and days to 50% flowering. Group 2 comprised of genotypes 8, 12, 16, 17, 19, 20, 36, 40, 42, 86, 105, 121, 122, 123 and 125 with the lowest mean of all the measured quantitative traits. Thirty-one accessions including genotypes 9, 23, 28, 35, 43, 52, 54, 56, 61, 62, 68, 70, 71, 72, 73, 74, 75, 81, 90, 96, 97, 99, 104, 112, 113, 115, 119, 120, 129, 132 and 136 were located in group 3. This group had the highest mean for the traits branch number, day to 50% podding,days to pod filling, plant height, plant weight, pod number per plant, pod weight per plant, number of seed per pod, seed number per plant, seed weight per plant and 100-seed weight. Group 4 comprised of genotypes 47, 69, 83, 91, 100, 106, 109, 130 and 133 and possessed the highest mean of days to podding, days to maturity, first pod height, pod length, pod width, grain yield and biomass.

Correlation between two proximity matrices of genotypes produced by qualitative and quantitative traits was estimated by mantel method and the significance of the coefficient of correlation was tested by the Monte Carlo simulation based on 999 replications. Monte Carlo simulation produced a reference distribution to test the significance of correlation between two proximity matrices of genotypes which had been calculated separately for quantitative and qualitative traits (Figure 6). The results indicated that the regarding coefficient of correlation (0.04) was small and non-significant.

Results of DAPC analysis successfully differentiated the quadruple groups of genotypes (Figure 7). The membership probabilities of genotypes were investigated and it became clear that a total of 112 genotypes were assigned to one certain group with a probability higher than 0.9 (Figure 8).

Relatedness of the genotypes was investigated by comparison of their pedigree and genetic distances. For this purpose, the value distribution of genetic distances (Figure 9) was divided into four parts based on the quartiles of the distribution, defining four ranges of A, B, C and D which were attributed to values of genetic distances lower than the first quartile, higher than the first quartile and lower than the second quartile (median), higher than the second quartile and lower than the third quartile and higher than the third quartile, respectively. The distribution

Table 8. Average values of quantitative traits in different groups of ICARDA lentil elite germplasm formed by cluster analysis.

Trait	Cluster			
	1	2	3	4
BN	8.06	7.18	9.30	8.33
DF	58.39	57.40	58.32	58.11
DF50	63.30	61.60	62.16	63.00
DP	66.60	65.80	66.26	67.00
DP50	70.65	69.80	71.00	70.33
DPF	76.98	75.87	77.23	76.89
DM	91.06	87.93	89.32	92.56
PH (cm)	25.53	23.40	26.95	26.67
FPH (cm)	9.34	8.67	9.97	10.61
PWI (mm)	1.66	1.02	2.46	2.06
PNP	7.05	6.12	8.16	7.57
PWP (g)	0.59	0.30	1.07	0.82
PL (mm)	10.90	10.13	11.25	11.58
PWE (g)	6.41	6.26	6.81	7.16
NSP	1.28	1.16	1.38	1.20
SNP	11.81	6.37	20.52	16.41
SWP (g)	0.53	0.21	0.78	0.63
SW (g)	3.69	2.27	4.55	4.36
GY (gm^{-2})	8.44	3.10	23.72	25.92
BMS(gm^{-2})	63.90	28.70	96.63	128.79

BN: branch number, DF: day to flowering,DF50: day to 50% flowering, DP: day to podding, DP50: days to 50% podding, DPF: days to pod filling, DM: days to maturity, PH: plant height, FPH: first pod height, PWE: plant weight, PNP: pod number per plant, PWP: pod weight per plant, PL: pod length, PWI: pod width, NSP: number of seed per pod, SNP: seed number per plant, SWP: seed weight per plant, SW: 100- seed weight, GY: grain yield, BMS: biomass.

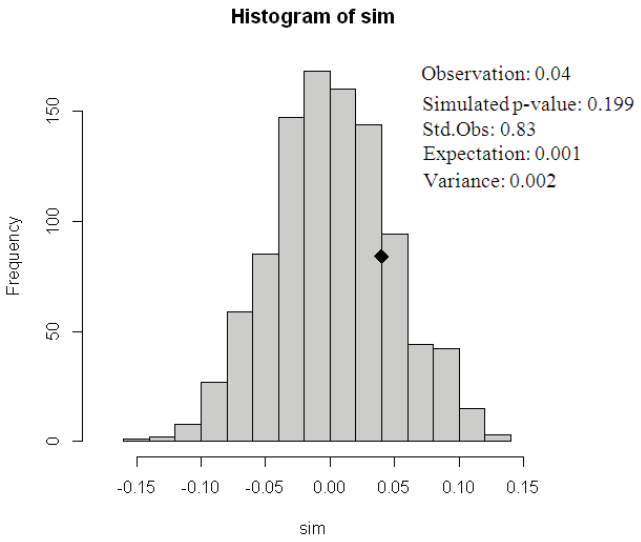

Figure 6. Reference distribution produced through the Monte Carlo simulation based on 999 replications to test the significance of correlation between two proximity matrices of quantitative and qualitative traits measured in ICARDA lentil elite germplasm.

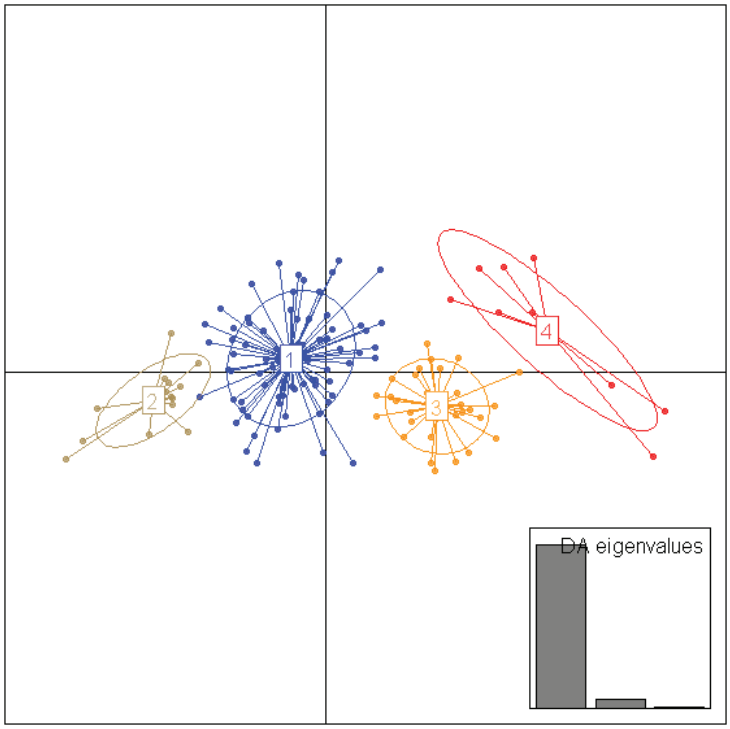

Figure 7. Distribution of ICARDA elite lentil germplasm in biplot of the first two discriminant functions developed by DAPC analysis.

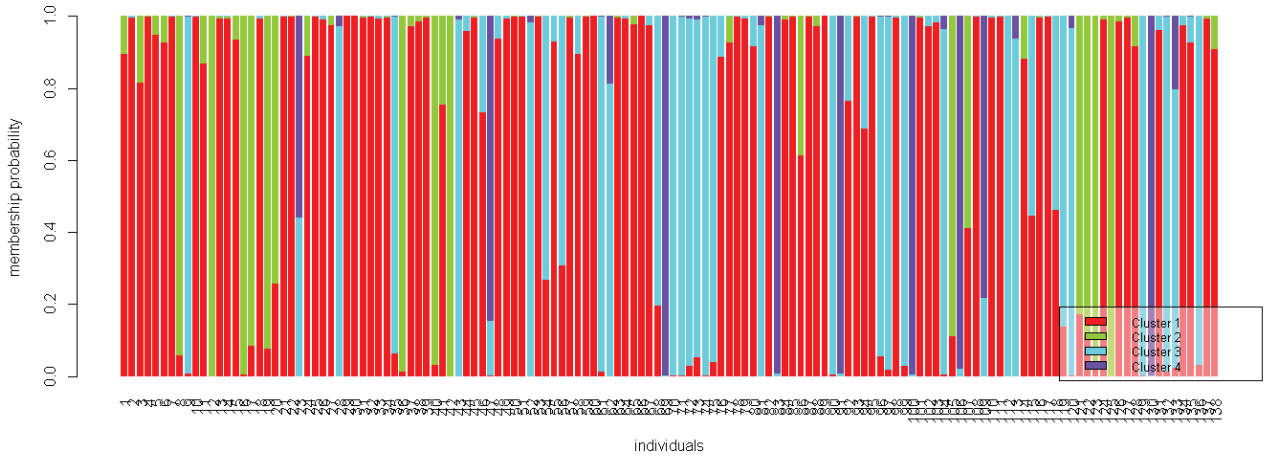

Figure 8. Membership probability of ICARDA lentil elite germplasm to the groups developed by cluster analysis.

of genetic distances showed positive skewness and kurtosis, however test of deviation from normality was non-significant. Genotypes 69,70, 71 and 138 shared the common parents ILL7620 and 91517. While two values of genetic distances including values for genotypes 70 and 71 (26.75) and for genotypes 70 and 138 (47.30) were in range B and C respectively, four values including genetic distances between 69 and 70 (68.18), between 69 and 71 (56.24), between 69 and 138 (106.64) and between 71 and 138 (54.08) were in range D.

The parents ILL4605 and ILL1005 were shared commonly by genotypes 96, 97, 105 and 106. For this set of genotypes, the only value of genetic distance in range A was for genotypes 96 and 97 (5.92), whereas the values for genotypes 96 and 106 (36.03) and between 97 and 106 (35.92) were in range C and the values for genotypes 96 and 105 (61.45), for genotypes 97 and 105 (62.00) and for genotypes 105 and 106 (95.10) were in range D.

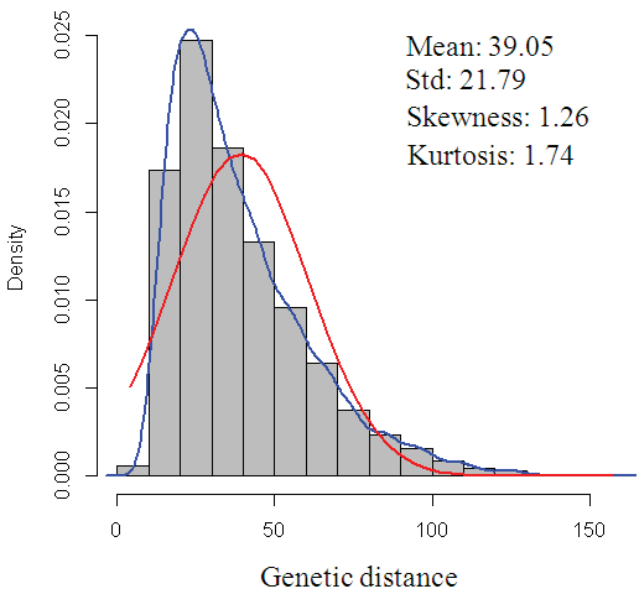

Figure 9. Distribution of genetic distance among ICARDA lentil elite germplasm.

The genotypes 108, 109, 110, 111 and 112 had similar pedigrees, comprising of the lines ILL7938 and ILL1005 as parents. Among genetic distances for these genotypes, only the value for genotypes 110 and 111 (9.87) was in range A. The other genetic distances in this set of genotypes were located in higher ranges so that values for genotypes 108 and 110 (29.71), 108 and 111 (29.24) and 109 and 112 (25.00) were in range B, for genotypes 108 and 109 (39.93), 108 and 112 (36.80), 110 and 112 (41.83) and 111 and 112 (44.90), were in range C and for genotypes 109 and 110 (56.38) and 109 and 111 (58.61) were in range D.

The lines ILL6994 and ILL5725 were commonly present in the pedigrees of genotypes 62, 63, 64 and 88. Genetic distances among these genotypes tended towards the extreme ranges of A and D, with two values of 12.55 (for genotypes 63 and 64) and 18.27 (for genotypes 63 and 68) in range A and two values of 56.73 (for genotypes 62 and 63) and 72.67 (for genotypes 62 and 88) in range D. Genetic distance of genotypes 64 and 88 (26.65) and that of genotypes 62 and 64 (49.28), were in range B and C, respectively.

The genotypes 78, 79, 80 and81 had also parental communality with the lines ILL7617 and ILL5883 in their pedigrees. This set of genotypes were the most genetically related groups among the studied germplasm with common parents as three values of regarding genetic distances were in range A. In fact, the values for genotypes 78 and 79 (13.66), 78 and 80 (22.39), and 79 and 80 (16.31) were lower than the first quartile of the total values of genetic distances. Furthermore, genetic distances of genotypes 79 and 81 (45.17), and 80 and 81 (45.74) were in range C and that for genotypes 78 and 81 (54.19) were in range D.

DISCUSSION

The total results of evaluations performed in this study including range of the measured traits, differences among genotypes and diversity indices indicated that a high level of variation exists in the studied germplasm which reveals the great potential of this genetic material in future breeding programs (Roy *et al.,* 2013). The comparison of the descriptive statistics of traits estimated in the present study with similar research studies shows improvement in most of these characteristics. For instance, the range, maximum and mean of the traits seed weight per plant and 100-seed weight was higher than the observations of Roy *et al.* (2013) or the mean of 100-seed weight was higher than the evaluations reported by Kumar (2015) and Sultana *et al.* (2010). These findings could be the achievement of the breeding activities which have been previously performed on the studied germplasm and could be of considerable value since in lentil, few genotypes have been used frequently in breeding program in order to develop improved cultivars (Kumar, 2015).

In the present study two statistics of Shannon index and coefficient of variation were used to evaluate the level of diversity within the germplasm under investigation for qualitative and quantitative traits. The traits ground color of testa and testa pattern and color of testa pattern had the highest variation among qualitative characteristics; therefore, these traits had the major role in differentiating the genotypes. In previous studies, on qualitative traits, the highest Shannon index was reported for the stipule shape, pod pigment, and arista (Pouresmael and Ghanavati, 2012) .Also, a high level of diversity was reported for pod pigmentation and cotyledon color in Iranian lentil germplasm (Saman *et al.,* 2012). In the study of 29 released Indian lentil cultivars by Dixit *et al.* (2011) a considerable variability was observed for leaflet size, whereas seed testa color had very low variation. Therefore, it seems that there is not a complete agreement between different studies in terms of the type of qualitative traits with the highest power of differentiation among lentil genotypes so that different traits with this potential are identified depending on the germplasm under study.

A high variation was also observed in some quantitative traits such as seed weight per plant, grain yield, pod weight per plant and seed number per plant.

This result is in line with the results of Toklu *et al* .(2009) who reported that the biological yield, pod weight per plant and pod number per plant and seed weight per plant showed the highest variation in the studied genotypes. Gaad *et al.* (2018) also reported pod and seed number per plant and seed yield per plant as more divergent traits in lentil accession of Algeria. Saman et al. (2012) reported that the number of pods per plant appeared to be the major differentiating trait among Iranian lentil germplasm. These traits are all related to grain yield. Therefore, the highest variation in the quantitative traits could be attributed to grain yield and its components. This feature is an advantage that provides an opportunity for breeder to improve grain yield by manipulating various components. Mekonnen *et al.* (2014) reported that genotypes introduced from ICARDA showed rich genetic bases for 100-seed weight, number of seeds per plant, seed weight per plant, resistance source for rust, and high yield in high yielding environment, where rainfall is not a major problem.

Phenological traits were also of considerable ranges. There was a 22 day difference, for instance, in maturity time of the earliest and the latest genotypes. Considering that lentil is mainly cultivated on marginal lands with unfavorable weather conditions, the earliness could be useful in avoidance of genotype from unfavorable circumstances especially at the end of the crop season. The highest value for Shannon-Weaver Index was determined in plant height, 100 seed weight and date of maturity in Iranian lentil germplasm by Jafar Aghaeei *et al.* (2005). Tahir and Omer (2017) also reported a high variability in number of days to 50% flowering, maturity and grain filling period in Iraq lentil germplasm.

In the present study six types of phenological traits including DF, DF50, DP, DP50, DPF and DM were evaluated in the germplasm under study. Although none of these traits showed significant correlation with grain yield, the group of genotypes with highest mean of grain yield formed by cluster analysis (group 4) had the highest mean of days to podding (DP) and days to maturity (DM) as well. These results indicate that these two traits could have a greater role than the other phenological traits in increasing the grain yield at least in the genotypes of this group (genotypes 47, 69, 83, 91, 100, 106, 109, 130 and 133). On the other hand group 4 with the highest mean for grain yield among other groups did not have the highest mean for the traits known as components of yield such as pod number per plant, pod weight per plant, number of seed per pod, seed number per plant, seed weight per plant or 100-seed weight, while group 3 was superior for these features. Therefore, it seems that some components of yield other than these traits contribute to higher grain yield of the genotypes of this group which would be interesting to be considered in future studies. On the other hand, members of these two groups (group 3 and 4) could be candidates for hybridization since, as it was above mentioned, each group alternatively comprises superior traits.

The results of conformity analysis of two distance matrices calculated by each set of qualitative and quantitative characteristics and investigated by Mantel test indicated that these two types of germplasm classification target different aspect of population diversity which in turn could be important. However, in this study qualitative traits were not as successful in separation of germplasm groups as quantitative features. In other words by using the approach of discriminant analysis of principal component, it was evident that cluster analysis based on quantitative characteristics successfully divided genotypes into different groups. The separation of genotypes into different groups could be another evidence of incidence of genetic diversity. In the study of 110 lentil germplasm, Roty *et al.* (2013) observed that the accessions segregated lentil accessions into six clusters by UPGMA dendrogarm and a high diversity was revealed in the accession from ICARDA gene bank. The classification of 317 accessions of lentil gave rise to 12 clusters with varying degrees of intercluster dissimilarity suggesting the selection of diverse superior parents for hybridization (Sultana *et al.,* 2010).

Parental communality is generally expected to result in higher levels of resemblance. The frequency distribution curve of total genetic distances showed positive skewness (towards lower distances) which is in accordance with this expectation as it indicates the presence of some degree of relatedness. However, it was also observed that groups of genotypes which shared common parents in their pedigree showed different levels of proximities so that some sets had higher level of similarity than the others. This observation could be attributed to different modes of genetic control in various parental lines and presence of different types of genetic effects such as epistasis and transgressive segregation.

CONCLUSSION

The germplasm under study showed a high level of genetic diversity and is a valuable genetic source in advanced breeding programs. Grain yield, seed weight

per plant, pod weight per plant and seed number per plant could be suggested as suitable criteria to be utilized for maximum differentiation in lentil population. Genotypes with high genetic distances with complementary features could be used for hybridization with the aim of new gene combination and selecting lines with higher performance. High yielding genotypes could be candidates for high yield potential in favorable conditions. The early matured lines could be tested in adverse environments in search of genetic resources with capability of completion their life cycle before confronting difficult conditions through mechanisms such as escape.

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