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Full Length Research Paper

Principal Component Analysis of Indigenous Lentil (*Lens culinaris*) Germplasm Collection

Nadiya A. Al-Saady[1], Saleem K. Nadaf*[1], Ali H. Al-Lawati[1] and Saleh A. Al-Hinai[2],

[1]Oman Animal & Plant Genetics Resources Center, The Research Council, PO Box 92, PC 123, Sultan Qaboos University Campus, Sultanate of Oman.

[2]Directorate General of Agriculture & Livestock Research, Ministry of Agriculture, PO Box 50 PC 121, Al-Seeb, Sultanate of Oman

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This paper presents the results of collecting missions undertaken from 2008 to 2011 and assessment of seed diversity of indigenous lentil (*Lens culinaris*) germplasm in Oman. 11 seed samples/accessions of lentil were collected of which Al-Dhahira & Buraimi governorates had the highest (6), followed by South Batinah-coastal governorate represented by mountains of Rustaq (3) and Al-Dakhliya (Interior) governorate (2) with no contribution from other agriculturally important Sharqiya (eastern), North Batinah and Southern Dhofar governorates. Seed accessions were diverse with respect to four seed traits investigated like seed length (cm) and width (cm), 1000-seed weight (g) and seed color. Seed length varied from 0.33 cm to 0.45 cm; seed width ranged from 0.320 cm to 0.405 cm and 1000-seed weight ranged from 22.5 g to 32.5 g. With respect to seed color, the accessions were classified into five groups based on simple to complexity of color combinations with scores from 1-5 with varying frequencies / numbers. These accessions were grouped into 4 genetically diverse clusters corresponding to their spread in four quadrants of the biplot graph based on the Principal Component Analysis (PCA) using seed traits which showed substantial contribution of seed length (38.095 %) followed by seed width (32.930%) and 1000-seed weight (28.733%), with the least contribution by seed color (0.242%) to the first principal component (PC1 or factor 1). Of the six-character combinations, only two correlation coefficients viz. between seed length and seed width (0.864*) and between seed length and seed weight (0.710*), were significant (p<0.05).

Keywords: Diversity, collections, seed characters, seed color, lentil

INTRODUCTION

The Sultanate of Oman is located in the characteristic location of the globe which has its northern part represent Asian countries and its southern part, to the African continent in physiography, climate, and culture. It is considered as the second largest country in the Arabian Peninsula with 85473.10 ha of agricultural land under cultivation (MAF, 2017) of which fruits occupy 36.11% followed by perennial and annual fodder crop species (39.40%), vegetables (19.72%) and field crops (4.77%). Of the field crops, lentil (*Lens culinaris*) is one among the few farmers who grow it these days in Oman for both food and green fodder (MAF,

*Corresponding Author's Email: saleem_nadaf@hotmail.com

2005). Lentil (Lens culinaris) is a self-pollinated crop and member of Fabaceae family with low percentage of natural outcrossing. The domestication of lentil occurred, along with that of emmer and einkorn wheats, barley, pea, chickpea, bitter vetch and flax, during the Neolithic Agricultural Revolution in the Eastern Mediterranean around the 8th and 7th millennia BC (Zohary and Hopf, 1973). Later, lentil spread rapidly to the Nile Valley, Europe and Central Asia. Lentil was introduced to South America via Chile by Spanish traders after 1500 AD (Solh and Erskine, 1984). It is under cultivation in Mexico, Canada, the USA, New Zealand and Australia, only recently. Lentil occupies fourth rank in pulses followed by bean (Phaseolus vulgaris), pea (Pisum sativum) and chickpea (Cicer arietinum) in the world (FAO 2016). Lentil is used for human consumption as food and green or dry fodder for livestock as it is rich in protein, carbohydrates, multivitamins, antioxidant, fibre etc. Being leguminous, it helps in improving fertility of cultivated land.

Important yield related quantitative characters are often useful to breeder in selection during crop improvement where genetic variability of quantitative traits of economic importance is exploited with the information on correlation between yield and its contributing characters as a guideline for better selection of quantitative traits (Hamdi et al., 1991; Younis et al., 2008).

In lentil, seed size is considered as an important trait as it directly influences productivity (Turk *et al.,* 2004; Ghassemi-Golezoni *et al.,* 2014; Gaad *et al.,* 2018) as larger seed cultivars had higher seed yield owing larger plants with increased expressions of yield and yield related components. There are varying reports of the seed sizes in lentil in terms of test weight per 1000 seeds in the range of 30 - 73g (Turk *et al.,* 2004; Ghassemi-Golezoni *et al.,* 2014; Gaad *et al.,* 2018; Noor *et al.,* 2017) on one hand and in the range 8.5 to 55.8 g on the other (Alam et al., 2011; Ahamed *et al*., 2014; Singh *et al.,* 2017; Mehra et al., 2018). In this respect, the consumer market in general shows preference for a seed from medium to large (30 to 60 g/1000-seed weight).

Genetic diversity in the crop species has foremost significance in improvement and development of effective conservation strategies (Hodgkin, 1997; Gaad *et al.,* 2018). The available information of genetic diversity concerning the indigenous germplasm is very useful in the efficient use of genetic variations in breeding programs through appropriate selection of parents for crossing. There are several studies undertaken on genetic diversity of lentil populations only recently using morphological traits (Toklu *et al.,* 2009; Singh *et al.,* 2017) or using both morphological and yield characters, and molecular markers (Erdogan, 2015).

In Oman, large number of indigenous ecotypes of field crops including that of lentil are grown mainly for food and fodder across the country due to diverse agro-climatic conditions. However, due to changing land use patterns and the gradual shift in cultivation of high-yielding commercial crops, the indigenous germplasm of several field crops including lentil is slowly getting extinct. Several collecting missions were carried out in Oman either independently or jointly with national and international organizations to collect and conserve germplasm of the most crops grown in the country (AlSaady *et al.,* 2014 and 2018a, 2018b). A series of joint collection missions between the Sultan Qaboos University and the Ministry of Agriculture & Fisheries of Oman were undertaken from the sites across all the governorates of Oman between 2008 and 2011 with an objective to conserve indigenous legume germplasm available in Oman. This paper presents the results of collecting missions of indigenous germplasm of lentil and its diversity in respect of four seed traits of marketing significance.

MATERIALS AND METHODS

Seven exploration trips were undertaken from April 2008 to March 2011 across all the Governorates of Oman involving the staff of Agriculture Development Centers of the Ministry of Agriculture and Fisheries according to the standard method (IPGRI, 1995; Hay and Probert, 2011; AlSaady *et al.,* 2014) for the collection of seed samples of indigenous germplasm of alfalfa and food legumes like chickpea, faba bean, cowpea, lentil, field pea, mung bean and pigeon pea, and the medicinal legume fenugreek. The seed samples were collected from individual farmers, farmers-fields and stores, and Agriculture Development Centers along with the information on the crop passport data and site descriptions including GPS data, electrical conductivity and pH of soil and water samples. In case of lentil, the sites covered during the trips were from non-coastal areas with the altitude ranging from 476 m to 1680 m. In all, 156 collecting sites were visited. 11 Indigenous lentil accessions were collected from 11 sites (Table 1). Seed traits such as seed length and width (cm) and test weight (1000 seeds) were measured, and seed color was determined on visual basis according to Dijkstra and van Soest (1986). The principal component analysis (PCA) was carried out with the extraction of the components using correlated matrix from the crop collection data on quantitative seed traits using XLSTAT software (XLSTAT, 2017).

RESULTS

During collecting missions of land races of legume crops, only 11 seed samples / accessions of lentil were collected of which Al-Dhahira & Buraimi governorates had the highest (6), followed by South Batinah-coastal governorate represented by mountains of Rustaq (3) and Al-Dakhliya (Interior) governorate (2) with no contribution from other

Table 1. Important characteristics of sites from where 11 lentil (*Lens culinaris* L.) samples were collected in Oman

Sl. No.	Site No	Collection No.	Governorate	Wilayat	Village/ Location	Latitude	Longitude	Altitude m	Soil texture	Soil hardness	Drainage	pH	EC dSm^{-1}	Color	Land form
1	22	MOA 62	Interior	Al Hamra	Jabel Shams	23^0 15.52'	57^0 00.57'	1680	Sand clay loam	Loose	Free	7	2.0	Light brown	Mountain
2	25	MOA 76	Dhahira	Ibri	Bilad Al-Shahoom	23^0 22.96'	57^0 09.95'	884	Sandy clay loam	Friable	Imperfect	8	2.1	Brown	Foothill
3	28	MOA 83	Dhahira	Ibri	Bilad Al-Shahoom	23^0 22.96'	57^0 00.57'	947	Sandy clay loam	Firm	Imperfect	7	1.3	Light brown	Plain
4	29	MOA 92	Dhahira	Ibri	Bat	23^0 15.22'	56^0 45.23'	508	Sandy clay loam	Firm	Imperfect	7	1.8	Brown	Plain
5	32	MOA 103	Interior	Bahla	Sint	23^0 07.96'	57^0 04.64'	952	Sandy loam	Loose	Free	9.5	0.4	Dark brown	Foothill
6	35	MOA 115	Dhahira	Yanqul	Dhahar Faris	23^0 38.72'	56^0 38.44'	480	Sandy loam	Loose	Imperfect	8	2.1	Brown	Mountain
7	36	MOA 116	Dhahira	Yanqul	Al-Bouwerdah	23^0 38.72'	56^0 38.33'	480	Sandy loam	Loose	Imperfect	8	2.3	Brown	Mountain
8	37	MOA 120	Dhahira	Yanqul	Al-Bouwerdah	23^0 38.06'	56^0 29.76'	586	Sandy loam	Friable	Imperfect	9	2.3	Brown	Plain
9	56	MOA 162	Batinah South	Rustaq	Atayeeb	23^0 25.40'	57^0 09.78'	557	Sandy loam	Loose	Imperfect	4.1	9	Dark brown	Mountain
10	57	MOA 171	Batinah South	Rustaq	Almahdooth	23^0 30.52'	57^0 11.42'	482	Sandy loam	Friable	Imperfect	3.1	9	Brown	Mountain
11	58	MOA 174	Batinah South	Rustaq	Almahdooth	23^0 30.57'	57^0 11.36'	476	Sandy loam	Friable	Imperfect	2.1	9	Brown	Mountain

agriculturally important Sharqiya (eastern), North Batinah and Southern Dhofar governorates. (Table 1). The collection sites varied in their characteristics and altitude. Altitude ranged from 476 m at site No.58 of Al Mahdooth, wilayat Rustaq to 1980 m at site No. 22 of Jabel Shams, wilayat Al Hamra of Interior (Al-Dakhiliya) governorate (Table 1). Soil characteristics were also varied. Sites ranged in soil texture from sandy loam to sandy clay loam. Soils were firm or loose or friable. With respect to drainage, soils were either imperfect or free. Soil pH ranged from 2.1 (Site No. 58 of Al Mahdooth of wilayat Rustaq) to 9.5 (Site No. 32, Sint, wilayat Bahla, Interior or Al-Dakhiliya governorate).

Soil EC varied from 0.4 dSm^{-1} (Site No.32, Sint, wilayat Bahla, Interior or Al-Dakhiliya governorate) to 9 dSm^{-1} (Sites No. 56 of Atayeeb and Nos. 57 & 58 of Al-Mahdoot, wilayat Rustaq, Batinah South governorate). Soil color ranged from light brown to dark brown (Table 1).

Variability in seed characters:

The indigenous lentil accessions, collected, had large variation with respect to all the seed characters studied, i.e. seed length (cm) and width (cm), 1000-seed weight (g) and seed color (Table 2). Seed length varied from 0.330 cm (Collection No. 76 of Bilad Al-Shahoom, Ibri, Dhahirah) to 0.45 cm (Collection No. 83 of Bilad

Table 2. Variation among seed characters of 11 indigenous lentil genotypes/accessions of Oman

Sl.No.	Collection No.	Length (cm)	Width (cm)	1000-seed weight (g)	Seed Color Pattern	Seed Color (Score)
1	MOA62	0.395	0.38	22.9	Green, tan, pink, brown, blood red	8
2	MOA76	0.33	0.32	22.5	Tan, green, mottled	1
3	MOA83	0.45	0.4	32.5	Green, tan, brown, mottled	3
4	MOA92	0.4	0.35	29.7	Green, tan, light brown, mottled	5
5	MOA103	0.39	0.37	29.1	Green, tan, brown, mottled	3
6	MOA115	0.405	0.385	23.1	Green, tan, pink, brown, blackish, mottled	9
7	MOA116	0.395	0.365	24.9	Green, tan, light brown, pinkish, mottled	7
8	MOA120	0.4	0.4	26.1	Tan, green, mottled	1
9	MOA162	0.42	0.4	32.2	Green, tan, pink, mottled	2
10	MOA171	0.42	0.39	27.6	Green, tan, pink, brown, mottled	6
11	MOA174	0.43	0.405	32.3	Green, tan, pink, brown	4
Statistical Parameters						
Minimum		0.330	0.320	22.5	-	1.0
Maximum		0.450	0.405	32.5	-	9.0
Mean		0.403	0.379	27.5	-	4.4
Std. Deviation (±)		0.030	0.026	3.9	-	2.8

Al-Shahoom, Ibri, Dhahirah); seed width ranged from 0.320 cm (Collection No. 76 of Bilad Al-Shahoom, Ibri, Dhahirah) to 0.405 cm (Collection No. 174 of Al-Mahdooth Hajer Bani Omer, Rustaq, Batinah South); 1000-seed weight ranged from 22.5 g (Collection No. 76 of Bilad Al-Shahoom, Ibri, Dhahirah) to 32.5 g (Collection No. 83 of Bilad Al-Shahoom, Ibri, Dhahirah). With respect to seed color, two seed accessions—Collection No. 76 of Bilad Al-Shahoom, Ibri, Dhahirah and Collection No. 120 of Al-Bouwerdah, Yanqul, Dhahirah—were homogenous (pure) with tan, green, mottled seeds which received score 1; the rest (9) were heterogeneous (mixture) with seeds of various colors ranging from green, tan, light brown, brown, pink, mottled. The critical analysis of seed colors of these samples indicated the presence of 8 groups of which one group had two seed accessions viz. collection Nos. 83 and 103 with green, tan, brown, mottled color having score of 3 and the remaining seven seed accessions—Collections Nos. 62 (score 8), 92 (score 5), 115 (score 9), 116 (score 7), 162 (score 2), 171 (score 6) and 174 (score 4)— formed groups of their own due to a unique combination of seed colors (Table 2).

Principal Component Analysis:

The Principal Component Analysis (PCA) was performed to understand which combination of four seed characters of the indigenous germplasm of Oman could contribute to attain high quality in lentil in terms of their commercial value. The PCA has potential in transforming the data of related characters into a smaller number of variables as principal components (PCs) towards simplification of the complex data. The scree plot of the PCA (Figure 1) indicated that the first two eigenvalues that correspond to PC1 (factor 1) and PC2 (factor 2) had major proportion of the variance in the dataset which is also depicted in Table 2.

The first two PCAs extracted from the complicated components accounted to 90.943 % with PC 1 having eigenvalue of 2.406 and PC 2, just 1.232 (Table 3). The first PC or factor accounts for maximum variability in the data in comparison with succeeding components or factors. The PCA grouped the estimated lentil variables into three main components of which PC 1 or factor 1 accounted for about 60.146% of the variation; PC 2 or factor 2 for 30.797% and PCA 3 or factor 3 for only 8.207%. The PC4 or factor 4 accounted for the least percent of variation (0.850%) (Table 3).

The PC1 was positively influenced by seed length with the value measuring 0.957 and seed width with 0.890 whereas the second PC was greatly and positively influenced by seed color (0.971). However, third PC was

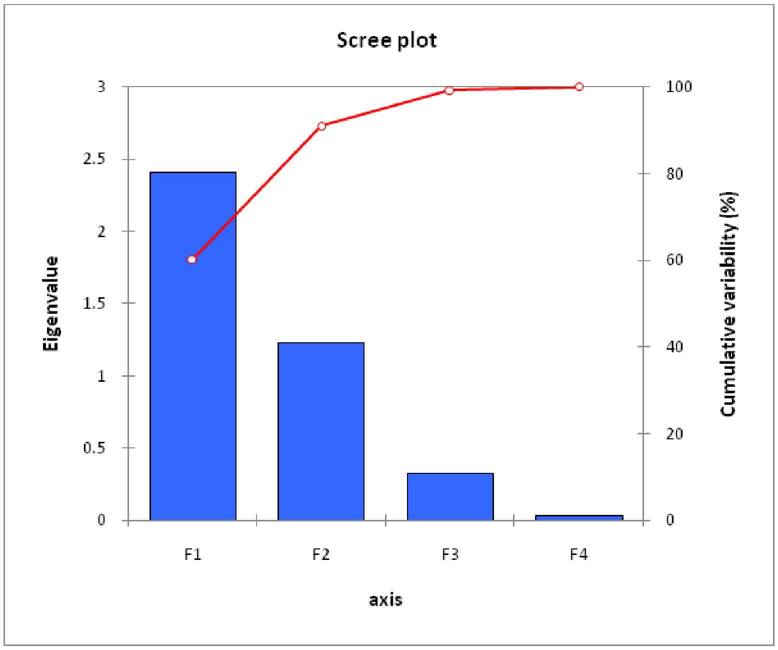

Figure 1. Scree plot showing eigen values in response to four principal components, PCs or Factors (F1 to F4) for four seed variables/ characters in indigenous lentil accessions

Table 3. Eigen values and percent variance of principal components to total variation in 11 indigenous lentil accessions

Principal Components (PC' s)	Eigen value	% Variance	Cumulative variance
PC 1	2.406	60.146	60.146
PC 2	1.232	30.797	90.943
PC 3	0.328	8.207	99.150
PC4	0.034	0.850	100.00

Table 4. The principal component values or factor loadings of four seed characters in 11 indigenous lentil accessions

Variables/Characters	PC 1	PC 2	PC 3	PC4
Seed length (cm)	0.957	0.241	-0.080	-0.137
Seed width (cm)	0.890	0.222	0.391	0.075
1000- Seed Weight (g)	0.831	-0.427	-0.346	0.082
Seed Color	-0.076	0.971	-0.222	0.053

also associated with seed width, which had low value (0.391) whereas PC4 had least influence of these seed characters (Table 4). This is reflected in terms of percent contribution of four variables (seed characters) to four PCs presented in Table 5.

In respect of associations between the traits, of the six character combinations, only two correlation coefficients were significant and were positive viz. seed length vs seed width (0.864*) and seed length vs 1000-seed weight (0.710*) (Table 6).

The scatter of 11 indigenous lentil accessions in biplot graph of the first two PCs (factors) as X and Y –axes clearly indicated that lentil accessions were spread over all the four quadrants of the graph to classify the accessions into four clusters corresponding to the four quadrants with which they were associated. These accessions of each cluster are considered genetically similar (Figure 2). The number of accessions in the Cluster IV / fourth quadrant of the biplot graph was the highest with four accessions namely MOA 83, MOA 120, MOA 162 and MOA 174, followed by that in the Cluster II of the second quadrant with 3 accessions namely MOA 62, MOA 115 and MOA 116. Similarly, the Cluster III of the third quadrant had also 3 accessions viz. MOA 76, MOA 92 and MOA 103. Cluster

Table 5. The percent contribution of four variables (seed characters) to four principal component values in 11 indigenous lentil accessions

Variables/Characters	PC 1	PC 2	PC 3	PC4
Seed length (cm)	38.095	4.730	1.969	55.206
Seed width (cm)	32.930	4.004	46.548	16.518
1000 Seed Weight (g)	28.733	14.778	36.519	19.970
Seed Color	0.242	76.488	14.964	8.307

Table 6. Correlation coefficients between four seed characters of 11 indigenous lentil accessions

	Seed length (cm)	Seed width (cm)	1000- Seed Weight (g)	Seed Color
Seed length (cm)	1	0.864*	0.710*	0.172
Seed width (cm)		1	0.516	0.065
1000- Seed Weight (g)			1	-0.396
Seed Color				1

*Significant at 5% level of significance (p<0.05)

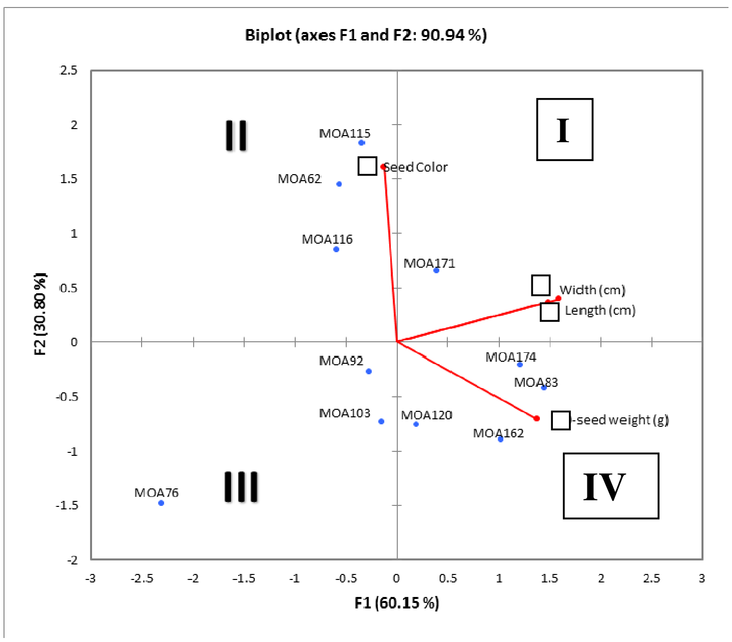

Figure 2. Principal component score of PC1 and PC2 describing the overall variation among indigenous lentil germplasm estimated using seed characters and their spread in four quadrants

I of the first quadrant had only one accession, MOA 171 of South Batinah governorate as solitary cluster. The accessions of Cluster I had features of higher seed length and width whereas that of Cluster IV and Cluster II had feature of higher seed weight (g) and higher scores of seed color, respectively. In respect of cluster composition, the accessions of the clusters II, III and IV belonged to different governorates.

DISCUSSION

In the 2008-2011 collecting missions of food and fodder legumes from the most governorates of the Sultanate only 11 accessions were collected of which Al-Dhahira & Buraimi governorates had the highest (6), followed by South Batinah-coastal governorate represented by mountains of Rustaq (3) and Al-Dakhliya (Interior) governorate (2) with no contribution from other agriculturally important Sharqiya (eastern), North Batinah and Southern Dhofar governorates owing to changes in the climatic abiotic and biotic factors. Al-Dhahira & Buraimi governorate contributed 54.54% of the collected accessions followed South Batinah- coastal governorate represented by Rustaq in the mountains which contributed 27.27% whereas Al-Dakhliyah (Interior) contributed the rest,18.19% Musandam governorate which is at Northern tip of the Sultanate had also no contribution to collections during the collecting missions possibly because of farmers' switch over to greenhouse cultivation of vegetables due to irrigation water shortage in the area.

The critical examination of lentil seed samples at the laboratory showed large variation in seed coat patterns (color) and seed weights to such an extent that even the adjoining locations of collecting sites had similar or different patterns of seed coat color in the collected accessions. This is attributed to possible mixing of seeds between the land races during exchange or at harvest during cultivation (Al-Maamari *et al.,* 2014). Therefore, samples of the accessions with heterogeneous seeds with respect to seed coat color required purification into sub-groups (AlSaady *et al.,* 2014). The transfer of landraces of lentil between wilayats and neighboring governorates of the Sultanate of Oman indicated that these landraces/accessions were resulted through centuries of selection to get adopted to local climatic, edaphic and cultural factors thus possessing unique gene complexes that arise in the course of evolution (Mathur, 2010; Al-Maamari *et al.,* 2014; AlSaady *et al.,* 2014, 2018a and b). The present collections in lentil were significantly lower than the ones collected during earlier collecting missions led by Guarino and local representatives of the Ministry in 1980s (Guarino, 1990). This is evident by the fact that there were no collections from any farmers of Al-Musandam, North Al-Batinah-coast and Sharqiyah governorates. This is attributed to the gradual loss of land races because of changes in the land use pattern and erratic droughts besides the signs of loss of interest in the farmers to grow less-economic field crops against highly profitable vegetables. However, constant availability of landraces with the farmers having interest to preserve with them for cultivation is an indication of local conservation strategy for sustainable production of lentil (AlSaady *et al.,* 2014).

The correlation analysis of seed characters showed significant ($p<0.05$) and positive associations only between seed length and seed width and between seed length and seed weight. Selection of strongly associated characters like seed length, seed width and 1000-seed weight can be used to improve seed quality characters that influence yield and their marketing value as suggested by earlier workers who studied both seed and yield traits in lentil for selection of parents in crossing program (Turk et al., 2004; Bicer, 2009; Ghassemi- Golezoni et al., 2014; Gaad et al., 2018; Erdogan, 2015).

The results of PCA analysis have been useful in identifying the phenotypic characters that contribute higher genetic variations among the genotypes for selection of potential parents for crossing to improve the characters of interest for productivity in quantity and quality (Mehra et al., 2018). In the present study, PCA clearly indicated that all the seed traits except seed color had positive and higher percent of contribution to PC1 component reflecting the seed size as potential parameter existing in variation of lentil accessions. However, seed color contributed the highest (97.10%) and positively to PC2 obtained in PCA analysis in the land races of the present study. The existence of wider phenotypic variability among the indigenous lentil germplasm was further explained by the location pattern of different lentil landraces spread over all the four quadrants of the biplot graph (Figure 2). This provided a characteristic pattern of the similarities and differences existed among the lentil accessions elucidated with the interrelationships found between the variables, studied. The graph characteristically demarcated the accessions about their scattering pattern based on the first two dimensions/ components into just four clusters based on seed characters corresponding to their positions the four quadrants, indicating wide genetic variability for the traits, studied. Interestingly, the accessions collected from the Al-Dhahirah governorate such as MOA 115 in the cluster II, MOA 76 in the Cluster III and MOA 83 in Cluster IV were placed at extreme positions from the origin of the graph in the respective quadrants II, III and IV indicating that they are genetically distinct accessions whereas other accessions were positioned around the origin of bi-plot graph, indicating their genetic similarity among the constituents of the clusters. The fact that accessions of three clusters II, III and IV were different in terms of their locations in the governorates showed the extent of inter-exchange of the accessions taken place among the farmers of different governorates located adjacent or far off from one another. The accessions of different clusters/ quadrants can be utilized in crossing program to improve seed characters with higher seed yield as these accessions would be genetically distant to provide wide range of variability in the F2 plants.

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