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Full Length Research Paper

Genetic Diversity of Indigenous Chickpea (*Cicerarietinum* L.) Germplasm Collection

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Chickpea (*Cicer arietinum* L.) is one of the important pulse crops cultivated for traditional consumption, in all the Arabian Peninsula countries including Oman. This paper highlights features of variation in seed characters found in only 13 accessions collected during the legume crops collecting missions across all the governorates of the Sultanate of Oman undertaken jointly by the staff of College of Agriculture, Sultan Qaboos University and the Ministry of Agriculture & Fisheries between 2008 and 2011. The indigenous chickpea accessions were only from three governorates viz. the highest of 7 accessions from interior(Al-Dakhliyah) governorate followed by South Batinah governorate represented by Rustaq (5) and Dhahirah governorate (1). Seed accessions were diverse with respect to all seed characters studied, i.e. seed length (cm) and width (cm), 100-seed weight (g) and seed color. Seed length varied from 0.645 cm to 1.210 cm whereas seed width ranged from 0.505 cm to 1.005 cm. 100-seed weight was found to vary from 12.6 to 67.9 g. Chickpea accessions were classified into five groups with scores from 1 to 5 on the basis of simple (1) to complexity (5) of seed coat color pattern. With respect to seed color, the accessions were grouped into five groups based on simple to complexity of color combinations with scores from 1-5 with varying frequencies / numbers. Principal Component Analysis (PCA) with seed characters divided indigenous chickpea accessions into 4 genetically diverse clusters corresponding to their scatter in four quadrants of the biplot graph. The PCA further revealed almost equal contribution of seed length (33.861 %), seed width (31.437%) and 100-seed weight (34.595%) coupled with the least of seed color (0.108%) to the first principal component (PC1 or factor 1). Of the six-character combinations, only three correlation coefficients viz. between seed length and seed width (0.857), between seed length and 100-seed weight (0.981**), and between seed width and seed weight (0.884**), were significant (p<0.05).**

Keywords: Landraces, indigenous, accession, seed characters, principal components, diversity, chickpea

INTRODUCTION

Pulses are important food crops for the food security of large proportions of populations, particularly in Latin America, Africa and Asia, where pulses are part of traditional diets and often grown by small farmers. Global efforts were initiated by the United Nationsin 2015to promote pulse production by declaring 2016 as the International Year of Pulses (UN, 2015; FAO,2016;) to position pulses as a primary source of protein and other essential nutrients under the title of nutritious seeds for sustainable future (FAO, 2016). Chickpea (*Cicerarientinum* L.) is one of the most important pulse crops widely grown in almost all the continents of world and help in providing nutritious food for the ever growing global population (Millan et al., 2015; Muehlbauer and Sarkar, 2017).

Chickpea (*Cicer arietinum* L) is one of the major legume crops in the world after dry beans and peas (Kumari and Van Leur, 2011). Chickpea is a highly nutritious pulse and occupies third in the importance list of the food legumes that are cultivated throughout the world. It has capacity to stand in drought conditions. It is used not only as an edible seed and but also in making several preparations from its flour in different continents. The Sultanate of Oman is one of the arid countries in the Arabian Peninsula with 85473.10 ha of cultivated agricultural land occupied in the cultivation of fruits (39.40 %) followed by perennial and annual fodder crops (36.11%), vegetables (19.72%) and field crops (4.77%) (MAF, 2017). As field crops area itself is limited, the area under chickpea is insignificant and not specifically documented. However, it is an important winter season crop between November and April among few interested farmers in the plains of North Oman grown primarily for its edible seeds in local preparations. A range of indigenous chickpea land races, which do not belong to *Kabuli* type, were grown earlier in Oman under varied ecological conditions for their food and dry fodder value. Of late, the indigenous germplasm of various field crop species like chickpea is slowly facing the problem of extinction due to gradual introduction of high yielding crops of commercial value and shift in land use patterns either to growing high profit yielding crops like vegetables under green houses, hydroponics, etc or to urbanization. The germplasm collections and their conservation would safeguard the rare available accessions from their extinct.

Several crop germplasm collecting missions were conducted in Oman with collaboration of international organizations like FAO in 1990's mainly to conserve indigenous plant genetic resources for future food security of Oman (Guarino, 1990; Osman et al., 2002; AlSaady *et al.,* 2014). Similarly, a series of collecting missions were conducted between April 2008 and March 2011 in collaboration with the staff of the Sultan Qaboos University and the Ministry of Agriculture & Fisheries from different sites across all the governorates of Oman to explore the indigenous germplasm/ land races of all the legume crops grown in Oman. Collection of diverse genetic materials in the crops and their characterization are one of the pre requisites for all the breeding programs (Rafiq et al., 2018; Mahmood et al., 2018).

The extent of genetic diversity existing among the genotypes of a crop species varies depending on the degree of variation in the characters considered for study (Karakoyet al., 2012; Govindaraj et al., 2015;Ye et al., 2018; Mahmood et al., 2018). The information on diversity with composition of clustering pattern among the genotypes is of great significance for the crop breeders in selecting parents for crossing for improvement towards the development of most suitable genotypes considering ideal plant types that are expected to provide higher yield of quality grains that suit to higher marketability (Ghafoor et al., 2003; Bicer, 2009). Multivariate analysis involves principal component analysis (PCA) which is efficient for assessment of genetic variation in chickpea genotypes (Upadhyay, 2003; Gupta et al., 2011; Nihal and Adak, 2012; Sharifi et al., 2018). In general, two types of chickpea are reported according to the size, shape and color of the seeds. *Kabuli* chickpeas are large seeded with salmon white test a, grown mainly in the Mediterranean area, central Asia and America whereas *desi* chickpeas are small seeded with a light brown test a, cultivated mostly in Indian sub-continent, Asia and east Africa (Rincon et al., 1998). It is generally accepted that the *kabuli* types were derived from desi type through mutation followed by conscious selection (Jana and Singh, 1993). Polymorphism has been reported between *Cicer arietinum* and its wild genotype *Cicerreticulatum* (Udupa et al., 1993). This paper presents the results of the analysis of chickpea (*Cicer arietinum*L.) germplasm diversity in respect of few seed traits.

MATERIALS AND METHODS

Seven collecting missions were conducted from April 2008 to March 2011 across different governorates of Oman jointly by the staff of the Sultan Qaboos University and Agriculture Development Centers of the Ministry of Agriculture and Fisheries following the procedures reported earlier (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 by simultaneously recording the crop passport data and site descriptions including GPS data, electrical conductivity and pH of soil and water samples. The sites covered during the trips lied between coastal and interior plains from 12 m to 1,983 m altitude. In all, 156 collecting sites were visited. Indigenous chickpea accessions were collected from 13sites which lied between 274 m to 1983 m altitude (Table 1). Seed traits such as seed length and width (cm) and test

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Table 1. Important characteristics of sites from where 13 chickpea (*Cicer arietinum* L.) samples were collected in Oman

Sl. No.	Site No	Collection No.	Governorate	Wilayat	Village/ Location	Latitude (N)	Longitude (E)	Altitude (m)	Soil texture	pH	EC	Color	Landform
1	1	OMA 1	Interior	Nizwa	Tanouf	23^0 02.00’	57^0 43.45’	604	Clay loam	8.75	6.275	Brown	Wadi
2	3	OMA 8	Interior	Nizwa	Nizwa city	22^0 57.80’	57^0 31.67’	508	Clay loam	9.5	4.524	Brown	Wadi
3	8	OMA 18	Interior	Manah	Manah Al Blaad	22^0 47.88’	57^0 35.98’	430	Clay loam	8.5	0.6	Brown	Wadi
4	13	OMA 34	Interior	Bahla	AL-Khatwah	22^0 59.32’	57^0 17.91’	363	Clay loam	9.5	2.1	Light brown	Plain
5	14	OMA 38	Interior	Bahla	Alfeth Old village	22^0 55.27’	57^0 18.51’	555	Sandy clay loam	9.5	1.3	Brown	Plain
6	15	OMA 42	Interior	Bahla	Tawee A Nuseif	22^0 57.66’	57^0 12.63’	583	Sandy loam	9	1.3	Brown	Foothill
7	21	OMA 58	Interior	Bahla	BiladSait	23^0 01.98’	57^0 16.00’	723	Clay loam	8.1	1.1	Brown	Wadi
8	24	OMA 69	Batinah South	Rustaq	BaladSait	23^0 11.16’	57^0 23.51’	1983	Sandy loam	7.8	0.9	Brown	Hill side
9	28	OMA 85	Dhahira	Ibri	Bilad Al-Shahoom	23^0 22.96’	57^0 00.57’	947	Sandy clay loam	9.0	2.2	Light brown	Plain
10	44	OMA 139	Batinah South	Rustaq	Al-Ayeer	23^0 12.79’	57^0 27.56’	723	Sandy loam	9	2.25	Brown	Mountain side
11	47	OMA 143	Batinah South	Rustaq	Al-Hajeer	22^0 12.32’	59^0 30.39’	655	Sandy loam	8.5	2.55	Light brown	Foothill
12	48	OMA 144	Batinah South	Rustaq	Al-Hajeer	22^0 12.38’	59^0 30.66’	659	Sandy loam	8.2	1.4	Brown	Foothill
13	51	OMA 151	Batinah South	Rustaq	Al Ghasahb	23^0 24.97’	57^0 25.92’	274	Sandy loam	9	22.7	Brown	Plain

weight (100 seeds) were measured. The seed color of each sample was determined on visual basis and scored from simple to complex color combinations. 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

13 seed samples/accessions were collected, with the highest of 7 accessions (53.84%) from Interior or Al-Dakhiliyah governorate represented mostly by mountainous areas of wilayats Nizwa (2), Manah (1) and Bahla (4) followed by South Batinah governorate (5) represented by mountains of Wilayat Al-Rustaq (38.46%) and the least of only one accession (7.70%) from the mountains of Wilayat Ibri in Al-Dhahirah governorate. There were no accessions collected from either Dhofar (South Oman) governorate or from coastal areas of North and South Batinah and other governorates of Oman viz. Musandam and Al-Buraimi.

Table 2. Variation among seed characters of 13 indigenous chickpea genotypes/accessions of Oman

No.	Collection	Length	Width	100 seed weight	Seed Color	Color	Governorate
1	OMA 1	0.710	0.570	13.9	Bright light straw	2	Interior
2	OMA 8	0.690	0.505	12.6	Bright light straw, green	3	Interior
3	OMA 18	0.735	0.605	22.6	Bright light straw, green	3	Interior
4	OMA 34	0.645	0.560	14.5	Bright light straw, green	3	Interior
5	OMA 38	0.665	0.590	17.1	Bright light straw	2	Interior
6	OMA 42	0.705	0.605	18.5	Bright light straw, green	3	Interior
7	OMA 58	0.675	0.550	13.5	Light brown	1	Interior
8	OMA 69	1.210	1.005	67.9	Bright light straw	2	Batinah South
9	OMA 85	0.885	0.800	36.4	Bright light straw, brownish, greenish	5	Dhahira
10	OMA 139	1.160	0.915	63.5	Bright light straw	2	Batinah South
11	OMA 143	0.805	0.760	37.8	Bright light straw, pinkish, greenish	4	Batinah South
12	OMA 144	0.925	0.520	39.3	Bright light straw, pinkish, greenish	4	Batinah South
13	OMA 151	0.745	0.650	18.8	Bright light straw, green	3	Batinah South
Statistical Parameters							
Minimum		0.645	0.505	12.6	-	1	-
Maximum		1.210	1.005	67.9	-	5	-
Mean		0.812	0.664	28.9	-	2.8	-
Std.Deviation (±)		0.186	0.158	18.9	-	1.1	-

Variation in collection sites

Collection sites were found varied in their soil and position characteristics and altitude. Altitude ranged from 274 m at site No.51 of Al-Ghasahb, Wilayat Rustaq of Batinah South to 1983 m at site No. 24 of BaladSait, wilayat Rustaq of Batinah South. The sites ranged in soil texture viz. sand, sandy loam, sandy clay, sandy clay loam, clay and loam. Soils were hard, firm or loose, variable-loose to crust and friable. With regard to drainage, soils were imperfect, free or variable. Soil pH ranged from 7.8 (site No. 24 of BaladSait, wilayat Rustaq of Batinah South) to 9.5 (sites No. 3, Nizwa city, Nizwa wilayat, Interior; No. 13, Al-Khatwa, Bahla wilayat, Interior; No. 14, Alfeth old village, Bahla, Interior). Soil EC varied from 0.9 dSm^{-1} (Site No.24, BaladSait, wilayat Rustaq of Batinah South) to 22.7 (Site No. 51, Al-Ghasahb, Rustaq, Batinah South). Soil color ranged from brown to light brown (Table 1).

Variation in seed characters:

Seed accessions were found diverse with respect to all but few quantitative seed characters studied like seed length (cm) and width (cm), and 100-seed weight (g) and qualitative trait like seed color (Table 2). Seed length was found varied from 0.645 cm (Collection No. OMA 34 of Al-Khatwa, Bahla wilayat, Interior) to 1.210 cm (Collection No. OMA 69 of BaladSait, Rustaq, wilayat South Al-Batinah); seed width ranged from 0.505 cm (Collection No. OMA 8 of Nizwa city, Nizwa wilayat, Interior) to 1.005 cm (Collection No. OMA 69of BaladSait, Rustaq, wilayat South Al-Batinah); 100-seed weight ranged from 12.6 g (Collection No.OMA 8 of Nizwa city, Nizwa wilayat, Interior) to 67.9 g (Collection No. OMA 69 of BaladSait, Rustaq, wilayat South Al-Batinah). In respect of seed color, the score ranged from 1 (light brown) (Collection No. 58 of Bilad Sait, wilayat Bahla, Interior/ Al-Dakhiliyah) to 5 (bright light straw, brownish, greenish) (Collection No. 85 of Balad Al-Shahoom, wilayat Ibri, Al-Dhahirah)(Table 2).

Principal Component Analysis:

The data on four quantitative seed characters were subjected to the Principal Component Analysis (PCA) to understand which combination seed traits contribute to obtaining optimum quality of the indigenous chickpea germplasm in terms of their importance and value in marketing. The scree plot of the PCA (Figure 1) indicated that the first two eigenvalues had major proportion of the variance in the dataset as evidenced by the first two PCAs extracted from the components contributing to 95.452 % with PC 1 having eigenvalue of 2.817 and PC 2, 1.001 (Table 3).

The PCA mainly transforms the complex data of the number of correlated quantitative traits into a simple and meaningful smaller number of principal components as

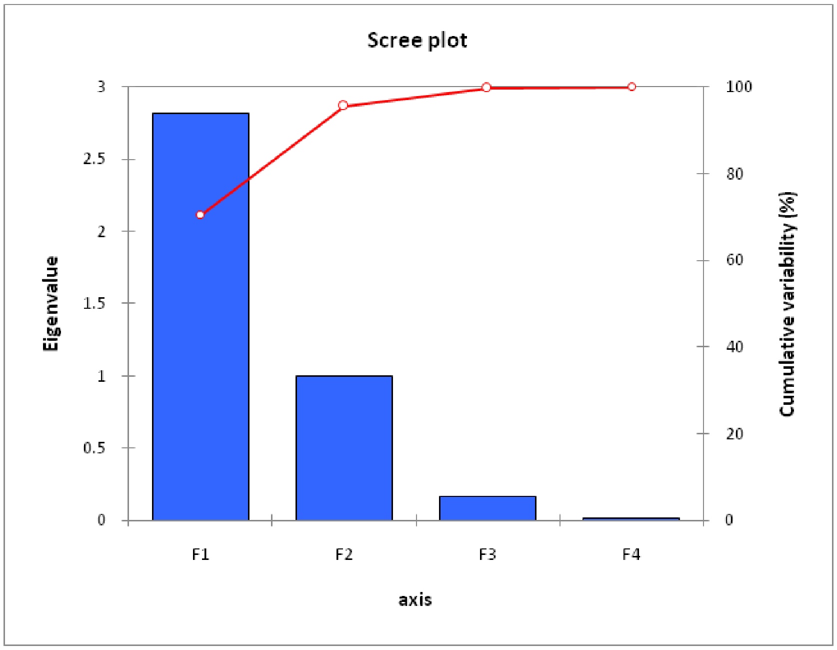

Figure 1. Scree plot showing eigenvalues in response to four principal components (F1 to F4) for four seed variables/ characters studied

Table 3. Eigen values and percent variance of principal components to total variation in 13 indigenous chickpea accessions

Principal Components (PC' s)	Eigen value	% Variance	Cumulative variance
PC 1	2.817	70.434	70.434
PC 2	1.001	25.018	95.452
PC 3	0.167	4.168	99.620
PC4	0.015	0.380	100.00

Table 4. The principal component values or factor loadings of four seed characters in 13 indigenous chickpea accessions

Variables/Characters	PC 1	PC 2	PC 3	PC4
Seed length (cm)	0.977	-0.041	-0.194	-0.081
Seed width (cm)	0.941	-0.041	0.335	-0.012
100 Seed-Weight (g)	0.987	-0.023	-0.128	0.092
Seed Color	0.055	0.998	0.009	-0.006

PCs or factors (Fs). In the present investigation the PCA transformed four seed related variables into four components of which PC 1 accounted for 70.434% of the variation; PC 2 for 25.018%, PC 3 for 4.168 % and PC 4 for the least 0.380 % of the variation in chickpea accessions of the present study (Table 3).

The PC 1 was positively and equally influenced by seed width (0.977) and 100-seed weight (0.987). However, the PC 2 was influenced positively and the highest by seed color (0.998) but negatively and the least by other seed traits. The influence of these seed traits to PC 3 and PC 4 was found to be insignificant or negligible (Table 4).

In terms of percent contribution of seed traits to the PCs, all except seed color (0.108 %) had contribution ranging from 31.437 % (seed width) to 34.595 % (seed weight) to PC1 and to the extent of 99.991% to PC3 whereas seed color alone contributed to the extent of 99.615 % to PC2. (Table5). Similarly, only positive and significant correlation values (r) were found between three seed characters studied viz. seed length vs seed width (0.857**), seed

Table 5. The percent contribution of four variables (seed characters) to four principal component values in 13 indigenous chickpea accessions

Variables/Characters	PC 1	PC 2	PC 3	PC4
Seed length (cm)	33.861	0.164	22.671	43.305
Seed width (cm)	31.437	0.167	67.474	0.922
100 Seed-Weight (g)	34.595	0.054	9.809	55.542
Seed Color	0.108	99.615	0.047	0.231

Table 6. Correlation coefficients between four seed characters of 13 indigenous chickpea accessions

	Seed length (cm)	Seed width (cm)	100 Seed-Weight (g)	Seed Color
Seed length (cm)	1	0.857*	0.981*	0.012
Seed width (cm)		1	0.884*	0.014
100 Seed-Weight (g)			1	0.076
Seed Color				1

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

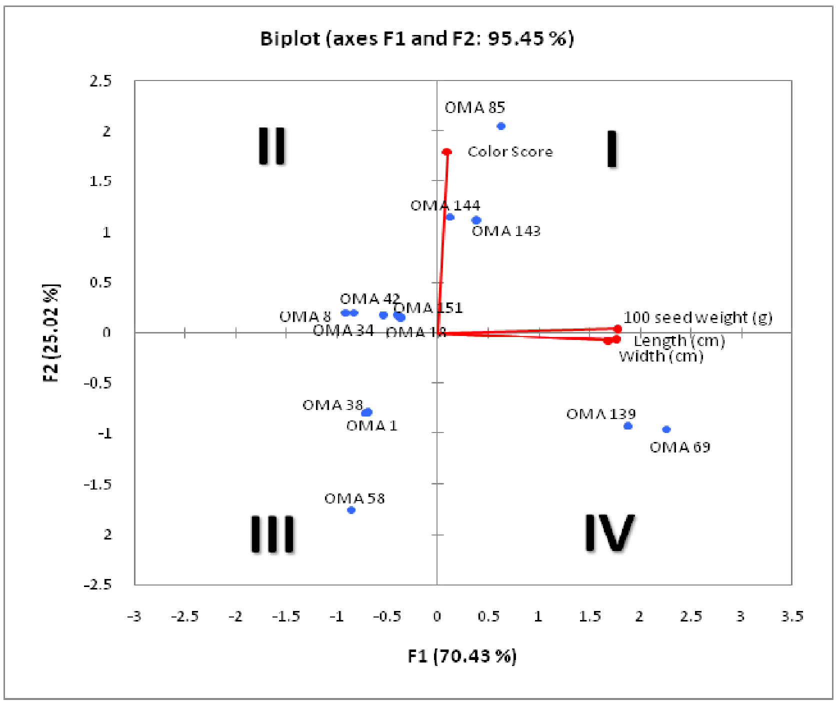

Figure 2. Principal component score of F1 (PC1) and F2 (PC2) estimated using seed characters describing the overall variation among indigenous chickpea germplasm

length vs 100-seed weight (0.981**) and seed width vs 100-seed weight (0.884**) (Table 6).

The spread of 13 indigenous chickpea accessions in biplot graph of the first two PCs (factors) as X and Y –axes clearly depicted that chickpea accessions were scattered over all the four quadrants of the graph to classify the accessions into four clusters corresponding to the four quadrants with which they were related. These accessions of each cluster are considered genetically alike (Figure 2) whereas those of different clusters are genetically diverse. The number of accessions in the Cluster II / second quadrant of the biplot graph was the highest with five accessions namely OMA8, OMA18, OMA34, OMA42 and OMA151, followed by that in the Clusters I(OMA 85, OMA 143 and OMA 144) and III (OMA 1, OMA 38 and OMA 58) of the first and third quadrants with each of 3 accessions. Cluster IV of the fourth quadrant had two accessions, OMA69 and OMA 139. The accessions of Cluster IV had features of quantitative seed traits like seed length and width, and seed weight whereas that of Cluster Ihad feature of higher scores of seed color. In respect of cluster composition, the accessions of the clusters III and IV belonged exclusively to Interior (Al-Dhakhliyah) and South Batinah governorates, respectively whereas clusters I and II had accessions of different governorates.

DISCUSSION

In contrast to earlier collections of chickpea during 1980s and 1990s (Guarino, 1990), the number of accessions collected in the current collecting missions was considered as the least. All the accessions were acquired from the farmers whose chickpea fields were at locations of altitudes higher than 274 m. Interior / Al-Dhakhliyah governorate represented by the mountainous areas of wilayat Nizwa, Manah and Bahla contributed the highest number followed by South Batinah governorate represented by the mountains of wilayat Rustaq. The third, Al-Dhahirah governorate contributed only one accession which was collected from the mountains of Balad Al-Shahoom of wilayat Ibri. Interestingly, Al-Sharqiya, easten governorates, North Batinah, Musandam and southern Dhofar governorates had no contribution to collections.

The results of critical analysis of seed color of chickpea germplasm accessions at the laboratory indicated complexity of variation in seed coat color pattern besides large variation in seed size characters (Table 2). These variations observed in seed size among the collected samples of indigenous Omani accessions are in conformity with those observed in previous studies that dealt with either local small or large germplasm collections (Williams and Singh, 1986; Bahl and Salimath, 1996; Hossain *et al.*, 2010; Rybinski *et al.*, 2019) wherein seed size was assessed only in terms of seed weight. In the present study 100-seed weight was found to vary from 12.6 g to 67.9 g which is comparable to the 100-seed weight reported earlier by Williams and Singh (1986) (mean-60 g/ 100 seed), Sastry *et al.* (2007) (9.6 g to 41 g/ 100-seed and Street *et al.* (2008) (7.5 g to 68 g/ 100-seed). In the present study, villages located closely with the collecting sites appeared to have interestingly similar patterns of seed coat color indicating the presence of large amount of homogeneity in the collected chickpea seed samples/ accessions whereas heterogenous seed samples were found in seed samples from different sites. In such cases, there is need for intensive purification of seed accessions into sub-homogenous groups with respect to seed color pattern (Cuberto, 1987; Singh and Diwakar, 1995; Jadav, 2015).

The extensive exchange of chickpea landraces among the farmers in the highlands of wilayats of different governorates like South Batinah and Interior governorates were presumably resulted from selections over centuries and adaptation to local climatic and edaphic factors. Constant availability of indigenous landraces with the farmers of mountain areas of the country indicates the existence of local conservation strategy for sustainable production. Genetic erosion of chickpea landraces was perhaps assumed in North Batinah, Al-Sharqiyah, Al-Burqaimi and Musandam as there were no collections and also in Al-Dhahira governorates where only one accession was collected as compared to the collections made in the past in Oman (Guarino, 1990; AlSaady *et al.,* 2018 a &b).This could be because of use of modern high-yielding crops like vegetables, changes in land use pattern for cultivation like greenhouses, hydroponics etc., urbanization, drought periods besides the lack of interest among the farmers to grow un-economical field crops like chickpeas .However, in case of Dhofar governorate chickpea is not traditionally cultivated and hence, not found.

The correlation analysis of seed characters showed their significant ($p<0.05$) and positive associations between each other. Selection of strongly associated character like 100-seed weight can be used to improve seed quality characters that influences yield and their value in marketing (Williams and Singh, 1986; Bahl and Salimath, 1996;Toker and Cagirgan, 2004).

The results of PCA analysis are of significance 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 (Shivwanshi and Babbar, 2017; Arora 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 chickpea accessions. Also, seed color had highest and positive contribution (97.10%) to PC2 in the land races of the present study. Earlier scientists reported that higher values of PC scores can be used for selection and for further utilization in future breeding program (Ghafoor *et al.,* 2003; Toker and Cagirgan, 2004; Miladinovic *et al.,* 2006; Iqbal *et al.,*2008; Ojo*et al.,* 2012; Amrita *et al.,* 2014).

The existence of wider phenotypic variability among the indigenous chickpea germplasm was further explained by the spread of different chick pea accessions over all the four quadrants of the biplot graph (Figure 2). This provided a unique pattern of the similarities and differences existed among the chickpea accessions revealed with the interrelationships found between the variables, studied. The graph characteristically defined 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 OMA85 in the cluster I, that from Interior (Al-Dhakhliyah) such as OMA 58 in the Cluster III and that from South Batinah such as OMA 69 in the Cluster IV were placed at extreme positions from the origin of the graph in the respective quadrants I, III and IV indicating that they were genetically distinct whereas other accessions were positioned around the origin of bi-plot graph such as those of cluster II corresponding to quadrant II which indicated their genetic similarity. The fact that accessions of three clusters I, 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 exploited in crop breeding program to improve characters with higher seed yield, as these accessions would be genetically distant to provide wide range of variability in the F_2 plants (Upadhyay, 2003; Gupta *et al*., 2011; Govindraj *et al*., 2015; Sharifi *et al*., 2018).

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