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Genetic Diversity of Indigenous Mungbean (*Vigna radiata* L. Wilczek) Germplasm Collection in Oman

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Abstract: Mungbean (*Vigna radiata* L. Wilczek) is a grain legume crop grown all over Arabian Peninsula countries including Oman often as a dual crop for both food and fodder during summer. Consumers and farmers prefer large size seed for food. This paper highlights the results of collecting missions of mungbean germplasm undertaken between 2008 and 2011 and analysis of genetic diversity of collected accessions based on seed traits. 25 seed samples/accessions were collected with the largest number from South Batinah coastal governorate represented by wilayat Rustaq (9) followed by southern Dhofar governorate (6), eastern Sharqiyah governorates (4), Dhahira & Buraimi governorates (3), interior Al-Dakhliyah governorate (2) and North Batinah coastal governorate (1). The seed samples of the accessions collected were highly diverse with respect to all the characters associated with seed such as seed length (cm) and width (cm), 100-seed weight (g) and seed color. Seed length varied from 0.35 cm to 0.76 cm; seed width ranged from 0.245 cm to 0.495 cm; 100-seed weight varied from 1.8 g to 7.3 g. The accessions were grouped into 16 genetically diverse clusters based on the Principal Component Analysis, which indicated the major contribution of seed width and 100-seed weight to the total variation existing in indigenous germplasm collected from all the governorates of Oman. With respect to seed color, six accessions numbering OMA 284, OMA 295, OMA 313, OMA 335, OMA 341 and OMA 345 were homogenous (pure) with their characteristic green color. The remaining 19 seed accessions were heterogeneous (mixture) with seeds of various colors such as green, brown and black. Critical analysis of seed colors of these samples indicated the presence of 4 groups of which the largest group had 12 seed accessions with green, black seed color followed by one group of three seed accessions with green, brown and black seed color and two groups of two accessions, each with green, black, brown, and green, brown seeds, respectively.

Keywords: Landraces, Accession, Seed Characters, Genetic Diversity, Mungbean

1. Introduction

Mungbean (*Vigna radiata* L. Wilczek) is one of the important grain legume species of the pan-tropical genus *Vigna* [1] which is native to Asia and cultivated in the tropics and sub-tropic areas of the world like Africa, Asia and Latin America [2]. It is considered as quality pulse due to its excellent digestibility and rich protein (25-28%), especially when combined with cereals and also as an important source of readily available proteins in cereal-based diet of the people in South Asia and Southeast Asian countries [3]. It is also regarded as fodder for livestock and incorporated in soil to enrich organic matter. It is consumed as boiled dry beans. It is adapted to many diversified cropping systems and rotations due to its short growth duration [4-5]. In mung bean, seed size in terms of test weight i.e. 100 or 1000 grain-weight is very important character as it directly influences productivity [6-10] along with seed colors [11-12], which determine grain quality for marketing. The seed sizes in mungbean ranged from < 2.5 g per 100 seeds to >7 g [7, 10, 13]. In this regard, the consumer market is expected to have preference for medium to large (35 to 70g/ 1000 seed [14-17]. In every crop species, the large seed size has been major component of seed yield in view of its importance in breeding and ease in selection as seed size is reported to be stable and highly heritable in comparison with other quantitative traits [7,18-19]. Existence of genetic diversity is essential not only for development of effective conservation strategies [20] but also for improvement in any crop species [4, 21-23]. Information about genetic diversity available in the indigenous mungbean germplasm can be exploited for promoting the efficient use of genetic variations in crop breeding programs through proper selection for cross combination among large sets of parental accessions / genotypes. There have been several studies undertaken on genetic diversity of mungbean populations not only in the past [20, 22, 24] but also recently [23, 25] using morphological and SSR markers.

Oman is the second largest country in the Arabian Peninsula region with about 85473.10 ha of agricultural land under cultivation of which fruits occupy the highest area of 36.11% followed by perennial and annual fodder crops (39.40%), vegetables (19.72%) and field crops (4.77%) [26]. Mungbean is grown throughout Oman as summer crop between April and June and its dried seeds are consumed with local diet preparations after cooking whereas its' whole plant is used as a green or dry fodder.

In Oman, a range of mungbean land races is grown for both grain and fodder, as a local affinity, under the fields of varied ecosystem. These days the local indigenous germplasm of various crop species, including that of mungbean is gradually getting extinct due to many factors like changing land use patterns and the gradual introduction of high-yielding crops of commercial value, etc. There have been several missions since 1980s to collect germplasm of different crops grown in Oman either independently or jointly with national and international organizations [27-29]. During these collecting missions several landraces of alfalfa, wheat, barley and grain legumes like chickpea, faba bean, cowpea, lentil, fenugreek, mungbean etc. were collected and conserved in local conservation facilities. In continuation of these, a series of joint collection missions between the Sultan Qaboos University and the Ministry of Agriculture & Fisheries of Oman were undertaken in different areas of all the governorates of Oman from June 2008 to December 2011 to conserve the vast indigenous germplasm available in legume crops of Oman. This paper highlights the results of mungbean germplasm collection and its diversity in respect of seed traits.

2. Materials and Methods

Seven exploration trips for the collection of indigenous legume crops germplasm such as of alfalfa, chickpea, faba bean, cowpea, lentil, field pea, mung bean and pigeon pea were undertaken from April 2008 to March 2011 in different Governorates of Oman in coordination with the staff of Agriculture Development Centers of the Ministry of Agriculture and Fisheries following standard guidelines of collecting missions [30-31]. The seed samples were collected from individual farmers, farmers-fields and stores, and Agriculture Development Centers along with passport data and site descriptions including GPS data, electrical conductivity and pH of soil and water samples (Table 1). Remote and unexplored localities were prioritized.

The area covered during the trip lied between coastal and interior plains from 325-1089 m altitude in case of mungbean germplasm. The collecting routes were systematically followed as per the plan in each of seven exploration trips in all the governorates. The collecting sites within the wilayats of governorates were at least 25 km apart to avoid collecting several seed samples of similar genotype.

A random sampling method was applied where populations were large, otherwise, it was selective. Sampling was done from farmers' household stores and from harvested lots, together with individual sampling from fields. Samples were in the form of pods or seed. Standard passport data for each accession were recorded at the time of collection, including crop name, botanical name, local name of the variety or landrace, village, wilayat and Governorate and sample type, sample method, farmer's name, and source of collection including indigenous knowledge (IK), if any. Each accession was subsequently assigned serially the collection number according to collection, irrespective of crop species.

Descriptions of each site were recorded with regard to important soil and edaphic characteristics. Soil EC and pH were measured using field EC and pH measuring meters. Slope angle and aspect were assessed using an inclinometer and compass. The longitude and latitude of each collecting site and its altitude were recorded using a Magellan 315 GPS meter. Indigenous food legume accessions were collected from 110 sites of which indigenous mungbean accessions were collected from 25 sites across all the governorates of the Sultanate.

Seed characters such as seed length and width (cm), test weight (1000 seed), seed color and nature of seed samples (pure or mixture) were determined in the laboratory [32-33]. The principal component analysis (PCA) was performed in the extraction of the components using correlated matrix from the crop collection data using XLSTAT software [34].

Table 1. *The sites/ locations in different villages, wilayats / districts and governorates/ states from where mungbean (Vigna radiata L. Wilczek) accessions were collected along with their latitudes, longitudes and altitudes.*

Sl.No.	Site No	Accession No.	Governorate	Wilayat	Village/location	Latitude (N)	Longitude (E)	Altitude (m)
1	4*	11	Interior	Manah	Mhuol	-	-	-
2	12	33	Interior	Adam	Seih A slam	22° 31.05'	57° 31.20'	325
3	25	75	Dhahira	Ibri	Bilad Al-Shahoom	23° 23.26'	56° 57.57'	884
4	28	84	Dhahira	Ibri	Bilad Al-Shahoom	23° 22.96'	57° 00.57'	947

Sl.No.	Site No	Accession No.	Governorate	Wilayat	Village/location	Latitude (N)	Longitude (E)	Altitude (m)
5	38	121	Dhahira	Yanqul	Al-Bouwerdah	23° 41.89'	56° 30.33'	623
6	44	138	Batinah South	Rustaq	Al-Ayeer	23° 12.79'	57° 27.56'	723
7	53	154b	Batinah South	Rustaq	Dhabaa	23° 26.99'	57° 06.82'	632
8	54	156b	Batinah South	Rusaq	Dhabaa	23° 26.86'	57° 06.86'	632
9	55*	165	Batinah South	Rusaq	Al-Dahir	-	-	-
10	56	176	Batinah South	Rustaq	Atayeeb	23° 25.40'	57° 09.78'	557
11	58	183	Batinah South	Rustaq	Almahdooth	23° 30.57'	57° 11.36'	476
12	62	188	Batinah South	Rustaq	Nezooh	23° 28.92'	57° 17.21'	344
13	63	191	Batinah South	Rustaq	Salm	23° 28.36'	57° 17.95	366
14	70	207	Batinah South	Rustaq	Alkhoof	23° 08.29'	57° 08.29'	579
15	99	255	Sharqiya	AL-Qabel	Al-Dubaha	22° 35.89'	58° 10.97'	625
16	114	284	Sharqiya	Wadi Atayeen	Asubal	23° 07.22'	58° 31.72'	439
17	118	292	Sharqiya	Wadi Atayeen	Maqtaa	22° 49.33'	58° 59.33'	1089
18	119	295	Sharqiya	Wadi bani Khalid	Al-Qaryah	22° 35.97'	59° 05.37'	611
19	129	313	Batinah North	Sohar	Wadi Aheer	23° 59.35'	56° 28.93'	467
20	149	335	Dhofar	Taqah	Shebdate	17° 11.27'	54° 40.73'	487
21	151	337	Dhofar	Taqah	Geloy	17° 05.85'	54° 16.71'	628
22	152	338	Dhofar	Mirbat	Qadeeh	17° 31.58'	54° 48.11'	358
23	153*	341	Dhofar	Mirbat	**	17° 58.37'	54° 34.82'	625
24	155	345	Dhofar	Mirbat	Tharbad	17° 07.78'	54° 53.03'	504
25	156*	346	Dhofar	Mirbat	**	17° 5.61'	54° 33.84'	630

- or * "information not available as the seed samples were acquired at Agriculture Development Centers "; ** Not recorded.

3. Results

During collecting missions of legume crops, 25 seed samples/accessions of mungbean were collected from different governorates of Oman with the most from South Batinah (coastal) governorate (9) represented by wilayat Rustaq followed by Dhofar (Southern) governorate (6), Sharqiya (Eastern) governorates (4), Dhahira & Buraimi governorate (3), Al-Dakhliyah (Interior) governorate (2), North Batinah (coastal) governorate (1).

3.1. Variation in Collection Sites

Collection sites were found varying in their characteristics and altitude. Altitude ranged from 325 m at site No. 12 of Seih A slam, Wilayat Adam of Al Dakhliyah (Interior) governorate to 1089 m at site No. 118 of Maqtaa, wilayat Wadi Atayeen of Sharqiyah. Soil characteristics were also found varied in in soil textures viz. sands, sandy loam, sandy clay, sandy clay loam, clay and loam. Soils were hard, firm or loose, variable-loose to crust and friable. With respect to drainage, soils were imperfect, free or variable. Soil pH was found varying from 2.1 (site No. 58, Al-Mahdooth Hajer Bani Omer, Rustaq, Batinah South) to 9.0 (sites No. 28, Bilad Al-Sahhoom, wilayat Ibri and and No. 38, Al-Bouwedah, wilayat Yanqul of Dhahira) whereas soil EC was found varied from 0.2 (Site No. 38, Al-Bouwedah, wilayat of Dhahira) to 9.0 dSm^{-1} (sites No. 56, Al-Tayeeb and No. 58, Almahdooth of Rustaq, Batinah South). Soil color ranged from light brown to dark brown).

3.2. Variability in Seed Characters

Seed accessions were diverse with respect to all the seed characters studied viz. seed length (cm) and width (cm), 100-seed weight (g) and seed color. Seed length varied from 0.350 cm (Collection No. 335 of Shebdate, Taqah, Dhofar) to 0.760 cm (Collection No. 345 of Tharbad, Mirbat, Dhofar); seed width ranged from 0.245 cm (Collection No. 33 of Seih A'slam, Adam, Interior) to 0.495 cm (Collection No. 345 of Tharbad, Mirbat, Dhofar); 100-seed weight ranged from 1.800 g (Collection No. 33 of Seih A'slam, Adam, Interior) to 12.400 g (Collection No. 345 of Tharbad, Mirbat, Dhofar) (Table 2).

With respect to seed color, six accessions—Collection No. 284 of Asubal, Wadi A'Tayeen, Sharqiya, Collection No. 295 of Al-Qarya, Wadi Bani Khalid, Sharqiya, Collection No. 313 of Al-Ghudafah, Wadi Aheer, Sohar, Batinah North, Collection No. 335 of Shebdate, Taqah, Dhofar, Collection No. 341 of Mirbat, Dhofar and Collection No. 345 of Tharbad, Mirbat, Dhofar—were homogenous (pure) with their characteristic green color. The remaining 19 seed accessions were heterogeneous (mixture) with seeds of various colors such as green, brown and black. The critical analysis of seed colors of these samples indicated the presence of 4 groups of which the largest group had 12 seed accessions with green, black seed color followed by one group of three seed accessions with green, brown and black seed color and two groups of two accessions, each with green, black, brown, and green, brown seeds, respectively (Table 2).

Table 2. *Variation among seed characteristics of 25 indigenous mungbean accessions collected.*

Sl. No.	Collection No.	Length (cm)	Width (cm)	100 seed weight (g)	Seed color	Color	Region
1	11	0.460	0.350	3.6	Heterogeneous	Green, black	Interior
2	33	0.475	0.245	1.8	Heterogeneous	Green, black, brown	Interior
3	75	0.435	0.325	4.1	Heterogeneous	Green, black	Dhahira

Sl. No.	Collection No.	Length (cm)	Width (cm)	100 seed weight (g)	Seed color	Color	Region
4	84	0.455	0.350	4.6	Heterogeneous	Green, black	Dhahira
5	121	0.440	0.320	4.8	Heterogeneous	Green, black	Dhahira
6	138	0.395	0.320	3.9	Heterogeneous	Green, black	Batinah South
7	154 b	0.420	0.325	3.9	Heterogeneous	Green, black	Batinah South
8	156 b	0.435	0.325	3.9	Heterogeneous	Green, black	Batinah South
9	165	0.455	0.310	3.5	Heterogeneous	Green, black	Batinah South
10	176	0.465	0.335	4.6	Heterogeneous	Green, black	Batinah South
11	183	0.480	0.365	4.4	Heterogeneous	Green, black, brown	Batinah South
12	188	0.420	0.335	4.0	Heterogeneous	Green, black	Batinah South
13	191	0.395	0.305	3.7	Heterogeneous	Green, black	Batinah South
14	207	0.450	0.320	3.8	Heterogeneous	Green, brown, black	Batinah South
15	255	0.465	0.320	3.3	Heterogeneous	Green, brown	Sharqiya
16	284	0.490	0.360	3.6	Homogeneous	Green	Sharqiya
17	292	0.410	0.300	3.7	Heterogeneous	Green, brown	Sharqiya
18	295	0.420	0.325	3.8	Homogeneous	Green	Sharqiya
19	313	0.465	0.335	4.5	Homogeneous	Green	Batinah North
20	335	0.350	0.280	2.3	Homogeneous	Green	Dhofar
21	337	0.450	0.355	5.0	Heterogeneous	Green, brown, black	Dhofar
22	338	0.435	0.365	5.1	Heterogeneous	Green, brown, black	Dhofar
23	341	0.455	0.375	4.6	Homogeneous	Green	Dhofar
24	345	0.760	0.495	7.3	Homogeneous	Green	Dhofar
25	346	0.410	0.390	5.6	Heterogeneous	Green, black	Dhofar
Statistical Parameters							
Minimum		0.350	0.245	1.8			
Maximum		0.760	0.495	7.3			
Mean		0.451	0.337	4.1			
S.E.(+)		0.015	0.009	0.388			

3.3. Principal Component Analysis

Principal Component Analysis was performed to reduce the original characters/ variables into a new set of uncorrelated variables known as principal components (PCs). The scree plot of the PCA (Figure 1) indicated that the first two eigenvalues correspond to the major proportion of the variance in the dataset. The first two PCAs extracted from the original correlated variables amounted to 93.129% with PC 1 having eigenvalue of 1.853 and PC 2, approximately half of PC1 (0.941) (Table 3).

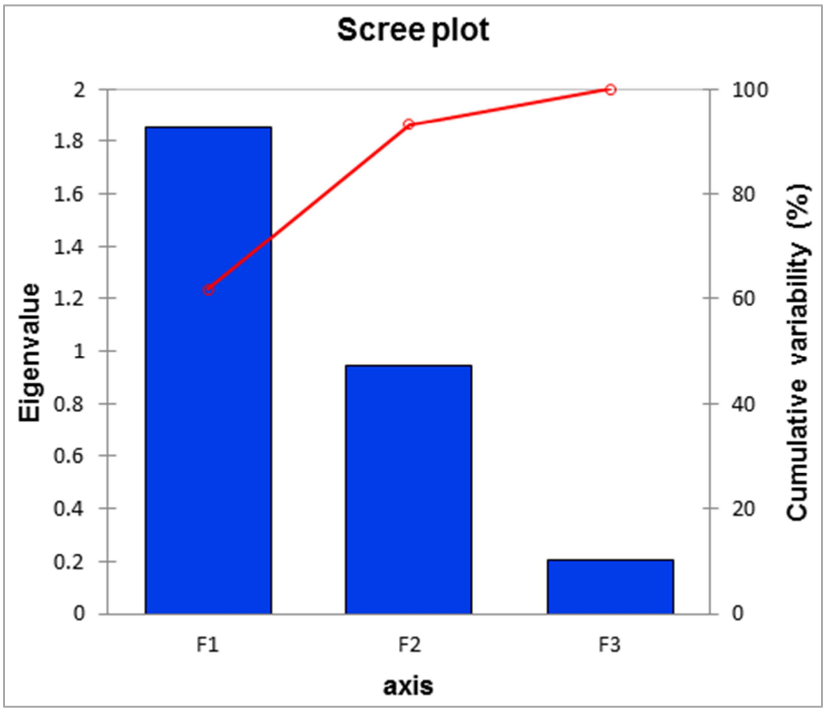

Figure 1. *Scree plot showing eigenvalues in response to three principal components (F1 to F3) for three seed variables/ characters studied in 25 indigenous mungbean accessions.*

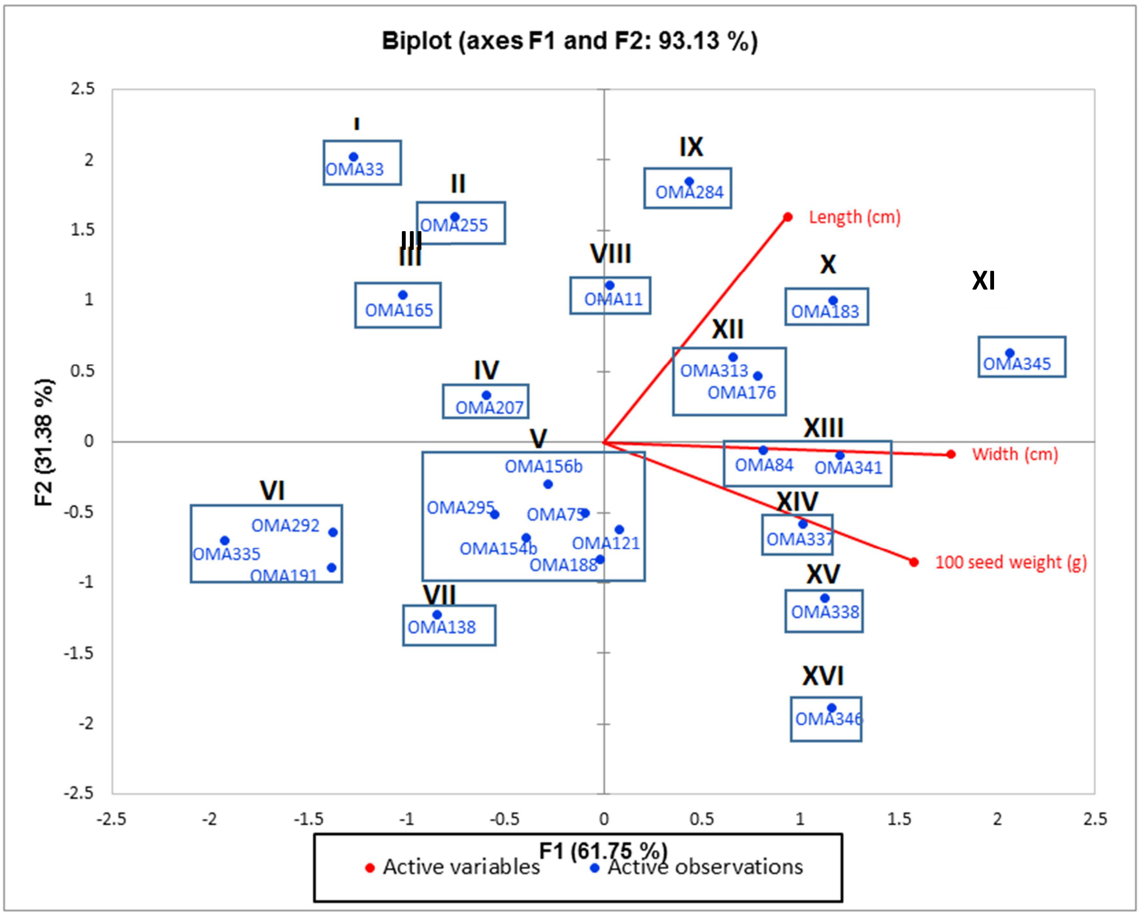

Figure 2. *Principal component scores of PC1(F1) and PC2(F2) showing the overall variation/scattering among indigenous mungbean germplasm for three seed traits.*

Table 3. *Eigen values and percent variance of principal components to total variation in 25 indigenous mungbean accessions.*

Principal Components (PC' s)	Eigen value	% Variance	Cumulative variance
PC 1	1.853	61.752	61.752
PC 2	0.941	31.378	93.129
PC 3	0.206	6.870	100.000

The PCA grouped the estimated mungbean variables into three main components of which PC 1 accounted for about 61.752% of the variation; PC 2, for 31.378% and PC 3, for 6.870% (Table 3). The first PC was influenced equally and positively by seed width (0.694) and 100 seed weight (0.620) whereas the second PC was influenced positively by only seed length (0.882). However, third PC was influenced positively by seed width (0.719) and negatively by (100-seed weight (-0.629) (Table 4). In respect of correlation between seed size characters, only correlation between seed width and 100-seed weight was found highly significant (0.724**) and positive (Table 5).

Table 4. *The principal component values of three seed size characters in 25 indigenous mungbean accessions.*

Variables/Characters	PC 1	PC 2	PC 3
Seed length (cm)	0.367	0.882	-0.296
Seed width (cm)	0.694	-0.048	0.719
100 Seed-Weight (g)	0.620	-0.469	-0.629

Table 5. *Correlation coefficients between seed size characters in 25 indigenous mungbean accessions.*

	Seed length (cm)	Seed width (cm)	100 Seed-Weight (g)
Seed length (cm)	1	0.388	0.071
Seed width (cm)		1	0.724*
100 Seed-Weight (g)			1

With respect to per cent contribution of seed characters to the PCs, both seed width and 100-seed weight together contributed to the extent of 86.51% to PC1 and 91.26% to PC3 whereas seed length alone had 77.77% contribution to PC2 (Table 6).

Table 6. *The percent contribution of variables (three seed size characters) to three principal component values in 25 indigenous mungbean accessions.*

Variables/Characters	PC 1	PC 2	PC 3
Seed length (cm)	13.490	77.771	8.739
Seed width (cm)	48.115	0.230	51.655
100 Seed-Weight (g)	38.395	21.999	39.606

The scatter of 25 indigenous mungbean accessions in biplot graph of the first two principle components as X and Y –axes clearly indicated their clustering pattern spreading wide apart to each other in all the four quadrants of the graph in such a way that as many as 12 of the accessions tended to form independent clusters on their own from as many as 16 clusters formulated where the accessions belonging to the same cluster are closely positioned to form clusters in whichever quadrants of the graph they belonged due to their similarities (Figure 1). These solitary clusters were (Clusters I (OMA-33), II (OMA 255), III (OMA 165), IV (OMA 207), VII (OMA 138), VIII (OMA 11), IX (OMA 284), X (OMA 183), XI (OMA 345), XIV (OMA 337), XV (OMA 338) and Cluster XVI (OMA 346)). Of the remaining four clusters, Clusters XII (OMA 176 and OMA 313) and XIII (OMA 84 and OMA 341) had two accessions each whereas Cluster VI had three accessions (OMA 191, OMA 292 and OMA 335) and Cluster V had the highest number of six accessions, as its members (OMA 75, OMA 121, OMA 154b, OMA 156b, OMA 188 and OMA 295) were closely positioned to each other.

4. Discussion

A range of indigenous mungbean samples was collected from different wilayats of the governorates of the country. South Batinah represented the most collections to the extent of 36% followed by the country's southern Dhofar governorate (24), eastern Sharqiya governorates (16%), Dhahirah & Buraimi (12%) and the interior, Al-Dakhliyah governorate (8%). The north coastal Batinah governorate had the least of 4%. The extent and distribution through availability of indigenous mungbean samples in different governorates showed that there existed local conservation of the crops among the farmers on their preferences of the crops to grow and consume. The changes in edaphic or biotic or climate factors did not seem to affect the availability of seed samples of mungbean with the farmers across the governorates. This is in contrast with observations made in other crops where seed samples of some crops were almost absent from governorates namely Southern Dhofar (chickpea, faba bean, lentil and field peas), Coastal North Batinah (Chickpea, faba bean and lentil) and eastern Sharqiyah governorates (Chickpea and lentil) [27-29]. Critical examination of mungbean seed samples at the laboratory indicated wide variation in seed size traits and seed coat pattern (color). The variation in mungbean germplasm in respect of seed size has been investigated previously using only seed test weight viz. 100 or 1000-seed weight [7, 25, 35] whereas in the present study seed length and width were additionally used as a measure of seed size as evidenced by strong positive correlation (r=0.724**) between seed weight and seed width. Seed test weights of the present study (1.8-7.3 g/ 100 seed) were comparable with those of previous studies in their mungbean accessions viz. 2.31-6.85 g [7], 2.63 to 7.02 g [35] and 2.4-5.0 g [25]. The present study for the first time indicated existence of enormous variation among the 25 indigenous Oman accessions in seed color ranging from green to blends of green, black and brown colors categorizing in four groups. Selection of strongly associated characters like seed width and 100-seed weight could be utilized along with desired seed color to improve seed size characters that influence yield and their value in marketing as suggested by earlier workers who studied both seed and yield traits in mungbean towards selection of parents in crossing program [7, 25, 36].

It was observed during the collecting missions that villages in the vicinity or far from collecting sites had either the same or different seed samples. Such widespread shift of landraces of mungbean between wilayats and neighboring areas indicated that these landraces/accessions are the outcomes of centuries of selection for adaptation to local climatic, edaphic and cultural selection forces and hold unique gene complexes which reflect local agro-climatic variation and evolution [27, 37].

The results of PCA analysis are very useful in crop breeding programs for selecting not only potential parents for crossing to improve the characters of interest for productivity in quantity and quality but also in identifying the phenotypic characters that contribute higher genetic variations among the genotypes/ accessions studied [7, 25, 35-36]. In the present study, PCA clearly revealed that all the seed characters positively influenced to PCA1 component reflecting potential of the seed size of each accession whereas only seed length contributed positively to PCA2. The biplot graph characteristically demarcated the accessions about their scattering pattern on the basis of first two dimensions/components into as many as 16 clusters based on seed characters in all the four quadrants. The existence of wide spectrum of phenotypic variability among the indigenous mungbean accessions was further explained by their pattern of distribution over all the four quadrants of the graph wherein as many as 12 accessions seemed to position themselves separately. Interestingly, these accessions belonged to either same (e.g. OMA 11 and OMA 33 from Interior/ Al-Dakhliyah; OMA 165 and OMA OMA 183 from Batinah South and OMA 337, OMA 338, OMA 345 and OMA 346 from Dhofar governorate) or different (e.g. OMA 75 from Dhahirah, OMA 183 from Batinah South; OMA 284 from Sharqiya and OMA 346 from Dhofar) governorates The accessions of clusters namely V, VI, XII and XIII belonged to either similar (OMA 75 and OMA 121 of Dhahirah and OMA 154b and OMA 156b of Batinah South in cluster V) or different (e.g. OMA 191 of Batinah South, OMA 292 of Sharqiyah and OMA 335 of Dhofar in cluster VI and the constituents of clusters XII and XIII) governorates. Such distributions of accessions showed the possibility of inter-exchange of the seed samples among the farmers from different governorates. It is suggested that the accessions of different clusters be involved in crossing for improvement of seed characters, as these accessions would be expectedly genetically different.

5. Conclusions

Twenty-five mungbean (*Vigna radiata* (L.) R. Wilczek) seed accessions collected were diverse with respect to seed related characters. These accessions were grouped into 16 genetically diverse clusters based on the Principal Component Analysis (PCA) using seed characters. The results of PCA indicated significant contributions of seed width (48.115%) and 100-seed weight (38.395%) to the PC 1 that influenced 61.752% to the total variation existing in indigenous mungbean germplasm collected from the most of the governorates of Oman. The accessions of different clusters could be involved in crossing programs for improvement of yield along with seed characters.

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