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Multivariate Analysis among Okra (*Abelmoschus esculentus* (L.) Moench) Collection in South Western Ethiopia

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ABSTRACT

Okra (*Abelmoschus esculentus* (L.) Moench) is an economically important vegetable crop grown in different part of Ethiopia particular in south western part of the country. The objective of the study was to evaluate genetic diversity among Okra accessions based on quantitative morphological traits. Twenty five Okra accessions were planted in 2011/2012 at Gambella in randomized complete block design with three replications. Data on 20 quantitative traits were collected and subjected to various statistical analyses. The analysis of variance showed significant differences ($p<0.01$) among the accessions for all quantitative characters measured. Cluster and distance analysis of quantitative characters based on multivariate analysis pointed out the existence of five divergent groups. The maximum distance was observed between cluster II and I (2846) while the minimum was between I and III (213.64). Principal component analysis indicated that six principal components explained about 83% of the total variation. Differentiation of germplasm into different cluster was because of cumulative effect of number of characters. Accessions like GM7, GM9 and GH13 from Gambella collection and AS4 and AS11 from Assosa collection are recommended for the next breeding work as they are high yielder accessions compared to the others. The present study indicated a considerable amount of variability for the majority of the quantitative characters in Okra for exploitation. However, it is recommended that the experiment should be repeated at more location and years with more collections to confirm the obtained results.

Key words: Okra, multivariate, clustering, principal component analysis, divergence, ethiopia

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is a warm-season annual herbaceous vegetable crop grown primarily for immature fruits. It is apparently originated in Ethiopia, the mountainous or plateau area of Eritrea and the eastern, higher part of the Anglo-Egyptian Sudan (Aladele *et al*., 2008). It is widely distributed from Africa to Asia, in Southern Europea, the Mediterranean and all of the America (Oyelade *et al*., 2003). Okra, commonly known as "lady finger", is primarily suitable for cultivation as a garden crop as well as on large commercial farms. The crop grows well in hot weather, especially in the regions with warm nights (>20°C) (Ndunguru and Rajabu, 2004). It is sensitive to frost, water logging and drought conditions. Although, there is no complete record on production area and productivity of the crop under

Ethiopian condition, it has high diversity in some parts of the country particularly in the southwestern low lands (550-650 m asl) region (PGRC, 1995). Okra is a multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods and stems, pods and seeds (Schippers, 2000). For generation, farmers in Gambella and Asossa have been cultivating for its fruit and leaf to use as a food and medicine of different diseases.

Despite its multi-directional importance and utility, only limited research has been conducted in the improvement of Okra in Ethiopia. Okra has been collected and maintained for long period of time by Institute of Biodiversity Conservation (IBC) and other research center like Gambella Agricultural Institute (GARI) but they are not yet characterized and their variability is not known. Moreover, little has been previous attempted by breeders in improving the crop in terms of developing core collections for higher yield and quality. The accessions under cultivation, over the years in the various regions across the country are landraces.

It is well known that adequate genetic diversity is necessary in breeding program for the development of high yielding varieties. The use of multivariate analysis and the use of generalized distance (D^2) as quantitative measure of genetic diversity are well illustrated. Moreover, information on the extent of genetic diversity in Okra germplasm is absolutely essential in parental selection to start hybridization. It is generally believed that crosses between parents with maximum genetic divergence are likely to produce desirable segregation and recombination in progeny (Reddy, 1988). Characterization of genetic resources therefore refers to the process by which accessions are identified, differentiated or distinguished according to their character. It provides information on diversity within and between crop collections. This enables the identification of unique accessions essential for curators of gene banks (Ren *et al*., 1995). Moreover, information obtained on genetic relatedness among genetic resources of crop plants is useful, both for breeding and for the purposes of germplasm conservation. Diversity analysis using morphological traits is therefore a highly recommended first step that should be undertaken before more in-depth biochemical or molecular studies are employed in any diversity studies. Hence, the objective of the study was to determine the extent of genetic diversity among Okra collections using multivariate analysis with the ultimate goal of providing genetically divergent parents for hybridization.

MATERIALS AND METHODS

Experimental site, materials and design: The experiment was conducted at Gambella (8°15' N, 34°35' E with an elevation of 526 m above sea level). The experiment was conducted on alluvial soil type with a pH of 6.5. The average annual rainfall of the study area was 1020.5 mm per year with the average annual minimum and maximum temperatures of 20.1 and 35.7°C, respectively (National Meteorology Agency, 2012). Twenty-five Okra accessions which have been collected by Gambella Agricultural Research Institute from Gambella and Asosa regions were used for the study (Table 1). The accessions were arranged in a Randomized Complete Block Design (RCBD) with three replications making a total of 75 plots.

Data collection: Data on 20 quantitative traits were recorded from nine randomly selected plants from the three middle rows using International Plant Genetic Resources Institute (IPGRI, 1991) descriptor list for Okra species. These includes: Days to emergence, days to 50% flowering, leaf length, leaf width, internodes length, stem diameter, peduncle length, number of epicalyxes,

Table 1: *Abelmoschus esculentus* accessions and their area of collection

Accession No.	Accession name	Zone	Woreda	Local name
1	GM-1	Agnuak	Bonga	Amula
2	GM-2	Agnuak	Bonga	Amula
3	GM-3	Agnuak	Bonga	Amula
4	GM-4	Agnuak	Chobokir	Amula
5	GM-5	Agnuak	Chobokir	Amula
6	GM-6	Agnuak	Chobokir	Amula
7	GM-7	Agnuak	Chobokir	Amula
8	GM-8	Agnuak	Pinkyo	Amula
9	GM-9	Agnuak	Tagni	Amula
10	GM-11	Agnuak	Jawe	Amula
11	GM-12	Agnuak	Abol	Amula
12	GM-12	Nuwer	Lare	Amula
13	GM-13	Agnuak	Eley	Amula
14	GM-14	Nuwer	Itang	Amula
15	AS-1	Assosa	Abrahamo	Qenqes
16	AS-2	Assosa	Abrahamo	Sharma
17	AS-3	Assosa	Furfur	Sharma
18	AS-4	Assosa	Furfur	Bamiya
19	AS-5	Assosa	Furfur	Sharma
20	AS-6	Assosa	Furfur	Qenqes
21	AS-7	Assosa	Afamagale	Qenqes
22	AS-8	Assosa	Kuldadine	Qenqes
23	AS-9	Assosa	Surqole	Qenqes
24	AS-10	Assosa	Bambasi	Qenqes
25	AS-11	Assosa	Bambasi	Sharma

number of primary branches per stem, days to maturity, plant height, fruit length, fruit diameter, average fruit weight, number of pod per plant, number of internodes, number of ridges on fruit, number of seeds per pod, hundred seed weight and yield per plot.

Data analysis: Quantitative data were subjected to analysis of variance (ANOVA) to examine the presence of statistically significant differences among accessions for the characters measured. Clustering was performed using the proc cluster procedure of SAS version 8.2 (SAS, 2001) by employing the method of average linkage clustering of hierarchical clustering called Un-weighted pair group methods with arithmetic average (UPGMA). The numbers of clusters were determined by following the approach suggested by Copper and Milligan (1988) by looking into three statics namely Pseudo F, Pseudo t^2 and cubic clustering criteria.

Genetic divergences between clusters were calculated using the generalized Mahalanobis's D^2 statistics (Mahalanobis, 1936) using the equation: Squared distance (D^2) for each pair of genotype combinations was computed using the following equation:

$$D^2_{ij} = (x_i - x_j)\, S^{-1}(x_i - x_j)$$

where, D^2ij = the square distance between any two genotypes i and j, X_i and X_j = the vectors for the values for genotype ith and jth genotypes and S^{-1} = the inverse of pooled variance covariance matrix.

The D^2 values obtained for pairs of clusters were tested for significance at 0.05 level of significance against the tabulated values of for p degrees of freedom, where p is the number of variables considered (Singh and Chaudhary, 1985). Finally Principal component analysis was performed using correlation matrix by employing SAS procedure.

RESULTS AND DISCUSSIONS

Analysis of variance: Analysis of variance for the measured quantitative characters showed highly significant differences for all the traits. Because of this all traits were included in multivariate analysis like cluster analysis, divergence analysis and principal component analysis.

Cluster analysis: Cluster analysis based on quantitative characters grouped 25 germplasm accessions in to 5 distinct clusters (Table 2, Fig. 1) in which the first cluster consisted of 13 accession (52%), the second cluster consisted of 6 accessions (24%), the third clusters consisted 3 accessions (12%), the fourth cluster contained of 2 accession (8%) and the fifth cluster consisted of only one accession (4%) from the total accessions. The distribution pattern of genotypes into 5 clusters confirmed the existence of diversity among the genotypes.

Cluster means analysis: The mean value of the 20 quantitative characters in each cluster is presented in Table 3. Cluster I consisted of 13 genotypes having the characteristic of relatively narrow leaf width (37.56 mm) and high number of epicalyxes (9.44). Cluster II could be characterized by relatively late fruit maturing (104.22) and had relatively long leave length (42.21 cm) and wider stem diameter (32.70 mm). It also known by having the short fruit length

Table 2: Clusters of Okra accessions based on quantitative characters studied at Gambella in 2011/2012

Cluster	No. of accessions	Accessions name
I	13	GM1, GM3, GM4, GM5, GM6, GM8, GM10, GM11, GM12, GM13, GM14, AS8, AS9
II	6	AS1, AS2, AS3, AS5, AS6, AS7
III	3	GM2, GM7, GM9
IV	2	AS4, AS11
V	1	AS10

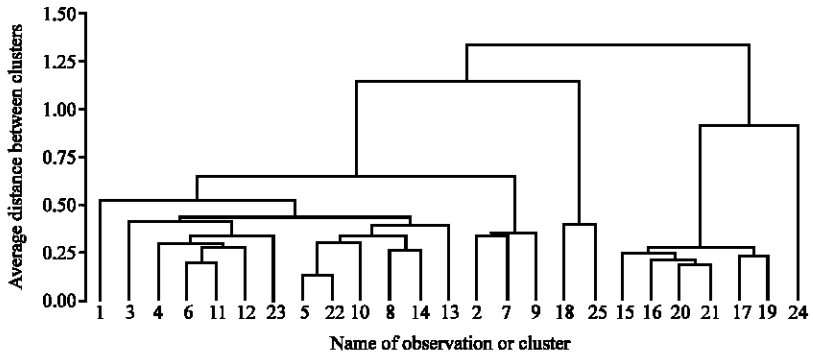

Fig. 1: Dendrogram depicting the genetic relationship of Okra germplasm based on 20 quantitative characters evaluated at Gambella in 2011/12 NB. The numbers representation is as indicated in Table 1

Table 3: Cluster means for 20 quantitative characters of Okra accessions studied at Gambella in 2011/2012

	Cluster				
Quantitative characters	1	2	3	4	5
Days to emergence	5.41	6.06	5.22	5.33	6.00
Days to 50% flowering	54.05	62.33	52.67	52.50	53.33
Days to maturity	85.44	104.22	84.00	93.67	84.00
Leaf length (cm)	31.09	29.28	33.10	31.76	24.21
Leaf width (cm)	37.56	42.21	38.06	40.67	44.07
Stem diameter (mm)	30.20	32.70	29.38	29.70	31.91
Internodes length (cm)	6.56	5.65	7.47	4.45	6.53
No. of branch	3.78	9.31	2.49	5.73	10.47
Peduncle length (cm)	4.75	3.02	5.21	4.45	4.20
Fruit length (cm)	25.39	12.30	28.81	43.43	45.07
Average fruit weight	79.48	22.31	113.81	148.32	22.37
Fruit diameter (mm)	26.86	9.73	29.15	43.81	45.10
No. of ridge	7.73	7.94	7.67	7.87	7.67
Seed pod^{-1}	100.85	84.72	105.00	112.87	113.13
100 seed weight	6.64	6.11	6.89	7.17	6.00
No. of epicalyxes	9.44	9.06	9.33	9.17	9.33
Internodes No.	26.14	28.77	23.03	24.23	29.65
Plant height (cm)	68.82	45.27	80.61	95.14	55.05
No. of pods plant^{-1}	20.08	24.16	16.91	18.56	29.65
Fruit yield (kg plot^{-1})	9.47	3.95	11.01	17.55	6.65

(12.30 cm), peduncle length (3.02 cm) and plant height (45.27 cm) with small average fruit weight, umber of seed per pod (84.72 No.) and fruit yield per plot (3.95 kg) (Table 3).

Cluster III consisted of three genotype. The shortest time to germinate (5.22) and relatively had narrow stem diameter (29.38 mm). These cluster also had longer peduncle (5.21 cm). Additionally it had fruits with high number of ridge (7.67), with small number of internodes (23.03). Cluster IV had two genotype having the characteristic of small internodes length (4.45 cm) with long plant height (95.14 cm), heavier average fruit weight (148.32 g). On the other hand it has relatively high number of seed per pod (107.933) and yield per plot (95.14 kg). The last cluster V had only one genotype having the characteristics of early harvesting date (84.00), short leaf length (24.21 cm) however it had wider leaf (44.07 cm) and fruit width (45.10 mm). On the other hand, it is known by high number of primary branches (10.47), internodes number (29.65) and seed per pod (113.13) as well as number of pod per plant (29.65). Based on cluster means, it is evident from the data (Table 3) that germplasm accessions falling in cluster IV and V showed higher performance for the characters of interest viz., internodes number, Internodes length, fruit length, average fruit weight and plant height. Furthermore, most of these characters also had positive genotypic association with fruit yield per plot except internodes length.

Hence, their potential as parents in heterotic breeding work seems possible. On the other hand, cluster II which is consisted of 5 germplasm accessions was the least in performance for the majority of quantitative characters studied. For example, all of the accessions grouped under this cluster gave the least fruit yield and average fruit weight. The result also pointed out that the importance of accessions in cluster II for their exploitation in fruit yield improvement appeared limited in view of their poor performance for the majority of the characters of interest. This indicated that different

Table 4: Average inter cluster (off diagonal) D^2 values among three clusters in *Abelmoschus esculentus* accessions studied at Gambella in 2011/2012

Clasture	I	II	III	IV	V
I		2513**	213.64**	1034**	931.53**
II			1698**	2846**	1421**
III				410.38**	1638**
IV					867.58**
V					

**Significant χ^2 = 28.87 and χ^2 = 34.81 at 5 and 1% probability level

clusters have different breeding values that enable breeders to improve different traits and parental selection should be made based on the relative merits of each cluster for each trait depending on the objective of the breeding program.

Divergence analysis: Test of significance for divergence analysis showed significance between all cluster distances (Table 4). The minimum squared distance was between clusters I and III (213.64) followed by cluster III and IV (410.38). Maximum squared distance was between cluster II and IV (2846) followed by cluster I and II (2513) and cluster II and IV (1698.0). Generally this study revealed that germplasm accessions included in this study are highly divergent. Reddy *et al.* (2012) revealed considerable genetic diversity among 100 genotypes of Okra (*Abelmoschus esculentus* (L.) Moench).

According to Ghaderi *et al.* (1984) increasing parental distance implies a great number of contrasting alleles at the desired loci and then to the extent that these loci recombine in the F2 and F3 generation following a cross of distantly related parents, the greater will be the opportunities for the effective selection for yield factors. Minimum inter cluster distance was observed between clusters I and III (213.64) indicating that genotypes in these clusters were not genetically diverse or there was little genetic diversity between these clusters. This signifies that crossing of genotypes from these two clusters might not give higher hetrotic value in F1 and narrow range of variability in the segregating F2 population. Maximum genetic recombination is expected from the hybridization of the parents selected from divergent cluster groups. Therefore, maximum recombination and segregation of the progenies is expected from crosses involving parents selected from cluster II and IV followed by cluster I and II and cluster II and IV. However, the breeder must specify his objectives in order to make best use of the characters where the characters are divergent. Thus, it can be concluded that selection of genotypes from the most divergent clusters may exhibit a high heterosis besides fruit yield. Therefore, hybridization between the genetically diverse parents in further breeding programs may produce large variability and better recombinants in the segregating generations.

Principal component analysis: The principal component analysis (Table 5) revealed that 6 principal components PC1, PC2, PC3, PC4, PCA5 and PCA6 with Eigen value 10.65, 30.4, 2.41, 1.7, 1.62 and 1.32, respectively, have accounted for 83% of the total variation. The first 2 principal components PC1 and PC2 with values of 43 and 12.9%, respectively, contributed more to the total variation. According to Chahal and Gosal (2002), characters with largest absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero. Therefore, in this study, differentiation of the genotypes in to

Table 5: Eigenvectors and eigen values of the first six principal components (PCs) for characters of *Abelmoschus esculentus* accessions studied at Gambella in 2011/2012

	PC					
Quantitative characters	1	2	3	4	5	6
Days to emergence	0.14	0.12	-0.09	-0.31	0.17	0.31
Days to 50% flowering	0.27	0.00	-0.05	0.16	0.05	-0.09
Days to maturity	0.21	-0.02	-0.25	0.28	0.16	0.14
Leaf length	-0.10	-0.09	0.08	0.53	-0.33	0.00
Leaf width	0.14	0.21	-0.09	0.30	0.26	0.29
Stem diameter	0.13	0.36	0.08	0.12	0.29	-0.19
Internodes length	0.01	-0.15	0.50	-0.14	0.11	0.25
No. of branch	0.21	0.18	-0.16	0.03	0.19	0.25
Peduncle length	-0.17	0.26	0.33	0.00	-0.17	-0.05
Fruit length	-0.25	0.18	-0.05	-0.03	0.08	0.21
Fruit weight	-0.27	0.10	0.02	0.16	-0.02	-0.04
Fruit diameter	-0.25	0.17	-0.01	-0.05	0.15	0.17
No. of ridge	0.09	0.10	0.20	0.39	-0.01	0.37
Seed pod^{-1}	-0.25	0.14	0.01	-0.08	0.14	0.11
100 seed weight	-0.28	0.12	0.03	0.11	0.01	-0.01
No. of epicalyxes	-0.05	0.06	0.45	-0.09	0.45	-0.10
Internodes No.	0.15	0.42	-0.08	-0.04	-0.16	-0.23
Plant height	-0.28	0.16	0.01	0.11	0.01	-0.03
No. of pods $plant^{-1}$	0.16	0.39	-0.16	-0.11	-0.08	-0.16
Fruit yield (kg $plot^{-1}$)	-0.21	0.31	-0.01	0.15	0.04	-0.22
Eigen value	10.65	3.04	2.41	1.70	1.62	1.32
Difference	7.61	0.62	0.72	0.08	0.30	0.41
Proportion	0.43	0.12	0.10	0.07	0.06	0.05
Cumulative	0.43	0.55	0.64	0.71	0.78	0.83

different cluster was because quantitative traits such as days to fifty percent flowering, stem diameter, number of seed per pod, number of pod per plants, internodes length, inter nodes number and plant height.

Agronomic characters having relatively higher value in the first principal component (PC1) were days to 50% flowering, fruit length, average fruit weight, fruit diameter, seed per pod, 100 seed weight and number of seed per pod and plant height had more contribution to the total diversity and they were responsible for the differentiation of the six clusters. Characters like stem diameter, internodes number, peduncle length had contributed a lot for principal component (PC2); peduncle length, internodes length, number of primary branch, days to maturity, plant height and number of epicalyxes had contributed in the third principal component (PC3).

Generally quantitative traits such as days to fifty percent flowering, stem diameter, number of seed per pod, number of pod per plants, internodes length, inter nodes number and plant height contributed to the variation in two PCs out of the six PCs. This result further confirmed the presence of genetic diversity for use in improvement program of Okra.

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