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Multivariate analysis in advance lines of okra (*Abelmoschus esculentus*)

SONIA SOOD[1], DEEPTI KAPOOR[2], JYOTI DEVI[3] and NIVEDITA GUPTA[4]

CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176 062

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ABSTRACT

An attempt was made to determine the degree of divergence among 37 genotypes of okra. The multivariate analysis (D^2) arranged 37 genotypes into 8 clusters in pooled over years and hence, showed reasonable variability. The cluster I was the largest, comprising of 54.54% genotypes , showing high degree of homogeneity among them or had least genetic variation. The intra-cluster distance was high in cluster VI and VIII followed by cluster II. Maximum inter-cluster distance was observed between cluster II and VI in pooled over years. Based on cluster means, cluster IV '(IC-169469-1 × PK)-1-2-1-2' for fruits/plant and nodes/plant and cluster III '(VRH-2 × VRO-6)-9-2-1-3' for fruit yield/plant were found high for pooled over years. The contribution of individual character toward diversity, calculated by using Mahalanobis D^2divergence, showed that the mineral content (58.71%), dry matter (22.07%) fruit yield/plant (12.31%) and internodal length (3.15%) was the most important characteristic for divergence in pooled over the years.

Key words: *Abelmoschus esculentus,* Clusters, Genetic divergence, Genotypes, Traits

Okra [*Abelmoschus esculentus* (L.) Moench] belongs to family Malvaceae and genera *Abelmoschus* (syn. *Hibiscus*) (Schippers 2000). It is grown primarily for its young immature green fruits (Sekyere *et al.* 2011). Okra has a prominent position among fruit vegetables due to its multiple virtues like high nutritive and medicinal value, ease of cultivation, wide adaptability, year round cultivation, good portability, export potential and bountiful returns (Reddy *et al*. 2012). The centre of origin of okra is uncertain, but centre of diversity exits in West Africa, India and South East Asia (Hamon and Van Sloten 1989, Sawadogo *et al*. 2009). Although, there has been considerable improvement in the yield of okra, further improvement appears to be possible with identification and development of new improveddisease-resistant cultivars. In the recent years, efforts have been made by the breeders for its genetic improvement with the basic objective of developing high yielding and Yellow Vein Mosaic Virus (YVMV) resistant varieties.

The classification of a range of genetic variability among genotypes is pivotal for the maintenance and further acquisition of germplasm resources. Even accessions from diverse origins are needed as parent stocks for the development of improved varieties (Aremu *et al*. 2007, Nwangburuka *et al*. 2011). It is particularly useful for characterizing individual accessions and cultivars and as a general guide in the selection of parents for hybridization. Better knowledge on genetic diversity or genetic similarity could help to sustain long-term selection gain. Further, improvement in cultivars lies in the fact that crosses which involve genetically divergent parent are the most suitable to bring forth high heterotic effects and also, a greater genetic variability in segregating generations (Rao *et al.* 1981, Cruz 1990). Thus, the primary need of the crop industry is the estimation of genetic diversity between cultivated accessions for identification and breeding purposes (Tam *et al*. 2005). The use of multivariate techniques is a feasible option for this purpose, since these techniques allow combinations of multiple information within the experimental unit, through genotype discrimination based on a variable complex (Cruz and Regazzi 2001). However, the genotype × environment interaction is a constant challenge for breeders as it causes complications in selecting accessions evaluated in different environments/seasons. Consequently, the evaluation of genetic diversity in more than one environment could bring forth more clarifying results on the behaviour of genotypes with a subsequent influence on their performance in future breeding programs (Teixeira *et al.* 2004). The aim of present study was to characterize okra genotypes collected from different regions of India and F_5 progenies to assess the genetic diversity within the germplasm.

MATERIALS AND METHODS

In pursuance of the envisaged objectives, the present study was undertaken at Department of Vegetable Science and Floriculture, CSK HPKV, Palampur (Himachal Pradesh), India, situated at 32° 6′N latitude, 76° 3′E longitude, and elevation of 1 290.8 m above sea level during the summer

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[1]Professor (e mail: soniasood2005@rediffmail.com), [2]Agriculture Extension Officer, [4]Ph D Student, Department of Vegetable Science and Floriculture, College of Agriculture, CSK HPKV, Palampur, Himachal Pradesh 176 062. [3]Scientist, IIVR, Varanasi.

rainy (May-September) season of year 2011–12. The experimental material comprised of 37 diverse genotypes of okra collected from different sources across the country (Table 1). The experiment was arranged in Completely Randomized Block Design (CRBD) with 3 replications. Each entry/progeny was accommodated in 2 rows spaced 45 cm apart with an intra-row spacing of 15 cm. Seeds were treated with Bavistin (2 g /kg seed) to avoid the attack of fungal diseases. Besides, the application of farm yard manure (@ 10 tonnes/ha), chemical fertilizers were applied as per the recommended package of practices (100 kg N, 50 kg P_2O_5, 50 kg K_2O/ha). The intercultural operations, irrigations and pest-control measures were carried out in accordance with the recommended schedule. A plant population of 10 plants per row, plot and genotype was maintained. Biometric data were recorded on five randomly selected plants in each genotype in each replication for eight quantitative and three quality traits, viz. days to first picking, harvest duration (days), plant height (cm), fruits/plant, average fruit weight (g), nodes/plant, internodal length (cm), fruit yield/plant (g). Among quality traits, analysis was done to estimate dry matter (%), mucilage (%) and mineral content (mg/kg) in fresh marketable pods.

Table 1 List of okra genotypes and their sources

Genotype	Source
Hissar Unnat	CCS Haryana Agricultural University, Hissar, Haryana
SKBS-11	SK University of Agricultural Sciences & Technology, Srinagar
P-8	Punjab Agricultural University, Ludhiana, Punjab
Varsha Uphar	CCS Haryana Agricultural University, Hissar, Haryana
Komal	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
VRO-3	IIVR, Varanasi
VRO-4	IIVR, Varanasi
VRO-5	IIVR, Varanasi
VRO-6	IIVR, Varanasi
S-51	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
IC-169468	NBPGR, Regional Station, Dr. PDKV Campus, Akola, Maharashtra
Arka Anamika	Indian Institute of Horticultural Research, Bangalore
Prabhani Kranti	Marathwada Agricultural University, Parbhani
Barkha Bahar	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
Kanchan	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(VRH-2 × VRO-6)-1-1-2-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(VRH-2 × VRO-6)-9-2-1-3	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-169469-1 × PK)-1-2-1-2	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-169469-1 × PK)-2-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-169469-1 × PK)-2-1-2-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-169469-1 × PK)-8-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-169469-1 × PK)-4-1-1-2	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-169469-1 × PK)-6-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × AA)-3-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × AA)-3-1-2-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × AA)-3-1-2-2	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × AA)-3-2-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × AA)-5-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × AA)-6-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × AA)7-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × P-8)-2-2-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × P-8)-2-2-1-2	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × P-8)-3-1-1-2	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(IC-31033-1 × P-8)-8-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(P-8 × AA)-4-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(P-8 × AA)-4-2-2-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
(P-8 × AA)-7-1-1-1	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur

WINDOSTAT 8.0 software PL was used for statistical analysis which employed multivariate analysis utilizing Mahalanobis D^2 statistic Mahalanobis 1936, Rao 1952. Using 'V' statistic which in turn utilizes Wilk's criteria, simultaneous test of difference in mean values of a number of correlated variables/characters was done as suggested by Rao (1952). Tochers method (Rao 1952) was followed for determining the group constellations.

RESULTS AND DISCUSSION

Multivariate analysis provides valuable information on the extent of genetic diversity present in the germplasm. List of okra genotypes collected from different regions of

Table 2 Analysis of variance for dispersion of quantitative and quality traits in okra germplasm

Sources of variations		df	Sum of squares	Mean squares	F ratio	Probability
Pooled over years	Varieties	36	-2.8167E18	-7.8242E16	-1.304E14	0.00000**
	Error	179	1.0739E05	5.9996E02		
	Total	215	-2.8167E18	-13101E16		

Significant at 1% level.

India and developed in the institute are given in Table 1. The simultaneous significance of mean differences was tested by analysis of dispersion (Table 2). The F-value is highly significant indicating large differences between the means of the populations based on pooled effect of all the twelve characters and may be continued for further analysis for computing D^2 estimates.

The composition of clusters on the basis of D^2 statistics differed between years which showed inconsistent clustering behaviour of some of genotypes due to influence of environmental variation. The multivariate analysis (D^2) arranged 37 genotypes into 8 clusters in 2011, 2012 and pooled over years respectively and hence, showed reasonable variability (Table 3). The cluster I was the largest comprising of 54.54% genotypes during pooled years, showing high homogeneity among them or had least genetic variation. Similar results were also found by Sharma *et al.* (2008), Pradip *et al.* (2010) and Yonas *et al.* (014). Clusters III (VRH-2 X× VRO-6)-9-2-1-3, IV ((IC-31033-1 ×X AA)-6-1-1-1), V ((IC-31033-1 ×X AA)-6-1-1-1) and VII 'Kanchan' were monogenotypic, i.e. contained only one genotype which indicated their independent identity and to various unique characters possessed by them falling in highly divergent group and would be expected to manifest maximum heterosis and release of desirable transgressive segregants (Table 3).

The clustering of the genotypes was random, thus indicated no parallelism between clustering pattern and geographical diversity rather genetic constitution of the genotypes influenced by the clustering pattern and indicated that geographical isolation need not be necessary related to genetic diversity. However, forces other than geographical distance like genetic architecture of population, heterogeneity, history of selection, proximity of development of traits and genetic drift etc may be another cause for genetic diversity (Duzyaman and Vural 2002, Singh *et al.* 2012). Similar results have also been presented by Rohit *et al.* (2011) and Pachiyappan and Saravanan (2012) in their respective studies on okra. Further, the advanced derived lines from a specific cross had a tendency to be grouped in several clusters and thus represented the different nature of recombinants from a specific cross. Similar findings have also been reported by Dhankhar *et al.* (2008).

Through this study, it is possible to conclude that no agreement of the clustering takes place over the years. Probably, the exact concordance of clustering never existed due to the fact of significant interaction of genotype for environment. However, 20 genotypes from cluster I viz., '(IC-169469-1 X× PK)-2-1-1-1, (P-8 ×X AA)-4-1-1-1, (IC-31033-1 ×X P-8)-8-1-1-1, Barkha Bahar, Varsha Uphar, (IC-169469-1 ×X PK)-2-1-2-1, (IC-31033-1 ×X P-8)-2-2-1-1, (P-8 ×X AA)-4-2-2-1, (IC-169469-1 ×X PK)-6-1-1-1, S-51, (IC-31033-1 ×X AA)-3-1-1-1, Arka Anamika, SKBS-11, P-8,(IC-31033-1 ×X AA)-3-2-1-1, (IC-31033-1 ×X AA)-3-1-2-1, (P-8 ×X AA)-7-1-1-1, Hissar Unnat,(VRH-2 ×X VRO-6)-1-1-2-1, (IC-31033-1 ×X AA)-3-1-2-2' showed consistency in clustering pattern by remaining in the same group in pooled over years and are supposed to exhibit higher external heterogeneity.

Average intra and inter-cluster D2 values are tabulated in (Table 4) providing an interesting information on the nature of genetic divergence at intra and inter-cluster levels, respectively. In general, intra-cluster distances were much lesser than inter-cluster distances. The intra-cluster distance was high in cluster VI and VIII followed by cluster II during pooled over years. The clusters with single genotype had zero intra-cluster divergence.

Since the intra-cluster distance was low, the chances of developing good segregants by hybridization among parents within cluster would be low. Therefore, it is logical to attempt crosses between genotypes falling in different clusters based on inter-cluster distance which indicated that genotypes belonging to these clusters are genetically more diverse from each other. Therefore, suggested that based upon larger clusters distances to attempt crosses of genotypes from all the clusters, which may lead to broad spectrum of favourable genetic variability for yield improvement (Singh and Dubey 2011). The inter-cluster distance ranged from 6.01-23.99 and \ maximum inter cluster distance was observed between cluster II and VI in pooled over years.

The cluster means for different characters (Table 5) indicated considerable differences among the clusters. From the data, it can be seen that considerable differences exist for all the traits studied. A high mean for important traits is fundamental for the selection of superior material, since it is important not only to account for the performance but also their genetic divergences (Palomino *et al.* 2005, Umrao *et al.* 2014). Based on cluster means, cluster IV (IC-169469-1 × PK)-1-2-1-2 for fruits/plant and nodes/plant and cluster III (VRH-2 × VRO-6)-9-2-1-3 for fruit yield/plant were found to be superior than other economic traits. Cluster V (SKBS-11 and (IC-31033-1 × AA)-6-1-1-1) had high mean value for plant height over the years. Hence, indicated that the genotypes included in these clusters had sufficient genetic diversity for above traits. The crossing among genotypes of these clusters selected for specific component traits may be helpful in bringing new gene pool and expanding the

Table 3 Cluster compositions in okra using multivariate analysis in 2011, 2012 and pooled over years

Years	Clusters	Number of genotypes	Name of genotypes
2011	I	16	(IC-169469-1 × PK)-2-1-1-1, (P-8 × AA)-4-1-1-1,(IC-31033-1 × P-8)-8-1-1-1, (IC-31033-1 × P-8)-2-2-1-1, Varsha Uphar, P-8, (IC-31033-1 × AA)-3-1-2-1, Hissar Unnat, (IC-31033-1 × AA)-3-1-1-1, Arka Anamika, Barkha Bahar, (P-8 × AA)-4-2-2-1, (IC-169469-1 X PK)-6-1-1-1, S-51, (IC-169469-1 × PK)-2-1-2-1, (VRH-2 × VRO-6)-1-1-2-1
	II	10	VRO-4, Prabhani Kranti, Komal, VRO-6, VRO-5, (P-8 × AA)-7-1-1-1, (IC-169469-1 × PK)-4-1-1-2, (IC-169469-1 × PK)-1-2-1-2, (IC-31033-1 × AA)-3-1-2-2, (VRH-2 × VRO-6)-9-2-1-3,
	III	3	VRO-3, (IC-31033-1 × P-8)-3-1-1-2, Kanchan
	IV	1	(IC-31033-1 × AA)-3-2-1-1
	V	2	SKBS-11, (IC-31033-1 × AA)-6-1-1-1
	VI	5	(IC-169469-1 × PK)-8-1-1-1, (IC-31033-1 × P-8)-2-2-1-2, IC-169468,(IC-31033-1 × AA)7-1-1-1, (IC-31033-1 × AA)-5-1-1-1
2012	I	20	(IC-169469-1 × PK)-6-1-1-1, (P-8 × AA)-4-2-2-1, (IC-169469-1 × PK)-2-1-1-1, S-51, (IC-31033-1 × P-8)-2-2-1-1, (IC-169469-1 × PK)-2-1-2-1,(IC-31033-1 × P-8)-8-1-1-1, (P-8 × AA)-7-1-1-1, Barkha Bahar, Varsha Uphar, Arka Anamika,(IC-31033-1 × AA)-3-1-1-1, P-8,(P-8 × AA)-4-1-1-1, SKBS-11, (VRH-2 × VRO-6)-1-1-2-1, Hissar Unnat, (IC-31033-1 × AA)-3-1-2-2,(IC-31033-1 × AA)-3-2-1-1, (IC-31033-1 × AA)-3-1-2-1
	II	8	VRO-4, Prabhani Kranti, Komal, (IC-169469-1 × PK)-4-1-1-2, VRO-6, VRO-5, (VRH-2 × VRO-6)-9-2-1-3, VRO-3
	III	1	(IC-31033-1 × P-8)-3-1-1-2
	IV	1	IC-169468
	V	1	(IC-31033-1 × AA)-6-1-1-1
	VI	1	(IC-31033-1 × P-8)-2-2-1-2
	VII	1	(IC-169469-1 × PK)-1-2-1-2
	VIII	1	(IC-169469-1 × PK)-8-1-1-1
	IX	1	Kanchan
	X	1	(IC-31033-1 × AA)7-1-1-1
	XI	1	(IC-31033-1 × AA)-5-1-1-1
Pooled over years	I	20	(IC-169469-1 × PK)-2-1-1-1, (P-8 × AA)-4-1-1-1, (IC-31033-1 × P-8)-8-1-1-1, Barkha Bahar, Varsha Uphar, (IC-169469-1 × PK)-2-1-2-1, (IC-31033-1 × P-8)-2-2-1-1, (P-8 × AA)-4-2-2-1, (IC-169469-1 × PK)-6-1-1-1, S-51, (IC-31033-1 × AA)-3-1-1-1, Arka Anamika, SKBS-11, P-8,(IC-31033-1 × AA)-3-2-1-1, (IC-31033-1 × AA)-3-1-2-1, (P-8 × AA)-7-1-1-1, Hissar Unnat,(VRH-2 × VRO-6)-1-1-2-1, (IC-31033-1 × AA)-3-1-2-2
	II	8	VRO-4, Prabhani Kranti, (IC-169469-1 × PK)-4-1-1-2, Komal, VRO-6, VRO-5, VRO-3, (IC-31033-1 × P-8)-3-1-1-2
	III	1	(VRH-2 × VRO-6)-9-2-1-3
	IV	1	(IC-169469-1 × PK)-1-2-1-2
	V	1	(IC-31033-1 × AA)-6-1-1-1
	VI	3	IC-169468, (IC-31033-1 × P-8)-2-2-1-2, (IC-169469-1 × PK)-8-1-1-1
	VII	1	Kanchan
	VIII	2	(IC-31033-1 × AA)-5-1-1-1, (IC-31033-1 × AA)7-1-1-1

Table 4 Intra-and inter-cluster distances in okra germplasm during pooled over the years (2011, 2012)

		Clusters							
		I	II	III	IV	V	VI	VII	VIII
Pooled over years	I	6.98	12.88	8.40	11.50	10.54	14.34	13.90	12.44
	II		7.44	10.32	11.02	13.69	23.99	9.32	18.56
	III			0.00	6.01	14.86	17.39	12.53	14.87
	IV				0.00	16.97	20.33	12.18	18.15
	V					0.00	18.49	12.86	14.01
	VI						7.68	23.73	12.05
	VII							0.00	17.06
	VIII								8.21

Table 5 Cluster means of different quantitative and quality traits in okra germplasm for pooled over years (2011, 2012)

Trait	I	II	III	IV	V	VI	VII	VIII	Mean	Max	Min
Days to first picking	56.10	56.90	54.60	56.70	58.10	56.10	54.50	57.90	56.40	58.05	54.50
Harvest duration (days)	59.70	59.00	61.30	58.90	58.00	59.50	61.20	58.90	59.60	61.32	58.00
Plant height (cm)	202.00	196.40	182.50	205.20	230.40	201.20	178.50	173.40	196.20	230.40	173.40
Fruits/plant	12.00	12.40	14.10	17.70	8.70	13.00	13.50	11.10	12.80	17.70	8.70
Average fruit weight (g)	18.40	18.10	18.20	13.90	18.30	17.40	12.10	17.50	16.70	18.40	12.10
Nodes/plant	12.90	12.80	15.40	18.10	10.40	13.40	13.60	12.00	13.60	18.10	10.40
Internodal length (cm)	16.10	15.50	12.20	11.30	22.40	15.20	13.00	14.50	15.00	22.40	11.30
Fruit length (cm)	13.90	14.50	13.00	13.40	14.40	13.70	13.40	12.40	13.60	14.50	12.40
Fruit diameter (cm)	1.70	1.70	1.50	1.70	1.90	1.80	1.80	1.80	1.70	1.90	1.70
Dry matter (%)	6.00	8.10	7.20	6.30	6.20	6.90	9.20	11.15	7.60	11.15	6.20
Mineral content (mg/kg)	9.00	3.20	7.10	5.10	7.80	16.10	2.80	12.10	7.90	16.10	2.80
Fruit yield/plant (g)	219.30	223.0	253.90	245.10	158.50	224.60	157.10	192.90	209.30	253.92	157.10

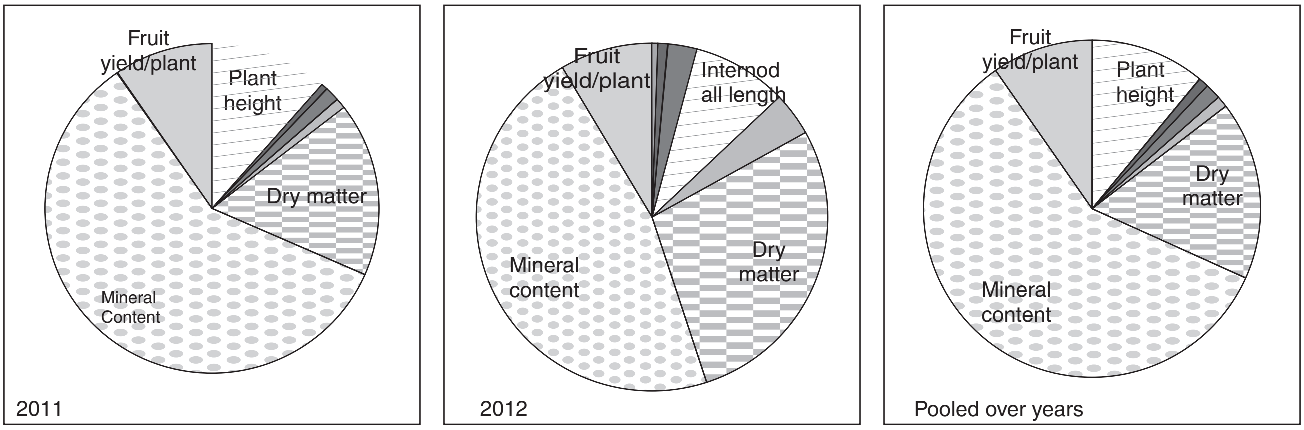

Fig 1 Contribution (%) towards genetic divergence of different characters

range of adaptation.

For quality parameters, highest means for mineral content were observed in cluster VI for pooled over years, which indicated that crosses attempted by utilising the parents (IC-169469-1 × PK)-8-1-1-1, (IC-31033-1 × P-8)-2-2-1-2, IC-169468, (IC-31033-1 × AA)7-1-1-1, (IC-31033-1 × AA)-5-1-1-1 and IC-169468 could improve quality of okra fruits.

The contribution of individual character toward diversity, calculated by using Mahalanobis D^2 divergence, showed (Fig 1) that the mineral content (58.71%), dry matter (22.07%) fruit yield/plant (12.31%) and intermodal length (3.15%) was the most important characteristic for divergence in pooled over the years.

Based upon above information, it can be concluded that genotypes, viz such as VRO-4, Prabhani Kranti, Komal, VRO-6, VRO-5, (P-8 × AA)-7-1-1-1, (IC-169469-1 × PK)-4-1-1-2, (IC-169469-1 × PK)-1-2-1-2, (IC-31033-1 × AA)-3-1-2-2, (VRH-2 × VRO-6)-9-2-1-3, (IC-169469-1 × PK)-4-1-1-2, (VRH-2 × VRO-6)-9-2-1-3, VRO-3, (IC-169469-1 × PK)-8-1-1-1, (IC-31033-1 × P-8)-2-2-1-2, IC-169468, (IC-31033-1 × AA)7-1-1-1 and (IC-31033-1 × AA)-5-1-1-1 have greater potentiality to be used as a breeding stock for good quality and high yielding okra genotypes and could be used as parents in hybridization for expecting transgressive segregants for further exploitation in okra improvement programme.

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