

Original Research Article

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## Genetic Divergence Analysis of Inbred Lines of Okra (*Abelmoschus esculentus* (L.) Moench)

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### ABSTRACT

#### Keywords

Clustering pattern, Genetic diversity, Mahalanobis D<sup>2</sup> statistics, Multivariate analysis, Tocher's method.

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Genetic divergence analysis following Mahalanobis D<sup>2</sup> statistics revealed considerable genetic diversity among 29 genotypes of okra [*Abelmoschus esculentus* (L.) Moench] for all the eighteen characters which pertaining to the growth, earliness and yield. Twenty nine genotypes were grouped into 5 distinct clusters depending upon the similarities of their D<sup>2</sup> values following Tocher's method. The characters YVMV infestation, fruit length and days to 50% flowering with high per cent of contribution to total divergence were the potent variables in differentiating the breeding material under study. The use of diverse inbred lines from the divergent clusters with high intercluster distance (cluster III and IV, I and III and clusters II and III) in hybridization is expected to result in high heterosis and throw desirable transgressive segregants. The genotypes of two solitary clusters (cluster II and IV) being diverge from others may also serve as potential inbred parents for breeding programmes.

### Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) belongs to the class dicotyledonae, order Malvales, family Malvaceae and genus *Abelmoschus* (Schippers, 2000). It is native to West Africa (Murdock, 1959). The crop was taken to other parts of the world by the Portuguese (Sinnadurai, 1992). It is one of the important vegetables grown for its immature green non-fibrous edible fruits in the tropical and subtropical parts of the world. 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, 2010). It is an excellent source of iodine, thus it could be used in the control of goitre (Purewal and Randhawa, 1947). Being an upright, quick growing and medium duration annual herb, it fits well into multiple cropping systems either as a sole crop or intercrop (Reddy, 2010). It is the main fruit vegetable crop in many parts of the world, predominantly in Asia and Africa. Okra is a drought and heat tolerant crop (Reddy *et al.*, 2013). It is an important cash crop for marginal, small and large farmers, with a potential to boost food, nutritional and health security, foster rural development and support sustainable land care (Reddy, 2010).

The average productivity of okra has remained very low and almost stagnant over the last few decades. A major constraint to okra productivity is the low genetic potential of the current varieties that have poor plant type, late maturity, early senescence, short fruiting period, long crop duration and susceptibility to a range of biotic and abiotic stresses. To meet the demand of ever growing human population in the country, it is thus imperative to find alternative means for increasing the yield potential of okra in a sustainable manner. A successful variety in any vegetable crop must meet minimal criteria for numerous traits that are potentially valued in the markets (Reddy *et al.*, 2014). Superiority for multiple 'yield' and 'quality' traits is essential for economic sustainability of a variety (Reddy *et al.*, 2014). The major emphasis in okra breeding is on the development of HYVs coupled with acceptable pod quality and resistance to yellow vein mosaic virus (Reddy *et al.*, 2012b).

Inbred lines are important plant genetic resources for the development of open-pollinated varieties and/or single cross hybrids. Germplasm is an indispensable material to vegetable breeders. Germplasm development plays a key role for genetic improvement of any crop. Inbred line development is an important means of new germplasm development. Characterization and evaluation of the newly developed inbred lines play a significant role in identification of genetic diversity, promising accessions, elite material and sources of resistance for utilization in vegetable crop improvement programmes.

Genetic diversity is an important factor for any heritable improvement. Divergence analysis generates valuable information on the nature and degree of genetic diversity, which is useful for selecting desirable lines from

germplasm for successful breeding programme. The determination of genetic diversity of accessions for agro-economic traits is extremely important in breeding of crop plants for the development of successful varieties (Reddy *et al.*, 2014). In okra, pod yield is a complex quantitative trait as it is governed by a large number of genes and considerably affected by the environment. The traditional approach to germplasm evaluation is based on morphological features. Evaluation of germplasm is done to provide information on genetic diversity within crop. Selection of lines based on individual attribute may not be as advantageous as the one based on a number of important traits collectively. Multivariate analysis provides valuable information on the extent of genetic diversity present in the germplasm. Mahalanobis  $D^2$  statistics is a unique tool for quantifying degree of divergence between biological populations at genetic level. Mahalanobis  $D^2$  statistics is based on multivariate analysis and serves to be a good index of genetic diversity.

The present investigation was therefore, undertaken to assess the nature and magnitude of genetic diversity available in the genotypes of okra on the basis of various growth, earliness and yield attributes.

### **Materials and Methods**

The experimental material for the present study comprised of twenty five inbred lines of okra (RNOYR-30 to RNOYR-54) developed at the Vegetable Research Station, Rajendranagar, Hyderabad along with one YVMV resistant check (RNOYR-16) one YVMV susceptible check (RNOYR-19) and two commercial checks (Pusa Sawani and Arka Anamika). The experiment was laid out in a randomized block design with 3 replications during summer, 2014. In each replication each inbred line was raised in a

single-row plot of 3.0 m length and 0.6 m width. A row-to-row spacing of 60 cm and a plant-to-plant spacing of 30 cm was adopted. A plant population of 10 plants per row, plot and inbred line was maintained. Recommended package of practices were followed and necessary plant protection measures were carried out uniformly to safeguard the crop from major pests and diseases. Biometric data on eighteen quantitative characters were recorded on five competitive and randomly selected plants in each replication for plant height (cm), number of branches per plant, internodal length (cm), first flowering node, first fruiting node, fruit length (cm), fruit width (cm) and fruit weight (g) except days to 50% flowering, first flowering node, days to first fruit harvest, days to last fruit harvest, fruiting period, total number of fruits per plant, number of marketable fruits per plant, total yield per plant (g), marketable yield per plant (g), YVMV infestation (%) and pod borer infestation (%) which were recorded on whole plot basis. The replicated mean values of pod borer infestation on fruits (%) were subjected to square root transformation and arcsin transformed values for per cent yellow vein mosaic virus infestation (YVMV) to restore the distribution to normality. The mean replicated data on various biometric traits were subjected analysis of variance of randomized block design as per the standard statistical procedure (Panse and Sukhatme, 1985). The genetic divergence in the germplasm was assessed following Mahalanobis  $D^2$  statistics (Mahalanobis, 1936). The genotypes were grouped on the basis of minimum generalized distance using Tocher's method as described by (Rao, 1952). The average intra and inter cluster distances were calculated by the formula given by Singh and Chaudhary (1979). The character contribution towards genetic divergence was computed using method given by Singh and Chaudhary (1979).

## Results and Discussion

The simultaneous significance of mean differences was tested by analysis of dispersion. The analysis of dispersion is presented in Table 1.

The F value is highly significant indicating large differences between the means of the populations based on pooled effect of all the eighteen characters and may be continued for further analysis for computing  $D^2$  estimates.

Since all the eighteen variables were correlated, they were transformed into uncorrelated linear combination through pivotal condensation method. The quantitative assessment of genetic divergence among 29 genotypes of okra was made by adopting Mahalanobis  $D^2$  statistics for eighteen characters following the procedure given by Rao (1952). The average  $D^2$  values have been used for constellation of genotypes into clusters in such a way that the genotypes in the cluster had smaller average  $D^2$  values than those belonging to different clusters.

The 29 genotypes of okra were grouped into five distinct clusters as evident from the clustering pattern (Table 2). The distribution of different genotypes into distinct clusters is shown in (Table 2). Out of the five clusters formed, cluster I was the largest comprising of 15 genotypes, followed by cluster III with 10 genotypes and cluster V with 2 genotypes, whereas cluster II and cluster IV were solitary with one genotype in each cluster.

Average intra and intercluster  $D^2$  values are tabulated in Table 3, providing an interesting information on the nature of genetic divergence at intra and intercluster levels, respectively. In general, intracluster distances were much lesser than intercluster distances. The intracluster  $D^2$  values ranged from 0.00 (cluster II and cluster IV) to 5.58 (cluster V).

The cluster V had the maximum intracluster  $D^2$  value (5.58) followed by cluster I (4.39) and cluster III (3.76). The intracluster distance of solitary clusters II and IV was zero. The intercluster distance was maximum between clusters III and IV (20.77) followed by clusters I and III (20.54) and clusters II and III (20.45), while minimum between clusters II and IV (5.12) followed by clusters I and IV (5.64) and clusters I and II (5.78).

The cluster means for different traits (Table 4) are indicated considerable differences among the clusters. From the data, it can be seen that considerable differences exist for all the traits studied. Highest plant height was recorded in cluster II (166.67 cm) followed by cluster IV (141.07 cm), while lowest plant height was recorded in cluster V (132.38 cm) followed by cluster I (136.89 cm) and cluster III (140.16 cm). Number of branches per plant was highest in cluster V (2.83) followed by cluster IV (2.33) and cluster II (2.07), while lowest in cluster I (1.93) followed by cluster III (2.05). Internodal length was minimum in cluster IV (5.06 cm) followed by cluster V (5.14 cm), while maximum in cluster II (5.54 cm) followed by cluster III (5.49 cm) and cluster I (5.39 cm). Number of nodes on main stem was maximum in cluster II (27.11) followed by cluster IV (24.85) and cluster V (22.74), while minimum in cluster III (22.30) and cluster I (22.50).

The genotypes of cluster IV took minimum number of days to 50% flowering (44 days) followed by cluster V (44.50 days) and cluster II (47.00 days), while the genotypes of cluster III took maximum number of days to 50% flowering (48.17 days) followed by cluster I (47.98 days). The genotypes of cluster IV produced their first flower at the lowest node (4.73) followed by cluster III and cluster V (4.80), while the genotypes of cluster II produced their first flower at the highest node (5.07) followed by cluster I (4.85).

The genotype of cluster IV took minimum number of days to first fruit harvest (51.00 days) followed by cluster V (51.50 days) and cluster II (54.00 days), while the genotypes of cluster I took maximum number of days to first fruit harvest (55.00 days) followed by cluster III (55.13 days). The genotype of cluster V took maximum number of days to last fruit harvest (127.50 days) followed by cluster III (125.27 days) and cluster I (115.64 days), while the genotypes of cluster II took minimum number of days to last fruit harvest (110.00 days) followed by cluster IV (114.67 days). The genotype of cluster V had highest fruiting period (76.00 days) followed by cluster III (70.13 days) and cluster IV (63.67 days), while the genotypes of cluster II had lowest fruiting period (56.00 days) and cluster I (60.64 days).

The longest fruits were produced by the genotypes of cluster II (16.60 cm) followed by cluster IV (15.37 cm) and cluster III (15.09 cm), whereas the shortest fruits were produced by the genotypes of cluster I (14.55 cm) followed by cluster V (14.48 cm).

The genotypes of cluster I, III and V produced the widest fruits (1.70 cm), while the genotypes of cluster II produced the narrowest fruits (1.66 cm) followed by cluster IV (1.68 cm). The heaviest fruits were produced by the genotypes of cluster II (19.33 g) followed by cluster IV (17.80 g) and cluster III (17.61 g), while the lightest fruits were produced by the genotypes of cluster I (17.28 g) followed cluster V (17.55 g).

Total number of fruits per plant produced by the genotypes of cluster II was highest (32.38) followed by cluster IV (31.78) and cluster V (32.11), while lowest by the genotypes of cluster I (27.75) and cluster III (27.92). The genotypes of cluster V (24.26) produced maximum number of marketable fruits per plant followed by cluster III (24.10) and

cluster II (11.00), while the genotypes of cluster IV (4.00) produced minimum number of marketable fruits per plant followed by cluster I (9.27).

**Table.1** Analysis of variance for dispersion in inbred lines of okra

Source of variations	Degrees of freedom	Sum of squares	Mean squares	F ratio	Probability
Varieties	28	2.0095+18	7.1769+16	5.765+19	0.00000**
Error	55	6.8472+02	1.2449+03		
Total	83	2.0095+18	2.4211+16		

\*\* Significant at 1% level of significance

**Table.2** Clustering pattern of inbred lines of okra

Cluster	Number of genotypes	Genotypes included in cluster
I	15	Arka Anamika, Pusa Sawani, RNOYR-43, RNOYR-32, RNOYR-41, RNOYR-19, RNOYR-45, RNOYR-39, RNOYR-36, RNOYR-40, RNOYR-52, RNOYR-42, RNOYR-48, RNOYR-33, RNOYR-47
II	1	RNOYR-53
III	10	RNOYR-37, RNOYR-49, RNOYR-30, RNOYR-31, RNOYR-50, RNOYR-16, RNOYR-54, RNOYR-38, RNOYR-34, RNOYR-51
IV	1	RNOYR-35
V	2	RNOYR-44, RNOYR-46

**Table.3** Inter and Intra cluster distances among inbred lines of okra

Cluster	I	II	III	IV	V
I	<b>4.39</b>	5.78	20.54	5.64	12.52
II		<b>0.00</b>	20.45	5.12	12.66
III			<b>3.76</b>	20.77	10.14
IV				<b>0.00</b>	12.60
V					<b>5.58</b>

**Table.4** Cluster means of inbred lines for 18 agro-economic traits of okra

Character	Cluster				
	I	II	III	IV	V
Plant height (cm)	136.89	166.67	140.16	141.07	132.38
Number of branches per plant	1.93	2.07	2.05	2.33	2.83
Internodal length (cm)	5.39	5.54	5.49	5.06	5.14
Number of nodes on main stem	22.50	27.11	22.30	24.85	22.74
Days to 50% flowering	47.98	47.00	48.17	44.00	44.50
First flowering node	4.85	5.07	4.80	4.73	4.80
Days to first fruit harvest	55.00	54.00	55.13	51.00	51.50
Days to last fruit harvest	115.64	110.00	125.27	114.67	127.50
Fruiting period (days)	60.64	56.00	70.13	63.67	76.00
Fruit length (cm)	14.55	16.60	15.09	15.37	14.48
Fruit width (cm)	1.70	1.66	1.70	1.68	1.70
Fruit weight (g)	17.28	19.33	17.61	17.80	17.55
Total number of fruits per plant	27.75	32.38	27.92	31.78	32.11
Number of marketable fruits per plant	9.27	11.00	24.10	4.00	24.26
Total yield per plant (g)	383.78	500.54	392.77	454.13	447.24
Marketable yield per plant (g)	126.79	167.69	335.08	56.67	332.26
Pod borer infestation (%)	14.53	12.03	14.35	13.24	12.46
Yellow vein mosaic virus infestation (%)	100.00	100.00	0.00	100.00	46.67



**Table.5** Per cent contribution of different traits towards divergence in okra

Character	Number of times ranked first	Contribution towards divergence (%)
Plant height (cm)	6	1.48
Number of branches per plant	0	0.00
Internodal length (cm)	4	0.99
Number of nodes on main stem	2	0.49
Days to 50% flowering	35	8.62
First flowering node	14	3.45
Days to first fruit harvest	0	0.00
Days to last fruit harvest	8	1.97
Fruiting period (days)	0	0.00
Fruit length (cm)	58	14.29
Fruit width (cm)	17	4.19
Fruit weight (g)	2	0.49
Total number of fruits per plant	1	0.25
Number of marketable fruits per plant	24	5.91
Total yield per plant (g)	3	0.74
Marketable yield per plant (g)	2	0.49
Pod borer infestation (%)	6	1.48
Yellow vein mosaic virus infestation (%)	224	55.17

The genotypes of cluster II (500.54 g) produced highest total yield per plant followed by cluster IV (454.13 g) and cluster V (447.24 g), while the genotypes of cluster I produced lowest total yield per plant (383.78 g) followed by cluster III (392.77 g). The mean marketable yield of the genotypes of cluster III (335.08 g) was highest followed by cluster V (332.26 g) and cluster II (167.69 g), while lowest for the genotypes of cluster IV (56.67 g) and cluster I (126.79 g). The per

cent pod borer infestation was lowest for the genotypes of cluster II (12.03%) followed by cluster V (12.46%), while highest for the genotypes of cluster I (14.53%) followed by cluster III (14.35%) and cluster IV (13.24%).

The incidence of yellow vein mosaic virus was zero for the genotypes of cluster III (0.00%) and lowest for the genotypes of cluster V (46.67%), while highest for the genotypes of cluster I, II and IV (100.00%).

An assessment of relative contribution of eighteen characters towards total genetic divergence (Table 5) revealed that yellow vein mosaic virus infestation had contributed highest (55.17%) by taking 224 times first ranking followed by fruit length (14.29%) by 58 times, days to 50% flowering (8.62%) by 35 times, number of marketable fruits per plant (5.91%) by 24 times, fruit width (4.19%) by 17 times, first flowering node (3.45%) by 14 times, days to last fruit harvest (1.97%) by 8 times, pod borer infestation (1.48%) by 6 times, internodal length (0.99%) by 4 times, number of nodes on main stem, fruit weight and marketable yield per plant (0.49%) by 2 times. In contrast, total number of fruits per plant had contributed least (0.25 %) by taking 1 time. However, the traits number of branches per plant, days to first fruit harvest and fruiting period did not contribute materially towards total diversity.

The traditional approach to germplasm evaluation is based on morphological features. Evaluation of germplasm is done to provide information on genetic diversity within crop. Genetic diversity is an important factor for any heritable improvement. Divergence analysis generates valuable information on the nature and degree of genetic diversity, which is useful for selecting desirable lines from germplasm for successful breeding programme. Selection of lines based on individual attribute may not be as advantageous as the one based on a number of important traits collectively. Multivariate analysis provides valuable information on the extent of genetic diversity present in the germplasm. Mahalanobis  $D^2$  statistics is a unique tool for quantifying degree of divergence between biological populations at genetic level. Mahalanobis  $D^2$  statistics is based on multivariate analysis and serves to be a good index of genetic diversity. Very often phenotypic diversity has been taken as an index of the genetic diversity. The results

of Mahalanobis  $D^2$  statistics revealed substantial and desirable genetic diversity among 29 genotypes included in the present study for all the eighteen characters under consideration collectively. Several authors also reported profound diversity in the germplasm of okra by assessing genetic divergence on the basis of quantitative traits following Mahalanobis  $D^2$  statistics (Bendale *et al.*, 2003; Reddy *et al.*, 2012a; Singh *et al.*, 2012; Shaikh *et al.*, 2013).

Mahalanobis  $D^2$  statistic was found to be a useful tool to assess the relative contribution of different characters to the total divergence both inter and intracluster levels. In general, the characters responsible for discrimination between populations can narrow down the problem of selecting divergent parents for breeding programmes. In the present study, of the eighteen characters, the characters yellow vein mosaic virus infestation (55.17%), fruit length (14.29%), days to 50% flowering (8.62%) and number of marketable fruits per plant (5.91%) were the major contributors towards divergence. These characters were the potent factors in differentiating the germplasm of okra under study. Kumari and Chaudhury (2006) and Reddy *et al.*, (2012a) also observed such maximum contribution of fruit length (43.08 and 40.02%, respectively) to total divergence of okra germplasm. Prakash and Pitchaimuthu (2010) observed maximum contribution of days to 50% flowering (35.62%) to total divergence in okra genotypes. De *et al.*, (1988) opined that traits contributing maximum towards the  $D^2$  values need to be given more emphasis for deciding the clusters to be taken for the purpose of choice of parents for hybridization. The characters that predominantly contributed to divergence in this study also happen to be the main components of yield. The results of the present study point out a positive contribution of genetic divergence and yield components; this can be of considerable help



in selecting for yield and other economic traits. It can be concluded that there was more divergence for these characters offering greater scope while making selection of horticulturally superior genotypes of okra.

The inbred lines used for present study consisted of 29 genotypes of okra which were grouped into five distinct clusters. Out of the five clusters formed, cluster I was the largest comprising of 15 genotypes, followed by cluster III with 10 genotypes and cluster V with 2 genotypes, whereas cluster II and cluster IV were solitary with one genotype in each cluster. The genotypes in these solitary clusters being diverge from others may serve as potential inbred parents for breeding programmes. They indicate their independent identity and importance due to various unique characters possessed by them.

In general, the genotypes grouped together in one cluster are less divergent than those which are placed in a different cluster. Further, higher intracluster distance indicates high degree of divergence within that cluster. In the present investigation, average intracluster distances revealed that the genotypes of cluster V (5.58) were highly divergent while the genotypes of cluster III (3.76) were least divergent, while the cluster means revealed that cluster V produced maximum number of branches per plant, days to last fruit harvest, fruiting period and number of marketable fruits per plant.

The choice of parents for heterosis breeding depends on genetic diversity of parents. The expression of heterosis is influenced by genetic diversity of parents. Cress (1966) demonstrated that 'genetic diversity is necessary for significant heterosis but not sufficient to guarantee it'. Several reports are available to show that hybrids between genetically diverse parents manifest greater heterosis than those between more closely

related parents (Dhaduk *et al.*, 2004; Sharma *et al.*, 2008 and Shaikh *et al.*, 2013). In fact, such a conclusion is based upon a rather restricted range of genetic diversity and may not hold over the entire range of divergence encountered in a species. In general, the level of heterosis increases with the increase in parental diversity up to some limit and decreases with further increase in parental diversity owing to cross ability barriers. Thus maximum heterosis occurs at an optimal or intermediate level of parental diversity. Further, the occurrence of heterosis cannot be predicted on the basis of genetic divergence alone (Matzinger and Werusman, 1958). Apart from the high degree of divergence, the mean performance of genotypes and the characters with maximum contribution towards divergences should also be given due consideration. The best combination of parents for improvement in various economic characters can be recommended on the basis of *per se* performance of the genotypes and intercluster divergence.

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