Original Research Article

Implication of multivariate analysis in breeding to obtain desired plant type of okra (*Abelmoschus esculentus* L. Moench)

Abstract

Twenty diverse okra genotypes were evaluated to assess the genetic diversity based on quantitative morphological and qualitative biochemical traits in a randomized block design during 2015-16**.** On the basis of D^2 analysis, the 20 genotypes were clustered into five groups. Cluster I constituted the largest group (10 genotypes) followed by cluster II (7 genotypes). The cluster III, IV and V contains only 1 genotype in each cluster. The phytochemical character carotenoids alone contribute highest percentage (39%) toward divergence, followed by chlorophyll a (31%) and anthocyanin (17%). The five principal components have accounted 81.19% of total variation and percent variation expected were 36.27% (PC1), 18.21% (PC2), 16.42% (PC3), 5.91% (PC4) and 4.38% (PC5), respectively. The PC1 has positive association with days to first flowering, followed by yield/plant, primary branches/plant, carotenoid and phenol content. However, PC1 has negative association for fruits length, fruit diameter and ascorbic acid content. Therefore, the traits *viz*., days to first flowering, first flowering node and yield per plant should be given top priority in diverse parent selection for attempting high yielding along with important phytochemical properties in okra. Allocations of germplasm into different clusters were because of cumulative effect of number of characters.

Keywords: Genetic diversity, okra, principal component, yield, biochemical

Introduction

Okra is a multipurpose and multifarious vegetable crop valued for its tender and delicious pods [1], rich in iodine and fibre which are cooked in curry and also in soup making. The dried seeds of okra are used as a coffee additives or substitutes and are reported to contain 18-20% oil and 20-25% crude protein [2]. It is cultivated in tropical, sub-tropical and mild temperate parts of the world and gaining importance in these regions of the world; especially in Egypt. The tender green pods have an average nutritive value of 3.21 which is higher than tomato, eggplant and most cucurbits except bitter gourd [3]. In addition to its usefulness as a vegetable, its fruits are also useful in curing ulcer and suppressing the pains and effects of haemorrhoid. Okra fruits have mucilage in latex form i.e. used as a plasma replacement or blood volume expander [4]. It is photo insensitive in nature, short duration and having high seed setting capacity which makes the crop ideal for genetical study in crop improvement. In spite of its multipurpose use, it is being neglected because of the non-availability of improved and high yielding locally adapted cultivars and reduction in yield due to frequent attack of shoot and fruit borer and yellow vein mosaic virus. At present, the major objective of okra breeding programs is improving yield and ensuring its sustainability under adverse conditions through various techniques, hence, it is very necessary to determine the factors or traits that influence fruit and seed yields of okra, directly and indirectly or both. The major limiting factor which is responsible for reduction of cultivation of okra is incidence of okra yellow vein mosaic virus and its vector whitefly (*Bemisia tabaci* Gen.). It affects the quality of the fruit which ultimately cause heavy loss of fruit yield [5]. Among the world, India secure the first rank in collection of cultivated okra (*Abelmoschus esculents* L. Moench) in the gene bank. Moreover, India share 72.9 % of the world okra production and among Indian states, West Bengal (14% share) is leading in okra producer followed by Bihar (12% share). The main reasons for wide genetic diversity are geographically separation, genetic barriers to crossability, and different parents of evolution [6]. Genetic diversity is a key factor for crop improvement from which useful characters can be selected for developing broad-based populations to be used in hybridization programme towards improvement [7] and [8]. Mahalanobis D^2 statistics which is based on multivariate analysis is a powerful tool to estimate the degree of divergence among genotypes in the population and nature of forces operating at different levels [9]. For selection of parent for hybridization programme it is very essential to know the information of genetic diversity of okra germplasm. It revealed rich genetic diversity for various growth, earliness and yield associated traits in the germplasm offering a great scope for improvement of okra (Ghai et al [10], Kumari and Chaudhury [11], Singh et al [12], Bendale et al [13]). Moreover, most frequent genetic diversity assessing methods are cluster analysis and principal component analysis (PCA). The cluster analysis has been most exploited for assessing family relationships [14]. The present investigation was therefore, undertaken to assess the nature and genetic diversity available in a large germplasm and the characters which play important role in genetic diversity of okra.

Materials and Methods

The present investigation was carried out using 20 okra genotypes including 4 checks i.e. Kashi Pragati (VRO 6), Kashi Kranti (VRO-22), Kashi Lalima and Arka Anamika procured from different national institutes *viz*., All India Coordinated Research Project on

Vegetable Crops, ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, India and ICAR-Indian Institute of Horticulture Research (IIHR), Bengaluru. This trial was performed in randomize block design with three replications and germplasm evaluated at research farm of the Department of Horticulture (Vegetable & Floriculture), Bihar Agricultural University, Sabour, Bhagalpur (Bihar) during Rainy season of 2015-16. The soil of the plot was sandy loam in texture having good fertility properly levelled and well drained. The rainfall of this region is mainly distributed between middle of June to middle of October. The total rainfall received during the crop period was 282.57 mm. The maximum temperature ranged from 23.9˚C - 35˚C during the plant growth and development phase. All the agronomic package and practices were adopted to raise the healthy crop. Observations were recorded on 22 economically important traits *viz*., 13 quantitative traits i.e. Days to first flowering, days to 50% flowering, first flowering node, plant canopy width (cm), number of primary branches per plant, plant height (cm), fruit length (cm), fruit diameter (cm),number of fruits per plant, average fruit weight (g), number of seeds per pod, yield per plant (kg),fruit yield (q/ha) and 7 biochemical characters i.e. Chlorophyll a, Chlorophyll b, total Chlorophyll, Carotenoids, anthocyanin, ascorbic acid, phenols, crude fibre and moisture. The genetic divergence among the okra genotypes was estimated by using D^2 statistics [15]. All genotypes were clustered into different groups accomplished by Tocher's method [16]. The average distance between the cluster and within the cluster was calculated by the statistical procedure given by Singh and Choudhary [17].

Results and Discussion

Grouping of genotypes into different cluster

Based on Mahalanobis D^2 statistics, clustering of all 20 okra genotypes for 22 quantitative traits was done into five different groups (Table 1). The maximum number of genotypes (10) were grouped into cluster I followed by cluster II (7 genotypes). The cluster III, IV and V each contains only 1 genotype. The genotypes present in a single group are genetically similar for most of the traits. However, they have different geographical area of origin, for example cluster II consists of Arka Anamika (from IIHR, Bangalore) and Azad Bhindi-1 (from CSAUA&T, Kanpur), in cluster I Pusa Sawani (from IARI, New Delhi) and Kashi Mohini (from IIVR, Varanasi) which are having different source of origin. Hence, geographical origins do not decide the grouping of genotypes in a group [18]. Oriyo [19] also has given the same statement. Therefore, the reason behind occurrence of genetic diversity among the genotypes might be some other factors like different genetic architecture of the population, heterogeneity, selection history, and genetic drift. The cause for greater diversity in genotypes may due to genetic drift and selection in different environments than the geographical distance [18]. It described that the geographically isolated genotypes in okra need not to show genetic diversity [20]. Shantha kumar and Salimath [21], Kumar and co-workers [22], Solankey and co-workers [23] also conveyed the similar results. Ramya and Senthilkumar [24] advocated dearth of clear relationship between geographical as well as genetic diversity in okra.

Table 1: Clustering pattern of diverse okra genotypes

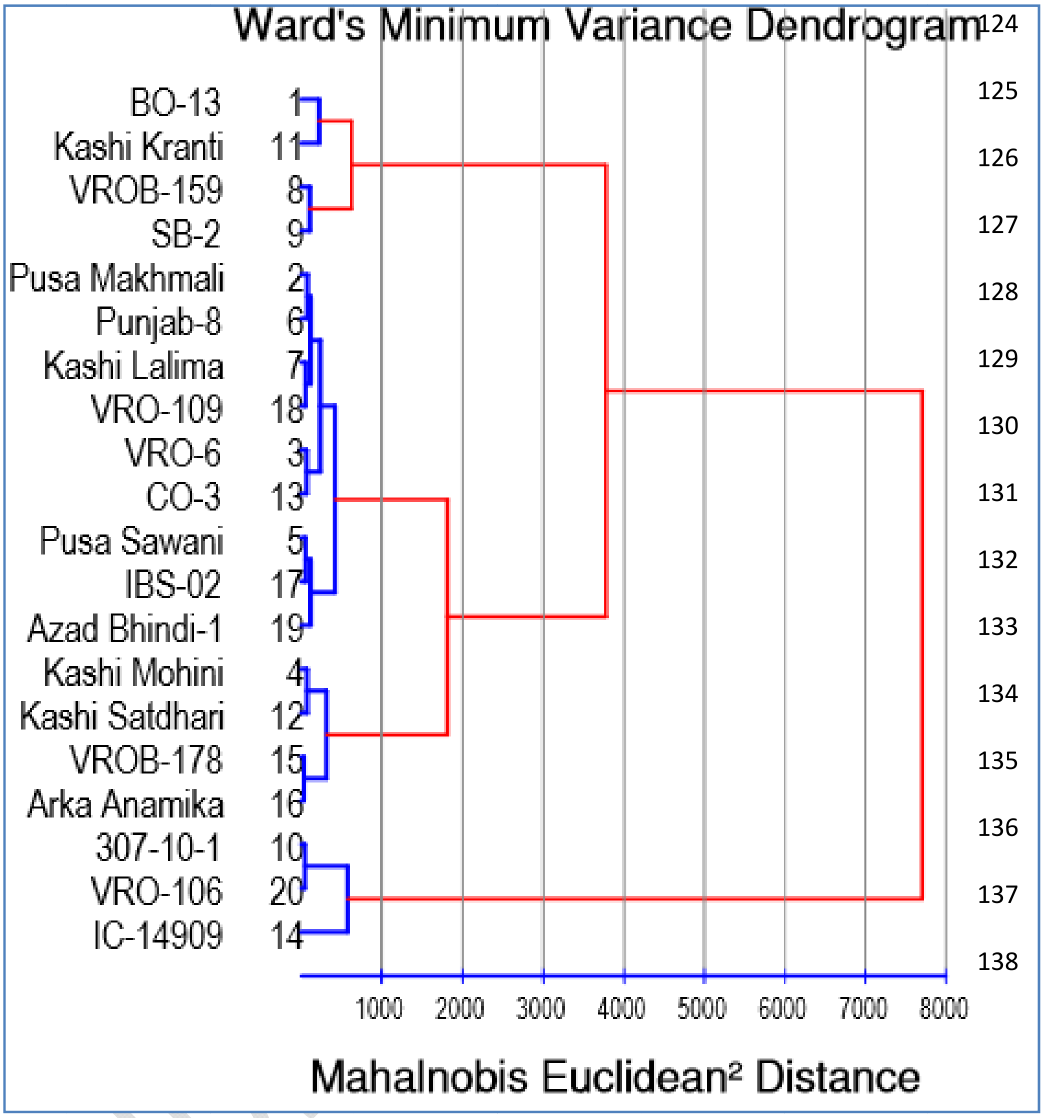
Clusters	Number of Genotypes	Name of genotypes
I	10	VROB-159, SB-2, VRO-6, Pusa Sawani, Punjab-8, BO-13, Kashi Mohini, VRO-109, Kashi Satdhar, Pusa Makhmali
II	7	Arka Anamika, Azad Bhindi-1, IBS-02, 307-10-1, IC-14909, CO-3, VROB-178
III	1	VRO-106
IV	1	Kashi Kranti
V	1	Kashi Lalima

The average D^2 values of intra and inter cluster are present in Table 2. The results showed that intra cluster distances varies from 221.525 (cluster II) to 624.030 (cluster V) with the highest inter cluster value between cluster II and V (8393.597). It was followed by cluster I and V (5708.096), cluster II and IV (3807.119), cluster III and V (3223.140), cluster II and III (1593.631).

Table 2: Intra and inter cluster values (Euclidean^2 cluster distance) for 20 okra genotypes

Cluster	I	II	III	IV	V
I	446.443	793.737	758.924	2184.159	5708.096
II		221.525	1593.631	3807.119	8393.597
II			288.685	888.870	3223.140
IV				282.383	1443.396
V					624.030

Fig. 1: Dendrogram (Tocher's method) showing clustering pattern among 20 okra genotypes



The cluster mean of 20 genotypes is presented in Table 3. The table values showed that cluster IV having only one entry (Kashi Kranti) but having highest contribution in mean of carotenoid, moisture content (91.891) and yield per plant (253.247) mainly due to highest fruit length, fruits per plant and high average fruit weight and less mean performance for days to first flowering, first flowering node, plant canopy width, plant height. Cluster V also contain one entry i.e. Kashi Lalima (red pod colour) were found highest mean performance for days to first flowering, days to 50% flowering, ascorbic acid and anthocyanin content mainly due to red colour of pod and less mean performance for chlorophyll a, chlorophyll b and total chlorophyll.

Table 3: Cluster means by using Tocher's method

Characters	Clusters				
	I	II	III	IV	V
Days to First Flowering	43.067	43.571	44.000	42.000	44.000
Days to 50 % Flowering	45.933	46.143	46.667	46.667	47.000
First Flowering Node	6.233	6.881	6.667	5.800	6.333
Plant Canopy Width (cm)	65.232	88.178	101.103	52.883	69.437
Primary Branches/ Plant	3.097	3.067	3.767	2.733	2.700
Plant Height (cm)	75.033	85.429	101.333	60.667	71.333
Fruit Length (cm)	9.718	9.896	9.767	10.833	10.533
Fruit Diameter (cm)	1.596	1.514	1.600	1.473	1.557
Fruits/ Plant	14.961	16.190	13.510	18.580	17.783
Average Fruit Weight (g)	11.821	10.489	9.303	13.627	10.723
Number of Seeds Per Pod	49.333	43.857	41.000	45.333	33.667
Yield/ Plant (g)	177.241	169.456	125.753	253.247	191.057
Fruit Yield (q/ha)	177.241	169.456	125.753	253.247	191.057
Chlorophyll a	0.452	1.631	4.841	3.847	0.000
Chlorophyll b	0.308	0.811	2.670	1.904	0.000
Total Chlorophyll	0.759	2.442	7.511	5.751	0.000
Carotenoids	1.518	1.833	2.558	5.705	0.742
Anthocyanin	0.000	0.005	0.000	0.000	0.142
Ascorbic Acid	12.385	14.171	13.847	18.367	19.671
Phenols	45.975	47.173	41.982	43.620	41.866
Crude Fiber (%)	0.290	0.265	0.230	0.210	0.127
Moisture (%)	86.958	87.978	87.419	91.891	81.851

Contribution of traits toward genetic diversity

The contribution percent towards genetic divergence showed that only nine yield and its contributing traits along with qualitative characters shares almost 100 % in genetic divergence (Fig. 2). Among these nine traits, highest contribution made by qualitative traits (carotenoids content, chlorophyll a and Anthocyanin content with percent share of 39%, 31% and 17% respectively) followed by quantitative traits such as plant canopy width (4%). Whereas, the traits like ascorbic acid, crude fibre (3%), phenols (2%), chlorophyll b and number of seeds per pod (1%) also having significant contribution towards divergence. It specifies that carotenoids content would be the important parameter for selecting diverse okra genotypes for nutritional purpose.

Fig. 2: Graphical representation of proportionate contribution of studied major traits (in parentheses value) towards genetic divergence in okra

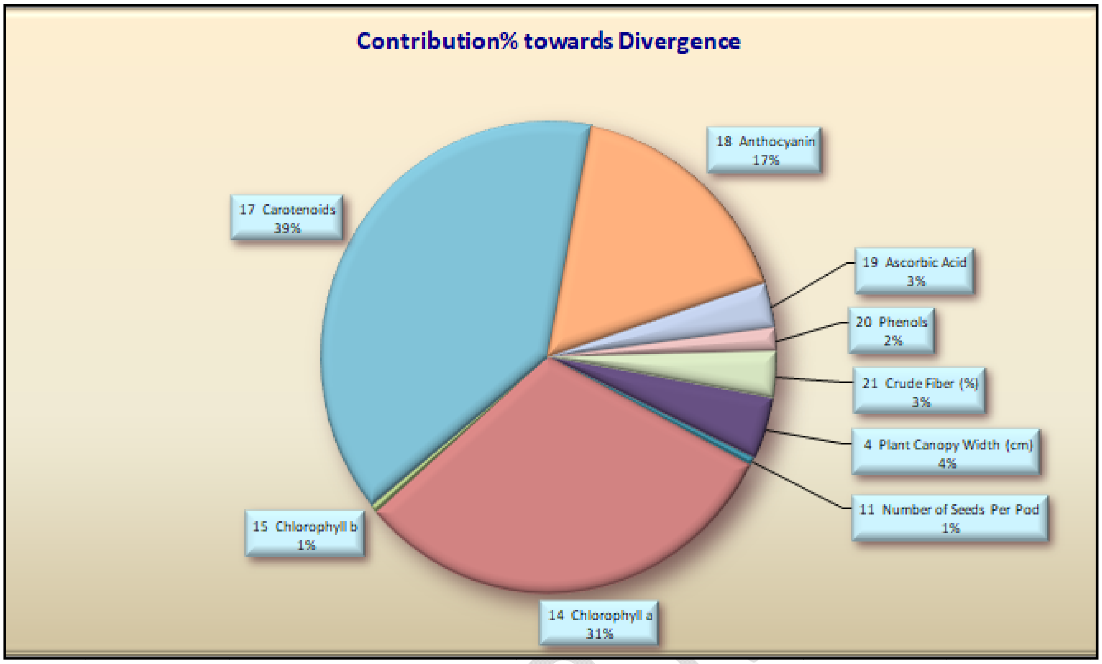
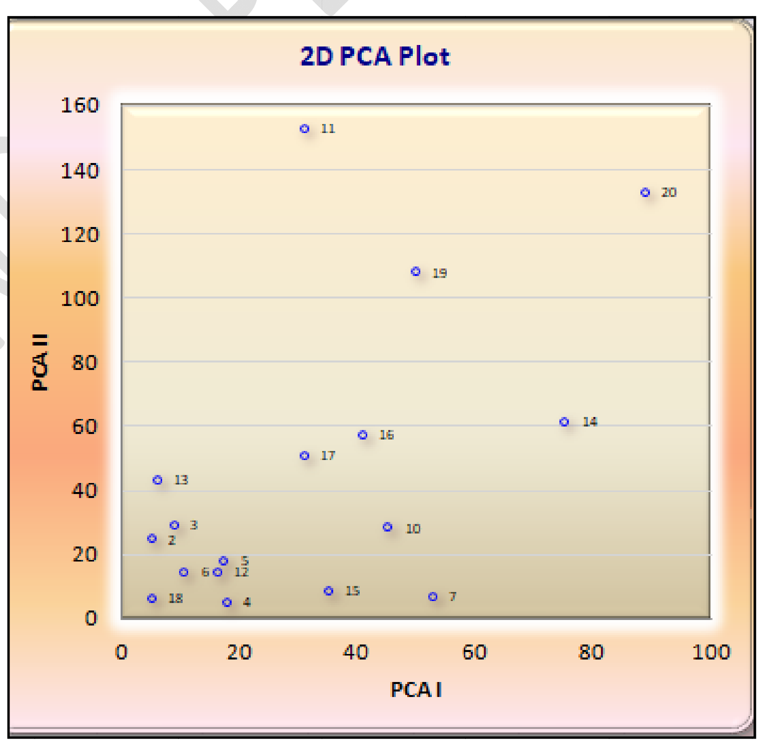


Fig. 3: Scattered diagram by using two dimensional ordinations of 20 okra genotypes based on PC (principal component) axis 1 and 2.



Principal component analysis

The principal component analysis (PCA) of 22 traits in 20 okra genotypes are mentioned in Table 4 & Fig. 3. The data displays the Eigen values for five principal components (PCs) *viz*., PC 1, PC 2, PC 3, PC 4 and PC 5 were 7.980, 4.007, 3.614, 1.302 and 0.964 respectively, which contributes about 81.19% of total genetic variation. These similar results were also reported by Yonas et al [25]. The maximum variations were contributed by PC 1 and PC 2 of about 36.27% and 18.21% of total variations. The two-dimensional ordinations of 20 okra genotypes on PC axis 1 and 2 (Fig. 3), revealed scattered diagram of genotypic distribution pattern on axis. The scattered diagram showed that 81.21% of cumulative total variations were contributed by first 5 principal components, collectively. The parameters like days to first flowering (0.113), first flowering node (0.175), plant canopy width (0.329), primary branches per plant (0.307), plant height (0.323), yield per plant (0.285), chlorophyll a (0.168), total chlorophyll (0.129), carotenoids (0.066), anthocyanin (0.034) and phenols content showed positive association with PC1 whereas traits such as fruit length (-0.152), fruit diameter (-0.328), number of fruits per plant (-0.245), average fruit weight (-0.225), number of seeds per pod (-0.321), chlorophyll b (-0.087), crude fibre (-0.215), moisture (-0.039) and ascorbic acid (-0.272) content having negative association for the same. The component PC2 displayed the positive association for days to first flowering (0.119), days to 50% flowering (0.277), number of fruits per plant (0.183), yield per plant and phenol content (0.332) while, plant height (-0.122), average fruit weight (-0.027), anthocyanin content (-0.077), crude fibre (-0.066) have negative association. The third PC has positive association with days to first flowering (0.174), days to 50% flowering (0.085), number of fruits per plant (0.186) and phenol content (0.163) while, negative association with primary branches per plant (-0.123), average fruit weight (-0.106) and crude fibre (-0.387) content. PC IV positively associated with days to first flowering (0.616), phenols (0.068), crude fibre (0.114) and moisture (0.288) content while, negative association with number of fruits per plant (-0.106), yield per plant (-0.275) and ascorbic acid (-0.054) content. The chief role in genetic divergence analysis demonstrated by positively associated characters for different PCs. The above results are in conformity with the works done by Singh et al [26], Koundinya et al [27], Kumar et al [22] and Solankey et al [23].

Table 4: Principal component analysis for 22 traits in 20 okra genotypes

Variables/ Characters	Eigen Vector				
	PC I	PC II	PC III	PC IV	PC V
Eigene Value (Root)	7.980	4.007	3.614	1.302	0.964
% Var. Exp.	36.273	18.212	16.426	5.917	4.383
Cum. Var. Exp.	36.273	54.485	70.911	76.828	81.212
Days to First Flowering	0.113	0.119	0.174	0.616	0.276
Days to 50 % Flowering	-0.122	0.277	0.085	-0.094	0.225
First Flowering Node	0.175	-0.175	-0.108	-0.092	-0.466
Plant Canopy Width (cm)	0.329	-0.104	-0.051	-0.065	0.064
Primary Branches/ Plant	0.307	-0.132	-0.123	-0.067	0.146
Plant Height (cm)	0.323	-0.122	-0.077	-0.055	0.007
Fruit Lenth (cm)	-0.152	0.136	0.178	-0.141	-0.536
Fruit Diameter (cm)	-0.328	0.072	0.039	0.107	0.122
Fruits/ Plant	-0.245	0.183	0.186	-0.106	-0.076
Average Fruit Weight (g)	-0.225	-0.027	-0.106	-0.471	0.316
Number of Seeds Per Pod	-0.321	0.043	-0.050	0.067	0.199
Yield/ Plant (g)	0.285	0.004	-0.100	-0.275	0.362
Fruit Yield (q/ha)	0.000	0.000	0.000	0.000	0.000
Chlorophyll a	0.168	0.405	-0.147	0.009	-0.058
Chlorophyll b	-0.087	-0.454	0.102	-0.058	0.158
Total Chlorophyll	0.129	0.382	-0.147	0.054	0.016
Carotenoids	0.066	0.362	-0.229	-0.341	0.018
Anthocyanin	0.034	-0.077	0.498	-0.148	0.114
Ascorbic Acid	-0.272	-0.030	-0.312	-0.054	0.000
Phenols	0.171	0.332	0.163	0.068	0.049
Crude Fiber (%)	-0.215	-0.066	-0.387	0.114	0.058
Moisture (%)	-0.039	-0.086	-0.455	0.288	-0.062

Conclusion

Desirable genetic diversity and principal component analysis are most reliable selection parameters for electing promising traits *viz*., days to first picking, first flowering node and days to first flowering in okra. These traits should be given top priority in okra breeding programme for diverse parent selection for attempting heterotic cross combination and development of high yielding and YVMV resistant hybrids/varieties in okra.

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