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Identification of diverse brinjal (*Solanum melongena* L.) genotypes through multivariate analysis

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Abstract
Twenty five brinjal (*Solanum melongena* L.) genotypes were evaluated under the Gangetic plains of West Bengal to determine genetic divergence with the help of multivariate analysis. The genotypes were logically grouped into six clusters on the basis of divergence analysis. The grouping pattern of genotypes indicated no direct relationship between geographical distribution and genetic distance. Inter-mating between the genotypes included in Clusters IV and VI or Cluster III and VI having high divergence will be expected to give heterotic response in F_1 generation. Among the characters, fruit weight contributed the maximum divergence towards total genetic divergence followed by plant height, days to 50% flowering and fruit yield per plant. Four out of 9 characters had eigenvalue more than 1 and together accounted for 100% of total variation. Based on D^2 statistics, principal component analysis and average values, five genotypes namely, BCB-40, KS-224, White Jhuri Begun, 10/BRBW/ RES-3 and 16/BRL var-8 possessed distinct differences of their genotypic characters and optimum combinations of all variables which could be used as parents in hybridization programme for the development of high yielding brinjal variety.

Keywords: brinjal, diversity, D^2 statistics, clustering pattern, principal component analysis

Introduction
Brinjal, eggplant or aubergine (*Solanum melongena* L.) is the one of the most popular and widely cultivated vegetable crops in the central, Southern and South East Asia and in some African Countries due to its high productivity, round the year availability, better transport qualities and good storability. It can be grown in almost all parts of India except in higher altitudes and is a major source of income for the small and marginal farmers as well. Due to its extreme variability in India, Vavilov (1928) [16] regarded the crop as being Indian origin. According to Zeaven and Zhukovsky (1975) [18], brinjal is originated from India, and secondary centre of origin is believed to be the China. Although the Gangetic new alluvial plain of West Bengal is the hot spot of brinjal diversity the farmers of this region are facing a problem of poor yield and quality produce. Non-availability of high yielding and good quality brinjal variety is the main reason of poor yield.

The basic step for crop improvement relies on characterization and identification of existing germplasm. It is generally agreed that genetically diverse parents will show the maximum heterosis and offer the maximum chance of isolating transgressive segregates. This serves the purpose of identifying probable parents for obtaining the best recombinants from the population. Although, Mahalanobis's generalized distance as a measure of genetic distance occupy a unique place in plant breeding yet, as it happens in biology, several problems under the influence of random unpredictable changes due to environment, evade the direct grip of the concept well proven in more exact fields like mathematical components. It suggests the determination of the genetic distance through multivariate analysis over environment, to fortify its reliability. The effectiveness of techniques like Multivariate analysis to analyze the genetic diversity of populations has been proved useful. Mahalanobis D^2 statistics and Principle component analysis (PCA) appear to be a meaningful approach based on multivariate analysis and serves to be a good index of genetic diversity. This experiment was planned to generate information on genetic diversity present in twenty five genotypes of brinjal in Gangetic new alluvial plains of West Bengal so as to help the breeder in selecting promising and

genetically diverse parents for bringing the desired improvement through hybridization.

Materials and Methods

Twenty five genotypes of brinjal, collected from different sources, were tested and evaluated at "C" Block Farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal under the research field of All India Coordinated Research project on Vegetable Crops. Topographic situation of the experimental site comes under the Gangetic new alluvial plains of West Bengal.

Seed beds were prepared in a sandy loam soil and were 20 cm high and 1.0 m wide. Fully decomposed cow dung manure @ 4 kg/m^2 was added into the beds. Drenching of beds was done with chlorothalonil (2g) + carbendazim (1 g) to control damping off disease. Seeds, after treated with Thiram (3 g/kg of seed), were sown during the 1st week of July, 2016 at a depth 5 cm apart and covered with finely sieved well rotten leaf mold (leaves left to decompose for two year) which acts as soil improver and also prevent moisture loss. After sowing, beds were covered with straw until germination which normally takes five to seven days and hand watered regularly. Nursery beds were covered with 200 µm ultraviolet (UV)-stabilized polyethylene film supported by bamboo poles with open sides to protect seedlings from rain and direct sunlight. Hardening of seedlings was done by withholding water 4 days before transplanting. Thirty days old seedlings were transplanted in the main field during 1st week of September, 2016 in the afternoon hours. Seedlings were transplanted at 75 cm in both ways in each plot measuring 3.75 m × 4.50 m accommodating 30 plants. Recommended fertilizer dose @ 150 kg N, 75 kg P_2O5 and 75 kg K_2O/ha was applied in the main field. Half of total 'N' and full dose of 'P' and 'K' was applied as basal and rest half of N were applies in two equal splits, first at 30 days after transplanting and the rest 25 days after first top dressing. Management practices as scheduled for cultivation were followed as per Chattopadhyay *et al*. (2007) [3].

Data were recorded from 15 randomly selected plants of each plot in each replication on days to first flowering, days to 50% flowering, plant height (cm), number of primary branches per plant, fruit length (cm), fruit diameter (cm), fruit weight (g), number of fruits per plant, fruit yield per plant (kg). D^2 statistic (Mahalanobis, 1936) [5] was used for assessing the genetic divergence of twenty five genotypes for nine quantitative traits. The grouping of the populations was done by using Tocher's method as described by Rao (1952) [11]. Hierarchical cluster analysis has been done with those same genotypes in order to observe the degree of association according to their characteristics that was expressed in dendrogram following Ward's (1963) [17] method. Principal component analysis (PCA), to identify the factor dimension of the data, was used to summarize varietal information in a reduced number of factors for selection of the best performing genotypes. Statistical analyses were done using statistics analytical software ver. 1.4, IRRI, Philippines, 2014.

Result and Discussion

The investigation was aimed at analyzing the genetic divergence of 25 genotypes employing nine important quantitative characters namely, days to 1st flowering, days to 50% flowering, plant height (cm), number of primary branches per plant, fruit length (cm), fruit diameter (cm), fruit weight (g), number of fruits per plant, and fruit yield (kg/plant). On the basis of degree of divergence (D^2 values) between any two genotypes a logical grouping of the genotypes with low D^2 value could be arrived at by Tocher's method as described by Rao (1952) [11]. Based on the determination of divergence, all the 25 genotypes could meaningfully be grouped into six clusters (Table-1). Cluster II had six genotypes followed by cluster VI which comprised of five genotypes, while cluster III and cluster IV had four genotypes each, and cluster I and cluster V had three genotypes each. Some earlier studies (Nand *et al*., 2018 and Sanga *et al*., 2018) [9, 13] also classified brinjal genotypes in number of clusters while conducting experiments in different environments utilizing various genotypes. The grouping pattern of genotypes was observed to be random, indicating that geographical diversity and genetic divergence were unrelated. Such observation has been reported by Banerjee *et al*. (2018) [2]. The absence of relationship between genetic diversity and geographical distance indicates that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous mutation, natural and artificial selection are responsible for genetic diversity. Therefore, selection of genotypes for hybridization should be based on genetic diversity rather than geographic divergence (Mehta *et al*., 2004) [7].

Table 1: Cluster classification and source of collection of brinjal genotypes.

Number of clusters	Name of the genotype/Source
I (3)*	16/BRL var-2 (U.P.), 16/BRL-3 (U.P.), BCB-40 (W.B.)
II (6)	16/BRL var-7 (U.P.), 16/BRL var-9 (U.P.), 10/BRBWRES-3 (U.P.), 15/ BRL Var-1 (U.P.), 15/ BRL var-3 (U.P.), 15/ BRL var-5 (U.P.)
III (4)	16/BRL var-1 (U.P.), 16/BRL var-4 (U.P.), 16/BRL var-6 (U.P.), White Jhuri begun (W.B.)
IV (4)	16/BRR var-2 (U.P.), 16/BRR var-3 (U.P.), 16/BRR var-7 (U.P.), 16/BRR var-8 (U.P.)
V (3)	16/BRR var-4 (U.P.), 16/BRR var-5 (U.P.), 16/BRR var-9 (U.P.)
VI (5)	16/BRL var-8 (U.P.), Swarna Mani (Jharkhand), KS-224 (U.P.), 15/ BRL var-2 (U.P.), 15/ BRL var-4 (U.P.)

*Figures in parentheses indicate number of genotypes

The intra-and inter-cluster distance represent the index of genetic diversity among clusters (Table-2). Cluster V showed the maximum intra-cluster value (205.30), while Cluster III recorded the minimum intra-cluster value (75.10). At inter-cluster level, minimum value was observed between Cluster 1 and II (377.89) indicating close relationship among the genotypes included in these clusters. The maximum inter-cluster values were observed between cluster IV and VI (2643.75) followed by (2343.00) between Cluster III and VI which indicated that the genotypes included in these clusters had the maximum divergence. Hence, intermating through different crossing fashions between the genotypes included in clusters IV and VI or Cluster III and VI would be expected to give heterotic response in F_1 generation. Kalloo *et al*. (1980) [4] suggested that the crosses between selected varieties from widely separated clusters were most likely to give desirable recombinants. Low level of intra-cluster distances was

indicative of narrow genetic variation within the cluster and would not yield desirable recombinants.

Table 2: Inter- and intra-cluster distances of twenty five genotypes of brinjal

Cluster Number	I	II	III	IV	V	VI
I	203.55	377.89	410.21	941.79	573.15	1377.76
II		148.75	383.15	471.71	589.54	1586.02
III			75.10	375.49	1043.57	2343.00
IV				144.84	1231.19	2643.75
V					205.30	511.16
VI						71.05

Cluster mean based estimations are very useful in targeting the genotypes for breeding programme, as they prevent the tedious efforts of screening the inferior germplasm lines. Hence, genotypes from desirable clusters could be directly used for final field evaluation in advanced breeding experiments. The character means were worked out for the genotypes falling in these six clusters (Table-3) showed that the mean values of the clusters varied in magnitude for all the 9 characters. Cluster I was the highest yielder followed by cluster III. Regarding fruit weight, cluster VI showed highest performance followed by cluster IV. Highest number of fruits per plant was produced in the genotypes belonging to cluster IV followed by the genotypes under cluster III. These clusters could be regarded as useful sources of gene for important yield component traits. However, Cluster III had lowest number of days to 1st flowering and days to 50% flowering which could be helpful for breeding an early plant type. Hence, it can be suggested from the present study that a high yielding early flowering type with appreciable fruit number could be bred by utilizing the genotypes from cluster I and III as parent in the future breeding programme.

The relative contribution of individual characters towards genetic divergence was computed in terms of number of times it ranked first (Table-3 and Fig-1). Fruit weight contributed the maximum (61.34 %) towards genetic divergence followed by plant height (13.33%), days to 50% flowering and fruit yield per plant (6.00%), number of fruits per plant (4.33%), days to 1st flowering (3.67%), fruit length (2.33%), fruit diameter (1.67%) and number of primary branches per plant (1.33%) showing the possibility for selection of these characters. Similar findings were also recorded earlier (Ravali *et al*., 2017; Nand *et al*., 2018; Sanga *et al*., 2018) [9, 12, 13] for fruit weight, days to 50 % flowering and fruit yield per plant. In further study of dendogram following Ward's method (Fig-2) by using squared Euclidean distance, it became clearly evident that there was high diversity among 25 genotypes of brinjal along with strong relationships among the genotypes.

Table 3: Cluster means and percent contribution of nine characters of brinjal.

Cluster/ Characters	Days to 1st flowering	Days to 50% flowering	Plant height (cm)	Number of primary branches per plant	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Number of fruits per plant	Fruit yield per plant (kg)
I	36.50	49.50	108.22	4.25	11.96	8.45	205.44	9.54	1.97
II	39.44	50.89	74.78	3.56	17.02	5.66	173.13	7.26	1.24
III	36.11	48.33	120.13	2.89	12.23	4.02	132.78	12.76	1.72
IV	41.54	52.33	87.37	4.12	15.91	4.42	104.52	14.61	1.51
V	47.40	59.40	88.80	3.73	10.57	10.18	229.07	5.23	1.18
VI	49.83	61.50	83.00	3.00	11.88	10.22	318.67	4.38	1.40
Percent Contribution towards divergence (%)	3.67	6.00	13.33	1.33	2.33	1.67	61.34	4.33	6.00

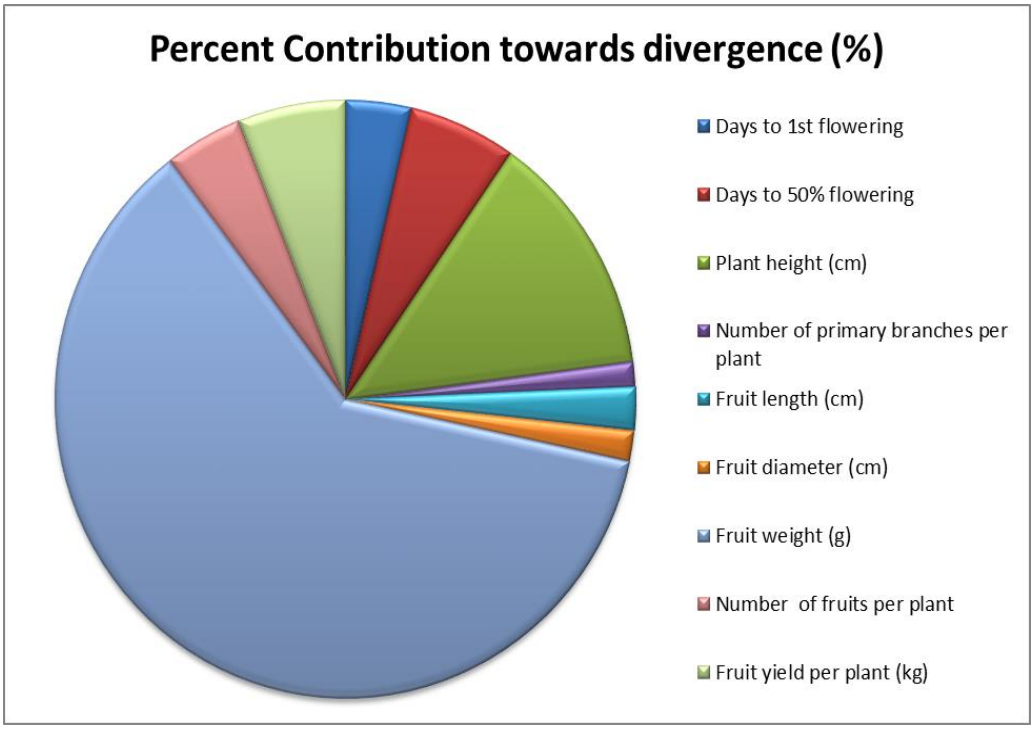

Fig 1: Percent contribution towards divergence (%) of different characters.

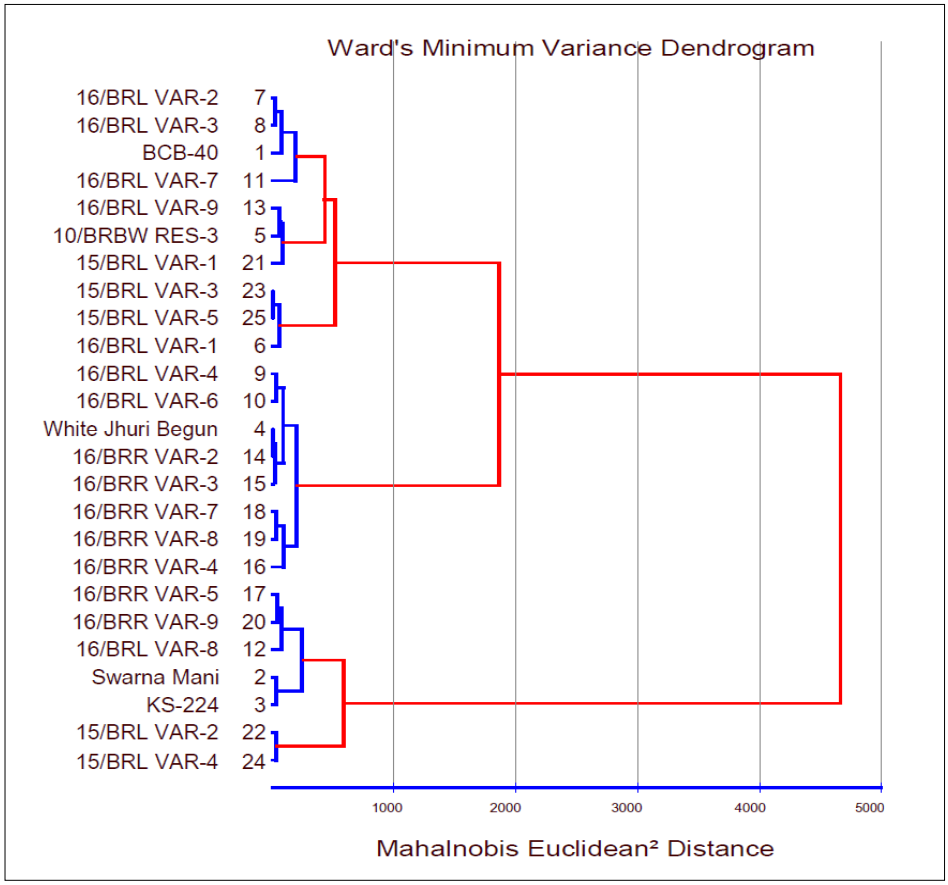

Fig 2: Dendogram of 25 genotypes of brinjal following Ward's method

Principal component analysis (PCA) involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. PCA was performed to obtain a simplified view of the relationship between four characters (days to 50% flowering, plant height, fruit weight and number of fruits per plant) having maximum contribution towards divergence, and variable loadings for components PC1 (days to 50% flowering), PC2 (plant height), PC3 (fruit weight) and PC4 (number of fruits per plant) were extracted in Table-4. These components were chosen as because their contribution towards divergence was the maximum and eigenvalues exceeded 1.0 and explained 100.00% of the total variance. The first component (PC1) explained 93.01% of total accounted for variance in which an increase in days to 50% flowering leads to increase in fruit weight and decrease in plant height and number of fruits per plant. The second component (PC2) explained an additional 99.57% of the variance in which an increase in plant height leads to decrease in days to 50% flowering and increase in fruit weight and number of fruits per plant. PCA was also analysed to identify parents for hybridization in brinjal by Shende *et al*. (2016) [14] and Patel *et al*. (2018) [10]. The scattered diagram (Fig-3) clearly depicted that five genotypes BCB-40, KS-224, White Jhuri Begun, 10/BRBW/ RES-3 and 16/BRL var-8 have registered distinct differences of their genotypic characters and belong to farthest distances and colours from the other genotypes in the plot. Rest of the genotypes have shown similar features and formed a separate cluster.

Table 4: Results of principal component analysis (PCA) for different characters in brinjal.

Principal component	**Eigenvalue %**	**% Variance**	**% Cumulative variance**	
Eigenvalues and variance accounted for (%) by PCA based on correlation matrix				
PC1	4747.41	93.01	93.01	
PC2	334.35	6.55	99.57	
PC3	16.35	0.32	99.89	
PC4	5.80	0.11	100.00	
Variables	**PC1**	**PC2**	**PC3**	**PC4**
Factor loadings due to PCs with eigenvalues greater than 1				
Days to 50% flowering	0.042355	-0.059670	0.993271	0.084264
Plant height (cm)	-0.026180	0.996894	0.064273	-0.036977
Fruit weight (g)	0.997340	0.030814	-0.044513	0.048152
Number of fruits per plant	-0.053237	0.039476	-0.074998	0.986466

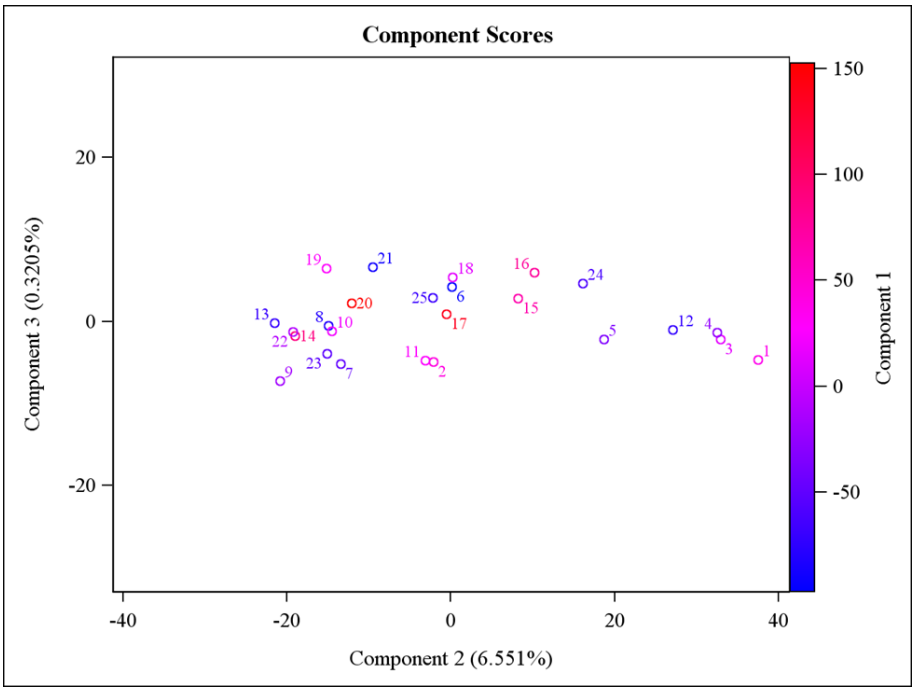

Fig 3: Scatter diagram of regression factor scores for the first and second components as determined by principal component analysis. Points in diagram closest to the intersection of 0 on the X- and Y-axes indicate similarity. Outliers on the X-axis, i.e., 1,3 4, 12 and 5 indicate diversity. Numbers correspond to name of the genotype (BCB-40 = 1, Swarna Mani = 2, KS-224 = 3, White Jhuri Begun = 4, 10/BRBW RES-3 = 5, 16/BRL VAR-1 = 6, 16/BRL VAR-2 = 7, 16/BRL VAR-3 = 8, 16/BRL VAR-4 = 9, 16/BRL VAR-6 = 10, 16/BRL VAR-7 = 11, 16/BRL VAR-8 = 12,16/BRL VAR-9 = 13, 16/BRR VAR-2 = 14, 16/BRR VAR-3 = 15, 16/BRR VAR-4 = 16, 16/BRR VAR-5 = 17, 16/BRR VAR-7 = 18, 16/BRR VAR-8 = 19, 16/BRR VAR-9 = 20, 15/BRL VAR-1 = 21, 15/BRL VAR-2 = 22, 15/BRL VAR-3 = 23, 15/BRL VAR-4 = 24, 15/BRLVAR-5=25)

The choice of parents for hybridization depends on genetic diversity of parents. Precise information on the nature and degree of genetic divergence would help the plant breeder in choosing the selective parents for hybridization. The expression of heterosis is influenced by genetic diversity of parents. It is general belief that more diverse the parents within overall limits of fitness, the greater are the chances of obtaining higher amount of heterosis expression in the F_1s and a broad spectrum of variability in segregating generations (Arunachalam, 1981) [1]. Several reports are available to show that hybrids between genetically diverse parents manifest greater heterosis than those between more closely related parents (Moll and Stuber, 1974 and Singh and Sharma, 1989) [8, 15]. 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 crossability 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) [6]. Apart from the high degree of divergence, the cluster mean performance and the characters with maximum contribution towards divergences should also be given due consideration for the selection of best combination of parents for improvement in various economic characters. The genotypes BCB-40, KS-224, White Jhuri Begun, 10/BRBW/ RES-3 and 16/BRL var-8 were selected from four diverse clusters having maximum values of yield and yield contributing traits and the minimum values of earliness. Therefore, variety with high yield and earliness could be breed by using these genotype through hybridization.

Conclusion

This study brought out the fact that there was no parallelism between genetic diversity and geographical divergence in brinjal. The maximum inter-cluster values were observed between Clusters IV and VI or Cluster III and VI having high divergence hence, emphasis should be given on these clusters, because hybridization between the genotypes included in these clusters would be expected to give either heterotic response in F_1 generation or transgressive segregates in segregating generation. On the basis of D^2 statistics and PCA, five genotypes namely, BCB-40, KS-224, White Jhuri Begun, 10/BRBW/ RES-3 and 16/BRL var-8 were identified highly diverse and suitable for cultivation under the region of Gangetic new alluvial plains of West Bengal. Hybridization between these genotypes would produce desirable F_1 and high yielding recombinants in the segregating generations.

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