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Genetic divergence in bitter gourd (*Momordica charantia* L.) under undulating topography of Tripura

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Abstract
Genetic divergence among nine genotypes of Bitter gourd (*Momordica charantia* L.) for fifteen important yield attributing traits and one qualitative trait was studied under the undulating topography of Tripura using multivariate analysis. The genotypes were logically grouped into three clusters on the basis of divergence analysis. The grouping pattern of genotypes indicated no direct relationship between geographical distribution and genetic distance. High inter cluster distance between cluster II and cluster III suggesting that the genotypes belonging to cluster II and cluster III will be taken in hybridization programme for evolving a good hybrid or segregate. Among the characters, vine length contributed the maximum divergence towards total genetic divergence followed by primary branches per plant, Internodal length, days to 1st male flower and days to 1st female flower. Five out of 16 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, two genotypes namely, Malda local and Pusa Do Mousumi 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 bitter gourd variety.

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

1. Introduction
Bitter gourd or balsam pear is one of the most important commercial and highly nutritive vegetables grown extensively throughout the country from the plain to an altitude of 1500 meter. Among the cucurbitaceous vegetables, it is considered a prized vegetable because of its high nutritive value especially ascorbic acid and iron contents (Behera, 2004) [2]. Bitter gourd is native to Asia with Eastern India and Southern China proposed as the centers of domestication (Yang and Walter, 1992) [23]. According to Young and Walters (1992)[23], there are threehorticultural groups (i) Small fruit type, 10-20 cm long (ii) Long fruit type most commonly grown in China, 30-60 cm. long, slightly bitter; (iii) Triangular fruit type, cone shaped, 9-12 cm. long light to dark green with prominent tubercles, moderately to strongly bitter. The typical Chinese phenotype is oblong with bluntly tapering ends and pale green in colours, with a gently undulating warty surface. The bitter gourd of India has a narrower shape with pointed ends and a surface covered with sagged, irregular teeth and ridges, either green or white in colour. Between these two extremes there are numbers of intermediate forms found in different parts of the world.

It is monoecious and a highly cross-pollinated crop in which large amount of variation is observed in many economically important traits. Selection of suitable parent is important to breed adaptable cultivars for wide agro-climatic zones. Genetic diversity is one of the important tools to quantify genetic variability, crop improvement and developments of new varieties in cross and self-pollinated crops. 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, 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 is 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 (Datta*et al*., 2018)[4]. This experiment was planned to generate information on genetic diversity present in nine genotypes of bitter gourd in sandy loam soil of Tripura, so as to help the breeder in selecting promising and genetically diverse parents for bringing the desired improvement through hybridization.

2. Materials and Methods

Nine genotypes of bitter gourd, collected from different sources, were tested and evaluated at Experimental Farm of College of Agriculture, Tripura, Lembucherra, Tripura. Topographic situation of the experimental site comes under undulating sandy loam soil of Tripura with warm and humid tropical climate.

The experimental land was ploughed and cross-ploughed 2-3 times by power tiller followed by laddering to obtain a good tilth. Stubbles and weeds were removed from the field. The entire experimental land was levelled and divided into 27 plots measuring 3.75m × 2.25m.The experiment was laid out in a randomized block design with 3 replications. The pre-soaked seeds were sown on ridges with a spacing of 0.75m in both ways ensuring 15 plants per plot during summer season of 2016. Full dose of FYM @ 20-25 t/ha along with half dose of Nitrogen (80 kg), full doses of P_2O_5 (40 kg) and full doses of K_2O (40 kg) was applied during field preparation. The remaining amount of Nitrogen (40 kg) was applied in two split doses, first at one month after sowing and the second split was given at the initiation of flowering. Nitrogen in the form of urea, phosphorus in the form of single super phosphate and potassium in the form of murate of potash was applied to the experimental plots. The crop was grown over trellises. Management practices as scheduled for its cultivation were followed as per Chattopadhyay*et al*. (2007) [3].

Data were recorded from 9 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) [10] 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) [13]. 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) [22] 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.

3. Result and Discussion

The nine accessions were grouped into three clusters (Table-1), which indicated optimum genetic diversity among the bitter gourd genotypes under taken for study. Cluster I was largest having six genotypes followed by clusters-II having two and cluster-III with one genotype. The monotypic genotype in cluster III indicated that the genotype from this cluster might have originated across the geographical location in breeding programs. Intra- and inter-cluster distances among genotypes (Table-2) indicated that cluster II had the most intra-cluster value indicating that the genotypes Pusa Do Mousumi and Ganapati seed included in the cluster were extremely diverse. Cluster II recorded maximum intra cluster (152.461) distance where as cluster- I had the minimum (44.869). With respect to inter cluster distance, the maximum distance was recorded between cluster II and cluster III, suggestingthat the genotypes belonging to cluster II and cluster III may be utlized in hybridization programme for evolving a good hybrid or segregate. Kalloo*et al*. (1980) [8] stated that crosses between selected varieties from widely separated clusters were most likely to give desirable recombinants.

Table 1: Grouping of nine Genotypes in Clusters

Clusters	Number of Varieties	Name of Varieties
I	6	Bolder uchhe, Tripura Local, Baruipur Local,Gangajali, Pearafulle, Singapuri
II	2	Pusa Do Mousumi, Ganapati seed
III	1	Malda local

Table 2: Average Inter and Intra Clusters D^2 values among three clusters in nine Bitter gourd genotypes

Clusters	I	II	III
I	44.869		
II	152.461	32.015	
III	96.991	194.648	0.00

Data clearly indicated that the genotypes did not cluster according to their geographical distribution. The grouping pattern of genotypes was random, indicating geographical diversity and genetic divergence was unrelated (Rasul*et al*., 2004) [15]. Similar observations have also been reported by Davmore*et al*. (2007) [5], Day *et al*. (2007) [6],Resmi and Sreelathakumary(2012) [14], Singh *et al*. (2014) [18]and Kumari*et al*. (2017) [9]. Somayajullu*et al*. (1970) [21] reported that the clustering revealed instability due to relatively lesser divergence, whereas widely divergent clusters remain distinct in different environments. The result has also supported by Raut*et al*. (1985) [16]. In this study, it was observed that the cluster II was highly diverged indicating more stability. The genotypes of the distant cluster could be used as potential source for obtaining wide range of variation among the segregates and crop improvement programmes to produce populations with wide variability with transgressive segregates possessing high yield (Singh *et al*. 2014) [18].

There was no definite correlation between geographic origin and genetic diversity of bitter gourd genotypes, indicating the parental selection should be made on the basis of a systemic assessment of genetic distance in any specific population rather than geography. According to Dias *et al*. (2003) [7], divergence between any two parents is caused by the allelic difference between them. Genotype in the same cluster diverge less from one another and as expected, hybrids between them result in fewer desirable segregate. Selection of parent material with maximum divergence is likely to produce higher heterosis and desirable genetic recombination (Roy *et al*. 2013) [17]. 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 variation, natural and artificial selection are responsible for genetic diversity. It may also be possible that causes of clustering pattern were much influenced by environment and genotype × environment interaction resulting in different expression. Another possibility may be that estimates of diversity based on the characters used in present investigation might not have been sufficient to account for variability caused by some other traits of physiological and biochemical nature which might have been important in depicting the total genetic diversity in the population. Thus, it is more appropriate to select genotypes for hybridization based on genetic diversity rather than geographical diversity (Solanki *et al*., 2000) [20].

The comparison of cluster means for the different characters indicated considerable differences between clusters for all characters. The clusters wise mean value (Table- 3) showed that the difference in cluster means were substantially high for yield per plant, average fruit weight and iron content. Cluster III which was having only one genotype had the highest values for vine length, (2.24), inter nodal length (7.38), node number to which first female flower appears (25.84), fruit length (9.33), fruit diameter (3.61), number of fruits per plant (5.02) and yield per plant (225.70). Cluster II had the highest values for traits like primary branches per plant (4.63), node number to which first male flower appear (16.78), days to first harvest (70.00) and in iron content (91.58). Cluster I possessed highest value for days to first male flower appearance (25.33), days to first female flower appearance (45.35), average fruit weight (47.77), number of seeds per fruit (7.87), and average seed weight (8.89). It can be conclusively decided that cluster III was deemed best for selecting diverse genotype followed by cluster I. In further study of dendogram following Ward's method (Fig- 1) by using squared Euclidean distance, it became clearly evident that there was high diversity among 9 genotypes of bitter gourd along with strong relationships among the genotypes.

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 five characters (vine length, primary branches per plant, inter nodal length, days to 1st male flower and days to 1st female flower) having maximum contribution towards divergence, and variable loadings for components PC1 (vine length), PC2 (primary branches per plant), PC3 (inter nodal length), PC4 (days to 1st male flower) and PC5 (days to 1st female flower) 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 99.20% of total accounted for variance in which an increase in vine length leads to increase in days to 1st female flowering and decrease in primary branches per plant, internodal length and days to 1st male flower. The second component (PC2) explained an additional 99.62% of the variance in which an increase in primary branches per plant leads to decrease internodal length and increase in days to 1st male flower and days to 1st female flowering (Table-5). PCA was also analysed to identify parents for hybridization in bitter gourd by Kumari*et al*. (2017) [9]. The scattered diagram (Fig-2) clearly depicted that 2 genotypes Malda Local and Pusa Do Mousumi 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 3: Clusters wise mean values of sixteen characters in Bitter gourd genotypes

Characters	Cluster-I	Cluster-II	Cluster-III
Vine length (cm)	1.71	1.79	2.24
Primary branches per plant	3.71	4.63	4.00
Inter nodal length (cm)	4.09	5.34	7.38
Days to1st male flower	25.33	22.18	22.93
Days to 1st female flower	45.35	42.93	41.27
Node at 1st male flower	15.91	16.78	15.67
Node at 1st female flower	24.85	24.25	25.84
Fruit length (cm)	7.65	4.03	9.33
Fruit diameter (cm)	3.18	2.34	3.61
Number of fruits per plant	4.46	4.63	5.02
Average weight of fruit (g)	47.77	21.08	42.10
Days to first harvest	65.82	70.00	67.33
Fruit yield per plant (kg)	210.78	95.48	225.70
Number of seeds per fruit	7.87	5.83	6.67
Average seed weight (g)	8.89	4.17	7.82
Iron content (mg/100 g)	1.29	1.58	0.22

Table 4: Results of principal component analysis (PCA) for quantitative characters contributing to divergence

Principal component (PC)	Eigenvalue (%)	% Variance	% Cumulative variance
Eigenvalues and variance accounted for (%) by PCA based on correlation matrix			
PC1	5111.55768	99.20	99.20
PC2	21.37707	0.41	99.62
PC3	8.66656	0.17	99.79
PC4	5.64966	0.11	99.90
PC5	3.63360	0.07	99.97

Table 5: Contribution of diverse traits in the principal components of bitter gourd

Variable	PC1	PC2	PC3	PC4	PC5
Factor loadings due to PCs with eigenvalues >1					
Vine length	0.001148	-.030699	0.002875	0.018911	-.046464
Primary branches per plant	-.003257	-.041476	-.000427	-.056459	-.057173
Inter nodal length	-.000445	-.209739	0.059034	0.154705	-.167204
Days to1st male flower	-.002727	0.515311	-.078342	0.694627	-.107335
Days to 1st female flower	0.005805	0.501122	0.172618	-.108928	-.653313

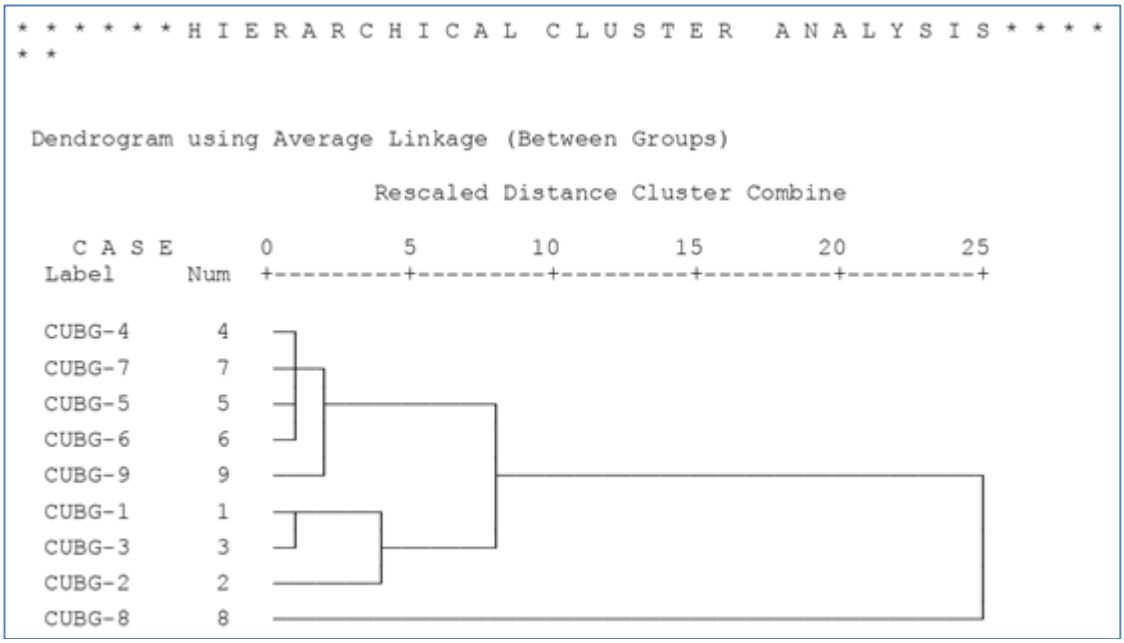

Fig 1: Dendrogram of 9 genotypes of bitter gourd following Ward's method

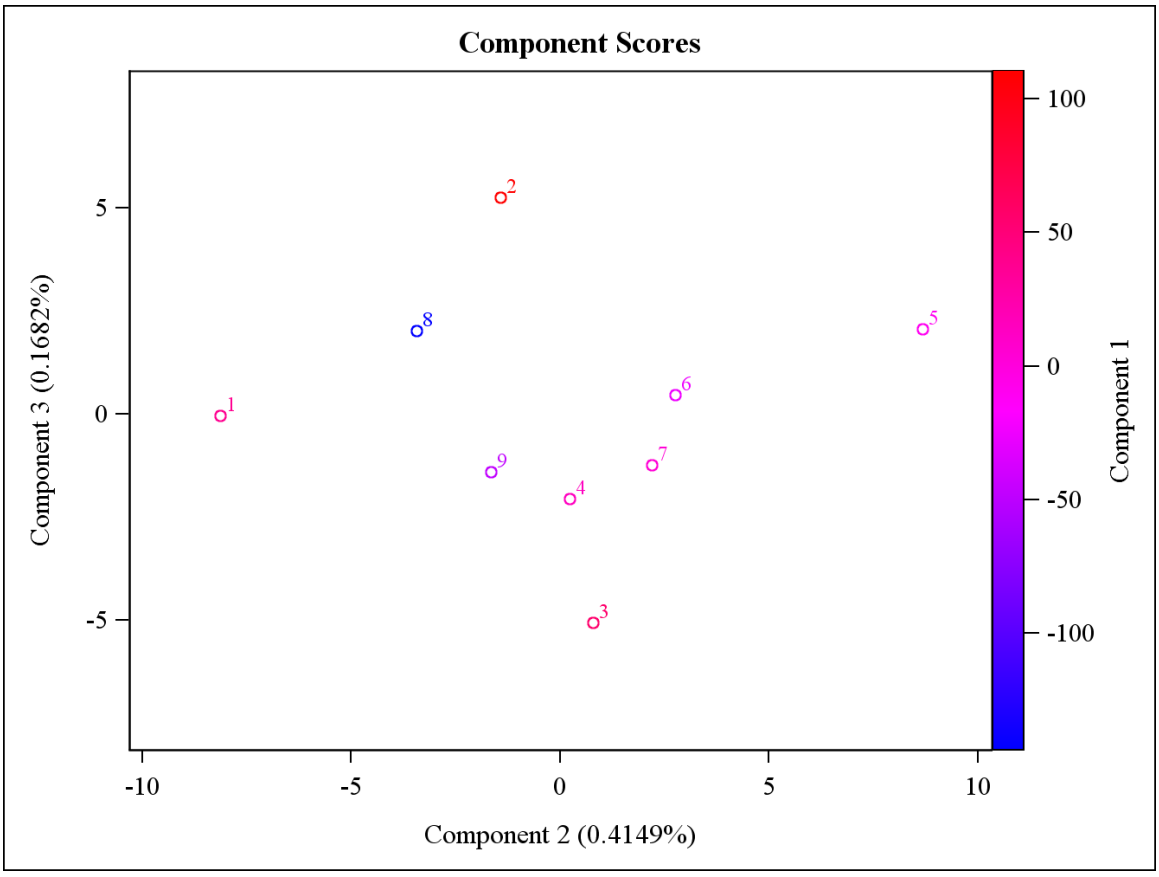

Fig 2: Scatter diagram of regression factor scores for the first and second components as determined by principal component analysis. Points in diagram closest to the intersection of 0 on the X- and Y-axes indicate similarity. Outliers on the X-axis, i.e., 1 and 8 indicate diversity. Numbers correspond to name of the genotype (Malda Local=1, Bolder uchhe=2, Tripura Local=3, Baruipur Local=4, Gangajali=5, Pearafulle=6, Singapuri=7, Pusa Do Mousumi=8 and Ganapati seed=9).

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 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 [12] and Singh and Sharma, 1989 [19]). Further, the occurrence of heterosis cannot be predicted on the basis of genetic divergence alone (Matzinger and Werusman, 1958) [11]. 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 Malda Local and Pusa Do Mousumi were selected from two 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 genotypes through hybridization.

4. Conclusion

This study brought out the fact that there was no parallelism between genetic diversity and geographical divergence in bitter gourd. The maximum inter-cluster values were observed between Clusters II and III 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, cluster mean and PCA, two genotypes namely, Malda Local and Pusa Do Mousumi were found highly diverse and suitable for cultivation under the sandy loam soil of Tripura. Hybridization between these genotypes would produce desirable F_1 and high yielding recombinants in the segregating generations.

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