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Genetic divergence analysis among indigenous temperate and tropical region adapted accessions of carrot (*Daucus carota* L.)

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Abstract
Carrot crop improvement depends on extent of genetic diversity in the asiatic and western gene pool. The nature and magnitude of genetic divergence in ninety-six indigenous temperate and tropical region adapted accessions of carrot (*Daucus carota* L.) was assessed for 18 quantitative traits using Mahalanobis D^2 analysis. Interestingly, five plants vegetative weight (g) (63.2%), followed by five plants root weight (33.57%), had higher contribution to the diversity among the selected genotypes. Tocher's method of cluster analysis grouped all the cultivars into twelve distinct clusters. The inter cluster distance was found to be highest between cluster X and XII (671114.10), followed by cluster I and XII (456310.80), Mean value for carrot root quality traits, like root length was highest in cluster II. Root weight was maximum for cluster XII. Whereas, cluster I recorded the highest root diameter and cluster X showed the highest mean value for TSS. Knowledge on extent of genetic divergence for different traits among these clusters would help the breeders for planning sound breeding programs.

Keywords: Genetic divergence, Mahalanobis D^2, clusters, quantitative traits

Introduction
Carrot is an important cool season vegetable and a rich source of provitamin A carotenoid. Number of varieties have been developed suitable to temperate climates. Carrots contain incredible antioxidants, detoxifying properties, anti-aging, anti-cancerous properties, good for the heart, and dental health, and also act as a natural liver cleanser (Alasalvar *et al.*, 2001; Simon *et al.*, 2008; Maksylewicz and Baranski 2013) [1, 15, 10]. Carrot (*Daucus carota* L.) belongs to one of the largest families of seed plants, Apiaceae (Judd *et al.*, 2016) [6], with the diploid chromosome number of 2n=2x=18 and an estimated genome size of 473 Mbp (Iorizzo *et al.*, 2016) [5]. In India, greater variation is present both in Asiatic and European types (Chaitra *et al.*, 2020) [3]. Many European varieties have been introduced in different parts of the country. Number of local varieties which are the selections by farmers adaptable to tropical and temperate climates are also available. Most of these Indian germplasm collections are least explored in breeding program as most of the breeding efforts are concentrated to development of temperate varieties (Kulkarni *et al.*, 2019) [7]. In India, due to a vast geographical diversity and varied agro-climatic conditions both Asiatic (tropical-type) and European (temperate-type) carrots are grown suitable to temperate and tropical conditions. Farmers have also made their own selections from these collections and maintained them as landraces or local types. An investigation was carried out involving 96 carrot germplasm containing local varieties, landraces, released varieties, public and private sector varieties, IIVR germplasm collections. This ninety-six-germplasm panel was subjected to phenotypic evaluation for root morphological traits, productivity traits and important biochemical traits with a total of 18 quantitative traits. Carrot being a highly cross-pollinating nature has a greater diversity for various roots morphological and biochemical components. Use of genetically diverse parents in breeding program helps to obtain heterotic gene combinations and superior recombinants (Haydar *et al.*, 2015) [4]. Therefore, prioritization of genetic divergence study before planning of important breeding program is prerequisite to assess the extent of genetic variability among genotypes. Keeping this in view, to exploit the extent of genetic divergence across genetic material for eighteen important various quantitative traits, genetic divergence and cluster analysis using D^2 statistics was undertaken. Using Mahalanobis D^2 statistics to estimate the net divergence in germplasm panel for crop improvement has been indicated by number of workers in different plants (Saran *et al.*, 2007; Santos *et al.*, 2011) [14, 13].

Material and methods

The present investigation was carried out in the department of Genetics and Plant breeding. College of Horticulture Bagalkot. The field experiment was conducted at Udyanagiri campus of University of Horticultural Sciences, Bagalkot, Karnataka, India during 2016. Bagalkot is located in the northern region of Karnataka and positioned at 16°12′N, 75°45′E the average elevation in this area reaches approximately 610 m. The climate is warm and dry throughout the year and rainfall is scarce with an average annual rainfall of 318 mm and belongs to semi-arid tropical region. Ninety-six Carrot (*Daucus carota* L.) germplasm lines were used, including Asiatic (Tropical) and European (Temperate) cultivated accessions. This panel represents a large diversity present in carrot especially for the colour *viz*., white, yellow, red, orange, Dark orange, purple and Black. The genotypes were collected from all over India, comprising of open-pollinated cultivars, local varieties, modern hybrid cultivars, released varieties. These germplasm lines have been evaluated in the present study after two years of sib-pollination (to constitute the homogeneity in the respective genotype). These genotypes were as UHSBC (University of Horticultural Sciences Bagalkot Carrot) collections. The nomenclature and the numbers are given as per the collection data. The details of the genotypes used in the present study are same as listed by Kulkarni *et al.*, 2019 [7]. Seeds of the ninety-six genotypes were planted and grown in Augmented Block Design. Three check varieties included in the design consisting of one tropical adapted released variety (Pusa Vrishti), one temperate adapted variety (Vigro Kuruda) and one local cultivar (Ghataprabha Local). These three check varieties were replicated and randomised in each of the six blocks. Ninety-six genotypes were evaluated across 18 quantitative traits as listed in Table 1.

Table 1: List of quantitative traits recorded in 96 genotypes in carrot

S. No	Characters	Details
1	Days to maturity	No. of days to harvest from the date of sowing
2	No. of petioles	Petioles Counted
3	Shoot length (cm)	Measuring scale
4	Plant height	Measuring scale
5	Root length (cm)	Measuring scale
6	Petiole length (cm)	Measuring scale
7	Root width (mm)	Digital Vernier Caliper-converted to cm
8	Shoulder width (mm)	Digital Vernier Caliper- converted to cm
9	Vegetative weight/plant (gms)	Weighing Balance
10	Five Plants Vegetative weight	Weighing Balance
11	Xylem width (cm)	Measuring scale
12	Phloem width (cm)	Measuring scale
13	Harvest index (%)	Economic yield/biological yield
14	Total Soluble Solids (^{0}Brix)	Digital Refractometer
15	Reducing Sugars (%)	Dinitro Salicylic Acid (DNS) method
16	Beta Carotene Content (µg/100 mg)	Acetone Extraction Method
17	Root yield (gms)/plant	Weighing Balance
18	Five plants root weight	Weighing Balance

Mahalanobis D^2 analysis for phenotypic data

Mahalanobis (1936) D^2 analysis was used for assessing the genetic divergence among the 96 genotypes involving 18 quantitative characters using the software package Window stat version 8.10. The generalized distance between any two populations is given by the formula as given below.

Since, the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized characters (X's) to standardized uncorrelated variables (Y's) was done to simplify the computational procedure. The D^2 values were obtained as the sum of squares of the differences between pairs of corresponding uncorrelated (s) values of any two uncorrelated genotypes (Rao, 1960) [11].

Cluster of D^2 values All n (n-1)/2 D^2 values were clustered using ward minimum method described by Rao (1960) [11].

Intra cluster distance-The intra cluster distances were calculated by the formula given by Singh and Choudhary (1977). The inter-cluster distances were calculated by the formula described by Singh and Choudhary (1977).

$D^2 = \Sigma\Sigma\lambda ij\sigma ai\sigma aj$

Where, D^2 = Square of generalized distance; λij = Reciprocal of the common dispersal matrix; $\sigma ai = (\mu i1 - \mu i2)$; $\sigma aj = (\mu j1 - \mu j2)$; μ = General mean.

In brief, the steps involved for the estimation of D^2 values are as follows (Rao, 1952) [12].

1. Pivotal condensation of error variance and co-variance matrix to obtain inverse matrix.
2. Transformation of original correlated data into un-correlated variables.
3. Calculation of mean values of the transformed characters.

Calculation of D^2 values: For each combination, deviation between the means was computed and the D^2 values were computed and arranged in the form of matrix.

Determination of group constellations: As such no standard rules are available for making the clusters because cluster is not a well-defined term. The only criterion appears to be that any two genotypes belonging to the same cluster should at least, on an average, show a smaller D^2 value than those belonging to two different clusters. The D^2 values for all the combinations presented in the matrix form were arranged in increasing order of magnitude and clustering was done according to the method suggested by (Rao, 1952) [12].

At first, the two most closely associated genotypes were chosen and then third genotype was located which had the smaller average D^2 value as compared to the first two

genotypes. Following this methodology, the subsequent genotypes were chosen which have smaller average D^2 value from the first three genotypes and change in D^2 value within a cluster due to inclusion of additional genotype was computed and so on. The new genotypes were added so long as the increase in average D^2 value became abruptly high, then this genotype was not included in the former groups. The genotypes of first cluster were omitted and rest were treated similarly for constructing new clusters.

Intra and inter cluster distance: The intra cluster D^2 value was calculated as the sum of n (n-1)/2 D^2 values among the genotypes within a cluster divided by n (n-1)/2. The single genotype always has zero intra cluster D^2 value. For calculating the inter cluster D^2 value, all possible D^2 values between genotypes of two clusters were added and then divided by n1×n2, where n1 and n2 represented the number of genotypes in the first and second cluster, respectively. The intra and inter cluster distances were calculated by taking the square root of respective D^2 value between genotypes of a particular cluster and between genotypes belonging to two clusters, respectively.

Clusters mean value: The cluster mean of a particular character is the summation of mean values of the genotypes included in a cluster divided by number of genotypes in the same cluster. Cluster mean values for all the 18 traits were estimated to identify the superior clusters for the economic root traits of carrot.

Results

To quantify genetic divergence between any two genotypes or group of genotypes, Mahalanobis' D^2 statistics (1936) as described by Rao (1952) [12] was used and the genotypes were grouped into different clusters on the basis of ward's minimum variance method.

The mean sum of squares due to genotypes for all the traits studied were highly significant from the analysis of variance as reported in our earlier paper (Kulkarni *et al.,* 2019) [7], thereby, revealing sufficient amount of genetic variation among the genotypes for all the eighteen characters studied. Hence, these 18 traits were considered for D^2 statistics to know the extent of diversity among these carrot genotypes representing broad genetic background.

Contribution of different characters towards divergence

The diversity among 96 genotypes was measured by employing D^2 statistics. Table 2 shows the contribution of 18 quantitative characters towards genetic divergence. Out of these characters, contribution of five plants vegetative weight was maximum (63.2%), followed by five plants whereas, the remaining characters like, beta carotenoid (1.05%), days to maturity (0.42%), harvest index % (0.31%) and few other traits contributed either very little or no contribution for divergence.

Table 2: Mahalanobis D^2 analysis showing percent contribution of root morphological characters to diversity among 96 carrot genotypes

S No	Source	Times ranked 1st	Contribution %
1	Days to Maturity	19	0.42%
2	No. of Petioles		0.0 %
3	Shoulder Length (cm)		0.0 %
4	Plant Height (cm)	3	0.07%
5	Root Length(cm)	4	0.09%
6	Petiole Length(cm)		0.0 %
7	Root width (mm)		0.0. %
8	Shoulder Width (mm)		0.0 %
9	Vegetative weight of single plant (g)	54	1.18%
10	Five plants vegetative weight (g)	2882	63.2%
11	Xylem Width (mm)		0.0 %
12	Phloem Width (mm)		0.0 %
13	Harvest Index	14	0.31%
14	Total Soluble Solids (^{0}Brix)		0.0 %
15	Reducing Sugars (%)		0.0 %
16	Beta Carotene (µg/gm)	48	1.05%
17	Root Weight of individual Plant (g)	5	0.11%
18	Five Plants Root Weight (g)	1531	33.57%

Cluster composition

All 96 carrot genotypes were grouped into clusters based on the relative magnitude of their D^2 values, in such a way that genotypes in each cluster had smaller D^2 value than between the clusters. Table 3 shows the distribution pattern of genotypes in different clusters. The genotypes were grouped into 12 different clusters. Cluster pattern revealed that cluster-I was the largest one with as high as 85 genotypes, and remaining 11 clusters formed solitary clusters. Most of these clusters which diverged from the cluster I were either IIVR collections or local varieties except for cluster 10 (Hybrid Kuruda-Public sector hybrid) and cluster 11 (IARI, released variety Pusa Payasa) indicating existence of higher diversity among the local cultivars and IIVR collections which are least explored in the breeding program for crop improvement.

Intra and inter cluster average D^2 values

The intra and inter cluster D^2 values among 96 genotypes are given in table 4. The results showed that inter cluster distances are more than intra cluster distance which indicates the presence of narrow genetic variation within a cluster, but very high divergence between the clusters as indicated by higher values of inter cluster D^2 values. The highest intra cluster D^2 value was observed for cluster number I as there were 85 different genotypes and remaining all other showed 0.00 intra cluster distance as they were solitary clusters consisting of single genotype. Diversity among the inter-clusters showed a very wide range of D^2 value ranging from 671114.10 to 456310.80. The inter cluster distance was found to be highest between cluster X and XII (671114.10), followed by cluster I and XII (456310.80), cluster X and XI

(411913.10). Whereas, the lower inter cluster distance was observed between cluster III and V, followed by cluster VI and II (3331.50). The higher inter cluster distance indicated the presence of more diversity among the genotypes included among these clusters.

Mean values of different clusters for 18 characters

The cluster means for all the 18 characters are presented in Table 5. The data revealed considerable differences among all the clusters for most of the characters studied. It was evident that carrot root length was highest in cluster II (23.83 cm) and lowest in cluster XII (12.52 cm). Cluster I recorded the highest root diameter (32.41 mm) while cluster III recorded the lowest (15.43 mm). Root weight was minimum for cluster VIII (32.03 g) and it was maximum for cluster XII (57.83 g). Cluster X revealed the highest mean value for TSS (9.29 ^{0}Brix), whereas cluster III had the lowest mean value (5.74 ^{0}Brix). For harvest index, cluster I (0.98) had the highest mean value while cluster XII had the lowest mean value (0.24).

Cluster III showed the maximum mean value (54.283) for phloem colour and cluster VII showed the lowest mean value (47.822). For xylem colour, the highest mean value was possessed by cluster III (12.600) and the lowest value was possessed by cluster VII (9.289). Root colour was the highest in cluster VII (86.44) and the lowest in cluster IV (82.303). Cluster VII recorded the highest mean value of days to maturity (114.667) and the lowest in cluster IV (107.212). Five plants root yield was recorded maximum for cluster V (3.327) and the minimum for cluster I (2.237).

Table 3: Cluster Composition showing the genotypes grouped into each cluster in Mahalanobis D^2 analysis using 18 quantitative traits of carrot

Clusters	No. of Genotypes	Genotype Composition
Cluster 1	85	UHSBC-1, UHSBC-2, UHSBC-3, UHSBC-7, UHSBC-14, UHSBC-15, UHSBC-16, UHSBC-17, UHSBC-19, UHSBC-20, UHSBC-21, UHSBC-22, UHSBC-23, UHSBC-25, UHSBC-27, UHSBC-28, UHSBC-29, UHSBC-30, UHSBC-31, UHSBC-32, UHSBC-32_2, UHSBC-33, UHSBC-34, UHSBC-36, UHSBC-37, UHSBC-38, UHSBC-39, UHSBC-40, UHSBC-42, UHSBC-43, UHSBC-43_1, UHSBC-44, UHSBC-45, UHSBC-46, UHSBC-48, UHSBC-49, UHSBC-50, UHSBC-51, UHSBC-52, UHSBC-53, UHSBC- 34_1, UHSBC- 34_2, UHSBC- 41_1, UHSBC-77, UHSBC-56, UHSBC-58, UHSBC-59, UHSBC-64, UHSBC-65, UHSBC-67, UHSBC-68, UHSBC-69, UHSBC-71, UHSBC-78, UHSBC-79, UHSBC-85, UHBC-89, UHSBC-90, UHSBC-93, UHSBC-94, UHSBC-95, UHSBC-96, UHSBC-97, UHSBC-98, UHSBC-99, UHSBC-101, UHSBC-102, UHSBC-106, UHSBC-107, UHSBC-108, UHSBC-110, UHSBC-111, UHSBC-112, UHSBC-113, UHSBC-114, UHSBC-115, UHSBC-54, UHSBC-66, UHSBC-92, UHSBC-103, UHSBC-104, UHSBC-105, UHSBC-116, UHSBC-117, UHSBC-23_1
Cluster 2	1	UHSBC-18-Local Cultivar (Hangaraki Local)
Cluster 3	1	UHSBC-47-IIVR Collection
Cluster 4	1	UHSBC-73-Local cultivar (Belgaum)
Cluster 5	1	UHSBC-41-IIVR collection
Cluster 6	1	UHSBC-24-IIVR collection
Cluster 7	1	UHSBC-76-Local cultivar (Naganur)
Cluster 8	1	UHSBC-35-IIVR collection
Cluster 9	1	UHSBC-55-IIVR collection
Cluster 10	1	UHSBC-100 (Kuruda)
Cluster 11	1	UHSBC-63-Released varieties (Pusa Payasa)
Cluster 12	1	UHSBC- 26- IIVR collection

Table 4: Mahalanobis D^2 Analysis showing inter-intra cluster distances among the twelve clusters analyzed for 96 carrot genotypes

Clusters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Cluster 10	Cluster 11	Cluster 12
Cluster 1	36227.53											
Cluster 2	91355.59	0.00										
Cluster 3	84352.95	29624.58	0.00									
Cluster 4	83582.09	4376.17	12787.12	0.00								
Cluster 5	79252.07	14530.97	3256.20	3477.88	0.00							
Cluster 6	112536.50	3331.50	36948.89	7504.90	19227.41	0.00						
Cluster 7	88565.80	5249.33	49384.45	13470.05	27838.94	5732.92	0.00					
Cluster 8	86820.52	51478.09	6048.01	28273.58	12491.81	58952.35	69638.55	0.00				
Cluster 9	163796.60	32538.49	22068.85	20269.64	18499.61	27221.05	53153.13	36317.88	0.00			
Cluster 10	87776.03	138674.80	210813.30	158188.90	178121.70	154027.10	102748.50	221859.00	283256.30	0.00		
Cluster 11	339242.70	93410.63	134827.70	99028.78	115300.60	71427.90	109410.00	166173.10	52730.13	411913.10	0.00	
Cluster 12	456310.80	209853.00	165322.80	184929.50	174429.50	191564.40	258766.80	180942.70	86098.05	671114.10	77268.62	0.00

Table 5: Cluster means of 12 clusters for 18 quantitative traits analyzed for 96 genotypes of carrot

Traits / Clusters	DM	NP	SL	PH	RL	PL	RWD	SWD	VW	FPVW	XW	PW	HI	TSS	RS	Beta	RW	FPRW
Cluster I	73.04	10.22	0.95	50.71	17.40	13.19	17.97	24.02	42.58	199.12	0.73	0.45	0.95	7.65	4.85	21.49	40.08	185.89
Cluster II	48.83	10.92	1.07	75.55	23.83	20.18	19.30	38.59	78.72	397.72	1.40	0.85	0.46	6.74	4.45	4.14	69.10	358.22
Cluster III	62.50	21.19	1.03	50.63	17.46	9.01	15.43	22.41	91.34	451.06	0.72	0.35	0.33	5.74	2.70	16.53	38.90	203.56
Cluster IV	75.50	11.66	1.19	67.47	16.33	19.02	21.26	36.34	83.80	419.72	1.13	0.44	0.42	8.45	6.36	5.86	60.63	304.89
Cluster V	73.50	13.59	1.15	56.63	18.06	11.95	17.42	27.65	86.14	433.06	0.91	0.25	0.39	9.19	3.90	21.86	49.30	253.56
Cluster VI	77.83	13.92	0.75	58.25	20.61	11.80	32.41	32.20	82.72	423.72	0.90	0.47	0.47	8.04	3.44	12.19	74.30	388.22
Cluster VII	75.50	8.86	0.87	68.67	19.35	17.58	21.84	42.95	71.40	353.72	1.21	0.71	0.53	9.20	5.16	27.53	78.63	394.89

Cluster VIII	73.50	14.46	0.99	53.02	13.84	12.97	15.59	22.37	92.54	448.72	0.87	0.20	0.26	7.80	3.11	79.97	32.03	161.56
Cluster IX	88.50	20.79	1.07	59.23	16.80	12.11	11.65	23.88	93.34	559.06	0.89	0.39	0.36	8.44	3.65	16.36	58.90	299.56
Cluster X	82.17	7.06	1.05	52.30	21.08	51.50	24.65	29.87	9.67	46.39	0.83	0.71	0.91	9.29	4.58	79.53	77.03	383.56
Cluster XI	63.50	21.06	1.03	61.57	22.13	11.98	26.52	34.76	132.20	661.72	0.98	0.62	0.43	8.60	4.34	22.69	97.43	494.89
Cluster XII	58.50	28.66	1.03	58.62	12.52	16.43	21.52	27.16	174.94	838.72	0.61	0.57	0.24	6.45	5.39	13.14	57.83	289.56

Discussion

Genetic diversity existing within and between groups of germplasm is important, and particularly, useful in proper choice of parents for realizing higher heterosis and obtaining useful recombinants. D^2 statistics is a useful tool for estimating the genetic divergence in plant breeding experiments. Based on the divergence study, 96 carrot genotypes involved in the present study were grouped into 12 clusters. Clustering pattern revealed the presence of considerable amount of genetic diversity in this material. In general, intra-cluster distances were relatively lower than inter cluster distances showing that genotypes included within a cluster were genetically less diverse than the genotypes included in different clusters. Cluster pattern revealed that cluster I was the largest one with 85 different genotypes and remaining other formed solitary clusters. Genotypes from different sources were grouped in the same cluster thereby, indicating that geographical diversity does not necessarily represent genetic diversity. These findings suggested that the pattern of clustering was independent to their geographical origin based on phenotypic observations. However, molecular marker-based diversity provides the clear genetic diversity and genetic differentiation (Chaitra *et al*., 2020) [3] in addition to phenotypic divergence. This implied that genetic material from same geographical region may provide substantial diversity. These finding are in conformity with earlier workers, Amin *et al.*, 2010 [2], and Kumar *et al*., 2014 [8] who have also reported significant differences in their breeding material for genetic diversity.

The inter cluster distance was found to be highest between cluster XII and X (D^2 value =671114.10), followed by cluster XII and I (D^2 value=456310.80), cluster XI and X (D^2 value =411913.10) indicating wide diversity between these two clusters, while the minimum inter-cluster distance with D^2 value of 3256.20 was observed between cluster V and III followed by cluster VI and II with D^2 value 3331.50 indicating their close relationship.

Inter-cluster distance was maximum between cluster XII and X which indicates that the genotypes included in these clusters are genetically diverse and would be utilized in future breeding program. So, it is desirable to select accessions from the clusters having high inter-cluster distance in the recombination breeding programs. The minimum inter-cluster distance was observed between cluster V and III indicating their narrow diversity.

The cluster means for all the 18 quantitative characters showed different quality traits of carrot had considerable variances amongst all the clusters for most of the characters studied. Root length was highest in cluster II, Root weight was supreme for cluster XII. Root diameter was found to be high in Cluster I. Cluster X revealed maximum mean value for TSS. For harvest index, cluster I (0.98) had the highest mean value.

In the above investigation, Asiatic (tropical adapted) genotypes are recognised as best in performance for almost all 18 quantitative traits with higher genetic divergence. Asiatic cultivars can be well utilized for future breeding efforts and commercial achievement of carrot production.

Conclusion

The genotypes collected and utilized in the present study consisting of larger genetic variation with broad genetic background including local cultivars, IIVR germplasm collections which were least explored in earlier carrot breeding programs. Germplasm panel also consisted of released varieties suitable to tropical and temperate conditions, Ooty, Kodaikenal (Karnataka), Mukteshwar (Sub-Himalayan Region) and other temperate climates of India. Despite this larger genetic background most of the genotypes (85) belonged to one cluster (1st) and remaining eleven genotypes diversified as eleven solitary clusters. Most of the genotypes in these solitary clusters are either IIVR collections or the local varieties indicating that these unexplored germplasm lines are highly diverse in nature and could be utilized in future breeding program concentrating on tropical region. One cluster consisted of hybrid Kuruda (Cluster-10) and the other released variety (Pusa Payasa).

Possible forces other than eco-geographical differentiation such as natural and human selection pressure would exert considerable influence on the genetic divergence. But to get more heterotic F1's and large number of desirable transgressive segregants, selection of parents for hybridization should be properly based on genetic diversity rather than geographic diversity (Chaitra *et al.*, 2020) [3]. An effective hybridization program may be initiated involving the genotypes belonging to diverse clusters with high mean for almost all component characters. The cluster means for all the characters revealed considerable differences among them for most of the characters studied. Further, the crosses involving diverse parents within compatible range could be done to obtain high heterotic expression or to recover desirable trans gressive segregants in subsequent generations.

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