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Genetic diversity in marigold genotypes

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ABSTRACT

The present investigation was carried out at the Research Farm of Department of Horticulture, CCS Haryana Agricultural University, Hisar, Haryana during two winter season of 2011-12 and 2012-13 in a randomized block design (RBD) with three replications to assess the genetic variability and diversity in thirty genotypes of marigold. The mean sums of squares were highly significant for all the characters studied, indicating the presence of variability. Highest range of variation was reported with fresh weight of plant followed by flower yield plant^{-1}, whereas dry weight of flower exhibited minimum range of variation. The PCV was higher in magnitude than the GCV for all the characters. During both the years of investigation, the high genotypic and phenotypic coefficient of variation were recorded for fresh and dry weight of plant, number of secondary branches plant^{-1} and number of buds and flowers plant^{-1}, whereas, low genotypic and phenotypic coefficient of variation were recorded for flower diameter and days taken to first flower opening. High heritability with high genetic advance was found with fresh and dry weight of plant, number of secondary branches plant^{-1} and number of buds and flowers plant^{-1}. High genetic advance as per cent was observed for fresh and dry weight of plant, number of flowers plant^{-1}, number of secondary branches plant^{-1} and number of buds plant^{-1} revealing the importance of additive gene effects for these traits. All the thirty genotypes of marigold were grouped into six clusters based on Mahalanobis D_2 statistics using Tocher's method. The clustering pattern of genotypes revealed that the genetic diversity was independent of the geographical diversity. Among the six clusters, cluster-II was largest with 12 genotypes, followed by cluster-IV (11), cluster-V (3), cluster-I (2) and clusters-III and VI with one genotype each. The maximum inter-cluster distance was observed between clusters-V and VI (D^2=13.94), closely followed by clusters-I and VI (12.73) and clusters-IV and VI (12.36). The intra- cluster distance was highest in cluster-V (4.88) and minimum in cluster-I (2.80).

Keywords : Diversity, genetic advance, GCV, heritability, marigold, PCV, variability

Marigold, a member of the family Asteraceae or Compositae, is a potential commercial flower crop that is gaining popularity on account of its easy culture, wide adaptability, and increasing demand in the subcontinent (Ahmad *et al*., 2011). It is popular among flower growers because of wide spectrum of attractive colours, shape, size and good keeping quality. They are extensively used for making garlands, beautification and other purpose *i.e*., pigment and oil extraction and therapeutic uses. Loose flowers are in great demand for garland making as well as in religious and social functions. Globular shaped flowers with long stalks are used for cut flower purposes. Apart from these uses, marigold is a widely grown plant in gardens and pots. It is highly suitable as a bedding plant, in herbaceous border and also ideal for newly planted shrubberies to provide colour and fill the space. French marigold is most ideal for rockery, edging, hanging baskets and window boxes. Marigold cultivation controls the nematode population in soil and is used for making mosquito repellent products (Gupta *et al*., 2001). Flower extract is used as a blood purifier as well as a good remedy for eye diseases and ulcer. The carotenoids extracted from petals of marigold are the major source of pigment for poultry industry as a feed additive to intensify the yellow colour of egg yolks and broiler skin (Narsude *et al*., 2010 and Dixit *et al*., 2013). Lutein, which is the major constituent of xanthophylls, is used for colouring foodstuffs (Singh, 2006). These wide uses depend on the variable performance of different genotypes.

The source of any breeding programme for developing suitable varieties depends largely on the availability of genetic variability in a given species. Hence, crop improvement is the need of the time to sustain the availability of desirable cultivars. Improvement through selection depends upon the variability existing in the available genotypes, which may be either due to different genetic constitution of cultivars or variations in the growing environments (Kavitha and Anburani, 2010). Selection is effective only when the observed variability in the population is heritable in nature. Heritability estimates give a measure of transmission of characters from one generation to the other, as consistency in the performance of the selection depends on the heritable portion of the variability, thus enable the plant breeder for isolating the elite selections in the crop. Hence, the magnitude of the variation and the estimates of the heritability and genetic advance are the important parameters on which the success of selection lies. The Mahalanobis D^2 statistics is the rational criterion to study the genetic variation in available genotypes (Mahalanobis, 1936). In the present investigation with D^2 statistic, an attempt

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was made to study the variability among the genotypes and also identify the suitable genotypes of marigold for hybridization programme on the basis of their clustering pattern. During any hybridization programme, the selection of parents using D^2 statistic provides the required potential parents, which are under study with respect to an array of characters. With the selection of genotypes, based on their genetic distance and yield potential, a breeder can formulate an appropriate crossing programme for the desired crop improvement. Therefore the present investigation was carried out to study the genetic variability and divergence in thirty genotypes of marigold.

MATERIALS AND METHODS

The present investigation was carried out at the Research Farm of Department of Horticulture, CCS Haryana Agricultural University, Hisar, Haryana, during two winter season of 2011-12 and 2012-13 to assess the genetic diversity and variability in 30 genotypes of marigold. The data of both the years were pooled and analysed. The materials utilized for the present study consisted of 28 genotypes of African marigold (*Tagetes erecta*) and two genotypes (Hisar Beauty and Hisar Jafari) of French marigold (*Tagetes patula*). Raised nursery beds of size 3.0 x 1.0 m were first prepared and drenched with Captan (0.01%). The seeds of different genotypes were sown in rows spaced at 6.0 to 8.0 cm and covered with thin layer of well rotten fine compost to ensure germination. The nursery beds were watered daily twice for first 10 days and daily once for the remaining period. The seedlings were ready for transplanting at 28-30 days after sowing. One month old, healthy, vigorous and uniform seedlings were selected and transplanted in 90 beds during September. The plot size was kept 3.2 x 1.2 m and in each plot consisted of 24 plants and they were transplanted at a spacing of 40 x 40 cm in randomized block design (RBD) with three replications. Five plants were selected in each replication of each genotype for taking observations after discarding the border plants at both the ends. Observations were recorded on growth, flowering and yield characters on five randomly selected plants in each replication of each genotype. The pooled data of both the years were analyzed to work out genetic variability and divergence. The genotypic and phenotypic coefficients of variation were calculated by using the formula suggested by Panse and Sukhatme (1978). Parameters of variability were calculated as per the formulae given by Burton and Devane (1953). Heritability in 'broad sense' was computed as the ratio between genotypic variance to total phenotypic variance and expressed in percentage (Allard, 1960). The expected genetic advance resulting from the selection of 5 per cent superior individuals were worked out as suggested by Lush (1940) and Johnson *et al.* (1955). The mean and standard and standard error worked out as per standard methods and coefficient of variation was computed. The Mahalanobis D^2 statistic was used to find out generalized distance between the genotypes as described by Rao (1952). The clustering was done by following Tochers method (Singh and Chaudhary, 2010). The criterion used in clustering by this method is that genotypes belonging to the same cluster should show smaller D^2 value than those belonging to different clusters.

RESULTS AND DISCUSSION

The extent of variability present in germplasm was estimated in terms of range, genotypic and phenotypic coefficient of variation (GCV and PCV), heritability, genetic advance and genetic advance as per cent of mean. The analyses of variance revealed that mean square of treatments were significant for all the characters (Table 1). This suggested the presence of wide range of variability for different characters in the material studied. In the present investigation, comparison of coefficients of variation indicated that the estimates of phenotypic coefficient of variation (PCV) were higher than genotypic coefficients of variation (GCV) for all the sixteen characters studied. The phenotypic coefficient of variation (PCV) exhibited nearby similar trend for the traits as in genotypic coefficient of variation (GCV) and had higher value than GCV indicating that genotypic expression was superimposed by the environmental influence and hence selection may be misleading. Similar findings were reported by Singh and Misra (2008), Sharma and Raghuvanshi (2011), Verma *et al*. (2002) in marigold.

In pooled analysis (Table 2), the highest range of variation was reported with fresh weight of plant (78.76-1122.42) followed by flower yield plant^{-1} (98.00-874.37). The highest values of GCV and PCV were recorded for fresh weight of plant (63.17 and 63.08%) followed by dry weight of plant, number of flowers plant^{-1}, number of secondary branches plant^{-1} and number of buds plant^{-1} which indicated that the selection for these traits would be effective. Namita *et al.* (2008) reported high GCV and PCV for number of flowers plant^{-1} and flower weight in marigold. Similar results were reported in marigold by Pattnaik and Mohanty (2002) for flower yield plant^{-1} and number of flowers plant^{-1}. Lowest values of GCV and PCV were obtained for days taken to first flower opening and flower diameter indicated that the genotypes used had less genetic variability for these characters. Panwar *et al.* (2013) also recorded lowest GCV and PCV for number of seeds and days to flowering. Similarly Sharma and Raghuvanshi (2011) observed similar results for days taken to bud formation

Table 1 : Analysis of variance for different characters of marigold genotypes

Sr. No.	Characters	Mean sum of square		
		Replication (d.f.=2)	Genotypes (d.f.=29)	Error (d.f.=58)
1.	Plant height (cm)	0.26	900.57**	7.45
2.	Plant spread (cm)	0.65	403.93**	3.51
3.	Stem diameter (cm)	0.00	0.30**	0.02
4.	Stalk length(cm)	0.14	97.51**	0.55
5.	No. of primary branches/plant	2.55	47.98**	0.67
6.	No. of secondary branches/plant	1.70	1487.92**	25.95
7.	Fresh weight of plant (g)	54.19	168728.61**	168.19
8.	Dry weight of plant (g)	1.27	2061.59**	10.74
9.	Days to first flower opening	4.81	106.01**	7.21
10.	Duration of flowering	17.22	434.49**	7.71
11.	No. of buds per plant	10.31	2781.39**	10.33
12.	No. of flowers per plant	13.22	2208.26**	13.59
13.	Flower diameter (cm)	0.10	3.04**	0.05
14.	Fresh weight of flower (g)	0.12	18.86**	0.09
15.	Dry weight of flower (g)	0.02	0.31**	0.01
16.	Flower yield per plant (g)	3220.14	95450.56**	1504.43

** *Significant at 1% level of probability*

Table 2: Estimation of variance and other genetic parameters in marigold in pooled study

Sl. No.	Characters	Range		Mean ± SE(m)	G.C.V.	P.C.V.	E.C.V.	Heritability (broad sense) (%)	Genetic advance	Genetic advance as % mean
		Min.	Max.							
1.	Plant height (cm)	32.40	103.75	73.79	23.07	23.36	3.65	97.56	35.11	46.94
2.	Plant spread (cm)	33.75	77.72	49.83	23.41	23.71	3.80	97.44	23.49	47.60
3.	Stem diameter (cm)	0.61	2.14	1.33	23.74	23.93	3.07	98.35	0.64	48.49
4.	Stalk length (cm)	4.38	27.03	15.03	37.83	38.15	4.94	98.33	11.61	77.28
5.	Number of primary	8.50	24.50	12.81	31.01	31.65	6.37	95.95	8.01	62.57
6.	Number of secondary	22.02	150.97	47.41	46.62	47.84	10.76	94.95	44.31	93.57
7.	Fresh weight of plant(g)	78.76	1122.42	375.79	63.08	63.17	3.45	99.70	487.57	129.75
8.	Dry weight of plant (g)	9.91	130.72	45.37	57.63	58.08	7.22	96.45	53.44	117.80
9.	Days taken to first flower opening	37.90	64.60	48.84	11.75	12.97	5.50	82.04	10.71	21.92
10.	Duration of flowering	31.93	76.53	50.29	23.72	24.36	5.52	94.86	23.93	47.59
11.	Number of buds plant^{-1}	37.87	217.10	68.33	44.48	44.73	4.70	98.89	62.26	91.12
12.	Number of flowers plant^{-1}	28.94	189.00	55.77	48.50	48.95	6.61	98.18	55.21	99.00
13.	Flower diameter (cm)	4.01	8.21	6.28	15.90	16.33	3.73	94.79	2.00	31.89
14.	Fresh weight of flower (g)	2.32	15.57	8.48	29.50	29.72	3.56	98.56	5.12	60.34
15.	Dry weight of flower (g)	0.28	2.04	1.04	30.64	32.20	9.91	90.54	0.62	60.06
16.	Flower yield plant^{-1} (g)	98.00	874.37	460.55	38.42	39.34	8.42	95.42	356.09	77.32

and flower diameter. Narrow difference between GCV and PCV revealed that variability existing among different genotypes of marigold was mainly due to genetic makeup and there was less environmental influence on the expression of this trait. Same results had also been recorded by Singh and Misra (2008) in marigold. GCV is helpful in the assessment of inherent variability. Marked difference between PCV and GCV values confirm the predominance of GxE interaction. GCV and PCV detect the amount of variability in the available genotypes.

The genotypic coefficient of variation alone does not provide reliable information about the assessment of variation that is heritable and therefore, estimation of heritability becomes imperative. Heritability recorded for all the characters under studied very high except days

Table 3 : Grouping of thirty genotypes of marigold into different clusters

Cluster number	Number of genotype (s)	Name of genotype (s)
I	2	MGH-09-271, MGH-09-276
II	12	MGH-09-301, MGH-09-304, MGH-148-8, MGH-09-303, MGH-133-1-3, MGH-133-3-2, MGH-09-305, MGH-160-9-1, MGH-160-5-1, MGH-160-5-2, MGH-09-276-1, MGH-09-302
III	1	MGH-148-3-3
IV	11	MGH-10-101, MGH-133-1-1, MGH-133-1-2, MGH-160-9, MGH-133-2, MGH-160-5-3, MGH-133-5, MGH-133-5-2, MGH-160-7-1, MGH-160-9-3, MGH-160-9-4
V	3	MGH-160-8-3-2, MGH-07-160-8-3-3, Hisar Beauty
VI	1	Hisar Jaffri-2

Table 4 : Average intra (diagonal) and inter-cluster D^2 values among six clusters in 30 genotypes of marigold

Cluster	I	II	III	IV	V	VI
I	2.80	4.99	6.72	6.40	7.95	12.73
II		3.79	6.18	4.22	6.15	12.02
III			0.00	7.30	9.10	9.95
IV				3.24	5.30	12.36
V					4.88	13.94
VI						0.00

taken to first flower opening. Sharma and Raghuvanshi (2011) also reported high heritability for all the characters excluding plant spread and number of lateral branches plant^{-1} in marigold. Similar results were reported by Anuja and Jahnavi (2013) in marigold. The heritability estimate of a quantitative character is very important since phenotypic expression of a genotype may be altered by environment at various stages of its development. Heritability indicates the effectiveness with which selection for genotypes can be done on the basis of its phenotypic variation. It expresses the extent to which individual phenotypes are determined by their genotypes.

The heritability estimates serve as a useful guide to the breeder because selection would be fairly easy for the characters with high heritability. Thus, a close correspondence between the genotype and phenotype will be attributed to a relatively smaller contribution of the environment to phenotype, but for a character with low heritability, selection may not be effective due to the masking effect of environment on genotypic effect. The response to selection depends upon the relative magnitude of heritable variation present in relation to the phenotypic variation. Therefore, it is desirable to partition observed variability into its heritable and non-heritable components. Burton (1952) suggested that genotypic coefficient of variation along with heritability would give a better idea about the efficiency of selection. Thus, a character with high genotypic coefficient of variation and high heritability will be more valuable in selection programme. The magnitude of heritable variability is the most important aspect of genetic constitution of the genetic material which has close bearing on the response of selection (Panse, 1957).

Genotypic coefficient of variation and heritability (broad sense) are not sufficient to determine the amount of variation which is heritable (Burton, 1952). Heritable variation can be determined with greater accuracy when heritability along with genetic advance is studied. Heritability along with genetic gain is more useful criterion in predicting the resultant effects of selecting the best individual (Johnson *et al.*, 1955). High heritability with high genetic advance tells that the character is governed by additive gene action, for that simple selection is advocated. In the present study high heritability with high genetic advance were observed for fresh weight of plant, flower yield plant^{-1} and flower yield ha^{-1} whereas low heritability with low genetic advance were observed for stem diameter, dry weight of flower and flower diameter. Kumari *et al.* (2011) recorded low heritability with low genetic advance for flower stalk diameter, flower dry weight and flower diameter in

Table 5 : Cluster mean values for various characters in marigold genotypes

Cluster	Plant height (cm)	Plant spread (cm)	Stem diameter (cm)	Stalk length (cm)	Number of primary branches plant^{-1}	Number of secondary branches plant^{-1}	Fresh weight of plant (g)	Dry weight of plant (g)	Days taken to first flower opening	Duration of flowering (days)	Number of buds plant^{-1}	Number of flowers plant^{-1}	Flower diameter (cm)	Fresh weight of flower (g)	Dry weight of flower (g)	Flower yield plant^{-1} (g)
I	67.95	56.63	1.50	9.49	12.18	45.63	306.20	38.52	51.85	54.22	71.53	53.94	7.87	15.08	1.77	812.94
II	82.85	51.42	1.29	14.93	11.14	45.73	399.87	48.02	50.40	55.77	64.58	51.73	6.71	9.34	1.16	483.45
III	66.39	66.82	2.14	15.65	11.07	57.03	1122.42	130.72	49.40	65.30	83.20	67.50	7.25	9.24	1.11	622.38
IV	78.36	45.74	1.28	19.07	13.69	44.27	275.19	34.57	46.06	40.78	63.46	53.22	5.78	7.30	0.88	392.70
V	40.92	34.08	1.13	5.44	13.36	28.56	199.19	23.82	45.37	46.81	44.49	34.13	5.73	5.98	0.70	190.89
VI	62.68	78.07	1.61	10.98	24.50	150.97	1115.58	125.36	64.60	76.53	217.10	189.00	4.01	4.64	0.56	874.37

gerbera. These results are in agreement with the findings of Yuvraj *et al*. (2012) and Karuppaiah and Kumar (2011), and Singh and Singh (2010) in marigold.

Higher expected genetic advance and genetic advance as per cent of mean was estimated for fresh weight of plant (487.57 and 129.57) followed by flower yield plant^{-1} (356.09 and 77.32), while low values were observed for dry weight of flower (0.62 and 60.06). These results are in conformity with the previous results as reported by Namita *et al*. (2008), Pattnaik and Mohanty (2002) and Singh and Sen (2001) in marigold. The studies revealed that genetically diverse genotypes should be further utilized as parents in crop improvement programme for the development of the varieties/hybrids with broad genetic base.

On the basis of D^2 value, all the thirty genotypes were grouped into six clusters (Table 3), indicating the presence of diversity for different traits among the various genotypes studied. The cluster-II had highest number of genotypes (12), followed by cluster-IV with 11 genotypes. The clusters-V and I included three and two genotypes, respectively, while the clusters-III and VI each accommodated one genotype. Grouping of genotypes into six clusters suggested presence of considerable diversity in the material under investigation. Kavitha and Anburani (2009) also reported genetic divergence in marigold and observed highly significant difference among the 30 genotypes which could be grouped into 8 clusters.

The average intra and inter cluster D^2 values represent the index of genetic diversity among clusters as given in table 4. The maximum intra-cluster distance was recorded within cluster-V (4.88) followed by cluster-II (3. 79) and cluster-IV (3. 24), while it was lowest for the genotypes of cluster-I (2.80), indicating that genotypes of cluster-V varied in genetic architecture and might have originated from different genetic pool. In cluster-I, the trend was exactly reverse of the cluster-V. With respect to inter-cluster, D^2 value ranged from 4.22 to 13.94. The widest inter-cluster distance was observed between cluster-V and VI (13.94) followed by cluster-I and VI (12.73), cluster-IV and VI (12.36), cluster-II and VI (12.02) and cluster-III and V (9.95), indicating that the genotypes included in these clusters were genetically diverse and might give rise to high heterotic response. The lowest inter-cluster D^2 value (4.22) was observed between cluster-II and IV, indicating close relationship among the genotypes included in these clusters. Present findings are in conformity with the results observed by Punitha *et al.* (2010) in sunflower, Sharma *et al*. (2013) in carnation and Sheikh and Khanday (2008) in gladiolus.

The comparison of clusters means for the different characters indicated considerable differences between clusters and all the characters (Table 5). The maximum

Table 6 : Contribution of each character to the divergence in marigold

Sr. No.	Characters	No. of times appearing 1st	Per cent contribution
1	Plant height	12	2.76
2	Plant spread	14	3.22
3	Stem diameter	28	6.44
4	Stalk length	40	9.20
5	No. of primary branches plant^{-1}	18	4.14
6	No. of secondary branches plant^{-1}	0	0.00
7	Fresh weight of plant	167	38.37
8	Dry weight of plant	1	0.23
9	Days taken to first flower opening	0	0.00
10	Duration of flowering	6	1.38
11	No. of buds plant^{-1}	9	2.07
12	No. of flowers plant^{-1}	0	0.00
13	Flower diameter	6	1.38
14	Fresh weight of flower	60	13.79
15	Dry weight of flower	37	8.51
16	Flower yield plant^{-1}	37	8.51
Total		**435**	**100**

plant height (82.85) was observed in cluster-II, followed by cluster-IV (78.36), while the minimum plant height was observed in cluster-V (40.42). The genotypes included in cluster-VI showed maximum plant spread (78.07) followed by cluster-III (66.82), whereas, the genotypes included in the cluster-V recorded the minimum plant spread (34.08). Cluster-III had the maximum (2.14), while cluster-V had the lowest (1.13) value for stem diameter. The highest cluster mean value for the stalk length was observed in cluster-IV (19.07) followed by cluster-III (15.65), whereas, the minimum value was recorded in cluster-V (5.44). The maximum number of primary (24.50) and secondary (150.97) braches plant^{-1} was recorded in cluster-VI, while cluster-III and V had the minimum (11.07 and 150.97, respectively) cluster mean value. The maximum fresh and dry weight of plant was recorded in cluster-III (1122.42 and 130.75, respectively) and minimum (199.19 and 23.82, respectively) in cluster-V.

The days taken to first flower opening showed earliest flowering mean performance in cluster-V (45.37) followed by cluster-IV (46.06) and the delayed flowering occurred in cluster-VI (64.60). Duration of flowering illustrated the highest cluster mean value (76.53) in cluster-VI followed by cluster-III (65.30). Number of buds and flowers plant^{-1} exhibited the highest cluster mean values (217.10 and 189.00, respectively) in cluster-VI, whereas, cluster-V showed the lowest cluster mean value (44.49 and 34.13, respectively) for both these characters. The biggest flower diameter (7.87) was observed in cluster-I followed by cluster III (7.25), whereas, the lowest flower diameter (4.01) was recorded in cluster-VI. Fresh and dry weight of flower revealed the highest cluster mean values (15.08 and 1.77, respectively) in cluster-I, whereas, cluster-VI showed the lowest cluster mean value (4.64 and 0.56, respectively) for both these characters. Flower yield plant^{-1} exhibited the highest mean performance (874.37) for cluster-VI followed by cluster-I (812.94), whereas, the lowest mean value (190.89) was in cluster-V. Similar results had also been suggested by Kavitha and Anburani (2009) in marigold, Nimbalkar *et al.* (2006), Bihari *et al*. (2009) and Manivannan *et al.* (2003) in gladiolus

While studying contribution of individual characters towards divergence, among 16 characters (Table 6), fresh weight of plant contributed maximum (38.37%) followed by fresh weight of flower (13.79%), stalk length (9.20%), dry weight of flower (8.51), flower yield plant^{-1} (8.51%) stem diameter (6.44%) and number of primary branches plant^{-1} (4.14%). However, the characters plant height, plant spread, dry weight of plant, duration of flowering, number of buds plant^{-1} and flower diameter exhibited very meagre contribution to the divergence. The variation may be due to the different genotypes studied and the environmental conditions. Since more than 85 per cent contribution to divergence was from fresh weight of plant, fresh weight of flower, stalk length, dry weight of flower, flower yield plant^{-1}, stem diameter and number of primary branches plant^{-1}, necessary attention should be paid to these characters in high yielding genotypes in marigold. Sharma *et al*. (2013) reported contribution of different quantitative characters in creating genetic diversity in carnation varieties and observed maximum contribution for stem length. Similar results were reported by Nimbalkar *et al.* (2006) for number of corms plant^{-1} and weight of corms plant^{-1} in gladiolus.

Keeping in view the above aspects, the genotypes 'Hisar Jaffri-2' from cluster-VI, 'MGH-09-271' and 'MGH-09-276' from cluster- I and 'MGH-148-3-3' from cluster-III, represents deserve to be considered as potent parents for further utilization in marigold improvement programme. Therefore, based on D^2 analysis, it has been understood that characters need to be given more weightage, while selecting parents for improvement (Swaroop, 2010 and Sheikh and Khanday, 2008 in gladiolus). It is increasingly realized that crosses between divergent parents usually produced greater heterotic effects than those between closely related ones (Patil and Apte, 2002). The results obtained indicated more relationship between clustering and ecogeographical origin (Patil and Bhapkar, 1987). Kavitha and Anburani (2009) and Srivastava and Sharma (2013) suggested that genotypes with greater divergence should be involved in hybridization programme.

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