Limits of parental divergence for the occurrence of heterosis through morphological and AFLP marker in chilli (*Capsicum annuum* L.)

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The present study was undertaken to assess the morphological and amplified fragment length polymorphism (AFLP) marker-based genetic diversity, to estimate mid-parent heterosis and to study the possible limits of the parental divergence for the occurrence of heterosis for yield and its contributing traits in chilli hybrids. Five CMS B lines and 30 testers were used for morphological (9 traits) and AFLP (8 primer combinations) marker-based genetic divergence analysis. One hundred fifty hybrids were synthesized through line × tester (5 × 30) mating design and were used to estimate the mid-parent heterosis for nine characters. More than 50% of hybrids showed significant mid-parent heterosis for both green and red fruit yield per plant. The nonlinear regression revealed intermediate parental divergence for the occurrence of higher frequency of heterotic crosses and extreme genetic divergence for occurrence of less heterotic crosses for green and red fruit yield per plant. Thus, the parental divergence should neither be too small nor very large for realizing higher frequencies of heterotic crosses in both green fruit yield per plant and red fruit yield per plant. It is worthwhile to involve parents with intermediate divergence than involving parents with extreme divergence to recover higher frequencies of heterotic hybrids for both green fruit yield per plant and red fruit yield per plant.

Keywords: AFLP, chilli, genetic diversity, heterosis.

CHILLI (*Capsicum annuum* L.) is a leading spice-cum-vegetable crop grown commercially in India, China, Ethiopia, Hungary, Indonesia, Japan, Spain, Mexico and other countries. India is the largest producer of chilli in the world, which is grown in an area of 9.15 m ha with production of 11 lakh tonnes. India accounts for 26% of global production followed by China. Although India is the largest producer, productivity is far less (1.1 tonnes ha^{-1}) compared to the global average productivity (4.0 tonnes ha^{-1}). Therefore, there is strong need to increase the productivity of chilli.

Genetic resources play a pivotal role in its economical utilization and desirable traits improvements. Genetic divergence existing in the population helps in the selection of suitable parents for utilization in chilli crop breeding programmes. Identification and characterization of desirable parental combinations provide the basis for selection in the follow-up breeding process for exploitation of heterosis. The study of genetic diversity and phenotypic variability for diverse morpho-economic traits in the available germplasms is a prelude to potential chilli crop improvement. Molecular markers are used to meet a number of objectives, including genetic diversity analysis and prediction of hybrid performances in different crop species[1]. Currently, several molecular marker techniques are available serving various purposes in several crops. Amplified fragment length polymorphism (AFLP) is one of the well-known molecular marker systems relying on polymerase chain reaction (PCR) technique to estimate genetic diversity. It requires no prior sequence knowledge and can detect large number of genetic loci than restrict fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers. These important features of the AFLP marker prompted us to use it in the genetic diversity analysis. The efficiency of hybrid breeding programme could be increased if the inbred/parental lines could be screened for genetic diversity using molecular markers and superior crosses are accurately predicted prior to field evaluation[2]. Molecular markers are not influenced by environmental factors, and are fast and more efficient than field testing to detect large number of distinct differences between genotypes.

Thus, it is necessary to explore the limits of parental distance that ensures the frequency of higher heterotic hybrids which is one of the most important steps in developing hybrids. However, this is one of the most

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costly and time-consuming steps in hybrid breeding programme as it is necessary to cross all the available parental lines and evaluate all hybrids in extensive yield trials. Development and evaluation of only a limited number of hybrids generated from a relatively fewer number of parents saves substantial resources[3]. Thus, it becomes necessary to identify relatively fewer number of parents that are likely to result in high frequency of heterotic hybrids. The selection of such fewer parents from among available ones is critical. The per se performance of a parent is not always a true indicator of its potential to exploit the hybrid vigour. In several crops, parental genetic diversity per se and parental combining ability have been successfully used to develop higher frequencies of heterotic hybrids. Parents with high general combining ability and a large genetic distance between them are known to produce hybrids with better yield performance[4]. Advances in genome research have generated interest in predicting hybrid performance using molecular markers as indicated by positive association between DNA marker-based genetic distance and heterosis[5,6]. The discovery of cytoplasmic male sterility (CMS) system provided the breakthrough for heterosis breeding in chilli[7–9]. Concerted efforts were made in this direction, to develop and evaluate hybrids using different CMS and fertility restorer lines. However, while taking the programme of hybrid chilli to logical ends, choice of suitable parents through careful and critical evaluation of current material is of paramount importance. This is because per se performance of a parent is not always a true indicator of its potential in hybrid combinations. There are several criteria by which a breeder can choose suitable parents for successful hybridization, of which the two important ones are (i) combining ability of parents and (ii) genetic diversity between the parents. Most often, combining ability has been extensively used by plant breeders to select suitable parents for realizing high frequency of heterotic hybrids. However, genetic diversity of parents is equally important, as found in corn[10]. There is a close association between the magnitude of genetic divergence and heterosis in *Brassica campestris*[11], sunflower[12] and sesame[13]. However, heterosis is not always found to occur when high divergent parents are crossed and lack of association between parental diversity and heterosis[14]. With this background, the present study was designed to elucidate the morphological and molecular marker-based genetic diversity among the parents, heterosis in F_1 crosses and to study possible limits of parental divergence to realize heterotic hybrids for economic traits in chilli.

Material and methods

Genetic diversity analysis

A total of 35 parents (5 CMS B lines and 30 testers) were used to analyse the morphological and AFLP marker-based genetic diversity (Table 1). The CMS A and B lines were received from the Asian Vegetable Research and Development Center (AVRDC), Taiwan. Testers were unrelated and have outstanding agronomic potential. The collected testers were maintained for 10 generations to get homozygous conditions at University of Agricultural Sciences (UAS), Bangalore.

Morphological marker-based genetic diversity

The experiment on genetic diversity was carried out by raising chilli plants of the 30 testers and 5 CMS B lines in kharif 2008 at UAS, Bangalore in randomized complete block design (RCBD). All the recommended package of practices were followed to raise a good crop. Ten plants in each genotype were tagged from each replication and nine characters were recorded, viz. days to 50% flowering, days to first fruit maturity, plant height (cm), fruits per plant, fruit length (cm), fruit width (cm), hundred seed weight (g), green fruit yield per plant (g) and red fruit yield per plant (g). The D^2-statistic was used for assessing the genetic divergence among the parents[15]. The genotypes were grouped into different clusters following Tocher's method[16]. Statistical analysis of the data was carried out using Generes statistical programme for morphological diversity.

AFLP marker-based genetic diversity analysis

Genomic DNA was extracted from young and healthy leaves of 40–50-day-old chilli genotypes with some modifications[17] and AFLP reactions were performed[18] with some modifications. After selective amplification, the PCR products were mixed with loading buffer, denatured and placed on ice. Four microlitres of the mixture was loaded on a polyacrylamide gel. For each primer combination, samples of 35 parents were run on the same gel. After electrophoresis, the gels were fixed and dried[19]. Fragment scoring was performed as present (1) and absent (0) on white luminous light. The AFLP marker-based genetic distance between all possible pairs of male and female lines was calculated using the software NTSYS-pc version 2.02i. The similarity matrix based on the AFLP data was used to construct a dendrogram by employing the unweighted pair-group method with arithmetic means (UPGMA)[20].

Association between morphological and molecular clustering

The test of association was carried out between morphological and molecular clustering to know whether a relationship is present or not. The two nominal variables (morphological and molecular clusters) form a contingency

Table 1. Source/geographical locations of the chilli CMS lines and restorers used as parents of hybrids in the present study

Genotype	Source/geographical locations
Testers	
Aparna	Released variety from HRS, Lamfarm, Guntur district, Andhra Pradesh
LCA 206	Released variety from HRS, Lamfarm, Guntur district, Andhra Pradesh
LCA 271	Elite line of HRS, Lamfarm, Guntur district, Andhra Pradesh
LCA 273	Elite line of HRS, Lamfarm, Guntur district, Andhra Pradesh
LCA 330	Elite line of HRS, Lamfarm, Guntur district, Andhra Pradesh
LAM 333	Released variety from HRS, Lamfarm, Guntur district, Andhra Pradesh
LCA 335	Elite line of HRS, Lamfarm, Guntur district, Andhra Pradesh
LCA 353	Elite line of HRS, Lamfarm, Guntur district, Andhra Pradesh
LCA 960	Released variety from HRS, Lamfarm, Guntur district, Andhra Pradesh
Vangara	Prakasham district, Andhra Pradesh
Arka Suphal	Released variety from IIHR, Bangalore district, Karnataka
Chitarachamba	Released variety for Bangalore district, Karnataka
Byadgi Dabbi	Released variety from RRS, Devihosur, Haveri district, Karnataka
Byadagi Kaddi	Released variety from RRS, Devihosur, Haveri district, Karnataka
D-379	Released variety from UAS Dharwad district, Karnataka
Chickballapur local	Commercial variety from Chikkaballapur district, Karnataka
Gowribidanur local	Commercial variety from Chikkaballapur district, Karnataka
Kunchangi local 1	Collected from Tumkur district, Karnataka
Kunchangi local 2	Collected from Tumkur district, Karnataka
CA 2	Received from AVRDC, Taiwan
CA 6	Received from AVRDC, Taiwan
CA 9	Received from AVRDC, Taiwan
CA 14	Received from AVRDC, Taiwan
PBC 142	Received from AVRDC, Taiwan
Susan's Joy	Received from AVRDC, Taiwan
Pant C-1	Released variety from G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand
Utkal Awa	Released variety from OUAT, Bubaneshvar
Pusa Jwaja	Released variety from IARI, New Dehli
Pusa Sadabahar	Released variety from IARI, New Dehli
Tiwari	Released variety from IARI, New Dehli
CMS lines	
CMS 1B	Received from AVRDC, Taiwan
CMS 2B	Received from AVRDC, Taiwan
CMS 3B	Received from AVRDC, Taiwan
CMS 5B	Received from AVRDC, Taiwan
CMS 8B	Received from AVRDC, Taiwan

table of cells. The contingency table (6 × 7) was prepared based on the common genotypes in both the morphological (6) and molecular (7) clustering. Each of the 35 genotypes was categorized by both morphological and molecular clustering and placed into one of 42 cells. The genotypes in cluster I of the morphological clustering and those that were common in cluster I of the molecular clustering were placed in the first cell of 6 × 7 contingency table. Similarly, 42 cells (6 × 7) were constructed by involving the 6 morphological clusters and 7 molecular clusters. The chi-square test serves both as a goodness of fit test, where the data are categorized along one dimension, and as a test for the more common contingency table, in which categorization is across two or more dimensions. The expected frequencies were computed as $R_i \times C_j/N$, where R_i and C_j represent row and column marginal totals and N is the grand total. The value of the test-statistic was computed according to the formula

$$X^2 = \sum_{i=1}^{r} \sum_{j=1}^{e} \frac{(O_{i,j} - E_{i,j})^2}{E_{i,j}}.$$

Fitting the model of independence reduces the number of degrees of freedom by $p = r + c - 1$. The number of degrees of freedom is equal to the number of cells rc, minus the reduction in degrees of freedom p, which reduces to $(r - 1)(c - 1)$. For the test of independence, also known as the test of homogeneity, a chi-squared probability of less than or equal to 0.05 (or the chi-squared statistic being at or larger than the 0.05 critical point) is rejecting the null hypothesis, i.e. morphological clustering is independent of the molecular clustering. The mantel test for molecular (A) and morphological (B) distance matrices was also carried out and p-value calculated from 10,000 permutations.

Line × tester (heterosis) analysis

The 30 testers were crossed manually with the 5 CMS A lines in line × tester mating design. The resulting 150 single cross hybrids were used for estimation of heterosis. The experiment for heterosis was carried out by raising chilli plants of the 30 male lines, 5 female lines and 150 hybrids in kharif 2008 at UAS, Bangalore in RCBD. Heterosis over mid-parent (average heterosis) was computed by taking the mean values of hybrids and parents using the formula

$$\text{Mid-parent heterosis} = \frac{\bar{F}_1 - \overline{MP}}{\overline{MP}} \times 100,$$

where $\bar{F}_1$ the mean value of F_1 hybrid and $\overline{MP}$ the mean value of both the parents. Mid-parent heterosis was calculated[21] and statistical analysis of the data was carried out using the statistical program Windowstat 8.0.

Parental divergence for the occurrence of heterosis

The linear regression was estimated between the pairwise total genetic divergence of the parents and mid-parent heterosis of the F_1 hybrids[22]. The coefficient of determent (R^2) of the linear regression of the relationship between genetic divergence of the parents and mid-parent heterosis of the F_1 hybrids was also estimated. The nonlinear regression of the total genetic divergence of the parents and mid-parent heterosis of the F_1 hybrids was estimated through Gaussian model using the SAS package.

Results and discussion

Genetic diversity analysis

Morphological marker-based genetic diversity: All 35 chilli genotypes were grouped into 6 clusters based on 9 morphological traits D^2 values. Cluster VI was the largest comprising 19 genotypes followed by cluster I with 8 genotypes, whereas clusters II–V had 2 genotypes each. Most of the chilli genotypes were collected from Guntur and all the CMS B lines grouped together in cluster VI (Table 2). The genotypes included were found to be diverse in the nature with maximum inter-cluster distance (D^2) of 30,412.29 between clusters I and VI, and the minimum D^2 value between clusters III and IV (3,643.71). All the clusters showed more intra-cluster distances and constituted more than one genotype. Intra-cluster distance was the highest in the cluster I (29,621.86) followed by cluster VI (23,296.37), cluster V (2,324.31), cluster IV (2,313.16), cluster III (1,212.97) and the lowest intra cluster distance was found in cluster II (596.40). The genotypes CMS 1B, CMS 2B, CMS 3B, CMS 5B and CMS 8B which are from the same geographical region fell in cluster VI. Gowribidanur local and Chickballapur local also fell in cluster VI. These two genotypes are from the same geographical region. The genotypes, viz. LCA 206, LCA 273, LCA 330, LAM 333, LCA 335 and LCA 960 which are from the same geographical region also fell into a same cluster (cluster VI). Thus, genotypes which share genetic background by virtue of their development from similar pedigree or because of their trait similarity driven by human or natural selection pressure in a particular geographical region. Similarly, Byadagi Kaddi and Byadagi Dabbi which are from the same geographical region fell in cluster I. Therefore, the present results support the findings in rice[23], cowpea[24] and chilli[25,26].

AFLP marker-based genetic diversity: A total of eight AFLP primer combinations (with three selective nucleotides) were used to amplify genomic DNA of 35 parental lines. The AFLP marker being dominant, locus was considered to be polymorphic due to presence or absence of the band. The 8 AFLP primer combinations generated a total of 335 amplicons, of which 316 were polymorphic with an average of 41.87 bands (Table 3). Among eight AFLP primer combinations, *Eco*RI + AGC and *Mse*I + GCT primers showed the highest percentage of polymorphic information content (PIC) of 94. Hence, these AFLP primer combinations are useful for analysis of divergence in chilli. Thirty-five chilli genotypes were grouped into seven clusters by Jaccard's similarity coefficient (Figure 1). Cluster IV had the largest number of 15 genotypes and cluster VII included all 5 CMS lines (CMS 1B, CMS 2B, CMS 3B, CMS 5B and CMS 8B). All CMS B lines were grouped into one cluster, which is confirmed with known geographical location. Among the testers, chilli genotypes collected from Taiwan were grouped into same cluster, except Susan's joy and PBC 142, which grouped with genotypes collected from Karnataka in two different clusters. Chilli genotypes collected from Guntur district, Andhra Pradesh were grouped in the same cluster, except Aparna genotype which was grouped with Arka Suphal genotype collected from Karnataka. This indicates that the grouping of genotypes collected from different locations in one group may be possible due to cross-fertilization at the location[27]. There are factors other than regional boundaries and taxonomic characters which are also responsible for divergence. The pairwise AFLP maker-based genetic distances between CMS B lines and testers ranged from 0.08 to 0.22. The two parental lines CMS 3B and Kunchangi local 2 were 22% different in terms of the portion of the genome surveyed by eight AFLP primer combinations. The parental lines CMS 3B and CA 6 were 8% different in terms of the portion of the genome surveyed by eight AFLP primer combinations. Similarily, seven genotypes were analysed using 53 RAPD markers and it was found that the pairwise RAPD maker-based genetic distances between parents ranged from 0.16 to 0.87 (ref. 28).

Table 2. Intra- (bold) and inter-cluster divergence (D^2 values) among six clusters in chilli

Clusters	I	II	III	IV	V	VI	Mean D^2	Genotypes included in the clusters
I (8)	**29621.86 (172.11)**	16587.21 (128.79)	21895.67 (147.97)	17716.72 (133.10)	22657.00 (150.52)	30412.29 (174.39)	23148.46	Aparana, Arka Suphal, Byadgi dabbi, Byadgi kaddi, CA 2, CA 6, PBC 142 and Pusa Jwala
II (2)		**596.40 (24.42)**	7052.52 (83.98)	4857.41 (69.70)	15237.09 (123.44)	23106.92 (152.01)	11239.59	Kunchangi local 1 and Vangara
III (2)			**1212.97 (34.83)**	3643.71 (60.36)	10915.90 (104.48)	28489.90 (168.79)	12201.78	CA 14 and LCA 271
IV (2)				**2313.16 (48.10)**	5631.87 (75.05)	22719.08 (150.73)	9480.325	LCA 353 and Susan's Joy
V (2)					**2324.31 (48.21)**	24937.84 (157.92)	13617.34	Tiwari and Utkal Awa
VI (19)						**23296.37 (152.63)**	25493.73	CA 9, Chickaballapur local, Chitara Chamba, D-379, Gowribidanur local, Kunchangi local 2, LCA 206, LCA 273, LCA 330, LAM 333, LCA 335, LCA 960, Pant C-1, Pusa Sadabahar, CMS 1B, CMA 2B, CMS 3B, CMS 5B and CMS 8B

Table 3. Selective primer combinations, number of polymorphic amplicons and polymorphic information content in amplified fragment length polymorphism analysis of parents in chilli

*Eco*RI primer selective nucleotides	*Mse*I primer selective nucleotides	Total bands obtained	Polymorphic bands obtained	Per cent polymorphic bands	Per cent PIC*
+AAT	+GTG	35	34	97.14	89.66
+AAT	+GCG	40	34	85.00	75.76
+AAT	+GCT	40	37	92.50	79.28
+AAT	+GAG	60	60	100.00	94.06
+AAT	+GCA	26	20	76.92	93.30
+AGC	+GCC	33	33	100.00	92.38
+AGC	+GCG	32	31	96.88	90.48
+AGC	+GCT	69	67	97.10	94.96
Total		335	316	94.32	

*PIC, Polymorphic information content.

Association between morphological and molecular clustering

The association of morphological and molecular clustering was estimated by chi-square test through 6×7 contingency table. The calculated chi-square value was 40.39 with a probability of 0.097 and was compared with the table chi-square value (43.77 at probability of 0.05 and 50.89 at probability of 0.01) at 30 error d*f*. The chi-square value of the test was non-significant (calculated chi square value was more than the table chi-square value) and the probability was 0.097, which is greater than 0.05. Therefore, the null hypothesis was accepted, i.e. the morphological and molecular clustering were independent. The association between molecular distance matrix (A) and morphological distance matrix (B) was also estimated using Mantel test (Figure 2). We noticed that the estimated *p*-value (0.35) was greater than the significance level $\alpha = 0.05$ hence, the both matrices were not correlated. The *p*-value was calculated using the distribution of *r*(AB) estimated from 10,000 permutations (Figure 3). The Mantel *r* statistic (0.041) indicates that there is relatively weak correlation between molecular distance matrix (A) and morphological distance matrix (B). Hence, both genetic distance matrices were combined to get the total genetic distance of parents for exploring the limits of parental divergence to get the more frequency of heterosis.

Heterosis analysis

Heterosis over mid-parent (average heterosis) was computed by taking the mean values of hybrids and parents for nine characters. There was significant and wide range of mid-parent heterosis for all nine characters. Among the

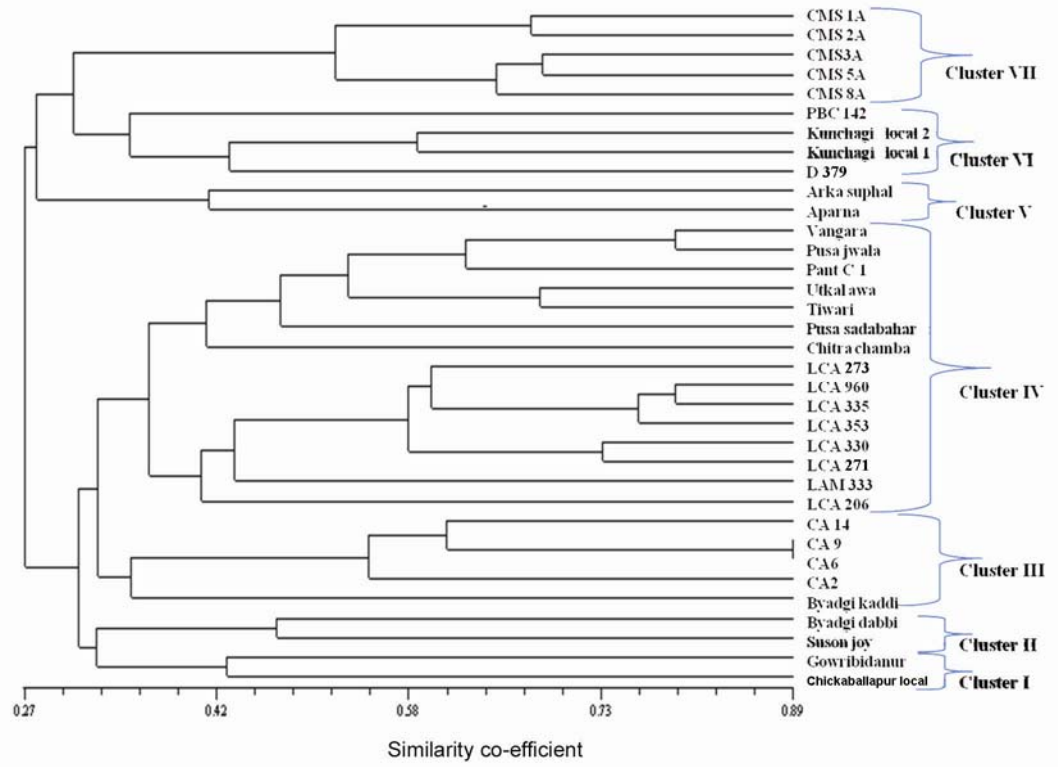

Figure 1. UPGMA dendogram of 35 chilli genotypes constructed based on AFLP marker data generated from eight selective primer combinations.

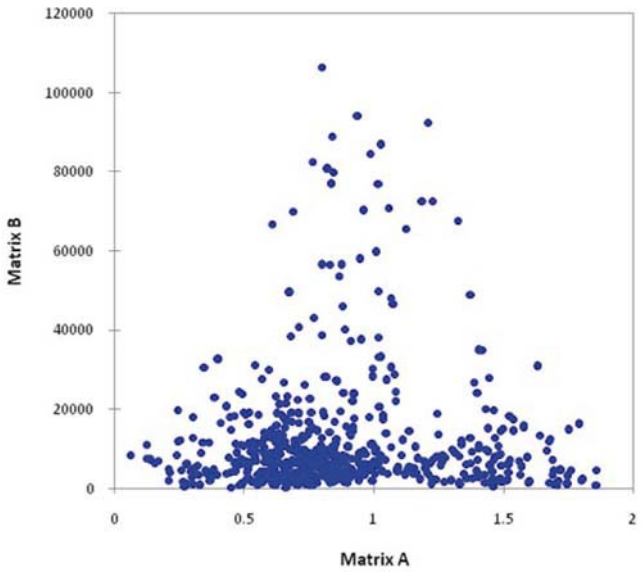

Figure 2. Scatter plot of molecular distance matrix (A) and morphological distance matrix (B) of 35 parents in chilli.

150 hybrids, 82 and 43 registered positive and negative significant mid-parent heterosis respectively, for green fruit yield per plant (Table 4). Majority of crosses, 142 out of the 150, exhibited significant mid-parent heterosis, of which 86 were positive and 56 were negative for red fruit yield per plant. More than 50% of hybrids showed significant mid-parent heterosis for both green and red fruit yield per plant. Hence, there is great potential for development of high yielding hybrids. The results indicated the variation on fruit yield and other characters in hybrids. Longest and widest fruits were observed in the hybrids compared to parental genotypes and the highest fruit width and fruit numbers per plant were also noticed in chilli hybrid[29]. Among the hybrids, some manifested higher positive heterosis and others exhibited low positive or negative heterosis. This is mainly due to the varying extent of genetic diversity between the parents of different crosses for fruit characters. For the most economically important character, green fruit yield per plant about 50% of the crosses were identified to be the desirable specific combinations. Among those crosses, CMS 8A × Pusa Sadabahar, CMS 8A × Tiwari, CMS 8A × LCA 273, CMS 2A × LAM 333, CMS 8A × Arka Suphal, CMS 3A × CA 9 and CMS 8A × Vangara exhibited the highest significant positive mid-parent heterosis. It is interesting to note that five out of the seven above-mentioned crosses involve CMS 8A as one of the parents. Expression of heterosis in F_1 hybrids of *Capsicum* species depends upon the involvement of the parents[30]. The observed positive heterosis for fruit number and fruit yield per plant in this study may be a breeding advantage to get higher yield. The highest amount of heterosis manifested in F_1 hybrids for the fruit yield indicated the prevalence of dominant gene action.

Table 4. Range and number of hybrids showing significant mid-parent heterosis among 150 hybrids and correlation between morphological and AFLP marker-based parental diversity and mid-parent heterosis for nine characters

Characters	Range	Number of hybrids showing significance over mid-parent heterosis			Correlation between total genetic divergence of parents and mid-parent heterosis
		Positive	Negative	Total	
Days to 50% flowering	78.5–88.5	38	21	59	0.02
Days to first fruit maturity	108.5–133.5	38	63	101	0.16*
Plant height (cm)	49.0–136.5	83	54	137	0.03
Fruits per plant	12–249.45	78	52	130	0.15*
Fruit length (cm)	4.5–16.75	98	28	126	–0.03
Fruit width (cm)	0.75–2.0	21	96	117	–0.13*
100 seed weight (g)	0.37–1.11	88	57	145	0.24**
Green fruit yield per plant (g)	105.1–796.5	86	56	142	–0.08
Red fruit yield per plant (g)	53.15–867.65	82	43	125	–0.01

*Significant at $p = 0.05$; **Significant at $p = 0.01$.

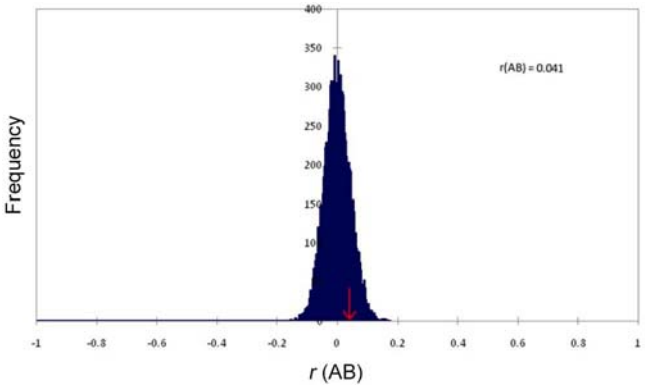

Figure 3. Histogram of Pearson correlation of molecular distance matrix and morphological distance matrix (rAB) with their frequecncy.

Accumulation of favourable dominant alleles and masking of deleterious effects of recessive alleles by their dominant alleles in the F_1 hybrids[31] and superiority of heterozygotes at some of the loci to both the relevant homozygotes were indicated in the heterosis[32,33]. Similar results were noticed for crosses with high and positive significant mid-parent heterosis for green fruit yield per plant in chilli[34–36].

Genetic divergence for the occurrence of heterosis

In the present study, pairwise genetic divergence of female to male parents was measured using nine morphometric traits and eight primer combinations of AFLP markers. Both genetic distance matrices were combined to get the total genetic distance of parents for exploring the limits of parental divergence to get the more frequency of heterosis. The estimated linear regression was mid-parent heterosis of hybrids $(H) = 30.32 + 0.0416 \times$ GD (total genetic divergence of the parents) for red fruit yield per plant and $H = 43.95 + 0.0058 \times$ GD for green fruit yield per plant. The correlation of the determinant was very low, i.e. 0.0012 (red fruit yield per plant) and 0.0042 (green fruit yield per plant). Hence, nonlinear regression was estimated and $H = 397.73 \times \exp(-(\text{GD} - 238.53)^2/(2 \times 75)^2)$ for red fruit yield per plant and $H = 256.83 \times \exp(-(\text{GD} - 171.89)^2/(2 \times 35)^2)$ for green fruit yield per plant. These results suggest that higher frequency of hererotic crosses was in the intermediate parental divergence of the parents for both green fruit yield and red fruit yield per plant (Figure 4 *a* and *b*) respectively. It is increasingly being realized that crosses between divergent parents usually produce higher heterosis than those between closely related ones. However, when divergent parents are crossed, heterosis was not always found to occur. It has also been demonstrated that genetic diversity is necessary for significant levels of heterosis, but is not sufficient to guarantee it[37]. Establishing the possible limits to parental divergence within which there are reasonably high chances for the occurrence of heterosis, therefore, is an important issue that needs to be addressed. The highest number of heterotic crosses fell under intermediate divergence of parents compared to the extreme divergence of parents, suggesting the crosses derived from parents with intermediate genetic diversity between them were more often heterotic than those derived from parents with extreme levels of genetic divergence between them for green and red fruit yield per plant. Thus, the parental divergence should neither be too small nor very large for realizing higher frequencies of heterotic crosses both in green fruit yield and red fruit yield per plant. The concept that there are limits to parental divergence for optimum expression of heterosis was also evident by past studies on crosses between divergent geographic races in chilli[38,39], maize[40], Triticale[41], groundnut[42] and sunflower[43]. Thus, the present study reveals the existence of limits to parental divergence for the occurrence of heterosis. It is worthwhile to involve parents with intermediate divergence than involving those with extreme divergence to recover higher frequencies of heterotic hybrids for both green fruit yield per plant and red fruit yield per plant.

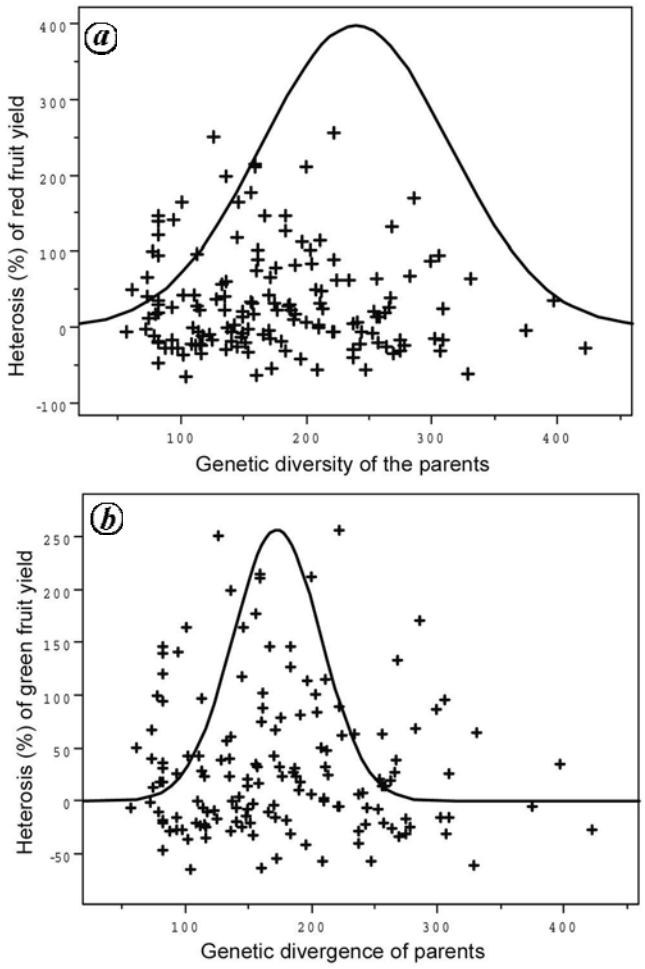

Figure 4. Relationship between parental total (morphological + AFLP-based) genetic divergence and mid-parent heterosis for (***a***) red fruit yield and (***b***) green fruit yield in chilli.

Conclusions

The crosses, viz. CMS 2A × LAM 333, CMS 3A × CA 9, CMS 3A × Pusa Jwala, CMS 3A × Arka Suphal, CMS 8A × Arka Suphal, CMS 8A × LCA 330, CMS 8A × Utkal Awa, CMS 8A × LCA 271, CMS 8A × Pusa Sadabahar, CMS 8A × Tiwari, CMS 8A × LCA 273 and CMS 8A × Vangara exhibited the highest significant positive mid-parent heterosis for fruit yield. Hence there is a great potential for development of good yielding hybrids. These crosses could be used for commercial exploitation. Studies of possible limits to total parental divergence for the occurrence of heterosis revealed the existence of limits to parental divergence. The nonlinear regression revealed that intermediate parental divergence for the occurrence of higher frequency of heterotic crosses and extreme genetic divergence for occurrence of less heterotic crosses. Parents with intermediate divergence between them than those with extreme divergence were useful to recover heterotic hybrids at a higher frequency for both green fruit yield and red fruit yield per plant.

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