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Full Length Research Paper

Relationship between morphological and amplified fragment length polymorphism (AFLP) marker based genetic distance with heterosis in hot pepper (*Capsicum annuum* L.)

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Identification of potential parents that produce the hybrids with superior yield is the most important step in developing hybrids to save the substantial resources. The present study was carried out to assess the morphological and amplified fragment length polymorphism (AFLP) marker based genetic diversity, to estimate mid-parent heterosis and to correlate the estimated parental genetic diversity with heterosis chilli. Five CMS B - lines and 30 testers were used for morphological and AFLP marker genetic divergence analysis. 150 hybrids were synthesized through Line × Tester (5 × 30) mating design and were used to estimate the mid-parent heterosis for nine characters at two locations. 35 parents were examined for nine morphological traits and were grouped in to six clusters. These parents were also examined for eight AFLP primers combinations and were grouped into seven clusters. More than 50% of hybrids showed significant mid-parent heterosis for both green and red fruit yield plant^{-1}. Hence, there is a much potential for development of good yielding hybrids. The positive significant correlation was found between morphological and AFLP marker distance of the parents with heterosis for plant height (r = 0.17 and 0.38), green fruit yield plant^{-1} (r = 0.19 and 0.25) and red fruit yield plant^{-1} (r = 0.20 and 0.34); however, the correlation coefficients were not strong in these traits. Genetic distance between parents was not strong enough to predict the performance of the hybrids and proved to be of no predictive value.

Key words: Correlation, molecular markers, genetic diversity, chilli.

INTRODUCTION

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

Genetic resources play a pivotal role in its economical

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Abbreviations: UPGMA, Unweighted pair-group method with arithmetic means; **AFLP,** amplified fragment length polymorphism; **UAS,** University of Agricultural Sciences; **IIHR,** Indian Institute of Horticultural Sciences.

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. Since, the study of genetic diversity and phenotypic variability for diverse morphoeconomic traits in the available germplasms is a prelude to potential chilli crop improvement. Molecular markers are use to meet the number of objectives including genetic diversity analysis and prediction of hybrid performances in different crop species (Melchinger 1999). 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 the 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 repeats (SSR) markers. These important features of the AFLP marker made us to use 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 (Melchinger et al., 1991). Molecular markers are not influenced by environmental factors and are fast and more efficient than field testing to detect large numbers of distinct differences between genotypes (Melchinger, 1999). However, one should not overlook the importance of field testing to identify phenotypically desirable hybrid combinations.

Thus, it is necessary to identify that the parental combinations that produce hybrids of superior yield is the most important step in developing hybrids. However, this is one of the most 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 (Bernardo, 1992).

Thus, it becomes necessary to identify relatively fewer numbers of parents that are likely to result the high frequency of heterotic hybrids. The selection of such fewer parents from among available ones is critical. The *per se* performance of 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 has 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 (Dier et al., 1996).

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 (Betran et al., 2003; Legesse et al., 2008; Krystkowiak et al., 2009). Thus, keeping these points in view, the present study was conducted with the following objectives: 1) to assess the genetic diversity of parents based on morphological and AFLP markers; 2) to estimate the mid-parent heterosis and 3) to correlate the estimated parental genetic distance with mid-parent heterosis in chilli.

MATERIALS AND METHODS

Genetic diversity analysis

A total of 35 parents (five CMS B - lines and 30 testers) were used to analyze 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 previously maintained for many generations 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 five CMS B - lines at UAS, Bangalore and Indian Institute of Horticultural Sciences (IIHR) Bangalore during, 2008 in randomized complete block design (RCBD). All the recommended package of practices was followed to raise a good crop. 10 plants in each genotype were tagged from each replication and recorded nine characters viz., days to 50%flowering, days to first fruit maturity, plant height (cm), fruits plant^{-1}, fruit length (cm), fruit width (cm), 100 seed weight (g), green fruit yield plant^{-1} (g) and red fruit yield plant^{-1} (g) in each location. Homogeneity of error variance across the two locations, UAS Bangalore and IIHR Bangalore during 2008, was tested by the F-test (Gomez and Gomez, 1983) and none of the error mean squares was significant for any of the traits. Combined analyses of variances for treatments (parents) across the two locations were performed to determine treatments × locations interaction for each trait using the General Linear Model (GLM) procedure of statistical analysis system (SAS). Significant levels were determined as suggested by McIntosh (1983) for combined analysis. Due to no significant difference among treatments × locations interaction (Table 2a), data of the UAS Bangalore and IIHR Bangalore were combined for genetic diversity analysis. Mahalanobis (1936) D^2-statistic was used for assessing the genetic divergence among the parents. The square root of D^2 provided general distance between the two genotypes. The D^2 values were arranged in a matrix form. The genotypes were grouped into different clusters following Tocher's method as described by Rao (1952). Statistical analysis of the data was carried out by using statistical programs Generes for morphological diversity analysis.

AFLP marker-based genetic diversity analysis

Genomic DNA was extracted from young and healthy leaves of 40 to 50 days old chilli genotypes as per the protocol of Prince et al. (1997) with some modifications. The AFLP reactions were

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

S/N	Genotype	Source/geographical location
Testers		
1	Aparna	Released variety from HRS, Lamfarm, Guntur district, Andra Pradesh
2	LCA 206	Released variety from HRS, Lamfarm, Guntur district, Andra Pradesh
3	LCA 271	Elite line of HRS, Lamfarm, Guntur district, Andra Pradesh
4	LCA 273	Elite line of HRS, Lamfarm, Guntur district, Andra Pradesh
5	LCA 330	Elite line of HRS, Lamfarm, Guntur district, Andra Pradesh
6	LAM 333	Released variety from HRS, Lamfarm, Guntur district, Andra Pradesh
7	LCA 335	Elite line of HRS, Lamfarm, Guntur district, Andra Pradesh
8	LCA 353	Elite line of HRS, Lamfarm, Guntur district, Andra Pradesh
9	LCA 960	Released variety from HRS, Lamfarm, Guntur district, Andra Pradesh
10	Vangara	Prakasham district, Andra Pradesh
11	Arka Suphal	Released variety from IIHR, Bangalore, district – Karnataka
12	Chitarachamba	Released variety for Bangalore district, Karnataka
13	Byadgi Dabbi	Released variety from RRS, Devihosur, Haveri district, Karnataka
14	Byadagi Kaddi	Released variety from RRS, Devihosur, Haveri district, Karnataka
15	D-379	Released variety from UAS Dharwad district, Karnataka
16	Chickballapur local	Commercial variety from Chikkaballapur district, Karnataka
17	Gowribidanur local	Commercial variety from Chikkaballapur district, Karnataka
18	Kunchanggi local 1	Collected from Tumkur district, Karnataka
19	Kunchanggi local 2	Collected from Tumkur district, Karnataka
20	CA 2	Received from AVRDC, Taiwan
21	CA 6	Received from AVRDC, Taiwan
22	CA 9	Received from AVRDC, Taiwan
23	CA 14	Received from AVRDC, Taiwan
24	PBC 142	Received from AVRDC, Taiwan
25	Susan's Joy	Received from AVRDC, Taiwan
26	Pant C-1	Released variety from GB Pant Agril. University, Uttar Pradesh
27	Utkal Awa	Released variety from OUAT, Bubaneshvar- Orissa
28	Pusa Jwaja	Released variety from IARI, New Dehli
29	Pusa Sadabahar	Released variety from IARI, New Dehli
30	Tiwari	Released variety from IARI, New Dehli
CMS Lines		
1	CMS 1B	Received from AVRDC, Taiwan
2	CMS 2B	Received from AVRDC, Taiwan
3	CMS 3B	Received from AVRDC, Taiwan
4	CMS 5B	Received from AVRDC, Taiwan
5	CMS 8B	Received from AVRDC, Taiwan

performed according to the protocol of Vos et al. (1995) with some modifications. After selective amplification, the PCR products were mixed with loading buffer, denatured and placed on ice. 4 μl of the mixture were loaded on a polyacrylamide gel. For each primer combinations, samples of 35 parents were run on the same gel. After electrophoresis, gels were fixed (Benbouza et al., 2006) and dried. 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 were calculated by using the software NTSYS-pc version 2.02i. The similarity matrix (Jaccard, 1908) based on the AFLP data was used to construct a dendrogram by employing the unweighted pair-group method with arithmetic means (UPGMA).

Line × Tester (heterosis) analysis

The 30 testers were crossed manually with the five CMS A - lines in Line × Tester mating design, resulted 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, five female lines and 150 hybrids at UAS, Bangalore and IIHR Bangalore during, 2008 in RCBD. All the recommended package of practices was followed to raise a healthy crop. 10 plants in each genotype

Table 2a. Combined analysis of variance for nine traits of 35 (5 Lines and 30 Testers) chilli genotypes at UAS Banlaore and IIHR Bangalore during 2008.

Source of variance	DF	50% flowering	Days to first fruit maturity	Plant height (cm)	Fruits plant^{-1}	Fruit length (cm)	Fruit width (cm)	100 seed weight (g)	Green fruit yield plant^{-1} (g)	Red fruit yield plant^{-1} (g)
Locations	1	0.45	0.57	239.20	93.35	0.41	0.012	0.0026	5575.52	1315.19
Blocks (locations)	2	17.85	118.86	36.00	1177.86	0.01	0.001	0.0173	252.73	95.29
Genotypes	34	23.29**	72.44**	803.87**	2475.82**	15.47**	0.168**	0.1125**	15736.98**	16825.28**
Genotypes x locations	34	0.05^{ns}	0.01^{ns}	32.06^{ns}	0.28^{ns}	0.24^{ns}	0.008^{ns}	0.0002^{ns}	69.87^{ns}	100.98^{ns}
Error	68	4.01	20.61	104.01	759.33	3.54	0.069	0.0265	3892.43	4073.36

^{ns}Non significant; *significant at $P < 0.05$ or **significant at $P < 0.01$.

Table 2b. Combined analysis of variance for nine traits of 185 (5 Lines, 30 Testers and 150 Crosses) chilli genotypes at UAS Banlaore and IIHR Bangalore during 2008.

Source of variance	DF	50% flowering	Days to first fruit maturity	Plant height (cm)	Fruits plant^{-1}	Fruit length (cm)	Fruit width (cm)	100 seed weight (g)	Green fruit yield plant^{-1} (g)	Red fruit yield plant^{-1} (g)
Locations	1	9.53^{ns}	4.54^{ns}	422.27^{ns}	61.70^{ns}	1.06^{ns}	0.0006^{ns}	0.0099^{ns}	1653.85^{ns}	5788.72^{ns}
Blocks (Locations)	2	22.83	319.18	664.05	9400.62	4.53	0.0071	0.0386	2461.67	1163.46
Genotypes	184	20.61**	72.02**	1016.50**	3961.19**	14.15**	0.106**	0.065**	27033.00**	22183.43**
Genotypes x locations	184	0.19^{ns}	0.14^{ns}	0.99^{ns}	32.73^{ns}	0.04^{ns}	0.0030^{ns}	0.0004^{ns}	209.16^{ns}	405.05^{ns}
Error	368	3.52	10.51	139.89	1037.98	2.11	0.0289	0.021	9712.62	5644.49

^{ns}Non significant; *significant at $P < 0.05$ or **significant at $P < 0.01$.

were tagged from each replication and recorded nine characters viz., days to 50% flowering, days to first fruit maturity, plant height (cm), fruits plant^{-1}, fruit length (cm), fruit width (cm), 100 seed weight (g), green fruit yield plant^{-1} (g) and red fruit yield plant^{-1} (g) in each location. Homogeneity of error variance across the two locations, UAS Bangalore and IIHR Bangalore, was tested by the F-test (Gomez and Gomez, 1983) and none of the error mean squares was significant for any of the traits. Combined analyses of variances for treatments (150 crosses, five lines and 30 testers) across the two locations were performed to determine treatments × locations interaction for each trait using the GLM procedure of SAS. Significant levels were determined as suggested by McIntosh (1983) for combined analysis. Due to no significant difference among treatments × locations interaction (Table 2b), data of the UAS Bangalore and IIHR Bangalore were combined for heterosis analysis. Heterosis over mid-parent (average heterosis) was computed by taking the mean values of hybrids and parents as per the method suggested by Fonesca and Patterson (1968).

Statistical analysis of the data was carried out using statistical program Windowstat 8.0.

Association of genetic divergence with heterosis

Simple correlation coefficients between morphological and AFLP marker-based parental distance with mid-parent heterosis of hybrids were computed for all the traits as per the method proposed by Panse and Sukhatme (1967). Statistical analysis of the data was carried out using statistical programs SPAR 2.0.

RESULTS

Genetic diversity analysis

The 35 chilli genotypes were grouped into six clusters and inter and intra cluster D and D^2 values are shown in the Table 3. The genotypes were found to be very diverse in the nature as they have shown maximum inter cluster distance (D^2) of 30412.29 between the cluster I and VI, the minimum D^2 values was between the clusters III and IV (3643.71). All the clusters showed more intra cluster distances and constituted more than one genotype. The highest intra cluster distance noticed in cluster I (29621.86) followed by cluster

Table 3. Intra (bold) and inter cluster divergence (D^2 values) among six clusters in chilli.

Cluster	I	II	III	IV	V	VI	Mean D^2	Genotypes included in the cluster
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, Chickabalapur 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, CMS3B, CMS 5B and CMS 8B

VI (23296.37), V (2324.31), cluster IV (2313.16), cluster III (1212.97) and the lowest intra cluster distance was expressed in the cluster II (596.40). Maximum number of genotypes in the cluster VI with 19 genotypes followed by cluster I with eight genotypes (Table 3). The genotypes CMS 1B, CMS 2B, CMS 3B, CMS 5B and CMS 8B fell in the cluster VI and LCA 206, LCA 273, LCA 330, LAM 333, LCA 335 and LCA 960 which are from same geographical region also in same cluster (cluster VI).

35 genotypes were grouped into seven clusters based on eight AFLP primer combinations. The eight AFLP primer combinations (with three selective nucleotides) were used to amplify genomic DNA of 35 parental lines. The eight AFLP primer combinations generated a total of 335 amplicons, out of which 316 were polymorphic with an average of 41.87 bands (Table 4).

The UPGMA based dendogram was obtained from the binary data deduced from the DNA profiles of the parents analyzed from eight AFLP primer combinations. 35 chilli genotypes were grouped in to seven clustered by Jaccard's similarity coefficient (Figure 1). The cluster IV had the largest number of 15 genotypes and the cluster VII included all five CMS lines (CMS 1B, CMS 2B, CMS 3B, CMS 5B and CMS 8B). Among testers, chilli genotypes of those collected from Taiwan were grouped in same cluster except Susan's joy and PBC 142 which were grouped with genotypes collected from the Karnataka in two different clusters. Chilli genotypes of those collected from the Guntur district of Andra Pradesh were grouped in the same cluster except Aparna genotype which was grouped with Arka Suphal genotype collected from Karnataka. The longest AFLP marker-based genetic distance of 0.22 was noticed between CMS 3 B and Kunchangi local 2 followed by CMS 5B and Kunchangi local 1(0.21), and CMS 5B and Kunchangi local 2 (0.21). On the other hand, the closest genetic distance of 0.08 corresponded to CMS 3B and CA6, and CMS 3B and CA14.

Heterosis analysis

Heterosis over mid-parent (average heterosis) was computed by taking the mean values of

Table 4. Selective primer combinations, number of polymorphic amplicons and polymorphic information content in AFLP analysis of parents in chilli.

S/N	*Eco* RI primer selective nucleotides	*Mse* I primer selective nucleotides	Total bands obtained	Polymorphic bands obtained	% Polymorphic bands	% PIC*
1	+AAT	+GTG	35	34	97.14	89.66
2	+AAT	+GCG	40	34	85.00	75.76
3	+AAT	+GCT	40	37	92.50	79.28
4	+AAT	+GAG	60	60	100.00	94.06
5	+AAT	+GCA	26	20	76.92	93.30
6	+AGC	+GCC	33	33	100.00	92.38
7	+AGC	+GCG	32	31	96.88	90.48
8	+AGC	+GCT	69	67	97.10	94.96
	Total		335	316	94.32	

*PIC, Polymorphic information content.

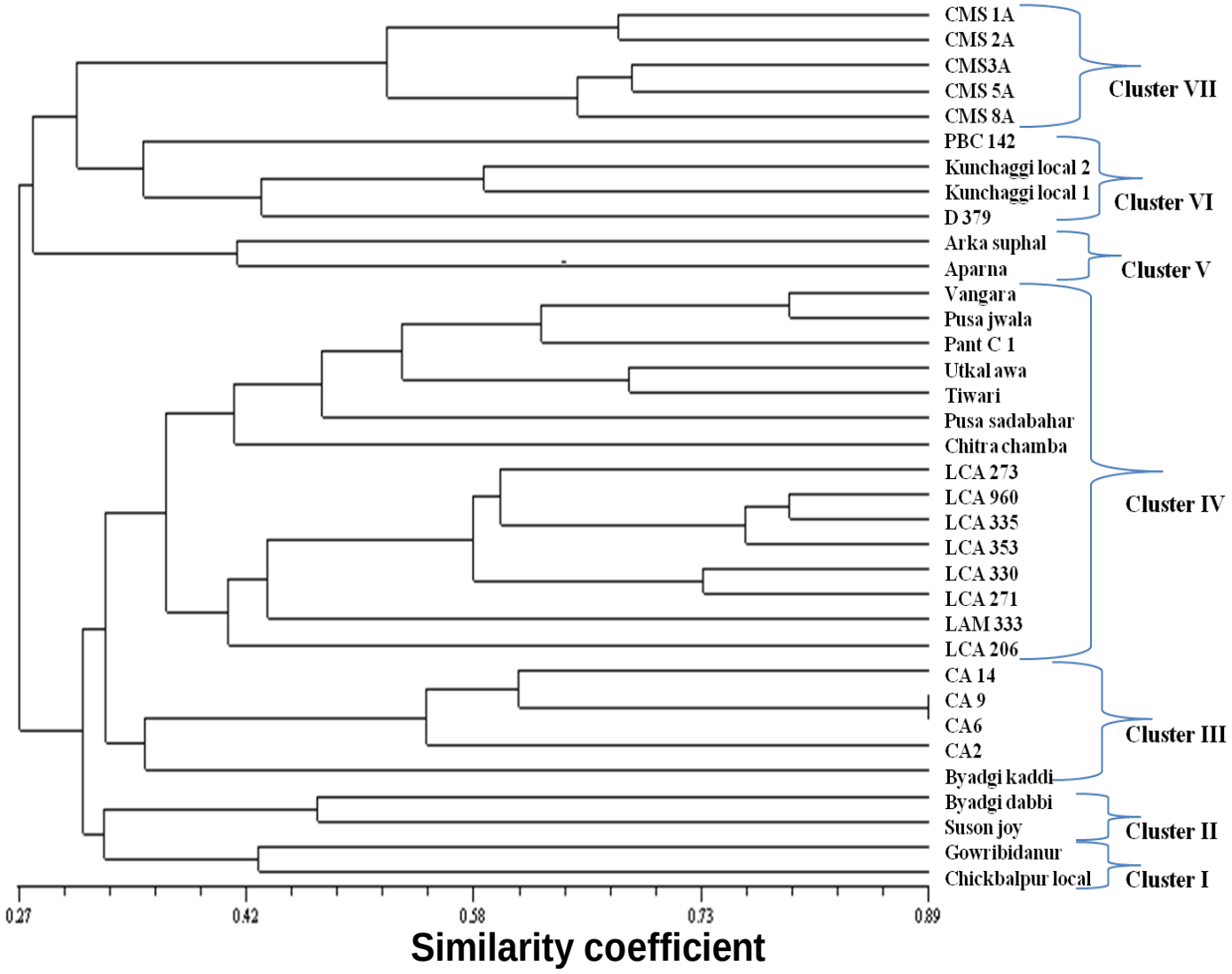

Figure 1. UPGMA dendogram of 35 chilli genotypes constructed based on AFLP marker data generated from 8 selective primer combinations.

Table 5. Range and number of hybrids that showed significant mid-parent heterosis of 150 hybrids and correlation between morphological and AFLP marker-based parental diversity and mid-parent heterosis for 9 characters.

Character	Range mean of performance of hybrid	Range (%) mid-parent heterosis	Number of hybrids showed significant over mid-parent heterosis			Correlation between AFLP marker-based parental diversity and mid-parent heterosis	Correlation between morpho-metric traits based parental diversity and mid-parent heterosis
			Positive	Negative	Total		
Days to 50% flowering	78.5 to 88.5	-6.19 to 8.75	38	21	59	0.02	- 0.04
Days to first fruit maturity	108.5 to 133.5	-13.56 to 9.96	38	63	101	0.14*	0.15*
Plant height (cm)	49.0 to 136.5	-38.88 to 90.18	83	54	137	0.38**	0.17*
Fruits plant^{-1}	12 to 249.45	-77.89 to 86.12	78	52	130	0.13*	0.13*
Fruit length (cm)	4.5 to 16.75	-31.03 to 76.17	98	28	126	0.13*	0.12*
Fruit width (cm)	0.75 to 2.0	-32.08 to 90.48	21	96	117	-0.01	-0.12*
100 seed weight (g)	0.37 to 1.11	-51.1 to 90.67	88	57	145	-0.03	- 0.23**
Green fruit yield plant^{-1} (g)	105.1 to 796.5	-71.62 to 70.72	86	56	142	0.25**	0.19**
Red fruit yield plant^{-1} (g)	53.15 to 867.65	-49.10 to 56.83	82	43	125	0.34**	0.20**

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

hybrids and parents for nine characters. There was significant and wide range of mid-parent heterosis for all nine characters. Hence, there is a much potential for development of good yielding hybrids. Among the 150 hybrids, 82 and 43 registered positive and negative significant mid-parent heterosis, respectively for green fruit yield plant^{-1} (Table 5). Majority of crosses, that is, 142 out of the 150 crosses exhibited significant mid-parent heterosis of which 86 were positive and 56 were negative for red fruit yield plant^{-1}. The 56% of hybrids showed significant mid-parent heterosis for both green and red fruit yield plant^{-1}. For the most economic important character green fruit yield plant^{-1}, 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 e×hibited highest significant positive mid-parent heterosis.

Association of genetic divergence with heterosis

The positive significant correlation was found between morphological marker genetic distance of the parents (Line-tester) and mid-parent heterosis for red fruit yield plant^{-1} (r = 0.20, $P < 0.01$), green fruit yield plant^{-1} (r = 0.19, $P < 0.01$), plant height (r = 0.17, $P < 0.05$), days to first fruit maturity (r = 0.15, $P < 0.05$), fruits plant^{-1} (0.13, $P < 0.05$) and fruit length (r = 0.12, $P < 0.05$) (Table 5). The positive significant correlation was found between AFLP marker-based genetic distance of the parents and mid-parent heterosis for plant height (r = 0.38, $P < 0.01$), green fruit yield plant^{-1} (r = 0.25, $P < 0.01$), red fruit yield plant^{-1} (r = 0.34, $P < 0.01$), days to first fruit maturity (r = 0.14, $P < 0.05$), fruits plant^{-1} (0.13, $P < 0.05$) and fruit length (r = 0.13, $P < 0.05$) (Table 5).

The correlations between the pair wise genetic distances and mid-parent heterosis were low in all characters (Table 5). However, CMS 3B and Kunchangi local 2 were more divergent parents and produced mid-parent heterosis of 63.74% in green fruit yield plant^{-1}. The next highest genetic divergent parents were CMS 5B and Kunchangi local 1, and produced mid-parent heterosis to -26.6 and -8.32% for green fruit yield plant^{-1} and red fruit yield plant^{-1}, respectively (Table 6a). The line CMS 3B and CA 14 were less divergent parents and produced 30.89 and 27.99% of mid-parent heterosis in green fruit yield plant^{-1} and red fruit yield plant^{-1}, respectively (Table 6b).

DISCUSSION

Genetic diversity analysis

The genotypes CMS 1B, CMS 2B, CMS 3B, CMS 5B and CMS 8B fell in the cluster VI which are from same geographical region. Gowribidanur local and Chickballapur local were also in the cluster VI. These two genotypes are from same

Table 6a. Highest AFLP maker genetic distances of line and testers and mid-parent heterosis of their crosses for 9 different characters.

S/N	Cross	Genetic distance	Mid-parent heterosis								
			50% flowering	Days to first fruit maturity	Plant height (cm)	Fruits plant^{-1}	Fruit length (cm)	Fruit width (cm)	100 seed weight (g)	Green fruit yield plant^{-1} (g)	Red fruit yield plant^{-1} (g)
1	CMS 3A x Kunchanggi local 2	0.22	-5.23 **	2.51 **	67.95 **	65.36 **	-22.03 **	-25**	-5.60 **	63.74 **	-2.06
2	CMS 5A x Kunchanggi local 1	0.21	-0.87	1.38	65.19 **	-49.20 **	70.63 **	-9.43 **	7.37 **	-26.60 **	-8.32 **
3	CMS 5A x Kunchanggi local 2	0.21	-2.54 **	6.12 **	50.00 **	9.81	32.49 **	1.69	11.86 **	56.78 **	36.43 **
4	CMS 3A x Kunchanggi local 1	0.20	-2.40 *	-4.63 **	52.29 **	16.82 **	17.39 **	4.76	-16.37 **	25.33 **	-8.23 **
5	CMS 8A x Kunchanggi local 2	0.20	-5.57 **	2.98 **	73.72 **	-1.82	-2.70	-4.35	-16.86 **	-14.77 *	-35.60 **
6	CMS 2A x Byadgi Dabbi	0.20	0.001	-4.44 **	-11.59 **	-32.22 **	3.23	-18.84 **	8.21 **	-6.00	18.31 **
7	CMS 8A x Kunchanggi local 1	0.19	-1.51	0.20	90.00 **	14.08 **	18.03 **	-5.00	2.25 **	68.88 **	10.97 **
8	CMS 1A x Byadgi Dabbi	0.19	3.05 **	-0.63	-34.20 **	-6.18	4.00 *	-26.67 **	96.44 **	-16.93 **	-46.10 **

Table 6b. Lowest AFLP marker genetic distances of line and testers and mid-parent heterosis of their crosses for 9 different characters.

S/N	Cross	Genetic distance	Mid-parent heterosis								
			50% flowering	Days to first fruit maturity	Plant height (cm)	Fruits plant^{-1}	Fruit length (cm)	Fruit width (cm)	100 seed weight (g)	Green fruit yield plant^{-1} (g)	Red fruit yield plant^{-1} (g)
1	CMS 3A x CA 14	0.08	0.61	-1.21	17.48 **	10.26 *	50.00 **	10.64 **	-18.64 **	30.89 **	27.99 **
2	CMS 3A x CA 6	0.08	3.09 **	-1.68 *	37.81 **	12.65 **	-13.41 **	-14.29 **	-2.67 **	-20.71 **	-16.85 **
3	CMS 3A x CA 2	0.09	1.23	3.83 **	25.48 **	54.83 **	27.87 **	-15.38 **	9.34 **	23.09 **	39.11 **
4	CMS 3A x CA 9	0.09	-1.23	-4.13 **	86.36 **	25.19 **	-8.57 **	2.56	21.89 **	78.37 **	30.28 **
5	CMS 3A x Tiwari	0.09	-1.51	-3.92 **	9.32 **	13.18 **	30.43 **	-6.38 **	-30.89 **	-8.18	-29.29 **
6	CMS 1A x Aparna	0.09	0.61	-2.51 **	-29.41 **	29.75 **	34.48 **	-16.67 **	-47.40 **	-9.59	-50.30 **
7	CMS 1A x Tiwari	0.09	1.20	-1.24	-13.70 **	78.13 **	16.36 **	-6.98 **	-24.21 **	27.59 **	-59.29 **
8	CMS 2A x Pant C1	0.09	3.05 **	-2.02 **	-7.04 **	-2.68	58.82 **	-10.20 **	105.91 **	41.15 **	41.76 **

geographical region. LCA 206, LCA 273, LCA 330, LAM 333, LCA 335 and LCA 960 which are from same geographical region are also in same cluster (cluster VI); thus, genotypes which share similar genetic background by virtue of their development from similar pedigree or because of their traits similarity driven by human or natural selection pressure in a particular geographical region. Similarly, Byadagi Kaddi and Byadagi Dabbi fell in the cluster I which are from the same geographical region. Therefore, the present results support the findings in rice, cowpea, tomato and chilli (Misra et al., 2004; Narayanankutty et al., 2005; Sreelathakumary and Rajamony, 2004; Thul et al., 2009) that the cluster pattern is not always related to geographical distribution.

The cluster IV had the largest number of 15 genotypes and the cluster VII included all five CMS lines (CMS 1B, CMS 2B, CMS 3B, CMS 5B and CMS 8B). All CMS B - lines were grouped in to one cluster which is confirmed with known geographical location.

Among testers, chilli genotypes of those collected from Taiwan were grouped in same cluster except Susan's joy and PBC 142 which were grouped with genotypes collected from the Karnataka in two different clusters. Chilli genotypes of those collected from the Guntur district of Andra Pradesh were grouped in the

same cluster except Aparna genotype which was grouped with Arka Suphal genotype collected from Karnataka. This is indicating that the grouping of genotypes which were collected from different location in one group may be possible due to cross-fertilization at the geographical location (Thul et al., 2006). There are many other factors other than regional boundaries and taxonomic characters are also responsible for divergence.

The pair wise AFLP maker based genetic distances between CMS B - lines and testers were 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. Similarly, Garcia et al. (2002) analyzed seven genotypes using 53 RAPD markers and found that the pair wise RAPD maker based genetic distances between parents is from 0.16 to 0.87.

Heterosis analysis

The 56% of hybrids showed significant mid-parent heterosis for both green and red fruit yield plant^{-1}. This indicates the variation on fruit yield and other characters in hybrids.

Longest and widest fruits were observed in the hybrids as compared to parental genotypes. Prasad et al. (2003) also reported the highest fruit width and fruit number plant^{-1} in chilli hybrid. Among the hybrids, some of them manifested higher positive heterosis whilst some hybrids 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.

Expression of heterosis in F_1 hybrids of chilli depends upon the involvement of the parents (Greenleaf, 1947). The observed positive heterosis for fruit number plant^{-1} and fruit yield plant^{-1} 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. Accumulation of favorable dominant alleles and masking of deleterious effects of recessive alleles by their dominant alleles in the F_1 (Hill et al., 1998) and superiority of heterozygotes at some of the loci to both the relevant homozygotes (Singh, 1993; Sprague, 1983) indicated the heterosis. Kumar et al. (2007), Prasath and Ponnuswami (2008) and Reddy et al. (2008) also reported crosses with high and positive significant mid-parent heterosis for green fruit yield plant^{-1} in chilli. On the contrary, negative heterosis in yield traits might be due to the recessive alleles acted towards the increased performance.

Association of genetic divergence with heterosis

Correlation coefficient between morphological and AFLP marker-based parental diversity and hybrid mid-parent heterosis was positive and significant for plant height, green fruit yield plant^{-1} and red fruit yield plant^{-1}. This is suggesting that increase in the morphological marker-based genetic distance between the parents' results in increased heterosis of their crosses of 17, 19 and 20%, for plant height, green fruit yield plant^{-1} and red fruit yield plant^{-1}, respectively. This is also suggesting that increase in the AFLP marker-based genetic distance between the parents results in increased heterosis of their crosses of 38% (plant height), 25% (green fruit yield plant^{-1}) and 34% (red fruit yield plant^{-1}), respectively. Hence, the detected correlations are not strong enough to predict the heterosis. The correlations of genetic distance involving the line testers and mid-parent heterosis were significantly positive but with low magnitude to be of predictive value (Legesse et al., 2008). The correlations of morphological and AFLP measured genetic distance of the parental lines with mid-parent heterosis of their hybrids were weak for all most all characters and proved to be of no predictive value in chilli (Geleta et al., 2004). However, wide and close divergent parental lines produced positive and significant mid-parent heterosis, this shows an isolated tendency in the chilli (Garcia et al., 2002; Geleta et al., 2004). Bernardo (1992) mentioned that it is essential to identify a specific marker related to the segments of the genome which determine the expression of the traits of interest to find a high correlation between genetic distance and heterosis. It may be expected that genetic distances calculated, using molecular markers, will become a useful way to predict heterosis until genes controlling important traits are placed on highly saturated genetic linkage maps and the adequate markers, those strongly linked, can be chosen to calculate the genetic distance.

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 highest significant positive mid-parent heterosis for fruit yield. Hence, there is a much potential for development of good yielding hybrids. These crosses could be used for the commercial exploitation. The genetic distance between the parents was not strong enough to predict the performance of the hybrid and proved no predictive value. However, the possibility that the molecular markers used are not close enough to the genes controlling traits in chilli, did not permit the extraction of final conclusions about the relationship between genetic distance among parents and the heterosis of their F_1s. It may be expected that genetic distances calculated, using molecular markers, will become a useful way to predict heterosis until genes

controlling important traits are placed on highly saturated genetic linkage maps and the adequate markers, those strongly linked, can be chosen to calculate the genetic distance. The crosses viz., CMS 3A × Pusa Jwala, CMS 8A × Arka Suphal, CMS 8A × LCA 330, CMS 3A × Arka Suphal and CMS 8A × Utkal Awa could be used in the breeding programme for the development of high yielding stable genotypes over environments for future use. Further investigations on G × E interactions at important crop growth stages for yield components and biochemical profiles would help to develop strategies that integrate traditional plant breeding with modern molecular marker-based selection for tailoring chilli hybrids for high yield and target environments.

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