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Potential of DNA markers in detecting divergence and in analysing heterosis in Indian elite chickpea cultivars

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Abstract Molecular markers such as RAPDs and microsatellites were used to study genetic diversity in 29 elite Indian chickpea genotypes. In general, microsatellites were more efficient than the RAPD markers in detecting polymorphism in these genotypes. Among the various microsatellites, $(AAC)_5$, $(ACT)_5$, $(AAG)_5$ and $(GATA)_4$ were able to differentiate all 29 chickpea cultivars. The mean value of probability of identical match by chance was 2.32×10^{-25} using *Dra*I-$(ACT)_5$, *Taq*I-$(AAC)_5$, *Taq*I-$(AAG)_5$ and *Taq*I-$(GATA)_4$ enzyme-probe combinations. The dendrogram, constructed on the basis of similarity index values, grouped the chickpea genotypes into five main clusters with 8 cultivars genetically distant and outgrouped from the main clusters. To investigate if DNA markers are useful in predicting F_1 performance and heterosis in chickpea, we crossed 8 genotypes having important agronomic characters in a diallel set. The F_1s and their parents in the diallel set were analysed for agronomic traits for better parent and midparent heterosis. Heterosis was found to be much higher for yield than for yield components that fit a multiplicative model. The analysis of genetic divergence using D^2 statistics clustered the 8 cultivars into two groups. Although molecular marker-based genetic distance did not linearly correlate to heterosis, two heterotic groups could be identified on the basis of the general marker heterozygosity.

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Introduction

Chickpea, one of the most important pulse crops in the world, is grown extensively throughout the Indian subcontinent, countries of North Africa, West Asia and the Mediterranean region. India is the largest producer of chickpea, accounting for 75% of the world production (Jodha and Subba Rao 1987). However, chickpea productivity is not high enough to fulfill the requirements of an increasing population. One major reason for the low productivity of cultivated chickpea, *Cicer arietinum*, is its narrow genetic base and its sexual incompatibility with other *Cicer* wild types in natural interspecific crosses.

A knowledge of genetic diversity and relatedness in the germplasm is a prerequisite for crop improvement programmes. Both morphological traits (Staub and Crubaugh 1995) and isozyme markers (Kazan et al. 1993) have been used for this purpose, but they have several disadvantages such as their limited number, environmental dependence and temporal and spatial expression. More recently, DNA markers have been reliably used in cultivar identification (Moser and Lee 1994), diversity analysis (Vasconcelos et al. 1996), construction of genetic maps (Song et al. 1991) and tagging agronomically important genes (Kelly 1995).

Another important application of DNA markers is the prediction of heterosis in hybrids. Evaluation of hybrids for heterosis or combining ability in the field is expensive and time-consuming. As a result, many parameters such as pedigree information, qualitative and quantitative traits (Smith et al. 1990; Wang et al. 1992) and biochemical data (Leonardi et al. 1991) are

being used to study heterosis. DNA-based markers have also been extensively used to correlate genetic diversity and heterosis in several crops such as maize (Ajmone Marsan et al. 1998), oat (Moser and Lee 1994; O'Donoughue et al. 1994), rice (Xiao et al. 1996; Zhang et al. 1994;) and wheat (Zhong and He 1991). It has been reported that measures of similarity based on restriction fragment length polymorphism (RFLP) and pedigree knowledge could be used to predict superior hybrid combinations in maize (Smith et al. 1990). However, both low and high correlations between heterosis and DNA-based genetic distance have been reported in various crops (Barbosa-Neto et al. 1996; Melchinger et al. 1990; Zhang et al. 1994).

In *Cicer arietinum*, commonly used markers such as isozymes (Kazan and Muehlbauer 1991), RFLPs and random amplified polymorphic DNAs (RAPDs) have not detected sufficient polymorphism (Sharma et al. 1995). However, intraspecific polymorphism in chickpea has been demonstrated using oligonucleotide fingerprinting (Weising et al. 1989, 1991, 1992). Recently, abundance and polymorphism of 38 different microsatellites have been studied in four chickpea accessions (Sharma et al. 1995). To our knowledge, no comprehensive effort has been made to investigate Indian elite chickpea genotypes using DNA markers. In the study presented here, RAPD and microsatellite markers were employed to assess genetic diversity in 29 elite chickpea genotypes in India. This investigation is a prerequisite for designing crosses for the pyramiding of desired traits to produce superior chickpea genotypes. The relationship of genetic diversity with hybrid performance and heterosis was also examined to determine whether these markers would be useful for predicting F_1 performance and heterosis in chickpea.

Materials and methods

Seed material and DNA isolation

Seeds of all the accessions of chickpea (*Cicer arietinum* L.) were obtained from the Pulses Research Centre of the Mahatma Phule Krishi Vidyapeeth, Rahuri, India. Table 1 lists the cultivars used in this study. The eight parental lines crossed to obtain a diallel set of 28 crosses (excluding reciprocals) during the rabi season of 1994–1995 are indicated in Table 1. The plants were grown in greenhouse, and leaf tissue was collected and subsequently frozen at − 80°C. Total DNA was extracted from the frozen tissue by the CTAB method (Rogers and Bendich 1988) with slight modification.

RAPD assay

RAPD assays were performed using random 10-mer oligonucleotide primers from Operon Technologies (USA). Amplification reactions were carried out in 25-µl volumes containing 10 ng of genomic DNA, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* TAPS [3-tri (hydroxylmethyl) methyl aminopropane sulfonic acid], 0.01% gelatin, 100 µ*M* each of dATP, dCTP, dGTP and dTTP (Amersham, UK), 5 pmoles primer and 0.6 U *Taq* DNA polymerase (Biogenei, India). Amplification was performed in a PTC-100 (Perkin Elmer) thermocycler programmed for 5 min at 94°C followed by 45 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. This was followed by a final extension at 72°C for 5 min. Amplification products were analysed by gel electrophoresis on 1.9% agarose in 0.5 × TAE buffer and visualised by ethidium bromide staining. All the reactions were repeated at least three times, and only reproducible bands were used in further analyses.

Hybridisation based microsatellite analysis

Genomic DNA was digested with various restriction enzymes such as *Alu*I, *Dra*I, *Hae*III, *Hin*fI and *Taq*I (Promega, USA) according to the manufacturer's instructions. The digested DNA was size-fractionated on agarose gels (0.8–1.2%), and the gels were dried

Table 1 *Cicer arietinum* L. germplasm used in the present study

Vijay,[a] Phule G-8505-7	High yield, wilt resistant, drought tolerant, temperature tolerant, late sown
Phule G-89224, GCP-102, Phule G-92014	High yield, wilt resistant
Phule G-92005, Phule G-12[a]	
Phule G-8501-1, Phule G-91025	
ICCV-10,[a] Phule G-89219,[a] Phule G-91028[a]	
JG-74, Phule G-93044, Phule G-92028	High yield, wilt resistant, temperature tolerant/late sown
Vishwas (Phule G-5)	High yield, bold seeded, wilt tolerant
Vishal (Phule G-87207)[a]	
Phule G-8505–10	High yield, drought tolerant, wilt resistant
Jaki-9324	High yield, bold seeded
Phule G-92007	High yield, bold seeded, wilt resistant, root rot resistant
ICC-410	High yield
ICC-31	Drought tolerant
ICC-4958[a]	Bold seeded, drought tolerant
JG-315	Temperature tolerant /late sown, wilt resistant
BG-372	Temperature tolerant/late sown, high yield
Bheema[a]	Bold seeded
C-235	High yield, wider adaptability, wilt susceptible
BG-390	High yield, wider adaptability, Wilt susceptible
JG-62	Wilt susceptible, twin podded

[a] Parental lines for diallel set

in-vacuo. The dry gels were denatured, neutralised and then equilibrated in 6 × SSPE. Oligonucleotides such as $(AAC)_5$, $(GATA)_4$ etc. were labelled with γ-[^{32}P]-ATP and purified on a DE52 column (Ali et al. 1986). The gels were hybridised with oligonucleotide probe at T_m-5°C (Miyada and Wallace 1987) overnight in hybridisation buffer containing 5 × SSPE, 5 × Denhardt's reagent, 0.1% milk powder and 0.1% SDS. Stringent washes were given, and the hybridized gels were autoradiographed with intensifying screens at −70°C.

Field evaluation and data collection

The F_1 hybrids obtained from the 28 crosses between the eight parents along with the parental lines were grown in the rabi season of 1995–1996 for phenotypic evaluation in a randomised complete block design with three replications, at Mahatma Phule Krishi Vidyapeeth, Rahuri, India. Each plot consisted of 3-m-long rows spaced 30 cm apart. The distance between 2 plants in a row was maintained at 15 cm. Five randomly selected competitive plants in each plot were used for recording field data. Various agronomic traits were examined for the experimental material. Means over replications were recorded for each trait and used in data analysis. The data obtained were used for combining ability analysis using Griffing's model I and method 2 (Griffing 1956). Analysis of variance for combining abilities and estimation of variance components were carried out. Yield potential was calculated as a function of its yield contributing components with the formula: Yield potential = 1/100 × pods/plant × grains/pod × 100-seed weight.

Statistical analysis

Pairwise comparisons of degree of band sharing were made, and similarity index values were calculated by Nei's (Nei and Li 1979) method as S.I. = $2N_{ab}/N_a + N_b$ where N_a = total no. of bands present in lane a, N_b = total no. of bands present in lane b, N_{ab} = no. of bands common to lanes a and b. A dendrogram was constructed using TAXAN version 4.0 software based on the degree of band sharing. Probability of identical match by chance by which 2 genotypes would show the identical band pattern was calculated as $(X_D)^n$ where X_D was the average similarity index value and n was the average number of total bands shared per probe/per primer (Wetton et al. 1987). Midparent and better-parent heterosis were estimated for seed yield/plant (g), pods/plant, number of seeds/pod, and 100-seed weight (g), and the significance of the percentage heterosis over better-parent and midparent was tested by the least significant difference method. The relationships between genetic distance and heterosis/hybrid performance were evaluated by regressing heterosis or trait values on the genetic distance in the F_1 hybrid.

Results

Assessment of genetic diversity in elite chickpea germplasm using RAPD and microsatellite DNA markers

RAPD- polymerase chain reaction (PCR) analysis

To obtain stable and reproducible DNA fingerprints which could discriminate chickpea cultivars, we deemed it necessary to optimize the PCR protocol with respect to concentrations of template DNA, primer and magnesium ions. Although, chickpea DNA was amplified with different concentrations of magnesium (1.5, 2.0, 2.5 m*M*), strong and reproducible bands were obtained at 1.5 m*M*, and this concentration was subsequently used in all experiments. Thirty-five random primers from Operon Technologies were used to amplify the DNA of chickpea cultivars. Each primer produced between 2 and 12 amplification products depending on the various cultivars, with an average number of 8.75 bands per primer per accession. The lengths of the amplification products varied from 0.26 kb to 3.0 kb. Out of the 35 primers used, 10 primers generated polymorphic patterns as listed in Table 2. As shown in Fig. 1, primer OPF09 generated a monomorphic pattern with the exception of 1 major band which was present only in cvs 'Vijay' (lane 1), 'ICC-4958' (lane 3), 'PG-89224' (lane 6), 'GCP-102' (lane 7), 'ICC-410' (lane 9), 'ICCV-10' (lane 15), 'PG-5' (lane 17), 'PG-87207' (lane 18), 'Jaki-9324' (lane 19), 'PG-93044' (lane 20), 'BG-390' (lane 25), 'PG-12' (lane 27) and 'Bheema' (lane 29) and 3 other minor bands. Based on the RAPD patterns, a pairwise comparison was made between all the genotypes for each primer. A total of 254 amplification products were scored out of which 14.56% were polymorphic in nature. The genetic distance values varied from 0.02 to 0.22 with an average value of 0.13.

Table 2 Primers producing polymorphic markers among the chickpea cultivars

Primer	Sequence	Number of polymorphic bands	Average number of bands
OPA05	AGGGGTCTTG	5	10
OPA07	GAAACGGGTG	4	9
OPA08	GTGACGTAGG	2	5
OPA10	GTGATCGCAG	5	12
OPA11	CAATCGCCGT	2	6
OPA12	TCGGCGATAG	4	12
OPA19	CAAACGTCGG	4	8
OPF09	CCAAGCTTCC	4	7
OPJ06	TCGTTCCGCA	4	11
OPJ07	CCTCTCGACA	2	5

Microsatellite-based diversity analysis

Genomic DNA of the 29 cultivars of chickpea were digested with restriction endonucleases *Alu*I, *Hinf*I, *Dra*I, *Taq*I and *Hae*III individually and then in-gel hybridised to various microsatellite probes. Table 3 lists the average number of bands per genotype obtained with each enzyme-probe combination. Most of the bands generated by the probes were polymorphic in the chickpea genotypes. Five microsatellites, namely $(AAC)_5$, $(ACT)_5$, $(AAG)_5$, $(ACG)_5$ and $(GATA)_4$, yielded clear polymorphic patterns with 12–23 bands on average per genotype which could distinguish all the genotypes in the present study. Probe $(ACG)_5$, however, yielded fewer than 6 bands upon hybridisation

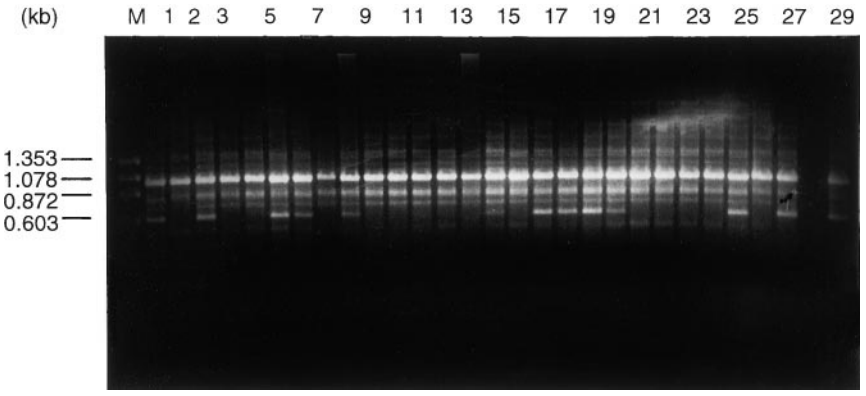

Fig. 1 RAPD fingerprints of the elite chickpea cultivars with primer OPF09. *M* ΦX-174/*Hae*III digest marker. Lanes 1–29 are: 1 'Vijay', 2 'PG-8505-7', 3 'ICC-4958', 4 'PG-8505-10', 5 'ICC-31', 6 'PG-89224', 7 'GCP-102', 8 'PG-92014', 9 'ICC-410', 10 'PG-92005', 11 'PG-8501-1', 12 'PG-92007', 13 'PG-91025', 14 'JG-74', 15 'ICCV-10', 16 'JG-315', 17 'PG-12', 18 'PG-87207', 19 'Jaki-9324', 20 'PG-93044', 21 'PG-92028', 22 'BG-372', 23 'JG-62', 24 'C-235', 25 'BG-390', 26 'PG-89219', 27 'PG-12', 28 'PG-91028', 29 'Bheema'

with *Taq*I. Figure 2a and b shows the polymorphic bands obtained with the *Taq*I-$(GATA)_4$ combination which can fingerprint all the 29 cultivars with bands ranging from 9 kb to 2 kb.

A total of 1916 loci were scored out of which 632 were similar on a pairwise comparison of the 29 cultivars. The average genetic distance for all the oligonucleotide-enzyme combinations listed in Table 3 was 0.560 with the lowest and highest genetic distance values being 0.39 and 0.82, respectively. The probability of identical match by chance, $(X_D)^n$, which denotes the probability of 2 genotypes having identical band profiles, was calculated to be 7.8×10^{-7}, 2.92×10^{-6}, 5.75×10^{-8} and 1.77×10^{-6} for probes $(AAC)_5$, $(ACT)_5$, $(AAG)_5$ and $(GATA)_4$, respectively, and 2.32×10^{-25} using all 4 enzyme-probe combinations (Table 4).

Table 3 Average number of bands produced by the enzyme-oligonucleotide probe used in fingerprinting the elite chickpea cultivars

Oligonucleotide probe	Restriction endonuclease	Average no. of bands
$(GATA)_4$	*Alu*I	13.7
	Hinf I	14.6
	*Dra*I	13.7
	*Taq*I	14.2
	*Hae*III	17.0
$(ACT)_5$	*Dra*I	23.0
	*Hae*III	18.0
$(AAC)_5$	*Dra*I	12.5
	*Taq*I	14.2
$(AAG)_5$	*Taq*I	12.3
$(ACG)_5$	*Taq*I	05.6
	*Hae*III	15.0

Fig. 2a Oligonucleotide fingerprint with *Taq*I-'GATA'$_4$. *M*: Lambda *Hin*dIII digest marker. *Lanes*. 1 to 25. 1 'Vijay', 2 'PG-8505-7', 3 'ICC-4958', 4 'PG-8505-10', 5 'ICC-31', 6 'PG-89224', 7 'GCP-102', 8 'PG-92014', 9 'ICC-410', 10 'JG-74', 11 'ICCV-10', 12 'JG-315', 13 'PG-12', 14 'PG-87207', 15 'Jaki-9324', 16 'PG-93044', 17 'PG-92028', 18 'BG-372', 19 'JG-62', 20 'C-235', 21 'BG-390', 22 'PG-92005', 23 'PG-8501-1', 24 'PG-92007', 25 'PG-91025'. **b** Oligonucleotide fingerprint with *Taq*I-'GATA'$_4$. *M* Lambda *Hin*dIII digest marker. *Lanes* 1 to 8. 1 'PG-89219', 2 'Vijay', 3 'ICCV-10', 4 'PG-12', 5 'PG-91028', 6 'PG-87207', 7 'ICC-4958', 8 'Bheema'

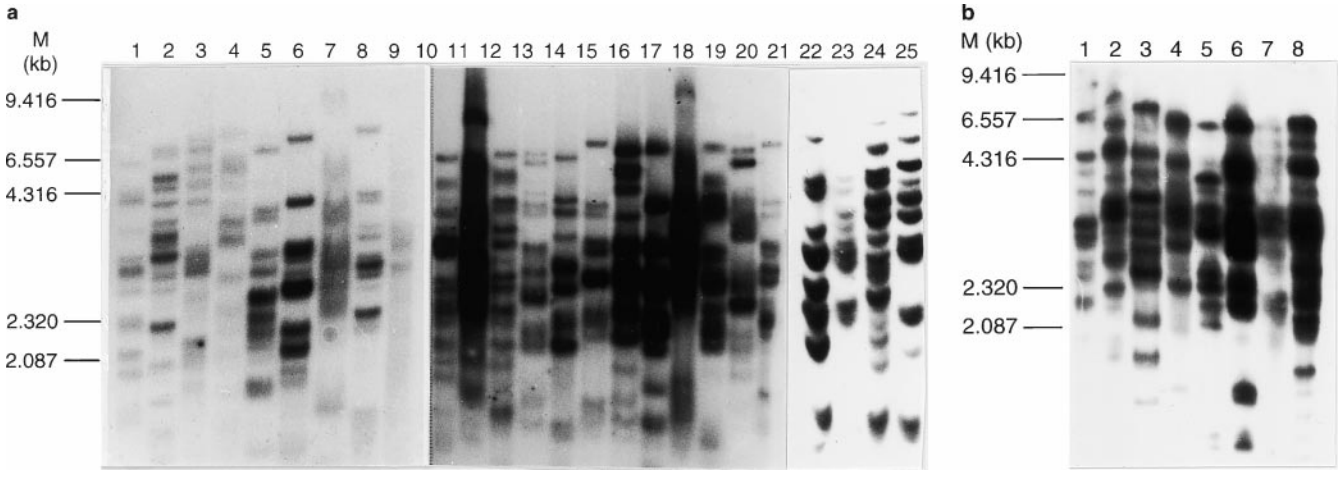

Table 4 Probability of identical match by chance (S.I. similarity index)

Probe	Highest S.I.	Lowest S.I.	X_D[a]	n[b]	$(X_D)^n$[c]
$(ACT)_5$	0.780	0.352	0.575	23.08	2.92×10^{-6}
$(AAC)_5$	0.710	0.078	0.337	13.36	7.8×10^{-7}
$(GATA)_4$	0.720	0.123	0.396	15.01	1.77×10^{-6}
$(AAG)_5$	0.577	0.000	0.260	12.37	5.75×10^{-8}

[a] X_D, Average similarity index for all pairwise comparisons
[b] n = Average no. of bands produced by the probe
[c] $(X_D)^n$ Probability of identical match by chance

Estimation of genetic relatedness

Computer software TAXAN version 4.0 was used to construct a dendrogram on the basis of similarity index values of the 29 chickpea genotypes (Fig. 3). Genetic distance as evidenced from the dendrogram showed a high level of genetic diversity in the range of 0.39 to 0.82 in chickpea elite germplasm. The chickpea genotypes formed five main clusters with 8 cultivars genetically distant and outgrouped from the main clusters. Cultivars 'Vijay', 'PG-92014', 'PG-92007' and 'PG-91025' formed the first cluster (cluster I) at a genetic distance of 0.47, while cvs 'GCP-102', 'JG- 74' and 'Vishal' together formed cluster II at a genetic distance of 0.49. Cluster III contained 'PG-8505-10', 'ICC-410', 'PG-8501-1' and 'ICCV-10' with cv 'PG-8505-7' loosely clustering in this group at a genetic distance of 0.58. Cluster IV consisted of 'ICC-4958', 'JG-315', 'Vishwas' and 'ICC-31', and Cluster V included cvs 'PG-93044', 'JG-62', 'BG-390' and 'PG-92028' at a genetic distance of 0.48 while cvs 'BG-372' was equidistant (0.49) from both of these clusters. Cultivars 'PG-89224', 'Jaki-9324', 'C-235', 'PG-91028', 'PG-92005', 'PG-89219', 'PG-12' and 'Bheema' were genetically distinct from the above clustered cultivars as well as from each other with the exception of group I 'Jaki-9324', 'C-235' and II 'PG-12', Bheema.

Potential of DNA markers in predicting F_1 performance and heterosis in chickpea

Polymorphism using molecular markers

Eight parents used in the diallel mating set (Table 1) were selected on the basis of their phenotypic characters and used for analysing DNA polymorphism. Twelve microsatellite-restriction enzyme combinations and 35 RAPD primers were used to generate polymorphic patterns that revealed 7.3% and 31.62% polymorphism among the parents, respectively. For increased genome coverage, 388 polymorphic loci, including 85 and 303 from RAPD and microsatellite analysis, respectively, were used to study genetic distance among the eight parents under consideration.

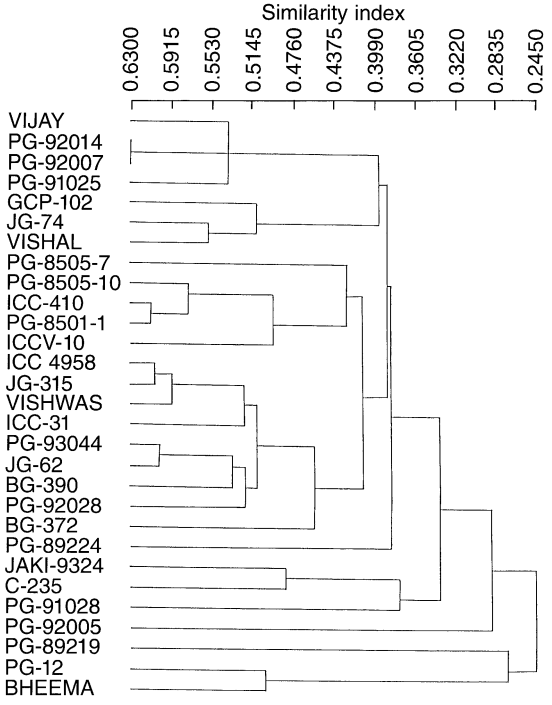

Fig. 3 Dendrogram of the 29 chickpea cultivars based on similarity index values using microsatellite markers

Genetic distance among parents and clustering of parental lines

Genetic distance was calculated from Nei's similarity index values for all 28 combinations of the eight parents considering RAPD and microsatellite approaches individually as well as together. Based on the RAPD markers alone, the genetic distance ranged from 0.09 to 0.27, while that calculated on the basis of microsatellite markers ranged from 0.42 to 0.61. However, genetic distance based on both molecular markers together ranged from 0.26 to 0.40, which was used to generate the dendrogram presented in Fig. 4. From the figure it can be seen that the eight parental lines clustered into one major group containing 6 cultivars, while the 2 cultivars 'Vijay' and 'ICCV-10' grouped out from the others. The major group consisted of two subgroups, 'PG-89219' and 'PG-12' forming one subgroup and 'PG-91028', 'PG-87207', 'Bheema' and 'ICC-4958' forming the other. 'PG-91028' and 'PG-87207' from the second subgroup share a common parent, 'K-850'. 'Bheema' and 'PG-87207', both bold seeded, were genetically closest at a distance of 0.26, however, the pedigree of 'Bheema' was not available, and therefore the clustering based on pedigree could not be commented upon.

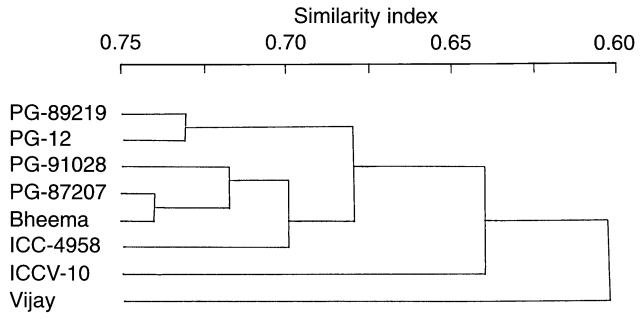

Fig. 4 Dendrogram of the 8 cultivars used in the diallel set based on the similarity index values using RAPD and microsatellite markers

When the same eight parents were analysed for their morphological and yield component traits, using D^2 statistics (Mahalanobis 1936), the 8 cultivars clustered into two groups. Here, 'Vijay', 'PG-91028' and 'ICC-4958' clustered together; 'ICCV-10', 'PG-87207' and 'Bheema' formed a second cluster while 'PG-12' and 'PG-89219' were outgrouped (data not shown).

Hybrid performance and heterosis

Table 5 gives the midparent heterosis values as well as specific combining ability effects for the agronomic traits. As observed in this table, the degree of heterosis and hybrid performance varied significantly for each trait. Highest midparent heterosis was obtained for seed yield (76.16%, $P = 0.01$) followed by number of pods/plant (57.64%, $P = 0.01$); number of secondary branches per plant (30.64%, $P = 0.01$) and 100-seed weight (9.91%, $P = 0.05$). Comparatively, number of basal branches per plant and 100-seed weight exhibited a low magnitude of heterosis. Among the other traits examined such as plant height, days to 50% flowering and days to maturity, no significant correlation was seen with grain yield. However, a correlation at 1% significance level was obtained between plant spread and heterosis for grain yield ($r = 0.507$, $P = 0.01$).

Midparent heterosis in seed yield per plant ranged from 21.76% to 76.16% in crosses with 'PG-89219' as one of the parents, which was the maximum value compared to all other crosses. Also, heterosis over

Table 5 Genetic distance, percentage midparent heterosis for different agronomic characters and SCA effects in crosses of the diallel set (*SCA* specific combining ability)

Cross	Genetic distance	Percentage midparent heterosis for						SCA (seed yield/plant)
		Number of secondary branches	Number of pods per plant	Number of basal branches	Plant spread	One-hundred seed weight	Yield per plant	
PG89219 × Vijay	0.3996	5.10	0.52	− 11.7	19.9	8.09	23.83**	0.329
PG89219 × ICCV10	0.3549	29.82**	17.88**	1.52	30.50	− 3.96	23.11**	0.118
PG89219 × PG12	0.2646	1.97	57.64**	8.60	32.27	9.91**	76.16**	13.78**
PG89219 × PG91028	0.3129	14.69**	15.40**	− 17.88	29.2	0.258	21.76**	0.449
PG89219 × PG87207	0.2919	9.21*	10.40**	− 13.45	− 6.85	− 12.46	22.23	5.38**
PG89219 × ICC4958	0.3540	1.00	46.86**	15.69**	11.98	− 20.98	36.74**	6.89**
PG89219 × Bheema	0.3299	21.88**	13.63**	7.19	14.34	− 7.19	10.57	− 0.723
Vijay × ICCV10	0.3556	− 13.44	7.26	− 6.99	− 16.59	1.74	11.31	0.348
Vijay × PG12	0.4051	16.20**	21.50**	0.00	4.37	3.10	31.96**	3.982*
Vijay × PG91028	0.3596	− 21.93	26.23**	− 8.33	− 13.40	6.54	37.72**	10.753**
Vijay × PG87207	0.3729	− 1.98	− 18.37	− 1.48	2.77	2.33	− 7.79	− 2.083
Vijay × ICC4958	0.3711	4.95	1.77	8.6	− 21.94	− 18.17	2.22	− 5.246
Vijay × Bheema	0.3631	− 24.11	− 0.48	1.23	− 29.15	− 14.12	− 11.27	1.334
ICCV10 × PG12	0.3471	18.11**	23.66**	− 9.46	− 5.96	− 5.16	23.08	− 2.904
ICCV10 × PG91028	0.3180	2.75	5.65	1.23	17.75	7.39	1.24	− 1.334
ICCV10 × PG87207	0.3131	− 12.76	− 8.41	− 8.14	− 3.01	− 18.32	− 4.94	− 1.325
ICCV10 × ICC4958	0.3467	9.00	56.94**	7.19	− 6.10	− 18.32	28.48**	8.217**
ICCV10 × Bheema	0.3146	− 4.58	10.22	− 2.43	− 23.63	− 30.03	8.98	3.022
PG12 × PG91028	0.3047	− 30.58	17.03	− 2.91	1.79	2.20	22.99	2.145
PG12 × PG87207	0.3044	− 6.62	− 7.17	− 4.44	− 15.69	− 6.50	− 12.30	− 15.544
PG12 × ICC4958	0.3153	30.64**	39.98**	− 0.22	− 4.20	− 11.71	25.92	3.605
PG12 × Bheema	0.3060	− 4.48	− 3.86	− 4.11	− 14.33	− 11.19	0.52	− 2.781
PG91028 × PG87207	0.2774	− 21.46	− 0.05	− 12.89	− 7.53	− 10.92	0.33	1.880
PG91028 × ICC4958	0.2952	− 14.28	3.27	− 0.22	21.11	− 21.99	− 10.92	− 6.97
PG91028 × Bheema	0.3031	− 11.37	10.84**	− 12.33	− 1.21	− 23.62	− 1.31	0.623
PG87207 × ICC4958	0.2923	− 14.68	− 24.07	1.34	− 13.24	− 5.86	22.54	− 8.211
PG87207 × Bheema	0.2587	7.97	− 19.50	− 16.49	− 15.43	− 5.22	− 13.24	− 2.213
ICC4958 × Bheema	0.2748	4.51	9.24	1.52	− 8.07	6.91	8.85	3.98*

* Indicates significance at $P = 0.05$; ** indicates significance at $P = 0.01$

Table 6 General combining ability effects of the parents for different characters

Character	PG-89219	Vijay	ICCV-10	PG-12	PG-91028	PG-87207	ICC-4958	Bheema
1. Days to 50% flowering	2.91**	− 1.783**	0.283	− 0.483	− 0.483	− 0.317	− 0.683*	− 0.58
2. Days to maturity	1.620**	− 1.583**	− 1.45**	1.217**	1.217**	− 0.25	− 0.517	− 1.35**
3. Plant height	0.816*	− 2.012**	− 0.727**	0.782*	0.782**	− 2.511**	− 0.181**	1.98**
4. Plant spread	0.148	0.148	− 1.655**	− 0.648**	− 0.648**	− 1.082**	0.695**	3.78**
5. Number of basal branches per plant	− 0.68**	0.039	0.006	0.056*	0.058**	− 0.094**	− 0.038	0.062
6. Number of secondary branches per plant	1.312**	− 1.222**	− 1.412**	0.968**	0.968**	− 0.872**	− 1.282**	0.36
7. Number of pods per plant	19.763**	3.629**	2.50*	− 1.95	7.542**	− 20.58**	− 12.80**	2.067
8. Number of seeds per pod	0.124**	− 0.073**	0.193**	− 0.003	− 0.003	0.029	− 0.132**	− 0.1**
9. 100 seed weight	− 2.707**	− 1.068**	− 1.843*	− 1.787**	− 1.787**	1.583**	5.485**	3.40**
10. Seed yield per plant	3.978**	2.715**	0.72	− 3.618	− 3.618**	− 2.325**	1.725**	3.30**

* Indicates significance at $P = 0.05$; ** indicates significance at $P = 0.01$

midparent in number of secondary branches, number of pods per plant and 100-seed weight was higher with 'PG-89219' as one of the parents than with all other crosses, (Table 5). As can be seen in Table 5, crosses of 'PG-12', 'PG-87207', 'ICC-4958' with 'PG-89219', 'Vijay × PG-91028' and 'ICCV-10' × ICC-4958' exihibited significant specific combining ability effects for seed yield per plant. General combining ability effects were calculated for each trait (Table 6). 'PG-89219' was a good combiner for seed weight per plant, number of seeds per pod, number of pods per plant and number of secondary branches; 'PG-87207', and 'ICC-4958' were good combiners for 100 seed-weight, whereas 'Vijay' showed a significant combining ability for number of pods per plant and seed yield per plant. 'Bheema' is a good combiner for 100-seed weight and seed yield per plant, and 'ICCV-10' showed significant combining ability for number of seeds per pod. 'PG-91028' showed good combining ability for number of basal branches/per plant, number of secondary branches per plant and number of pods per plant.

Correlation of hybrid performance with molecular marker heterozygosity

Significant positive heterosis resulted when crosses were performed between two subgroups, for example, 'PG-89219' and 'PG-12' from one subgroup with other parents, 'PG-91028', 'PG-87207', 'Bheema', 'ICC-4958' and 'ICCV-10', and 'Vijay' for the yield per plant, as shown in Table 5. However, the correlation between genetic distance and heterosis for any of the traits was not linear. For example, the highest midparent heterosis of 76.16% was obtained on crossing 'PG-89219' and 'PG-12' when the genetic distance between them was 0.2646, whereas a midparent heterosis of −13.24% was obtained on crossing 'PG-87207' and 'Bheema' which had a genetic distance of 0.2587. It is evident from Table 5 that there is no correlation between genetic distance and heterosis in different traits.

Discussion

Usefulness of microsatellites in fingerprinting chickpea genotypes

Our data revealed low polymorphism in chickpea germplasm with RAPD markers, and hence these markers may not be suitable in revealing genetic diversity in 'desi' chickpea genotypes. Extensive DNA polymorphism, however, has been reported using RAPD markers in several other crops plants (Hilu and Stalker, 1995; Morell et al. 1994; Ranade and Sane 1996). In an earlier work on pigeon pea from our laboratory, it has been shown that RAPDs could reveal a high degree of polymorphism in wild species of pigeon pea (Ratnaparkhe et al. 1995).

Oligonucleotides representing microsatellites detected high levels of polymorphism, and all the chickpea cultivars studied here could be fingerprinted. We used 25 microsatellite-enzyme combinations on 29 elite chickpea germplasm to identify those microsatellites which can fingerprint elite chickpea cultivars having a narrow genetic base. Four microsatellites namely, $(AAC)_5$, $(ACT)_5$, $(AAG)_5$ and $(GATA)_4$ gave distinct fingerprints for the 29 cultivars. From the $(X_D)^n$ values (Table 3), it is clear that $(AAG)_5$, which was the most polymorphic microsatellite, could distinguish the maximum number of cultivars even though it gave a fewer number of bands than the other microsatellites. Microsatellite hybridisation in this study revealed a genetic diversity in the range of 39% to 82%. Such a detailed analysis of microsatellites in chickpea genotypes indicates the overwhelming potential of these oligonucleotide probes in cultivar identification, genetic characterisation and relatedness in the chickpea germplasm.

Divergence among chickpea cultivars based on microsatellite markers

Cultivars 'PG-92014', 'PG-92007' and 'GCP-102', and Vishal clustered together as they shared a common parent, KPG-36 and K-850, respectively, which probably resulted in similar fingerprinting profiles. Cultivars 'PG-8505–7' and 'PG-8505–10', selected from the same population of a cross between WR-315 × Sel.436, clustered together at a genetic distance of 0.59. 'PG-8505–7' is temperature-tolerant/late sown as against 'PG-8505–10', thus differing in the phenotypic characters. 'PG-92014' and 'PG-92005', selected from the population [(KPG-36 × P-326) × ICC12271], and 'ICCV-10' and 'BG-372', selected from the population (P-1231 × P-1265), were separated by a genetic distance of 0.76 and 0.60, respectively thus indicating a divergence of the cultivars from each other during succeeding cycles of selfing. The diversity thus observed with microsatellites in the chickpea germplasm is probably due to the use of landraces throughout most of the Indian subcontinent (Malhotra et al. 1987), and even today these landraces are being used for the development of elite cultivars. However, the genetic diversity between the various landraces still remains to be studied, and molecular markers will be greatly useful in quantifying this diversity.

Diallel mating: a molecular approach

The prediction of hybrid performance has always been a primary objective in all hybrid crop breeding programmes (Hallauer and Miranda 1988). Earlier analyses demonstrated important features regarding the correlations of molecular marker heterozygosity with heterosis and F_1 performance. Therefore, we attempted here to make a diallel set and to correlate the hybrid performance with molecular marker heterozygosity, which is the first study of its kind in elite chickpea lines.

We observed that the hybrids were more heterotic for seed yield than for yield component traits. This is obvious because yield (Y) is generally a multiplicative function of three component traits, number of pods per plant (P), number of grains per pod (G), and 100-seed weight (W); ($Y = P \times G \times W$). Heterosis of component traits would multiplicatively amplify each other to produce a much larger heterosis in the ultimate trait. On applying the *t*-test for the observed and predicted yield in the hybrids, we obtained a '*t*' value of 2.329 at $P = 0.05$. Also, a significant positive correlation between plant spread and grain yield indicated a potential avenue for increasing grain yield in chickpea. Secondly, it was observed that one of the parents, 'PG-89219', when crossed with others consistently gave significant heterosis. The analysis of variance for combining ability and the estimation of variance components indicated that additive × dominance gene interaction played an important role in the inheritance of seed yield and all other characters except 100-seed weight. Hence, 'PG-89219' can be used in a crossing programme for developing a new line with high performance.

Although no linear correlation was obtained between genetic diversity and heterosis, it was evident that significant heterosis resulted for hybrids (Table 5) by crossing parents from two different subgroups (Fig 4). Hybrids obtained after crossing parents from the same group gave poor heterosis for yield. Thus, our results suggest that the concept of genetic divergence for maximum expression of heterosis has certain limitations in chickpea. It was suggested earlier that hybrids showing heterosis were usually developed from parental lines diverse in relatedness, ecotype, geographic origin etc. (Yuan 1985) However, heterosis manifested by hybrids developed from genetically diverse varieties was less than that between varieties which were to be genetically less diverse in a crop like maize (Moll et al. 1965). In wheat, a low correlation was reported between heterosis and DNA marker-based genetic distance (Barbosa-Neto et al. 1996). With different sets of maize cultivars, low correlation was detected between combining ability and RFLP-based genetic distance (Melchinger at al. 1990; Boppenmaier et al. 1993) Apparently, crosses between extremely divergent parents create a situation in which the harmonious functioning of alleles is disrupted. Consequently, the physiological functions are not efficient, resulting in low heterosis. In fact, doubts have been expressed about the usefulness of increased genome coverage for calculating marker distance and correlating it with hybrid performance to improve the efficiency of the prediction (Melchinger et al. 1990; Boppenmaier et al. 1993). Alternatively, the identification of marker loci and genotypes significantly associated with traits of interest was suggested. Thus, correlations calculated using specific heterozygosity based on the positive markers would be more significant than those based on general heterozygosity.

In summary, the diversity analysis based on microsatellites in our study has revealed the usefulness of these markers in the identification of polymorphism in chickpea genome, which was earlier thought to be less polymorphic. Such markers will be highly efficient in identifying specific markers linked to the trait of interest. Secondly, although the PCR-based RAPD markers and microsatellite markers could cluster the 8 genotypes under the diallel studies into heterotic groups, genetic distance and heterosis were not in linear correlation. It is, therefore, essential that specific DNA markers be developed in this system for an efficient and reliable estimation of genetic distance for predicting heterosis.

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