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Estimation of genetic diversity in fieldpea (*Pisum sativum* L.) based on analysis of hyper-variable regions of the genome

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Abstract

The genetic diversity present in the widely adapted Indian fieldpea varieties, many of which are from exotic background, has rarely been studied with DNA based markers. Forty-five microsatellite markers were used to assess the genetic variation within twenty four elite pea cultivars grown extensively in India. Out of total 45 markers, 39 markers amplified a total of 55 alleles with an average of 1.4 alleles per marker. Maximum diversity was recorded between cultivars KPMR 44-1 and Ambika. The average similarity coefficient value was found to be 0.84. Cluster analysis based on Dice similarity coefficient using UPGMA, grouped tall type and dwarf type varieties into two different clusters based upon their pedigree. Very low polymorphism within the studied genotypes indicates an urgent need to include diverse parents in fieldpea breeding programmes. The present study also generated valuable information about the comparative usefulness of genic and genomic microsatellite markers. Genomic microsatellite markers showed higher degree of polymorphism compared to the genic microsatellite markers.

Key words: Genetic diversity, Markers, Microsatellite, *Pisum sativum*.

The development of cultivated species and breeding of new varieties have always relied on the availability of biological diversity, issuing from the long term evolution of species. Estimates of genetic relations among parental lines may be useful for determining which material should be combined in crosses to maximize genetic gain. In a study with soybean, Manjarrez-Sandovel *et al*. (1997) found that genetic variance for yield was positively associated with parental genetic distance and that genetic variance declined to near zero when the coefficient of parentage was above 0.27. Other studies with oat (Kisha *et al*. 1997) and wheat (Souza and Sorrells 1991, Cox and Murphy 1990) showed the relation between genetic distance and variance varied among traits and populations.

The development of PCR based markers has opened new avenues for molecular differentiation of closely related strains in a species. Simple Sequence Repeats (SSR) marker system revealed higher genetic diversity level than Random Amplified Polymorphic DNA (RAPD) marker system (Zietkiewicz *et al*. 1994). The successful development of locus-specific SSR markers in pea (Burstin *et al*. 2001, Loridon *et al*. 2005) allow us using pea SSR marker system for systematic studies of genetic diversity, population structure and genetic relationship within *Pisum* genus. Recent diversity studies in pea have focused on assessment the genetic diversity within *Pisum* using, different molecular markers (Posvee and Griga 2000, Burstin *et al*. 2001, Simioniuc *et al*. 2002, Tar'an *et al*. 2005, Choudhary *et al*. 2007, Zong *et al*. 2008, Nasiri *et al*. 2009, Gowhar *et al*. 2010), DNA transposable elements (Vershinin *et al*. 2003), and numerical taxonomy (Muhammad *et al*. 2009). Despite being one of the most important winter pulse crop, with the exception of few reports (Choudhary *et al*. 2007, Yadav *et al*. 2007, Gowhar *et al*. 2010) the genetic diversity in elite cultivars of field pea (*Pisum sativum* L.) in India has rarely been studied with genome wide molecular markers. This has necessitated in depth characterization of molecular diversity in the leading pea cultivars with markers derived from both expressed and unexpressed parts of the genome. The availability of highly polymorphic, locus specific, easily transferable and cost effective molecular markers distributed throughout the genome is of great value. Microsatellite markers have been developed from plant genomes from both coding and non coding sequences containing simple repeats. Microsatellite loci that are found in gene coding sequences are referred to as genic microsatellites. Sequence data obtained from several crop plants indicate sufficient homology existing between genomes in the region flanking the SSR loci. This allows primer pairs designed on the basis of the sequence obtained from one crop to detect SSRs in related crop species. Such homology in the flanking region of SSRs loci has extended the utility of these markers to related species or genera where no and/or very little information on SSR is available. This phenomenon is sometimes described as transferability of microsatellite primers across species/genera and Datta et al. (2010a, b, 2011, 2012) analyzed the transferability of microsatellite markers across different legume taxa and reported marker transferability from 36-95%.

The purpose of the present study was to investigate and quantify the magnitude of genetic diversity at molecular level between 24 pea cultivars and will help in selecting better parents for future breeding programs.

MATERIALS AND METHODS

Plant materials and DNA isolation

Twenty-four fieldpea cultivars used in the present study were developed and released in India over the past 50 years

using different breeding methods. Total genomic DNA was extracted from the leaves of three-week old plants of each genotype, grown in the net house, following the modified CTAB method (Abdelnoor *et al*. 1995). The extracted DNA was purified with RNase treatment (10 mg/ml) for 1 hour at 37°C followed by treatment with phenol: chloroform: isoamyl alcohol (25:24:1). The pellet was dissolved in appropriate amount of $T_{10}E_1$ (Tris10mM, EDTA 1mM) buffer. DNA from different samples was quantified both by visual quantification and UV spectrophotometer (Smart Spec Plus, BioRad, Hercules, USA) and finally diluted to a concentration of 25 ng/ml.

Microsatellite markers and PCR amplification

A total of 45 pea specific microsatellite markers were used for PCR amplification to study genetic diversity within the cultivars. Microsatellite markers were based on the sequences published by Burstin *et al*. (2001) and details are provided in Table 2. Length of the primers varied from 18 to 24 nucleotides. Markers were custom synthesized from Integrated DNA Technologies, USA. Amplification of SSR motif was conducted in 200 ml thin-wall PCR tubes using a "touch down" programme (Don *et al*.1991) in a PTC-200 gradient thermocycler (MJ Research, USA). PCR amplifications were carried out in total volume of 5 µl containing 5 ng genomic DNA, 1X PCR buffer, 0.1mM dNTPs (Bangalore Genei, Bengaluru), 0.1 unit of *Taq* DNA polymerase (Bangalore Genei) and 2.5 pM of each primer. An initial denaturation was given for 3 min at 95°C. Subsequently, five touch-down PCR cycles comprising of 94°C for 20 s, 60-56°C (depending on the marker as given in Table 1) for 20 s, and 72°C for 30 s were performed. These cycles were followed by 40 cycles of 94°C for 20 s with constant annealing temperature (depending on marker) for 20 s, and 72°C for 20 s, and a final extension was carried out at 72°C for 20 min. PCR products were checked by agarose gel (3%) electrophoresis and were separated in 1X TBE buffer. Digital images of gels were made using gel documentation system (Alpha Digi Doc, Alpha Innotech Corporation) (Fig.1). The sizes of alleles were determined by comparing with Gene Ruler 100 bp ladder (MBI Fermentas).

Statistical analysis

Markers were scored based on the band pattern generated from the gel imaging system for the presence or absence of the corresponding band among the genotypes. Using the binary coding system '1' indicating the presence of clear and unambiguous bands and '0' indicating the absence of bands. Polymorphism Information Content (PIC) (Anderson *et al*. 1993) was calculated for each marker using the following equation:

Polymorphism information content

$$(PIC_i) = 1 - \sum_{j=1}^{n} P_{ij^2}$$

Where, Pij is the frequency of the j^{th} allele for i^{th} marker and summation extends over 'n' alleles. The 0/1matrix was used to calculate genetic similarity as Dice coefficient (Dice 1945, Sorensen 1948) using SIMQUAL subprogram and the resultant similarity matrix was employed to construct dendrogram using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method of Arithmetic Means (UPGMA) as implemented in NTSYS-PC version 2.1 (Rohlf 1998) to infer genetic relationships and phylogeny.

In order to estimate the congruence among dendrograms, product moment correlation (r) was computed and compared using Mantel statistics (t) in MXCOMP program (Mantel 1967).

RESULTS AND DISCUSSION

Microsatellite polymorphism

A set of 45 microsatellite markers (27 genic and 18 genomic) were used to amplify 24 genotypes of pea. Of the 45 markers, 18 contained loci for di-nucleotide repeats and 25 amplified tri- nucleotide repeats, whereas, motifs for two markers was unknown. Allelic differences were determined by relative mobility in 3% agarose gel and the size of alleles was estimated by reference to a 100 base pair DNA ladder. Where a PCR product was not obtained, data for the relevant sample were treated as null allele. Out of total 45 markers, 39 markers amplified easily scorable alleles ranging from 110 to 1100 bp size in all the cultivars. Out of 39 markers, 20 (51%) were polymorphic and 19 (49%) were monomorphic (Table 2). Thirty-nine markers amplified a total of 55 alleles with an average of 1.4 alleles per marker. Among the total alleles amplified, 33 (61%) alleles showed polymorphism whereas, 21 (39%) alleles were found to be monomorphic. The highest number of alleles (6) was amplified by marker PEACPLHPPS, followed by PSBLOX13.1 and PSGDPP which produced three alleles each. Two alleles each were amplified by PEARHOGTPP, PEARHOGTPP, PSAJ3318, PSCAB66, PSBT2AGEN and PEAOM14A. Rest of the markers amplified single alleles. Eighteen markers PSBLOX13.1, PEACPLHPPS, AF016458, PEAATPASE, PEARHOGTPP, PSGDPP, PSP4OSG, AA430902, PSAS, PSGSRI, PEAPHTAP, PATRG31A, PSBLOX13.2, PSY14558, PSAJ3318, PSCAB66, PSLEGJP, PSLEGKL showed 100% polymorphism whereas 50% polymorphism was shown by PSBT2AGEN and PEAOM14A (Table 2). Among the polymorphic markers, maximum PIC value of 0.99 was shown by PSY14558 and minimum (0.12) with PSGDPP and PSLEGJP with the average value being 0.48. Earlier, Burstin *et al*. (2001) used the same set of markers in their study on 12 pea genotypes and they found 31 markers to be polymorphic. The higher number of polymorphic markers obtained by them may be due to the reason they selected more diverse genotype in their study which comprised of wide range of cultivated as well as exotic types.

Table 1. Pedigree and morphological descriptions of 24 pea genotypes used in the present study.

S. No.	Variety	Parentage	No. of seeds / Pod	Plant type	Yield per plant(g)	Days to flower	Days to maturity	100 seed weight (g)
1	HFP-4	T 163x EC 109196	6.0	dwarf	21.7	66	123	21.5
2	KPMR 144-1	Rachna x HFP 4	5.0	dwarf	23.6	62	121	18.9
3	HFP 8909	EC 109185 x HFP 4	6.0	dwarf	17.7	68	115	16.3
4	HUDP-15	(PG 3 x S143) x FC 1	6.0	dwarf	24.3	66	122	22.3
5	KPMR 400	Rachna x HFP 4	6.0	dwarf	25.3	62	120	23.0
6	KPMR 522	KPMR 156 x HFP 4	6.0	dwarf	24.7	62	128	19.2
7	IPFD 99-13	HFP 4 x LFP 80	5.0	dwarf	35.6	58	110	22.4
8	DDR 44	HFP 4 x KPMR 157	6.0	dwarf	20.7	64	122	20.8
9	SWATI	Flavanda x HFP 4	5.0	dwarf	15.3	62	119	21.4
10	JAYANTI	HFP 4 x PG 3	5.0	dwarf	27.0	64	124	20.0
11	RACHNA	T 163 x T 10	7.0	tall	24.3	68	126	23.3
12	HUP 2	(Alfaknud x C 5064) x S143	5.0	tall	39.3	66	126	17.6
13	KFP 103	KPMR 83 x KPMR 9	5.0	tall	39.7	68	127	20.1
14	JP 885	(T 163 x 6588-1) x 46C	4.0	tall	37.6	64	129	19.7
15	DMR 7	6587 x L 116	5.0	tall	28.6	68	125	22.0
16	PANT P5	T 10 x T 163	4.0	tall	28.3	68	128	25.2
17	VL 1	Selection from Miller	6.0	tall	24.6	66	126	17.4
18	Ambika	DMR 22 x HUP 7	4.0	tall	40.0	64	126	17.5
19	B 22	Selection of local material from Berhampore (W.B.)	5.0	tall	12.3	72	126	16.0
20	IPF 99-25	PDPD 8 x Pant P5	4.0	tall	32.3	60	118	20.0
21	Subrita	Rachna x JP 885	4.0	tall	36.4	63	125	18
22	PG 3	T 163 x Bonnevilla	6.0	dwarf	19.8	60	121	19.5
23	VL 3	Old Sugar x Wrinkled Dwarf	5.0	dwarf	20.1	61	126	17.8
24	JM 6	Local yellow Botri x (6588-1x 46C)	5.0	tall	18.9	67	125	17.3

In order to quantify the level of polymorphism, Dice estimate of similarity coefficients was used to generate a similarity matrix which is based on the probability that an amplified fragment from one plant will also be found in another. Similarity coefficient among pea genotypes varied from 0.75 to 0.96, the average being 0.84. Similarity coefficient values were highest (0.96) between the two pair of genotypes JM 6 and Jayanti and KPM 400 and HFP 4, followed by (0.94) among VL-3 and Subrita and DDR 44 and HFP 8909. Minimum values of similarity coefficients were observed between KPMR44-1 and Ambika (0.75) followed by KPF 103 and KPMR 44-1(0.76). Rachna and HFP 8909, and IPF 99-25 and KPMR 522 had coefficient values of 0.77. Tar'an *et al*. (2004) studied diversity within 65 pea varieties and 21 accessions from wild *Pisum* subspecies using RAPD and SSR markers. The pair wise genetic similarity value among the 65 varieties ranged from 0.34 to 1.0 in their study. Earlier, Yadav *et al*. (2007) conducted similar study in fifteen germplasm line of *Pisum sativum* with 12 RAPD markers. They observed a similarity coefficient value ranges from 0.263 to 0.793.

Polymorphism with genomic microsatellites

Out of the 45 microsatellite markers used in the present study, 18 (PSBLOX13.1, PEACHLROPH, PSADH1, CHPSTZPP, PEALCTN, PSRBCS3C, PEAATPASE, PSJ000640A, PSP4OSG, PEAEGL1, PSGSRI, PATRG31A, PSBLOX13.2, PSCAB66, PSLEGJL, PSLEGJP, PSLEGKL, PSLEGKP) were genomic. No amplification could be observed with the marker PEACHLROPH and PEAEGL1. These 16 markers amplified alleles of size range 110-800 bp. All the markers showed the polymorphic alleles except PSADH1, CHPSTZPP, PEALCTN, PSRBCS3C, PSJ000640A, PSLEGJL, and PSLEGKP. A total of 21 alleles were amplified by the 16 markers with an average of 1.31 alleles per marker. The highest number of allele (3) was amplified by the marker PSBLOX13.1, two alleles each was amplified by PSP4OSG, PSCAB66 and PSLEGJP; rest all the markers amplified one allele each. Maximum PIC (0.95) was obtained with PSGSRI whereas PSLEGJP showed the minimum PIC value i.e. 0.12 with the average value being 0.53. The genetic similarity coefficient value with the genomic microsatellite markers ranged from 0.70 to 0.97 and the average value was found to be 0.86 (Table 3). Genomic microsatellites are found to be more polymorphic as they are mostly developed from non transcribed regions of genome, thus, they are ideal for mapping and diversity studies. The utility and effectiveness of genomic microsatellites have been proven in many legumes like pigeonpea (Odeny *et al*. 2009, Saxena *et al*. 2009), chickpea (Winter *et al*. 1995, Buhariwalla *et al*. 2005) and common bean (Blair *et al*. 2003).

Polymorphism with genic microsatellite markers

A total of 27 genic microsatellite markers were used, out of which four markers (PEADRR230B, PSU81288, PEALEGBC, and AF029243) did not amplify any scorable bands. The 23 markers amplified scorable bands of the size range 110-1100 bp. Markers PEACPLHPPS, AF016458, PEARHOGTPP, PSGDPP, AA430902, PSAS, PEAPHTAP, PSY14558, PSAJ3318, PSBT2AGEN and PEAOM14A were found to be polymorphic

Table 2. Details of the properties of different primers used to evaluate genetic diversity and summary of their amplification in pea genotypes

Primer Name/ locus	Forward primer (5'-3')	Reverse primer (5'-3')	Mean Tm (^{0}C) used	Nature	Motif	No. of alleles	Allele size	PIC
PEAATPSYND	CTCCAGCCCATCATAGTCGAAG	TCACAACCGAAGTCACAACC	58	Genic	$(AC)_6$	1	200	-
AA427337	GCTAGCTAGACTAGTCTTTACAG	CTGTTCATAACTAAAAAACATCTC	50	Genic	$(AC)_5$	1	200	-
PSBLOX13.1	GAACTAGAGCTGATAGCATGT	GCATGCAAAAGAACGAAACAGG	54	Genomic	$(AT)_{17}$	3	270-300	0.94
PSGAPA1	GACATTGTTGCCAATAACTGG	GGTTCTGTTCTCAATACAAG	51	Genic	$(AT)_{17}$	1	200	-
PSADH1	GATGTGATAGGCCTAGAACAAGC	CAGTCACACACTACAAGAGATC	54	Genomic	$(AT)_{10}$	1	400	-
PEACPLHPPS	GTGGCTGATCCTGTCAACAA	CAACAACCAAGAGCAAAGAAAA	58	Genic	$(AT)_6$	6	260-1100	0.17
CHPSTZPP	TGAATAAAGGGCAGAGTTAATACA	GAATCACGGGACCAAAACC	55	Genomic	$(AT)_6$	1	350	-
PEALCTN	TATGCTTCCTCCTCGCGTTA	TTTTGCCCCTATTTCACTATTTA	50	Genomic	$(AT)_6$	1	210	-
PSRBCS3C	CCCAGTGAAGAAGGTCAACA	CAATGGTGGCAAATAGGAAA	58	Genomic	$(AT)_6$	1	210	-
PSY14273	AATTCGGCACGAGGAGAGA	TGCAGCCTTGAGCTGGTTAT	50	Genic	$(TC)_{18}$	1	300	-
AF016458	CACTCATAACATCAACTATCTTTC	CGAATCTTGGCATGAGAGTTGC	54	Genic	$(TC)_9$	1	170	0.94
PSU58830	CACACTCCATTTTCACCACCT	AGCATTGAAGAACAAAAGCACT	55	Genic	$(TC)_8$	1	220	-
AF004843	CCATTTCTGGTTATGAAACCG	CTGTTCCTCATTTTCAGTGGG	54	Genic	$(TC)_7$	1	220	-
PSARGDECA	CTGTTCCTCTTTCAAGCACTCC	GGGAAAGCAAAGCATGCGGATC	58	Genic	$(TC)_6$	1	250	-
PEAATPASE	TGCAACATTCTATCTCTCTCTTT	AGTAGCCACATCGGTGGAGA	55	Genomic	$(TC)_6$	1	200	0.39
PEARHOGTPP	ACGCTTCAACGGCAAAAT	AGGACCCCAATCACTCTCAC	58	Genic	$(TC)_5$	2	200,300	0.24
PSJ000640A	GTCCACCTCCCGGGTTCGAA	CGGCTAGAAGAACCACCCCCAT	60	Genomic	$(AAC)_7$	1	200	-
PSZINCFIN	CGCGGAGTTTACATCAGGTC	CTGGCCTAATAATGGCAACC	60	Genic	$(AAC)_5$	1	200	-
PSGDPP	AAACCGTGCAACTCTGAAGC	AAGAAACCCACCAACACGTC	60	Genic	$(AAC)_5$	3	200-500	0.12
PSP4OSG	CAACCAGCCATTATACACAAACA	GGCAATAAAGCAAAAGCAGA	58	Genomic	$(AAT)_{36}$	2	250,350	0.49
AA430902	CTGGAATTCTTGCGGTTTAAC	CGTTTTGGTTACGTCGAGCTA	54	Genic	$(AAT)_7$	1	200	0.44
PSAS	GGTGATAACTATTTGGCTCATC	GTAGATTTCTCCATTCACCTG	54	Genic	$(AAT)_6$	1	250	0.5
PSGSRI	TGGATTGGATTGGATGATGA	TGGAGCCCTTAGTCCACAAC	60	Genomic	$(AAT)_{14}$	1	200	0.95
PEAPHTAP	TGAAACCACCATTCTCTGGA	AAGACCCCACTTGAAAATTACTTC	58	Genic	$(AAT)_5$	1	200	0.16
PATRG31A	CATGAAATGGAATAATCTTATG	CAGTCTAGTTGGCATATACC	48	Genomic	$(AAT)_4$	1	500	0.39
PSBLOX13.2	CTGCTATGCTATGTTTCACATC	CTTTGCTTGCAACTTAGTAACAG	54	Genomic	$(CAT)_8$	1	110	0.8
PSY14558	ACATGTCTCTGTTAGTGTG	GCCAATATCTTCTTTGTTGAAG	48	Genic	$(CAT)_7$	1	150	0.99
PSAJ3318	CAGTGGTGACAGCAGGGCCAAG	CCTACATGGTGTACGTAGACAC	58	Genic	$(CAT)_6$	2	180,650	0.66
PSCAB66	CACACGATAAGAGCATCTGC	GCTTGAGTTGCTTGCCAGCC	55	Genomic	$(CAT)_5$	2	300,800	0.28
PSBT2AGEN	GCAGCAGAGCTTGTCTTTGAG	GGAATCAGAAACAGCCTTGGG	58	Genic	$(CCT)_5$	2	110,290	0.37
PEAOM14A	GGTGCCCTAGCATTTGCTG	TAGTAACAACCGCGCTCAAA	60	Genic	$(CCT)_5$	2	200,500	0.15
PSLEGJL	GGTTCGTCGATTCAGAAAAGG	CACATTAGTTTAATAGTTACC	49	Genomic	$(GAA)_8$	1	200	-
PSLEGJP	GCGAGTTGAGGGAGGTCTCCGC	GTCGGCACGTGCAGCGTCCGC	61	_	_	2	250, 280	0.12
PSLEGKL	CCATTCATACAGTATGCTCT	ATAGTTAGTACTATACACACC	50	Genomic	$(GAA)_8$	1	700	0.44
PSLEGKP	GCGAGTTGAGGGAGGTCTCCGC	CTGATACGACCAGCACGTGGG	61	_	_	1	250	-
PSU51918	GTCGTAACAGATCAATATGGC	CGATAGTGAGAGTGGCGGTTG	54	Genic	$(GAA)_6$	1	150	-
PSY17134	GAGGCAATCCTTCGTTTCTC	CGAGTAAAGCCGCATAGAGC	58	Genic	$(TGG)_5$	1	380	-
PSU81287	AGAGACACCGGAAGATCGAG	CATCCCCATAGCCACCAC	58	Genic	$(TGG)_7$	1	280	-
PS11824	ACCACCACCACCGAGAAGAT	TTTGTGGCAATGGAGAAACA	60	Genic	$(TGG)_5$	1	200	-

whereas rest markers showed monomorphic bands. Total 34 alleles were amplified by the 23 markers with an average of 1.48 alleles per marker. The marker PEACPLHPPS amplified the highest number of six alleles. Three alleles were amplified with PSGDPP, whereas two alleles each were amplified with the markers PEARHOGTPP, PSAJ3318, PSBT2AGEN, PEAOM14A. Rest of the markers amplified only one allele each. Maximum PIC (0.99) was obtained with PSY14558 and minimum with PSGDPP (0.124). Dice similarity coefficient value for genic microsatellites ranged from 0.71 to 0.98 and the average coefficient being the 0.84 quite close to genomic microsatellites (Table 3). Evaluation of germplasm with microsatellite markers derived from genes or ESTs might enhance the role of genetic markers by assaying the variation in transcribed and known-function genes, although there is a higher probability of bias owing to selection. Expansion and contraction of microsatellite repeats in genes of known function can be tested for association with phenotypic variation or, more desirably, biological function (Varsheney *et al*. 2005). The microsatellite markers derived from EST are considered less powerful in the discrimination of genotypes than other sources. Eujayl *et al*. (2001) compared genic and genomic SSR markers to investigate genotypic variation of 64 durum wheat lines, land races, and varieties obtaining 255 polymorphic loci among 137 EST microsatellite markers and 505 among 108 genomic microsatellite markers, with an average of 4.1 and 5 alleles per locus, respectively. Earlier studies by many researchers also reported that genic markers are less polymorphic (Scott *et al*. 2000, Rungis *et al*. 2004) because of greater sequence conservation in transcribed region however, several studies have found that genic SSRs are useful for estimating genetic relationship (Hempel *et al*. 2007) and at the same time provide opportunities to examine functional diversity in relation to adaptive variations (Eujayl *et al*. 2001). The low level of polymorphism detected with genic microsatellites may be compensated by their higher potential for cross species/genus transferability.

Our study find that genomic microsatellites were more efficient in detecting polymorphism between the 24 pea

Table 3. Comparison between genomic and genic SSRs in terms of their ability to reveal polymorphism

	Genic markers	Genomic markers	Total
Markers used	27	18	45
Marker amplified	23(85%)	16(89%)	39 (87%)
No of monomorphic markers	12 (52%)	7 (44%)	19 (49%)
No of polymorphic markers	11 (48%)	9 (56%)	20 (51%)
Average PIC value	0.43	0.53	0.48
No. of alleles amplified	34	21	54
Similarity coefficient value (Avg)	0.84	0.86	0.85
Size range (bp)	110-1100	110-800	110-1100

genotypes as compared to their genic counterparts, however, genic microsatellites amplified more alleles. The average PIC value of genomic microsatellite markers was higher (0.53) than that of genic one (0.43). The Mantel matrix correspondence test used to compare the similarity matrices and the correlation coefficient was found to be 0.819. The test indicated that clusters produced based on genic and genomic microsatellite markers were conserved since the minimum required matrix correlation value was 0.80. The finding of this study showed that genic microsatellite are equally good for polymorphism studies along with genomic SSRs. Hanai *et al*. (2007) observed similar results while comparing genic and genomic microsatellites in common bean.

In the present investigation three DNM (di-nucleotide motif) and six TNM (tri-nucleotide motif) of varied repeats length were used to survey the level of polymorphism (Table 4). Most of the markers used (21) were from TNM whereas rest had DNM. The maximum no. of alleles (28) was amplified by TNM which ranged from 3-10 with an average of 4.66 alleles/ tri-nucleotide motif. Among the TNMs, CAT repeat was found to be most informative in observing polymorphism in *Pisum* genome which is followed by AAT. Similarly, 24 alleles were amplified by DNM with an average of eight alleles/dinucleotide motif. The average PIC value of DNM was 0.127 whereas TNM revealed a higher average PIC value of 0.26. In this study no correlation was observed between number of repeats with either the alleles amplified or with the PIC value however, it was found that TNMs were more useful in detecting polymorphism when compared with DNMs. This result contrasts the earlier findings of Cupic *et al*. (2009) where a

Table 4. The efficiency of different microsatellite repeat motif in detecting polymorphism in pea

Repeat Motif	No. of repeats	No. of markers	No. of alleles	Average PIC
(AC)	5-6	2	2	0
(AT)	6-17	7	14	0.158
(TC)	5-18	7	8	0.224
(AAC)	5-7	3	5	0.04
(AAT)	4-36	6	7	0.48
(CAT)	5-8	4	6	0.682
(CCT)	5	2	4	0.245
(GAA)	6-8	3	3	0.146
(TGG)	5-7	3	3	0

significant correlation between number of alleles and PIC values in *Pisum* genome was found.

Cluster analysis

Determining the relatedness among potential parents forms the basis for choosing genetically distant parents in a breeding programme. Cluster analysis indicated the ability and usefulness of SSR markers for studying the differentiation and relatedness among pea genotype. The genetic relationship among the 24 pea genotypes has been investigated using SSR profiles. It is evident from the cluster analysis that the field pea cultivars can be broadly grouped into two clusters (A and B) (Fig 2.). The cluster A includes all the tall type cultivars except HUDP 15 (a dwarf cultivar generated from an exotic line S 143). Three tall cultivars *viz*. PG 3, Rachna and DMR 7 positioned themselves away from any core cluster because of wide geographical distribution of their parents. Most of these tall cultivars have "T 163" as a parent directly or indirectly in their pedigree. Cluster B is constituted by all the dwarf cultivars except HUP 2 (a tall cultivar generated from an exotic line Alfakund) and Subrita (a cultivar generated from diverse background). HFP 4 has been involved as one of the parent in the pedigree of most of the dwarf type cultivars. Again, if the pedigree analysis of HFP 4 is done, an obsolete cultivar "T 163" is one the parent.

In a recent study, the pedigree analysis of released cultivars in India has been traced back to 26 ancestors (Dixit and Katiyar 2006). Out of these 26 ancestors, three ancestors contributed 49% of the genetic base. T 163 was the most

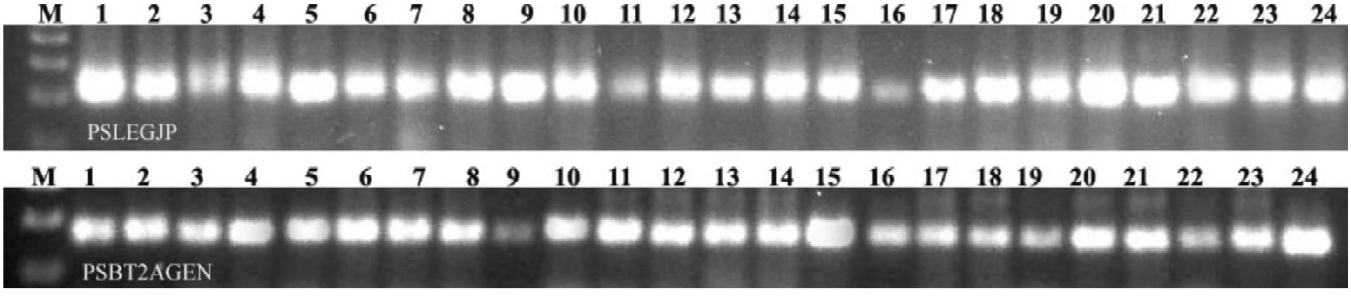

Fig. 1. Amplification profile of 24 pea cultivars obtained with microsatellite markers PSLEGJP and PSBT2AGEN. Lanes M; Molecular weight marker, 100 bp DNA ladder, Lanes 1-24; 24 pea genotypes as per the serial in Table 1.

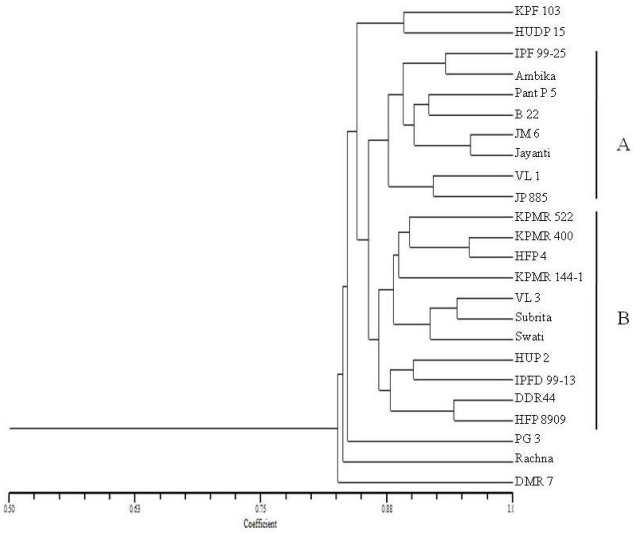

Fig. 2. UPGMA dendrogram based on the 54 alleles amplified in 24 pea genotypes

frequently used parent followed by EC 109196 and T 10. T 163 was mostly used for its wide adaptability whereas T 10 was used as donor parent for powdery mildew resistance. EC 109196 was used as a source of *afila* gene and dwarf plant type. T 163 contributed maximum to the genetic base of field pea with occurrence more than 51%. In other words, at least 51% cultivars of field pea released so far in India are more or less related due to involvement of T 163 in their pedigree. This has led towards genetic erosion and the narrowing of genetic base in this crop. So, it is desirable to have more diverse and usable genetic backgrounds in future varieties to provide protection against biotic and abiotic stresses. Furthermore this study highlighted the importance of genic microsatellite markers for use in resolving diversity.

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