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Assessment of genetic diversity in pea (*Pisum sativum* L.) using morphological and molecular markers

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Abstract

A total of 34 pea (*Pisum sativum* L.) genotypes including 7 adapted varieties, 6 popular local cultivars and 21 advanced breeding lines developed through crossing of elite cultivars were evaluated for genetic diversity and relatedness with 16 morphological traits and 15 SSR markers. Genotypes *viz*., DDR-23, E-6, *Makuchabi* and KPMR-885 were identified as early flowering while Rachna, IPFD 09-2, CAU FP-1, IPFD 1-10 and Pant P-136 were identified as high yielding. The number of alleles per SSR marker varied from 2 to 5 per locus. Polymorphic information content values (PIC) ranged from 0.105 to 0.560 per locus. Variability among groups (FIS=0.938) and variability within individuals (FIT=0.948) was low. The minimum and maximum molecular genetic distances were found to be 0.12 (Pant P-136 with VL-51) and 0.78 (E-6 with LP-4) respectively. Genotypes IPFD 09-2, HFP-620, Azad P-1, *Matek*, IPFD 1-10, CAU FP-1, IPFD 09-3, Pant P-136, Rachna, E-6, *Matek* and LP-3 showed high level of genetic diversity. Pea improvement through hybridization by utilizing diverse genotypes is suggested for breeding suitable genotypes for North Eastern region.

Key words: Fixation index, Genetic diversity, genetic distances, SSR, PIC

Introduction

Pea (*Pisum sativum* L.) is considered to be one of the world's oldest crops, since it was first cultivated with cereals like barley and wheat, 9000 years ago [1]. It is a native crop of Syria, Turkey, Israel, Ethiopia, and has been cultivated in India for several thousand years for its versatile use as vegetables, pulses and livestock feeds [2]. Its production ranks second amongst the cool season pulses in the world and the third largest area in pea cultivation is occupied by India with 6.3 lakhs ha with a production of 36.7 lakh tonnes and productivity of only 9.6 t/ha [3] after Canada and Russia. It is mostly grown in Uttar Pradesh, Madhya Pradesh, Bihar, Assam, and Orissa which together accounts for about 95% of the total area and production. Land races of pea have been grown in North Eastern India since time immemorial. Domestication of *Pisum sativum* L. for different end uses has led to emergence of morphologically distinct types. These different morphological types are commonly utilized in the form of above ground mass for livestock feeding and as immature pods or seeds for human consumption. Work in India through AICRP on pulse crop has led to introduction of diverse genotypes in this area. But due to varied agro-ecological niches, only a few of these got adapted in all niches/environments.

A replacement of landraces and traditional population with modern cultivars, particularly replacement with cultivars characterized by superior tolerance to biotic and abiotic stress is widespread and caused loss of genetic variability hence, narrowing down the genetic base. This may lead to danger of genetic uniformity and lack of diversity for further improvement in future. Genetic diversity is a prerequisite, for increasing yields and for stabilizing production in the face of disease epidemic and fluctuating environmental condition. In a plant breeding program, estimates of genetic relations among parental lines may be useful for determining which material

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should be combined in crosses to maximize genetic gain. Diverse genetic background among parental lines provides an ample supply of allelic variation that can be used to create new favorable gene combinations [4]. Therefore, the aim of the present study was to assess the level of genetic diversity within the collection of pea genotypes to aid in the selection and efficient utilization of diverse genotypes in breeding.

Materials and methods

Plant materials

A total of 34 pea (*Pisum sativum* L.) genotypes including 7 adapted varieties, 6 local cultivars and 21 advanced breeding lines were taken to evaluate their genetic diversity and relatedness. All the advanced breeding lines meant for evaluation in the North Eastern Hill region were collected from Indian Institute of Pulse Research (IIPR), Kanpur through AICRP's (All India Coordinated Research Project) Centre, ICAR-NEH region. The adapted varieties of this region and local cultivars were collected from the Directorate of Research, Central Agricultural University, Imphal. The details of the genotypes along with their pedigree and origin/source are given in Table 1. The genotypes were sown along with 4 checks *viz*., IPFD 1-10, HUDP-15, DMR-7, and Rachna in an augmented block design consisting of five blocks to evaluate their morphological characteristics during *rabi* 2010-11.

Morphological traits

The morphological traits and their status were scored in codes ranging from 1 to 9 as per the national test guidelines for the conduct of tests for distinctness, uniformity, and stability [5] of Pea (*Pisum sativum* L.). Five random plants from each plot were used to record the data on qualitative and quantitative characters. For analysis of morphological data, each character is treated to be a locus and the corresponding score for the phenotype is considered as an allele. Then, the data is used as an input to find out the Rogers' genetic distance and constructing corresponding tree using UPGMA method with Power Marker Software Version 3.25 [6].

Molecular study

Total genomic DNA was isolated from the young leaf tissues collected from 5 randomly selected healthy plants per genotype using the CTAB (Cetyl trimethylammonium bromide) method [8] with minor modifications. The leaf sample was collected before flowering and stored at –80^{o}C until DNA extraction. A total of 32 SSR markers were randomly selected with a probable total coverage of the pea chromosomes.The primers were synthesized by GCC BIOTECH Pvt. Ltd., New Delhi.

PCR amplification was performed on 10 µl final volume reactions containing 10 ng template DNA, including 0.5 unit Taq polymerase (5 units/µl, Fermentas), 1µl of 10 X PCR buffer (Fermentas) 0.5 ìl of DMSO, 0.5 mM of dNTP, 5 pmol of the forward and reverse oligonucleotide primers. The PCR conditions were: 95°C for 3 min. followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 55-60°C (depending upon GC Content of the primers) and 45 seconds at 72°C. Final elongation step was at 72°C for 3 min. The samples of PCR amplification were stored at 4°C before loading. The amplified products were electrophoretically separated at 90 V in a 2.5-3% agarose gels (Sigma, Ultra Pure Agarose 1000) for 2 h along with the 100 bp Plus Ladder (Fermentas, Generuler), stained with ethidium bromide, visualized by Biorad Gel Documentation System and documented thereafter.

Genotype score for SSR data

For each SSR marker and each sample, fragment sizes were visualized by comparison with the 100 bp Plus Ladder (Generuler, Fermentas) and genotype scoring was carried out manually as a/a for the single (homozygous) band and b/b, c/c, d/d for the next higher bands. Also the alleles are assigned band size relative to the molecular size ladder. Genotype data in numerical digit of the respective band size was archived in Excel tables for further analysis with the software Power Marker Version 3.25 [6] and Arlequin Version 3.5.1.2 [7].

Results and discussion

Morphological variation

Within the collection studied, morphological variation was observed in terms of seed, plant type, pod, stipule, flower colour and time of flowering which are important traits for identification, characterisation and grouping of genotypes. Stem anthocyanin colouration was observed in five genotypes (LP-1, LP-3, *Matek*, *Makuchabi* and *Apakpi*). All the advanced breeding lines and the already adapted varieties do not have stem anthocyanin pigmentation, however the pigmentation is present on five out of the six local cultivars. Four genotypes *viz*., HUDP-15, KPMR-871, HUDP-904 and IPFD 09-2 were leafless. HUDP-904 and IPFD 09-2 involved HUDP-15 in their pedigree.

Table 1. Details of the 34 pea genotypes including name of the genotype, their pedigree and origin/source

Code	Variety	Pedigree	Origin/Source
A.	**Adapted varieties**		
1.	IPFD 1-10	PDPD 8 x HUDP 7	IIPR, Kanpur
2.	HUDP-15	PG 3 (PG 3 x S-143) FC1	BHU, Varanasi
3.	DMR-7	6588 x T-163	IARI, New Delhi
4.	Rachna	T-163 x T-10	CSA, Kanpur
12.	E-6	Massey Gem x Harabora	Ludhiana, Punjab
17.	Azad P-1	6416 x 6405	CSA, Kanpur
27.	Arkel	Exotic variety	France
B.	**Advanced breeding lines**		
5.	TRC P-8	T-163 x DMR 7	Tripura
6.	Pant P-142	FC 1 x Pant P 11	GBPUA&T, Pantnagar
7.	HFP-620	Uttara x Arkel	HAU, Hissar
8.	DDR-88	HFP 4 x Pusa 10	IARI, New Delhi
9.	DDR-87	P 1563 x P 1556	IARI, New Delhi
10.	Pant P-136	Pant P 14 x FC 1	GBPUA&T, Pantnagar
11.	NDP 9-408	NDP 62-5 x Selection NDP 13-2	NDUA&T, Faizabad
13.	DMR-61	KPMR x DDR 23	IARI, New Delhi
14.	IPFD 09-3	KPMR 552 x IPFD 99-13	IIPR, Kanpur
15.	DDR-50	HFP-4 x P 1709	IARI, New Delhi
16.	KNS-8	Mutant from Makhayatmubi (local cultivar)	CAU, Imphal
18.	IPFD 09-2	IPFD 98-1 x HUDP 15	IIPR, Kanpur
19.	VL-51	LFP 303 x DMR 11	VPKAS, Almora
20.	KPMR-747	DMR 11 x HFP 4	CSA, Kanpur
21.	VL-52	DMR 11 x DMR 12	VPKAS, Almora
22.	KPMR-728	HUP 11 X KPMR 157	CSA, Kanpur
23.	DDR-23	HFP 4 x Pusa 10	IARI, New Delhi
24.	KPMR-871	Sapna x HFP 8909	CSA, Kanpur
25.	HUDP-904	HUDP 15 x Pusa 10	BHU, Varanasi
26.	KPMR-885	HFP 4 x DDR 13	CSA, Kanpur
28.	CAU FP-1	Selection from Makhayatmubi (local cultivar)	CAU, Imphal
C.	**Local cultivars**		
29-34.	LP-1, LP-3, LP-4, Matek, Makuchabi, Apakpi	Local pea cultivars	CAU, Imphal

Purple petal colour was observed only in the 6 local cultivars and the rest were recorded to have white colour petals. Seed testa mottling was also observed only in the 6 local cultivars and absent in other genotypes. Early flowering genotypes were DDR-23 (45 days), E-6 (53 days), *Makuchabi* (55 days), KPMR-885 (58 days) and late flowering were Pant P-142, IPFD 09-3 and HUDP-15 (> 80 days). IPFD 1-10, Pant P-136, IPFD 09-2, KPMR-747, CAU FP-1, E-6, HUDP-904, KPMR-871, LP-3, LP-4, and *Matek* produced large seeds whereas small seeded genotypes were VL-51 and VL-52. The genotype *Matek* was tallest with plant height of 117 cm and DDR-88 had the shortest mean plant height (19 cm) (Table 2). High genetic variation among the individual genotypes might be due to the use of diverse germplasm in breeding programmes adopted by different institutes.

Morphological marker-based genetic distance and UPGMA cluster analysis

The morphological distance analysis did not include the traits for yield per plant and seed number per plant because no codes are provided for scoring as per the National Test Guidelines for the Conduct of Tests for DUS. The minimum genetic distance based on morphological marker was zero which was observed in DDR-50 with DMR-61, KPMR-871 with IPFD 1-10 and Pant P-142 with NDP 9-408. This showed their close morphological relationship to each other and inability of morphological markers to differentiate among these genotypes. The maximum genetic distance observed was 0.81 in the genotype E-6 with *Apakpi* and Pant P 09-2 with *Makuchabi*. The average genetic distance based on morphological data was 0.33.

On the basis of Rogers' distance [8], five major clusters were obtained by truncating the dendrogram at the distance value of 0.30 (Fig. 1). High variation for most of the morphological characteristics, namely plant height, flowering time, seed weight, flower colour, seed surface and seeds per plant was recorded. Arkel and Azad P-1 have low mean seed weight as they produced wrinkled matured seeds. The genotypes which showed relatively high yield were Rachna, IPFD O9-2, CAU FP-1, IPFD 1-10 and Pant P-136.

Local cultivars were grouped together in one extreme group labelled as A with LP-3 and LP-4 as more closely related genotypes. Among the local landraces, genotype LP-3 and Matek were separated by a maximum morphological distance of 0.44. In group B, 3 adapted varieties (IPFD 1-10, Arkel, HUDP-15) and the 12 advanced breeding lines including DDR-23 and DDR-88 formed the cluster. Genotype HUDP-15 and HUDP-904 formed a sub-group in group B showing that their relatedness for HUDP-904 descended from HUDP-15. Similarly, DMR-7 and Rachna which were on the same branch in the dendrogram might be due to a common parent T-163 in their pedigree. Likewise, genotype KNS-8 and CAU FP-1 were also grouped together in group C because of *Makhayatmubi* is the common parent in their origin. E-6 formed a separate group from the rest of the genotypes indicating its distinct morphological variability with others. E-6 showed early flowering, short plant height, single pod/axil, pointed pod, bold and wrinkled seed shape. The clustering of varieties based on morphological traits was partially explained by the association between the markers/traits and the

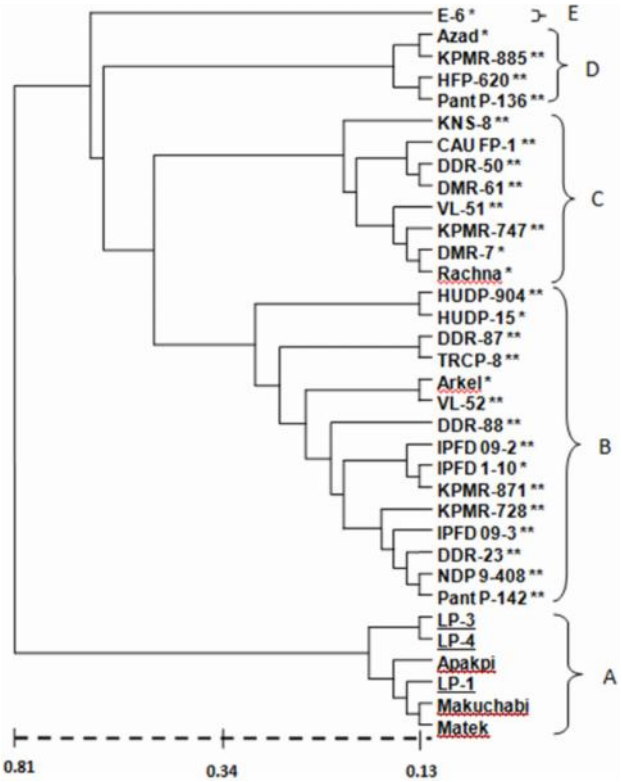

Fig. 1. Dendrogram based on Rogers' genetic distance among 34 pea genotypes using 16 morphological markers. Note: (*) = adapted varieties, (**) = advanced breeding lines, underlined = local cultivars

involvement of specific parents in their pedigree [9, 10]. Therefore, agreement between marker-based clusters and the pedigrees of the varieties is expected; for example, KPMR-747 and VL-52 along with VL-51 which were found in one sub-group of group II, were related to the common parent DMR 11 (Table 1 & Fig. 4); Rachna and TRCP-8 which formed sub-group in group II, were related to the common parent T-163; KNS-8 and CAU FP-1, found in sub-group of group III, were related to the common parent Makhayatmubi (Table 1 & Fig. 1). The trend observed in the results was in agreement with the previous findings [2, 9].

Molecular variation

Out of 32 SSR primer-pairs applied, amplification was not observed for 11, unclear fragments or faint bands were observed for 5 primer-pairs and monomorphic fragment was observed for one primer-pair. Hence, 15 SSR markers which were found to be polymorphic with clear and distinct fragments were used in further analysis. Total number of alleles observed was 43 and number of alleles per locus varied from 2 to 5,

with an average number of 2.866 alleles per locus (Table 3). Heterozygosity was observed in the two loci i.e., PSMPAA 31 and PAMPAD 99 (Fig. 2) with 33% and 12%, respectively with the overall mean of 3% which was low. Polymorphic information content values (PIC) ranged from 0.105 to 0.560 per locus with an average 0.401 indicating low gene diversity. The number of alleles observed suggested that the most informative loci were PSMPAD 99 with 5 alleles, PSMPSAA 205 with 4 and PSMPA 8, PSMPA 9, PSMPA 14, PSMPA 20, PSMPAA 31, PSMPA 5, PSMPAA 67, PSMPAA 476 with 3 alleles each. The low PIC values and less number of alleles per marker obtained in the study may be due to small population studied and few polymorphic markers because both the number of alleles detected per marker and gene diversity of markers depend on the number of genotypes analyzed [11]. SSR primer PSMPAD 99 has shown amplification in almost all the genotypes and indicated polymorphism with amplicon size ranging from 305 to 450 base pairs. The primer has showed amplification of homozygous and heterozygous loci. Heterozygous loci were observed in the genotypes IPFD 09-2, KPMR-871, Arkel and *Apakpi*. Fixation index ranged from 0.438 to 1.000 with an average of 0.946 which was found to be high. High fixation index in most of the loci was expected in the study as the genotypes under study were self-pollinated already adapted varieties, advanced breeding lines and local cultivars.

In analysis of molecular variance to assess variability among, within suggested-groups and within individuals, variability among groups was very low due to high fixation index (FIS=0.938). It was also observed that the variance within individuals was low as there were only few heterozygous loci observed across the genotypes (Table 3) due to high fixation index (FIT=0.948). However, high variability among the individual genotypes within groups was also recorded which may be due to low fixation index of the alleles (FST=0.163). This high variability among individuals within groups was likely due to the fact that genotypes were different and diverse purelines, homozygous at almost all the loci having different genetic constitution from one another. The findings of the present study are in conformity with the results presented earlier [12, 13].

Molecular marker (SSR)-based genetic distance and UPGMA cluster analysis

The minimum and maximum genetic distances based on molecular markers were 0.12 (Pant P-136 with VL-51) and 0.78 (E-6 with LP-4) with an average of 0.41. The UPGMA cluster analysis revealed five major groups. The adapted varieties were clustered in group II and III. The local cultivars were clustered in group I and group III. LP-1, LP-4, LP-3, were grouped together with KPMR-885 and KPMR-871 in cluster I; KPMR-747 and VL-52 along with VL-51 were found in one sub-group of group II; Rachna and TRCP-8 formed sub-group in group II. KNS-8 and CAU FP-1 were found in sub-group of group III. Group III consisted 5 adapted varieties (DMR-7, IPFD 1-10, Azad P-1, HUDP-15, and Arkel), 2 advanced breeding lines (KPMR-728 and HUDP-904) and 3 local cultivars (*Apakpi*, *Matek* and *Makuchabi*). HUDP-15 and HUDP-904 were grouped in one sub-group of group III. The advanced breeding lines DDR-23 and IPFD 09-2 were clustered in separate major group labelled as IV and V (Fig. 3). The clustering of varieties according to molecular marker was partially explained by the association between the markers and specific parents in their pedigree. Therefore, one would expect agreement between marker-based clusters and pedigrees of the varieties for example, KPMR-747 and VL-52 along with VL-51 found in one sub-group of group II were related to the common parent DMR 11. Rachna and TRCP-8 formed sub-group in group II were related to common parent T-163; KNS-8 and CAU FP-1 found in sub-group of group III were related to the common parent *Makhayatmubi* (Table 1). However, discrepancies have also been reported between pedigree information and marker-based clusters, for example, IPFD 09-2 and HUDP-904 which shared HUDP-15 as a common parent, were grouped in different clusters. Such exceptional case may be explained by pedigree analysis of any cultivar which inherits 50% of its alleles from each of the parents, however, due to intense selection this value might deviate by 20% [14].

Correlation between the genetic distance matrices derived from morphological and molecular markers

Genetic similarity/dissimilarity was often evaluated by combination of pedigree, morphological and molecular data as reported earlier [4, 9, 12, 15]. Comparison of Mantel testing of matrices resulted in rather low correlations between different marker systems. In present study, a joint analysis of molecular markers compared to morphological markers showed a low but positive significant correlation (r = 0.219). Similar results of low to medium positive correlation was also reported in pea (r=0.353) [15, 16] and in pepper (r =

Table 2. Morphological traits of the pea genotypes with their scores/indices

Sl.	Genotype	SA	Sc	FC	Sc	L	Sc	LAC	Sc	PC	Sc	SRE	Sc	PDE	Sc	PP	Sc	SS	Sc	SSR	Sc	CC	Sc	STM	Sc	DF	Sc	NFF	PH	Sc	MPA	TW	Sc	YP
1	IPFD 1-10	A	1	Dg	7	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	74	4	14	47	3	2	234.39	7	9.98
2	HUDP-15	A	1	Dg	7	A	1	G	1	W	1	P	9	B	2	P	9	Sp	1	S	1	G	5	A	1	93	4	17	35	3	2	200.84	7	5.30
3	DMR-7	A	1	G	5	P	9	G	1	W	1	P	9	B	2	P	9	Sp	1	S	1	C	3	A	1	78	4	14	67	5	2	188.73	5	5.51
4	Rachna	A	1	G	5	P	9	G	1	W	1	P	9	B	2	P	9	Sp	1	S	1	C	3	A	1	79	4	14	77	5	2	216.21	7	9.84
5	TRCP-8	A	1	G	5	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	Wr	2	C	3	A	1	73	4	9	74	5	2	191.86	5	5.70
6	Pant P-142	A	1	Dg	7	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	84	4	15	50	3	2	183.18	5	4.74
7	HFP-620	A	1	Dg	7	P	9	G	1	W	1	A	1	Pt	1	P	9	Sp	1	S	1	G	5	A	1	70	3	11	38	3	2	180.78	5	11.80
8	DDR-88	A	1	Dg	7	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	Wr	2	C	3	A	1	79	4	10	19	3	1	187.44	5	4.15
9	DDR-87	A	1	G	5	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	Wr	2	C	3	A	1	73	4	11	20	3	2	186.90	5	5.80
10	Pant P-136	A	1	Dg	7	P	9	G	1	W	1	P	9	B	2	P	9	Sp	1	S	1	G	5	A	1	69	3	15	46	3	2	252.00	7	10.19
11	NDP 9-408	A	1	Dg	7	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	75	4	13	37	3	2	210.50	7	7.47
12	E-6	A	1	G	5	P	9	G	1	W	1	A	1	Pt	1	P	9	Sp	1	Wr	2	G	5	A	1	53	3	9	45	3	1	233.21	7	6.03
13	DMR-61	A	1	G	5	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	65	3	12	77	5	2	206.35	7	6.55
14	IPFD 09-3	A	1	G	5	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	100	4	12	32	3	2	188.91	5	8.00
15	DDR-50	A	1	G	5	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	70	3	13	79	5	2	211.05	7	8.14
16	KNS-8	A	1	G	5	P	9	G	1	W	1	P	9	Pt	1	P	9	Sp	1	S	1	C	3	A	1	67	3	12	90	7	2	221.92	7	8.66
17	Azad P-1	A	1	Dg	7	P	9	G	1	W	1	P	9	B	2	P	9	Sp	1	Wr	2	G	5	A	1	69	3	12	42	3	2	200.77	7	7.30
18	IPFD 09-2	A	1	Dg	7	A	1	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	81	4	14	48	3	2	202.30	7	12.82
19	VL-51	A	1	Dg	7	P	9	G	1	W	1	P	9	Pt	1	P	9	Sp	1	S	1	C	3	A	1	76	4	12	69	5	2	155.32	5	6.03
20	KPMR-747	A	1	G	5	P	9	G	1	W	1	P	9	B	2	P	9	Sp	1	S	1	C	3	A	1	72	4	14	62	5	2	271.57	7	7.20
21	VL-52	A	1	Dg	7	P	9	G	1	W	1	A	1	Pt	1	P	9	Sp	1	S	1	C	3	A	1	79	4	13	32	3	2	134.49	3	6.22
22	KPMR-728	A	1	Dg	7	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	G	5	A	1	79	4	16	34	3	2	160.00	5	4.65
23	DDR-23	A	1	Dg	7	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	45	2	10	47	3	2	162.92	5	5.51
24	KPMR-871	A	1	Dg	7	A	1	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	77	4	12	44	3	2	179.66	5	9.83
25	HUDP-904	A	1	G	5	A	1	G	1	W	1	P	9	B	2	P	9	Sp	1	S	1	C	3	A	1	79	4	16	37	3	2	200.43	7	11.71
26	KPMR-885	A	1	Dg	7	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	Wr	2	G	5	A	1	58	3	10	74	5	2	167.19	5	6.14
27	Arkel	A	1	Dg	7	P	9	G	1	W	1	A	1	Pt	1	P	9	Sp	1	Wr	2	G	5	A	1	71	4	11	41	3	2	148.21	3	4.90
28	CAU FP-1	A	1	Lg	3	P	9	G	1	W	1	A	1	B	2	P	9	Sp	1	S	1	C	3	A	1	66	3	15	69	5	2	280.00	7	12.00
29	LP-1	P	9	G	5	P	9	Pr	2	Pr	5	P	9	B	2	P	9	Sp	1	S	1	G	5	P	9	75	4	16	74	5	2	206.02	7	6.03
30	LP-3	P	9	G	5	P	9	Pr	2	Pr	5	A	1	B	2	P	9	Sp	1	Wr	2	W	7	P	9	73	4	12	78	5	1	263.67	7	6.13
31	LP-4	A	1	G	5	P	9	G	1	Pr	5	A	1	B	2	A	1	Sp	1	Wr	2	W	7	P	9	63	3	9	72	5	1	213.24	7	4.97
32	Matek	P	9	G	5	P	9	Pr	2	Pr	5	P	9	B	2	A	1	Sp	1	S	1	G	5	P	9	61	3	12	117	7	2	238.79	7	8.25
33	Makuchabi	P	9	G	5	P	9	Pr	2	Pr	5	P	9	B	2	A	1	Sp	1	Wr	2	G	5	P	9	55	3	7	105	7	2	186.16	5	8.49
34	Apakpi	P	9	Lg	3	P	9	Pr	2	Pr	5	A	1	B	2	A	1	Sp	1	S	1	C	3	P	9	62	4	15	95	7	2	220.18	7	5.42

SA= Stem Anthocyanin; Sc=Score, FC=Foliage colour; L=Leaflets, LAC=Leaf Axil Colour; PC=Petal Colour; SRE=Stipule Rabbit-Ear; PDE=Pod Distal End; PP=Pod Parchment; SS=Seed Shape; SSR=Seed Surface; CC=Cotyledon Colour; STM=Seed Testa Mottling; DF=Days to Flowering; NFF=Nodes to First Flower; PH=Plant Height; MPA=Maximum Pods/Axil; TW=Test Weight; YP=Yield /Plant(g); W=White; P=Present; A=Absent; G=Green;Dg=Dark Green; Lg=Light Green; Pr=Purple; Pt=Pointed; Sp=Spherical; S=Smooth; Wr=Wrinkled; C=Creamy; B=Blunt

0.682) [17], however the correlations were of low magnitudes.

Mean genetic distance estimate for molecular markers was higher than that of the morphological markers indicating that the SSR marker data set had higher discriminating power compared to the morphological data set. It was evident from the molecular analysis that the zero genetic distances based on morphological marker found in between the paired genotypes DDR-50 and DMR-61, KPMR-871 and IPFD 1-10, Pant P-142 and NDP 9-408 showing their close relationship to each other were 0.47, 0.60 and 0.27, respectively. A significant positive correlation between the morphological data and SSR marker-based matrices indicates that SSR genetic distance tended to reflect morphological distance. Results of this study were in agreement with the findings of Smykal *et al*. [15-16] and Tihomir *et al*. [12], who suggested low to medium correlations between morphological and molecular markers. The lack of significant correlation between molecular and morphological data may arise due to the limited number of molecular markers examined.

Table 3. Molecular diversity of the 15 SSR loci across 34 pea genotypes

Marker	Allele no.	Gene diversity	Hetero-zygosity	PIC value	f
PSMPA 6	2	0.358	0	0.294	1
PSMPA 8	3	0.625	0	0.554	1
PSMPA 9	3	0.621	0	0.541	1
PSMPB 14	3	0.611	0	0.530	1
PSMPB 16	2	0.493	0	0.372	1
PSMPC 20	3	0.517	0	0.411	1
PSMPAA 31	3	0.636	0.333	0.560	0.488
PSMPAD 99	5	0.418	0.125	0.397	0.709
PSMPAD 100	2	0.251	0	0.219	1
PSMPAD 141	2	0.389	0	0.314	1
PSMPA 5	3	0.591	0	0.511	1
PSMPAA 67	3	0.443	0	0.384	1
PSMPSAA 205	4	0.465	0	0.429	1
PSMSAA 473	2	0.111	0	0.105	1
PSMSAA 476	3	0.452	0	0.401	1
Mean	2.866	0.465	0.031	0.401	0.946
Total	43.00				

where PIC = Polymorphism Information Content and f = fixation index

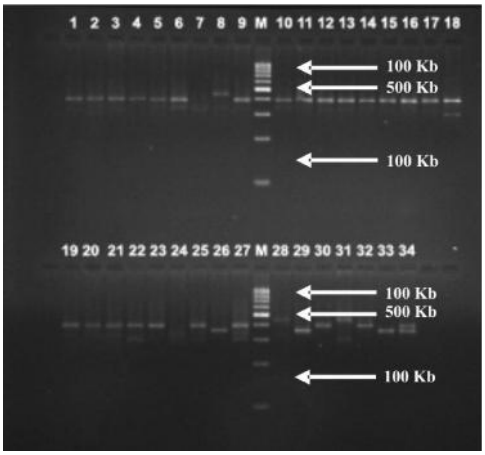

Fig. 2: SSR pattern for the locus PSMPAD 99, M=molecular size ladder 100 base pairs plus 3 kb. Serial codes of the genotypes corresponds to Table 1

Identification of diverse genotypes

The results of cluster analysis may be used to design a strategy to generate the genetic diversity in future varieties by crossing the local cultivars with the adapted ones. Another approach is to cross high yielding parents that possessed many random genetic differences which may increase the number of transgressive segregants. From morphological-based dendrogram, diverse genotypes identified were E-6, Azad P-1, HUDP 904, IPFD-09-2, Pant P 136, HFP-620, IPFD 1-10, LP-3, CAU FP-1, Rachna, and *Matek*. The genotypes Rachna, IPFD O9-2, CAU FP-1, IPFD 1-10 and Pant P-136 showed relatively high yield. From marker-based dendrogram, diverse genotypes identified were DDR-23, IPFD 09-2, Arkel, Azad P-1, IPFD 1-10, Rachna, CAU FP-1, E-6 and HFP-620. The zero genetic distances as indicated between the paired genotypes, DDR-50 and DMR-61, KPMR-871 and IPFD 1-10, Pant P-142 and NDP 9-408 by morphological markers were further differentiated to a genetic distance of 0.47, 0.60 and 0.27 respectively indicating robustness of molecular marker system. This study revealed that both the marker systems are important and supplemented each other as they have separate list of genotypes identified as diverse in which some genotypes are common.

On the basis of the present finding, it can be concluded that although only 34 genotypes were studied, estimated genetic variability among genotypes was high enough for creation of new favourable gene combinations. It is further suggested that the advance breeding materials of pea from IIPR, Kanpur had

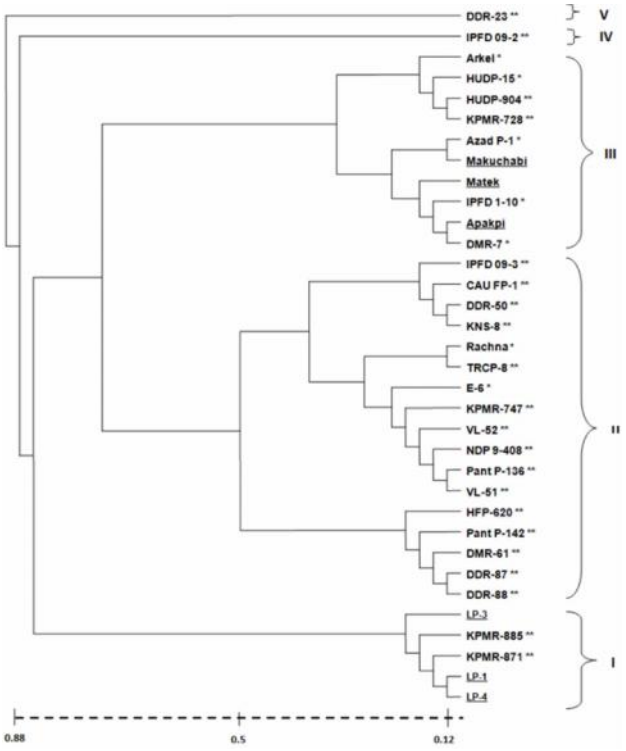

Fig. 3. Dendrogram based on Rogers' genetic distance (Rogers, 1972) among 34 pea genotypes using 15 SSR markers. * = already adapted varieties, ** = advanced breeding lines, underlined=local cultivars

sufficient genetic diversity to suit varied agro-ecological situations. Results indicated that the inter-crosses between already adapted varieties and advanced breeding lines as well as inclusion of valuable landraces into breeding programmes might prevent loss of diversity in the *Pisum* gene pool. The diverse genotypes identified by the morphological and molecular analysis may be used in pea improvement programmes.

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