Indonesian Journal of Agricultural Science
Vol. 19 No. 2 December 2018: 57–66
DOI: http//dx.doi.org/10.21082/ijas.v.19.n2.2018.p.57–66

ANALYSIS OF GENETIC DIVERSITY IN TWELVE CULTIVARS OF PEA BASED ON MORPHOLOGICAL AND SIMPLE SEQUENCE REPEAT MARKERS

Analisis Keragaman Genetik Dua Belas Kultivar Kacang Ercis Berdasarkan Marka Morfologi dan Simple Sequence Repeat

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Submitted 18 November 2017; Revised 30 September 2018 ; Accepted 03 October 2018

ABSTRACT

Pea *(Pisum sativum* L.*)* is the second most important legume crop worldwide after chickpea **(***Cicer arietinum* L**.)** and valuable resources for their genetic improvement. This study aimed to analyze genetic diversity of pea cultivars through morphological and molecular markers. The present investigation was carried out with 12 pea cultivars using 28 simple sequence repeat markers. A total of 60 polymorphic bands with an average of 2.31 bands per primer were obtained. The polymorphic information content, diversity index and resolving power were ranged from 0.50 to 0.33, 0.61 to 0.86 and 0.44 to 1.0 with an average of 0.46, 0.73 and 0.76, respectively. The 12 pea cultivars were grouped into 3 clusters obtained from cluster analysis with a Jaccardd's similarity coefficient range of 0.47-0.78, indicating the sufficient genetic divergence among these cultivars of pea. The principal component analysis showed that first three principal components explained 86.97% of the total variation, suggesting the contribution of quantitative traits in genetic variability. The contribution of 32.59% for number of seeds per plant, stem circumference, number of pods per plant and number of seeds per pod in the PC1 leads to the conclusion that these traits contribute more to the total variation observed in the 12 pea cultivars and would make a good parental stock material. Overall, this SSR analysis complements morphological characters of initial selection of these pea germplasms for future breeding program.

[Keywords: Genetic diversity, pea cultivars, *pisum sativum*, simple sequence repeat]

ABSTRAK

*Kacang ercis (*Pisum sativum L.*) merupakan tanaman legum kedua terpenting di dunia setelah kacang arab (*Cicer arietinum L.*) dan sumber daya yang berharga untuk program perbaikan genetik tanaman. Penelitian bertujuan untuk menganalisis keragaman genetik kultivar kacang ercis melalui penanda morfologi dan molekuler. Penelitian dilakukan terhadap 12 kultivar kacang ercis dengan menggunakan 28 marka* simple sequence repeat *(SSR). Penelitian berhasil memperoleh 60 pita polimorfis dengan rata-rata 2,31 pita per primer. Kandungan informasi polimorfis (*polymorphic information content*), indeks keragaman, dan* resolving power *berkisar 0,50–0,33; 0,61–0,86; dan 0,44–1,0 dengan rata-rata masing-masing 0,46, 0,73, dan 0,76. Dua belas kultivar kacang ercis yang diteliti dapat dikelompokkan menjadi tiga berdasarkan analisis klaster dengan nilai koefisien kesamaan Jaccardd berkisar 0,47–0,78, yang memperlihatkan adanya perbedaan genetik yang cukup di antara kultivar-kultivar yang diteliti. Analisis komponen utama menunjukkan bahwa tiga komponen utama pertama dapat menjelaskan 86,97% dari total variasi, yang memperlihatkan kontribusi sifat kuantitatif terhadap keragaman genetik. Kontribusi 32,59% untuk jumlah biji per tanaman, lingkar batang, jumlah polong per tanaman, dan jumlah biji per polong di PC1 mengarah pada kesimpulan bahwa sifat-sifat tersebut berkontribusi lebih banyak terhadap keragaman total 12 kultivar kacang ercis yang diamati, yang selanjutnya dapat menjadi tetua yang baik. Secara keseluruhan, analisis SSR dapat melengkapi karakter morfologis dalam seleksi awal plasma nutfah kacang ercis untuk kegiatan pemuliaan pada masa yang akan datang.*

[Kata kunci: *Keragaman genetik*, kultivar, *Pisum sativum*, simple sequence repeat]

INTRODUCTION

Pea (*Pisum sativum* L.) is an economically valuable cool-season pulse crop grown worldwide for its seed and soil fertility benefits (McPhee 2003). Being a short duration legume crop belonging to Leguminosae or Fabaceae family, it fixes its own nitrogen and therefore, can become an excellent candidate for bio-energy especially in temperate regions. In India, it is an important legume crop after chickpea and pigeon pea due to its high nutritive value, particularly proteins; 7.2 g 100^{-1} g (Singh 2007). Because of its important source of vegetable protein (21–32%) in major part of the world, recent studies have underlined the latent health profits of pulses in reduction of type II diabetes as well as cardiovascular

diseases (Boye et al. 2010).In Asian countries, this crop is consumed as green vegetable (whole pods or immature seeds), whereas dry seeds are consumed in Europe, Australia, America and Mediterranean regions (Ghafoor and Arshad 2008). An increasing demand for protein-rich food and feed around the world has highlighted the commercial importance of pulses as protein source (Santalla et al. 2001).

The comparatively narrow gene pool (Hebblethwaite et al. 1985) as well as the hefty use of a petty numbers of cultivars as parents in competing breeding programs have directed to a little genetic mixture among pea cultivars (Simioniuc et al. 2002; Baranger et al. 2004), resulting in vulnerability to pests and diseases (Duvick 1984; Cox et al. 1986). This study reported that at proliferation of grain legume production by thorough utilization of high yielding cultivars enriched with tolerance to biotic and abiotic stresses.

The major pea diversity analysis is based mainly on pedigree data, morphological characters and molecular markers (Simioniuc et al. 2002). Since morphological characters are commonly influenced by environmental factors and in some species, adequate level of morphological polymorphism is inaccessible, they are of restricted status in an evaluation of genetic diversity (Patto et al. 2004).

There are several methods present to harness genetic diversity among the genotypes in crop improvement which includes allele mining. The sequence based method entails detection of variation in DNA sequences of various lines by PCR amplification of alleles. Another technique to identify DNA sequence polymorphism is targeted induced local lesions in genomes (TILLING). However, these approaches are costly and time intense. Alternatively, molecular markers can assist to study the genetic diversity in crops. Molecular markers are suitable to supplement the morphological and phonological characterization because they are abundant, free from tissue or environmental effects and permit genotype identification in the early stages of development.

Microsatellites or simple sequence repeats (SSR) are generally used for assessing genetic diversity in peas due to their precision, consistency, co-dominance and reproducibility. Moreover, easy detection of high polymorphism markers with PCR procedure, performs as the best existing choice of markers for pea diversity assessment and characterization (Loridon et al. 2005). For these reasons, microsatellites have been extensively used in gene tagging, genome fingerprinting, genome mapping and marker-assisted selection for numerous crops including pea (Burstin et al. 2001; Loridon et al. 2005; Nasiri et al. 2009; Cupic et al. 2009; Sarıkamış et al. 2010; Gong et al. 2010).

The objective of this study was to characterize pea cultivars on molecular level to support morphology in order to give information potentially utilized for selection of better parents for effective breeding programmes.

MATERIALS AND METHODS

Plant Material

A total of 12 pea cultivars were used in this study. The genetically pure nucleus seed were acquired from the pea breeder Chandrasekhar, Azad Agricultural University, Kanpur (U.P), India. The 12 pea cultivars were grown under greenhouse and average day temperature was adjusted around 18–20 °C for 24 h.

Genomic DNA Isolation, Quality and Quantity Determination

Genomic DNA was extracted from each plant, selecting fresh, young disease free leaves at 8–10 leaf stage. Fresh young leaves were grinded into powder with liquid N_2 using a mortar and pestle. After that, plant genomic DNA was extracted following the method of Doyle and Doyle (1987) with slightly modified by Bhattacharyya and Mandal (1999).

The quality of extracted DNA was analyzed by agarose gel electrophoresis (0.8%), followed by ethidium bromide staining. The purity of the DNA was estimated by spectrophotometer using A260/A280 ratio, and the yield was estimated by measuring absorbance at 260 nm.

PCR Amplification Using SSR Markers

Twenty eight SSR primer sets developed by Loridon et al. (2005) and Kumari et al. (2013) were used for this study (Table 1). All SSR primers were synthesized from Xcelris Labs Limited, Ahmedabad. PCR amplification was conducted in a volume of 25 µl containing 2 µl of 10 ng μl^{-1} DNA, 1 µl of each primer (100 ng μl^{-1}), 1 µl of 2.5 mM dNTPs, 1 µl of 25 mM $MgCl_2$, 2.5 µl of 10X reaction buffer (Xcelris), 0.2 µl of 5 U μl^{-1} Taq polymerase (Xcelris) and 16.3 µl distilled water. PCR reactions were performed in Veriti Thermal Cycler (Applied Biosystems), an initial step of 3 min at 94 °C, 35 cycles of 30 s at 94 °C, annealing at 50-60 °C for 45 s and 1 min at 72 °C, and a final step of 7 min at 72 °C. The PCR products were separated and visualized on 2% agarose gel by ethidium bromide staining.

Table 1. Twenty eight simple sequence repeat (SSR) markers together with their characteristics used in this study.

Sl. No.	Primer Id	Primer sequence (5′-3′)	bp	Tm (°C)	Allele size (bp)
1	AD147	AGCCCAAGTTTCTTCTGAATCC	22	58.57	300-325
		AAATTCGCAGAGCGTTTGTTAC	22	58.45	
2	D21	TATTCTCCTCCAAAATTTCCTT	22	53.00	200-300
		GTCAAAATTAGCCAAATTCCTC	22	54.00	
3	AB91	CGAGACGACAACGGTAGTGAAA	22	60.60	153
		ACCATTACCATAGCGAGAGAGAG	23	59.18	
4	AD148	GAAACATCATTGTGTCTTCTTG	22	53.99	190
		TTCCATCACTTGATTGATAAAC	22	52.48	
5	AA504	TGAGTGCAGTTGCAATTTCG	20	57.60	375-400
		TCAGATGAAGAGCATGTGGG	20	57.30	
6	AA205	TACGCAATCATAGAGTTTGGAA	22	55.56	190-215
		AATCAAGTCAATGAAACAAGCA	22	55.10	
7	AA5	TGCCAATCCTGAGGTATTAACACC	24	60.63	225-240
		CATTTTTGCAGTTGCAATTTCGT	23	58.16	
8	AD174	GGAGGGATGATTCTAACAAGGT	22	57.22	190-215
		AACTCCCACTCCTCGAACTATT	22	58.56	
9	AA355	AGAAAAATTCTAGCATGATACTG	23	52.63	200-215
		GGAAATATAACCTCAATAACACA	23	52.26	
10	AD270	CTCATCTGATGCGTTGGATTAG	22	57.01	245-290
		AGGTTGGATTTGTTGTTTGTTG	22	56.18	
11	AA122	GGGTCTGCATAAGTAGAAGCCA	22	59.83	180-210
		AAGGTGTTTCCCCTAGACATCA	22	58.74	
12	AB45	ATTACACCAACAATCTCCCACT	22	57.34	140-230
		TGTAGAAGCATTTGGGTAGTTG	22	56.35	
13	AD61	CTCATTCAATGATGATAATCCTA	23	51.60	120-300
		ATGAGGTACTTGTGTGAGATAAA	23	55.08	
14	AB23	TCAGCCTTTATCCTCCGAACTA	22	58.36	200-225
		GAACCCTTGTGCAGAAGCATTA	22	59.18	
15	AD79	ACAAGACTTCCAGAAATTTTGCAT	24	57.83	300-325
		AGGACTGATGACGGAGACAAAG	22	59.77	
16	AC58	TCCGCAATTTGGTAACACTG	20	56.64	200-225
		CGTCCATTTCTTTTATGCTGAG	22	55.8	
17	AA416	TTACTGTTACTTTGCGACATCA	22	56.06	240-290
		ATAGTGTCGAAATTTTCCATCC	22	54.53	
18	AD56	GAAACATTGGTTGAAGAGCGAG	22	58.19	200-225
		GTTGTCGCGTGAACACAAGTAA	22	59.97	
19	AD60	CTGAAGCACTTTTGACAACTAC	22	55.87	216
		ATCATATAGCGACGAATACACC	22	55.7	
20	AD146	TGCTCAAGTCAATATATGAAGA	22	52.72	375-450
		CAAGCAAATAGTTGTTTTGTTA	22	51.86	
21	AA446	TTAGCTTGCAGCCCACTC	18	57.28	315-900
		ATCCGACCCATGGATTTA	18	52.35	
22	AA505	ATTCACACGCGCCCA	15	55.92	140-210
		CAATTAAGCCCTCATCCAGA	22	54.79	
23	AD237	AGATCATTTGGTGTCATCAGTG	22	56.42	275-300
		TGTTTAATACAACGTGCTCCTC	22	56.74	
24	D21	TATTCTCCTCCAAAATTTCCTT	22	53.00	200
		GTCAAAATTAGCCAAATTCCTC	22	54.00	

Table 1. (Continued).

Sl. No.	Primer Id	Primer sequence (5′-3′)	bp	Tm (°C)	Allele size (bp)
25	B16	GCATTTGTGCAGTTTCAATTTCG	23	58.55	150
		CCAATTACGGACAATGTTTGATCA	24	58.05	
26	AA 278	CCAAGAAAGGCTTATCAACAGG	22	57.29	155
		TGCTTGTGTCAAGTGATCAGTG	22	59.13	
27	AD135	TGGCATTAGATTCTCCAGCACA	22	59.76	206
		TGAGGAGGTGAACGTAAAAGCA	22	59.90	
28	AA1	TCCATAATGCAGCGGAATGG	20	58.39	235
		GCATTTAGTGCAGTTGCAATCTCA	24	60.62	

Phenotypic Performances

The cultivars were raised with recommended agronomical practices and observations were recorded on a randomly selected five competitive plants from each replication for morphological characters viz. plant height at maturity (cm), germination percentage, number of nodes per plant, number of leaves per plant, length of branch from main axis (cm), internode distance (cm), number of branches per plant, stem circumference (cm), number of seeds per plant, number of pods per plant, weight of 100 seeds (g) and number of seeds per pod. The collected data were mean of five randomly selected plants from each replication.

Data Analysis

For SSR marker data the presence or absence of the bands was scored as 1 or 0, respectively, obtaining the molecular identification profile for each individual. Cluster analysis was implemented by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and the corresponding dendrogram was constructed. To estimate the goodness of fit between similarity matrix and the dendrogram, the coefficient of cophenetic correlation was calculated using the NTSYSpc (Rohlf 2009) software. The capacity of each primer to distinguish among the cultivars studied was evaluated by the resolving power (RP) (Prevost and Wilkinson 1999) and the polymorphic information content (PIC) (Weising et al. 2005). PIC of dominant bi-allelic data was estimated by the formula: PIC = 1-pi2-qi 2, where "p" is frequency of visual alleles and "q" is the frequency of null alleles. PIC for the SSR marker was estimated by using the formula PICi = 2fi (1- fi). Where, fi is the frequency of the marker fragments that were present and (1-fi) is the frequency of the marker fragments that were absent. RP is defined per primer as: RP = Σ Ib, where "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band.

The phenotypic data of the 12 pea cultivars were analyzed using XLSTAT software. The PC was used to determine the extent of genetic variation. Eigen-values were obtained from PC, which were used to determine the relative discriminative power of the axes and their associated characters. Ward method was used to group the twelve accessions based on their genetic relationship.

RESULTS AND DISCUSSION

SSR Polymorphism

The DNA amplification of 12 pea cultivars using 28 SSR primers (Loridon et al. 2005; Kumari et al. 2013) revealed that only 26 primers were polymorphic, but not for AB91 and AD60. They showed different abilities to identify unique multiband among 12 cultivars. The example of DNA banding pattern on gel electrophoresis using primers AD148 and AA1 was illustrated in Figure 1. The primers produced 1–4 bands, of which, AD148 yielded the highest.

The polymorphism information content (PIC) differed between SSR primers, highest for AD174 and AA416 (0.50), and lowest for AA355 (0.33) with mean value of 0.46 (Table 2), suggesting their good indicators of the pea genetic diversity. The twenty six SSR markers revealed the usefulness of a marker in distinguishing accessions with DI values ranging from 0.61 (D21) to 0.86 (AA205) with an average of 0.73 (Table 3). The high DI values (more than 0.50) indicated that the SSR markers were informative. The estimates of RP were found to be the highest for AD174 and AA416 (1.0). The number of alleles and PIC value in our study are comparable to those reported by Loridon et al. (2005); Kumari et al. (2013); Wani et al. (2013); Ahmad et al. (2015). The above results provided an overview of the genetic diversity in all the cultivars. The polymorphism

information content ranged from 0.657 to 0.309 with an average of 0.493. The variation in genetic diversity among these cultivars ranged from 0.11 to 0.73. Cluster analysis based on Jaccard's similarity coefficient using the unweighted pair-group method with arithmetic mean (UPGMA; knowledge about genetic diversity and lipid content in field pea is limited. An understanding of genetic diversity and population structure in diverse germplasm is important and a prerequisite for genetic dissection of complex characteristics and marker-trait associations. Fifty polymorphic microsatellite markers detecting a total of 207 alleles were used to obtain information on genetic diversity, population structure and marker-trait associations. Cluster analysis was performed using UPGMA to construct a dendrogram from a pairwise similarity matrix. Pea genotypes were divided into five major clusters. A model-based population structure analysis divided the pea accessions into four groups. Percentage lipid content in 35 diverse pea accessions was used to find potential associations with the SSR markers. Markers AD73, D21, and AA5 were significantly associated with lipid content using a mixed linear model (MLM).

The genetic coefficients calculated from the molecular data on 26 polymorphic SSR markers revealed a wide

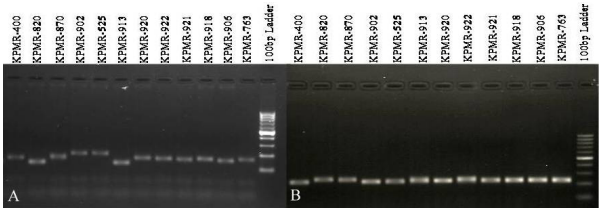

Fig. 1. Electrophoretic banding profile among 12 pea cultivars obtained by AD148 primer (A) and AA1 primes (B)

Table 2. The profiles of 26 simple sequence repeat (SSR) markers used in this study.

SSR marker	Total bands observed	Polymorphic bands	Polymorphism (%)	PIC* value	Diversity index	Resolving power
AD147	3	2	66.7	0.48	0.72	0.82
D21	2	2	100	0.47	0.61	0.75
AD148	4	4	100	0.47	0.75	0.81
AA504	2	2	100	0.43	0.63	0.67
AA205	3	3	100	0.38	0.86	0.67
AA5	3	3	100	0.36	0.85	0.56
AD174	2	2	100	0.50	0.75	1.00
AA355	3	3	100	0.33	0.83	0.44
AD270	2	2	100	0.38	0.69	0.50
AA122	2	2	100	0.49	0.74	0.83
AB45	2	2	100	0.49	0.74	0.83
AD61	2	2	100	0.44	0.72	0.67
AB23	2	2	100	0.49	0.74	0.83
AD79	2	2	100	0.49	0.74	0.83
AC58	2	2	100	0.47	0.77	0.75
AA416	2	2	100	0.50	0.75	1.00
AD56	2	2	100	0.47	0.69	0.75
AD146	2	2	100	0.44	0.72	0.67
AA446	2	2	100	0.49	0.74	0.83
AA505	4	3	75	0.49	0.74	0.83
AD237	2	2	100	0.47	0.69	0.75
D21	2	2	100	0.49	0.74	0.83
B16	3	3	100	0.48	0.69	0.80
AA 278	2	2	100	0.49	0.74	0.83
AD135	3	3	100	0.46	0.75	0.75
AA1	2	2	100	0.47	0.69	0.75
Total	62	60	2541.667	11.88	19.11	19.76
Mean	2.38	2.31	97.76	0.46	0.73	0.76

*PIC = polymorphic information content

Table 3. Jaccard's similarity coefficient of 12 pea cultivars based on 26 polymorphic SSR markers.

Cultivars	KPMR-763	KPMR-906	KPMR-918	KPMR-921	KPMR-922	KPMR-920	KPMR-913	KPMR-525	KPMR-902	KPMR-870	KPMR-820	KPMR-400
KPMR-763	1.000											
KPMR-906	0.709	1.000										
KPMR-918	0.582	0.618	1.000									
KPMR-921	0.582	0.546	0.600	1.000								
KPMR-922	0.364	0.364	0.346	0.491	1.000							
KPMR-920	0.509	0.582	0.564	0.455	0.382	1.000						
KPMR-913	0.655	0.509	0.527	0.491	0.491	0.455	1.000					
KPMR-525	0.509	0.436	0.455	0.564	0.636	0.309	0.564	1.000				
KPMR-902	0.455	0.709	0.582	0.473	0.618	0.436	0.436	0.509	1.000			
KPMR-870	0.491	0.527	0.509	0.364	0.436	0.546	0.509	0.473	0.527	1.000		
KPMR-820	0.473	0.436	0.600	0.564	0.564	0.527	0.782	0.418	0.509	0.400	1.000	
KPMR-400	0.509	0.473	0.491	0.527	0.673	0.564	0.527	0.455	0.618	0.291	0.673	1.000

array of genetic distances between the studied pea cultivars. The Jaccard similarity coefficient ranged from 0.291 to 0.782 owing to diversification in morphology and pedigree between the pea cultivars. The cultivars pairs KPMR-913 and KPMR-820 revealed the highest similarity of 0.782, followed by KPMR-906 and KPMR-902 (0.709), while between KPMR-870 and KPMR-400 showed the lowest (0.291) (Table 3). This distinguishable genetic similarity among cultivars demonstrated their different genetic background.

Cluster Analysis Using SSR Markers

Based on the constructed dendrogram, with NTSYSpc (Rohlf 2009), the 12 pea cultivars were grouped into three major clusters ranging from 0.47 to 0.78 coefficient scale (Figure 2A). Clusters I contains the highest number cultivars including KPMR-763, KPMR-906, KPMR-918, KPMR-921, KPMR-913 and KPMR-820. The cluster II and III grouped four and two cultivars, respectively. Dendrogram summarized the existing genetic similarity/ dissimilarity among pea cultivars within the cluster based on molecular marker (SSR) characteristics. The PCA analysis of the SSR marker data from twelve pea cultivars also supported the cluster analysis. A three dimensional plot (3D plot) diagram was prepared using the first three principal components (Figure 2B). Similar to the dendrogram, six cultivars preferentially were grouped together (cluster I). Whereas, the cultivars KPMR-922, KPMR-400 and KPMR-902 were in cluster II, and the remaining cultivars were in cluster III. These distinctive clusters demonstrated their varied genetics in support to phenotypes.

Phenotypic Performances

All of the quantitatively measured traits showed a high range of variation among 12 pea cultivars. The analysis of variance revealed significant variation among the cultivars for all of the studied morphological parameters at 1% critical level. The mean performance of the cultivars based on the phenotypic parameters indicated their cumulative performance with regards to multiple parameters (Table 4).

The number of seeds per plant among the cultivars varied from 164 to 299.66 with an average of 236.32. The cultivars KPMR-906, KPMR-922, KPMR-525, KPMR-870 and KPMR-400 had above average (>236.32) number of seeds. The weight of 100 seeds among the cultivars varied from 22.60 to 30 g. The cultivars KPMR-763, KPMR-918, KPMR-921, KPMR-913, KPMR-525 and KPMR-870 had above average (>25.94 g) seed weigh. Among the cultivars, KPMR-400 performed better than average mean in terms of plant height, germination percentage, number of pods per plant and number of seed per pods. Whereas KPMR-870 was better in case of parameters as number of nodes per plant, lenght of branch from main axis, stem circumference, number of seeds per plant, number of pods per plant, weight of 100 seeds and number of seeds per pods. The rest of the cultivars including KPMR-763, KPMR-906, KPMR-922, KPMR-920 and KPMR-525 showed better than average performance in at least three parameters among the four important traits as number of pods per plant, weight of 100 seeds and number of seed per pods.

The Pearson correlation analysis (Table 5) indicated significant correlation of plant height with germination percentage and stem circumference. The number of seeds per plant was positively correlated with number

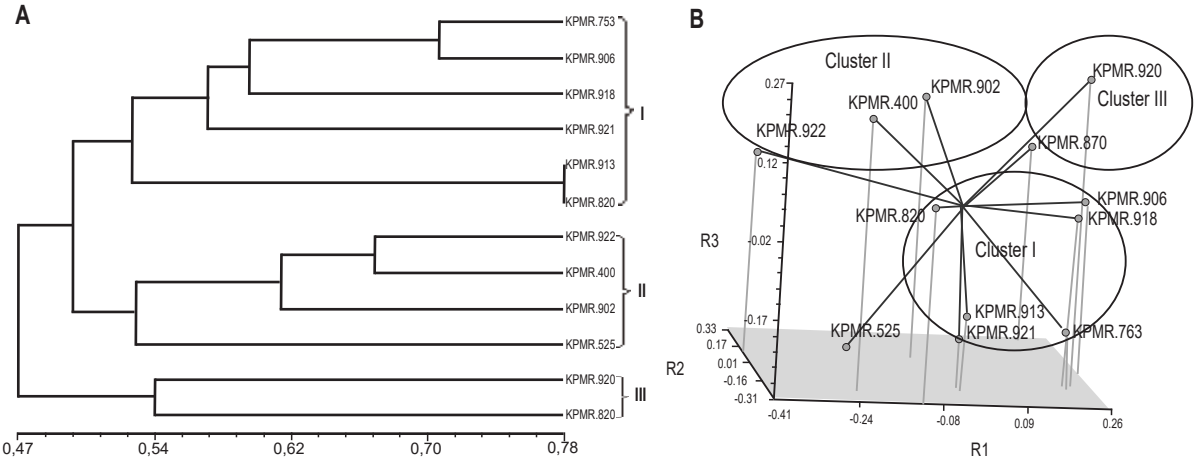

Fig. 2. UPGMA-based dendrogram showing genetic relationship among twelve pea cultivars (A), Three-dimensional plot of principal components (PC) 1, 2 and 3 based on pea SSR markers (B).

Table 4. Mean performance of 12 pea cultivars based on selected trait parameters.

Cultivars	PH (cm)	GP (%)	NNPP	NLPP	LBMA (cm)	ID (cm)	NBPP	SC (cm)	NSPP	NPPP	WS (g)	NSPD
KPMR-763	21.33	81.33	6.00	8.66	1.76	3.13	4.33	1.16	220.33	54.67	28.00	4.00
KPMR-906	24.66	82.33	5.33	8.66	1.83	3.60	6.00	1.74	299.66	52.67	23.00	5.67
KPMR-918	22.33	80.33	3.00	7.66	1.33	3.90	4.67	1.28	218.00	43.32	30.00	5.00
KPMR-921	25.66	87.66	4.33	8.66	1.66	3.66	4.66	1.45	186.66	42.67	28.00	4.33
KPMR-922	27.00	89.66	5.66	10.00	1.36	4.23	3.66	1.80	278.00	55.67	24.00	5.00
KPMR-920	23.33	81.00	4.66	6.66	1.06	4.10	3.33	1.40	164.00	41.00	24.50	4.00
KPMR-913	29.00	89.33	5.33	10.00	1.53	3.26	5.66	1.71	234.34	47.00	27.33	5.00
KPMR-525	22.00	81.00	5.33	9.66	1.66	4.43	5.33	1.53	269.32	48.34	26.29	5.67
KPMR-902	25.33	87.00	5.00	7.66	2.00	4.46	3.34	1.70	196.00	45.67	24.99	4.32
KPMR-870	24.66	81.00	7.00	8.00	0.96	3.90	5.66	2.00	298.66	60.00	26.68	5.00
KPMR-820	20.33	81.33	6.66	9.66	1.93	4.00	4.34	1.56	215.66	46.33	22.60	4.67
KPMR-400	29.66	91.33	5.33	8.66	1.80	3.86	4.67	1.74	255.34	51.00	25.89	5.00
Grand mean	24.61	84.44	5.30	8.83	1.57	3.88	4.69	1.59	236.32	49.02	25.94	4.80
Standard deviation	2.938	4.177	1.049	0.834	0.331	0.416	0.905	0.239	44.237	5.831	2.218	0.56

PH = plant height, GP = germination, NNPP = number of nodes per plant, NLPP = number of leaves per plant, LBMA = length of branch from main axis, ID = internode distance, NBPP = number of branches per plant, SC = stem circumference, NSPP = number of seeds per plant, NPPP = number of pods per plant, WS = weight of 100 seeds, NSPD = number of seeds per pod.

Table 5. Pearson correlation among the morphological characters for all of the studied pea cultivars.

	PH	GP	NNPP	NLPP	LBMA	ID	NBPP	SC	NSPP	NPPP	WT	NSPD
PH	1	0.889*	-0.064	0.135	-0.034	-0.123	0.128	0.575*	0.212	0.142	0.025	0.17
GP		1	-0.051	0.281	0.257	-0.038	-0.134	0.411	0.026	0.03	-0.047	-0.001
NN			1	0.351	0.051	-0.068	0.188	0.513	0.459	0.695*	-0.499	0.059
NL				1	0.111	-0.072	0.127	0.14	0.203	0.083	-0.36	0.272
LBA					1	-0.037	-0.037	-0.134	-0.086	-0.143	-0.26	0.043
ID						1	-0.442	0.275	-0.016	-0.18	-0.372	0.162
NB							1	0.305	0.655*	0.361	0.182	0.708*
SC								1	0.642*	0.533*	-0.443	0.478
NSPL									1	0.816*	-0.199	0.809*
NPP										1	-0.137	0.327
WT											1	-0.146
NSPD												1

'*' significant at P = 0.05 with critical R = 0.532

PH = plant height, GP = germination, NNPP = number of nodes per plant, NLPP = number of leaves per plant, LBMA = length of branch from main axis, ID = internode distance, NBPP = number of branches per plant, SC = stem circumference, NSPP = number of seeds per plant, NPPP = number of pods per plant, WS = weight of 100 seeds, NSPD = number of seeds per pod.

of branches and stem circumference. The number of pods per plant was positively correlated with number of nodes, stem circumference and number of seeds per plant. A significant positive correlation was also found for number of seeds per pod with number of branches and number of seeds per plant.

Eigen vectors indicate the degree of association among the original data and each principal component. The first three PC axes accounted for 86.97% (Table 6) of the multivariate variation among the entries indicating a higher degree of correlation among characters for these entries. The first principal component (PC 1) accounted for 32.59% of variation, mostly influenced number of seeds per plant, stem circumference, number of pods per plant and number of seeds per pod. The second principle component (PC 2) accounted for 17.4% of variation, mostly influenced by germination percentage and plant height. The third principle component (PC 3) accounted for 15.56% of variation which mostly influenced weight of 100 seeds, plant height and internode distance. The fourth principle component (PC 4) with 11.162% variance influenced length of branch from main axis and fifth principle component (PC 5) with 10.29% variance

Table 6. Eigen values and percentage of variation for corresponding five component characters in 12 cultivars of pea.

Component	PC1	PC2	PC3	PC4	PC5
Eigen value	3.91	2.088	1.864	1.339	1.235
% variance	32.586	17.398	15.531	11.162	10.294
Characters			Eigen vectors		
Plant height (cm)	0.419	0.672	0.568	-0.106	-0.133
Germination (%)	0.264	0.859	0.396	0.099	-0.044
Number of nodes per plant	0.624	-0.086	-0.488	0.377	-0.395
Number of leaves per plant	0.384	0.253	-0.169	0.472	0.279
Lenght of branch	-0.023	0.3	-0.153	0.509	0.516
Internode distance	-0.024	0.284	-0.572	-0.69	0.209
Number of branches per plant	0.605	-0.504	0.433	0.111	0.289
Stem circumference	0.827	0.311	-0.072	-0.32	-0.17
Number of seeds per plant	0.917	-0.282	0.044	-0.126	0.07
Number of pods per plant	0.767	-0.26	-0.052	0.092	-0.442
Weight of 100 seeds	-0.373	-0.332	0.746	-0.082	-0.119
Number of seeds per pod	0.714	-0.218	0.12	-0.269	0.588

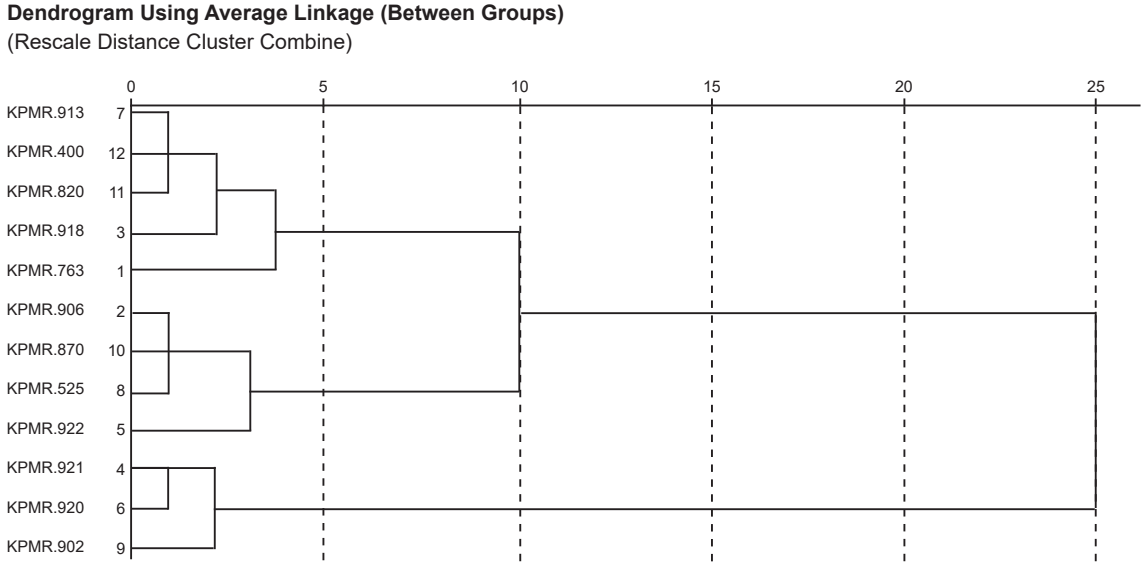

Fig. 3. Dendrogram showing relationship of pea cultivars based on Euclidian distance and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering using morphological characters.

influenced number of seeds per pod. High magnitude of negative relationships were also observed for number of branches (PC2) and internode distance (PC3). It is interesting to note that few characters as plant height, length of branches from main axis and number of seeds per pod influenced multiple principle components most of which did not influence principle component 1. These characters have a maximum variability among cultivars.

Hierarchical clustering using Euclidean distance as a distance measure was done for grouping the germplasm suitably. On the basis of dendrogram constructed by average linkage between groups (Figure 3), three clusters were formed consisting different cultivars. Cluster I consists the cultivars namely KPMR-913, KPMR-400, KPMR-820, KPMR-918 and KPMR-763. Cluster II contains KPMR-906, KPMR-870, KPMR-525 and KPMR-922. Cluster III contains only 3 cultivars namely 921, KPMR-920 and KPMR-902. These clusters clearly indicate the relation among the cultivars and the distance based on their overall parameter variables.

CONCLUSION

The diversity among cultivars may be assessed based on morphological and molecular markers. However, systematic studies regarding the genetic diversity of pea through molecular markers in India are meagre. Hence, in-depth studies based on morphological and molecular markers SSR will help in understanding the genetic diversity of germplasm as well as identification, conservation and utilization of authentic and superior crop materials. The results indicate the presence of moderate genetic variability among the elite green pea cultivars. Among all the cultivars, the KPMR-870 and KPMR-920 genotypes from cluster III have wider genetic diversity and suggested to utilize in crop improvement program.

ACKNOWLEDGEMENT

The authors are thankful to the DBT for providing grant (BT/04/NE/2009) under the Project of Advanced Level of Institutional Biotech Hub, at College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh.

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