AATCC Review

28 July 2024: Received
29 August 2024: Revised
06 October 2024: Accepted
07 November 2024: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Molecular characterization of pea (*Pisum sativum*) genotypes by utilization of RAPD markers

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ABSTRACT

A Random Amplified Polymorphic DNA (RAPD) marker was used for identifying and mapping the population in pea (Pisum sativum). The presence of multiple polymorphisms between cultivars and lines revealed at least one fragment for any given primer was present in the DNA of one form of pea and absent in the DNA of another line or cultivar. Polymerase chain reaction (PCR) based molecular marker viz. random amplified polymorphic DNA was applied to 20 germplasm of Pea to assess the degree of polymorphism within the genes and to investigate the genetic studies in Pea. This study, using 20 germplasm of pea was evaluated for variability using a panel of 14 random l0-mer primers. The polymorphisms in PCR amplification products were subjected to the unweighted pair group method for arithmetic averages (UPGMA) and plotted in a dendrogram based on similarity data showing that all the cultivars analyzed were related. Eleven out of 14 primers revealed scorable 60-polymorphic bands between cultivars of Pisum sativum and the rest did not show polymorphism in their genetic level. All the 60 amplified bands were polymorphic and the numbers of bands produced per primer ranged from band 3 to 11 bands. PIC, EMR, and MI values ranged from 0.22 to 0.37, 1.00 to 5.20, and 0.34 to 1.92 with the average of PIC, EMR, and MI values being 0.34, 2.86, and 0.95 respectively. In addition, the value of resolving power (RP) ranged from 0.80 to 6.20 with an average value of 2.59. GS (Genetic similarity) value ranged from 0.13 between genotypes VL-3 and Arka Ajit and 0.90 between genotype AP-3 and Arka Priya.

Keywords: *RAPD, Molecular Markers, Mapping, PCR, Genetic Similarity and Pisum sativum*

Introduction

Pea (*Pisum sativum* L.) is an important legume vegetable grown throughout the world. It is a native of the Mediterranean region with Near East and Ethiopia as secondary centers [1]. *Pisum sativum* L. is a self-pollinated crop that belongs to the family Leguminaceae and had chromosome number (2n = 14). There are two sub-species in the genus Pisum, namely *Pisum arvense* known as the field pea having colored flowers, and *Pisum sativum*, the white-flowered horticultural or vegetable pea which is also known as the sweet pea. Pea is an herbaceous winter annual; having an angular stem, glaucous, alternate leaves, distichous, rachis terminates into a simple or branched tendril, 1-3 flowers per raceme, white, pink or purple corolla, diadelphous stamens, straight or curved pods, and smooth or wrinkled seeds. For the development of improved varieties, classification of genetic variability, among the genotypes is valuable for maintenance, and further acquisition of germplasm as an accession from different origins is essential as parent stock [2].

The advent of molecular biology made possible the use of molecular genetic marker technology to better understand the genetic diversity in various crop species. DNA markers are defined as DNA fragments that reveal mutation/variation, to recognize polymorphism between dissimilar alleles and genotypes of a gene for a certain sequence of DNA in a population or gene pool. Such fragments are correlated with a definite location within the genome and can be determined by means of specific molecular technology. Primers are short fragments of DNA with defined segments that complement the target DNA that is detected and amplified. The success of DNA marker technology in bringing genetic improvement to crops would depend on close interaction between plant breeders, Agricultural biotechnology, skilled labour, and significant financial investments in research [3].

RAPD has resolved most of the technical obstacles owing to its cost-effective and easy-to-perform approach [4]. Therefore, RAPD has been extensively used to assess genetic relationships amongst various accessions of different plant species [5]. RAPD markers are useful for the evaluation of genetic diversity due to

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DOI: https://doi.org/10.21276/AATCCReview.2024.12.04.212

easy application and less expensive as compared to other molecular markers [6]. Molecular characterization by RAPD markers is easy and rapid. RAPD is used to identify the genetic relationship among cultivars [7] [8]. It is a modification of the PCR in which a single, short, and arbitrary oligonucleotide primer, able to anneal and prime at multiple locations throughout the genome can produce a spectrum of amplification products that are characteristics of the template DNA [9].

RAPD markers have a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics, and plant as well as animal breeding and improvement of varieties as well as traits. This is mainly due to the speed, cost, and efficiency of the technique to generate large numbers of markers in a short period compared with previous methods [10]. Therefore, the RAPD technique can be performed in a moderate laboratory for most of its applications. It also has the advantage that no prior knowledge of the genome under research is necessary [11] [12].

MATERIALS AND METHODS

The present research was conducted in 2021-22 and molecular analysis was performed at the Molecular Biology Laboratory (MBL), College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (250110) Uttar Pradesh, India. The details of all twenty pea genotypes included in the present study along with their sources are given in Table 1.

Table 1: List of genotypes included in the study

S.No.	Genotypes	Developed by
1.	Kashi Shakti	Indian Institute of Vegetable Research, Varanasi
2.	Kashi Mukti	Indian Institute of Vegetable Research, Varanasi
3.	Kashi Samrath	Indian Institute of Vegetable Research, Varanasi
4.	Kashi Nandini	Indian Institute of Vegetable Research, Varanasi
5.	Bonneville	USA
6.	Pant Matar-2	Govind Ballabh Pant University of Agriculture and Technology (Pantnagar)
7.	Arka Ajit	Indian Institute of Horticultural Research (Bangalore).
8.	Arkel	England
9.	Arka Sampoorna	Indian Institute of Horticultural Research (Bangalore).
10.	Kashi Samridhi (VRPMR-11)	Indian Institute of Vegetable Research, Varanasi
11.	Mithi Fali	PAU, Ludhiyana
12.	Arka Priya (IIHR-1)	Indian Institute of Horticultural Research (Bangalore).
13.	Azad Pea -3	Chandra Shekhar Azad University of Agriculture and Technology (Kanpur)
14.	Pusa Pragati	Indian Agricultural Research Institute (New Delhi).
15.	PC-531	Panjab Agriculture University (Ludhiyana)
16.	Kashi Ageti	Indian Institute of Vegetable Research, Varanasi
17.	Solan Nirog	Dr. Yashwant Singh Parmar University of Horticulture & Forestry (Solan)
18.	Arka Kartik	Indian Institute of Horticultural Research (Bangalore).
19.	Kashi Uday	Indian Institute of Vegetable Research, Varanasi
20.	VL-3	Vivekananda Parvatiya Krishi Anusandhan Sansthan (Almora).

Study of Morphological Traits

Selected morphological traits *viz* Days to germination, Days to 50 % flowering, Plant height (cm), Total pods/ Plant, Seeds per pods, Length of pods (cm), Width of pods (cm), Days to maturity, Pod yield /plants (g) and Pod yield (g/ha) studied in RBD design on the controlled climatic conditions. The selected morphological traits are regulated by environmental factors like high temperature, water, salt, fog, pollutants, etc., and other biotic stress.

Chemical composition of extraction buffer and DNA extraction

DNA was extracted from 20 genotypes of pea (Table 1), using the method described by Murray and Thompson with minor modifications [13] [14]. Fresh 0.15 g leaf tissues were ground by mortar-pastel in liquid nitrogen. The homogenized mixed with 3x extraction buffer [3% cetyl trimethyl ammonium bromide (CTAB), 100 mM Tris–HCl, 2.5M NaCl, 20 mM Ethylene Diamine Tetra Acetic acid (EDTA), 200 µl β-mercaptoethanol, and 2 % poly vinylpyrrolidone (PVP) at PH 8.0 (Table 2) were gently by swirling and inverting the tube and incubated at 65 °C in hot water bath for 40-45 minutes with mixing at 15 to 20 min intervals. The Eppendorf centrifuged tubes were taken out and centrifuged at 10,000 rpm for 10 min.

The supernatant was transferred to fresh Eppendorf tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The content was mixed by inversion for 3 min and centrifuged at 10,000 rpm for 10 min repeat this step until the aqueous layer is clear. The above clear aqueous layer is transferred in a fresh tube and mixed with 2/3 volume of ice-cold isopropanol was added and centrifuged tubes placed at -20 °C for 30 min. Total genomic DNA was pelleted by centrifuging at 10,000 rpm for 10 min, after that pelleted washed with 300µl [70 % (v/v)] ethanol and centrifuged at 10000 rpm for 5 min. The palleted was dissolved in 40 µl molecular grade water. The sample was treated with RNase enzymes and put on 30 min at 38°C in a water bath. After that the repeat CI to final step for the purification of isolated DNA. The palleted was dissolved in 40 µl molecular grade distill water or TE buffer by tapping and storing at -20 °C for future use. Purified total DNA was quantified and its quality was verified by spectrophotometer [15] [16] [14].

Table 2.0 Chemical composition of 3X CTAB extraction buffer for DNA extraction.

S.N.	Reagent Name	Final Concentration	Final Volume
1	Tris-HCl	100 mM	100 ml
2	EDTA	20 mM	
3	NaCl	2.5 M	
4	CTAB	3 % (W/V)	
5	PVP	2 % (W/V)	
6	β-mercaptoethanol	200 µl	
7	Double Distil Water	As per volume	

RAPD amplification

Amplification of RAPD fragments was performed according to Williams, *et al*. [4] using decamer arbitrary primers (Eurofins Genomics India). Amplifications were performed in a 25 µl reaction volume in Table 3. Amplification was performed in a programmed thermocycler (BIO-RAD My Cycler™ Thermal cycler) with initial denaturation at 94 °C for 4 min, denaturation at 94 °C for 60 sec, annealing of all primer pairs for 37 °C and extension 72 °C for 60 sec; final extension at 72 °C for 10 min, and all step repeat 35 cycles except initial denaturation as well as final extension. Amplified products were electrophoresed in 1.5% agarose in 1x TBE buffer. The gels were stained with ethidium bromide and documented using a gel documentation system (Bio-Rad, Hercules, California).

Table-3. Composition and preparations of primer amplification protocols

S.N.	Compound	Amount
1	Hichrom Master mix	12.5 µl
2	Primer	1.2 µl
3	DNA	2.5 µl
4	Molecular grade distill water	8.8 µl
Total		**25 µl**

Table -4. Panel of RAPD primer sequence with suitable annealing temperature

S.N.	Primer code	Sequence	Annealing temperature (˚C)
1.	OPM-2	ACAACGCCTC	---
2.	OPM-3	GAGA	---
3.	OPM-04	GGCGGTTGTC	37^0 C
4.	OPM-05	GGGAACGTGT	37^0 C
5.	OPM-06	CTGGGCAACT	37^0 C
6.	OPM-07	CCGTGACTCA	37^0 C
7.	OPM-8	TCTGTTCCCC	----
8.	OPM-09	GTCTTGCGGA	37^0 C
9.	OPM-10	TCTGGCGCAC	37^0 C
10.	OPM-12	GGGACGTTGG	37^0 C
11.	OPM-15	GACCTACCAC	37^0 C
12.	OPM-16	GTAACCAGCC	37^0 C
13.	OPM-18	CACCATCCGT	37^0 C
14.	OPM-19	CCTTCAGGCA	37^0 C

RAPD Analysis

The RAPD bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. By comparing the banding patterns of genotypes for a specific primer, genotype-specific bands were identified. Unclear bands were not considered. The binary data generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. The polymorphism information content (PIC) was calculated by the formula: PIC = 2Pi (1 − Pi) [17] where Pi is the frequency of occurrence of polymorphic bandsi in different primers. Pairwise similarity matrices were generated by Jaccard's coefficient of similarity [18] by using the SIMQUAL format of NTSYSpc [19]. MI and EMR the power of each primer to distinguish among the studied genotypes was evaluated by the Resolving Power (RP) [20].

A dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSYS-pc to show a phenetic representation of genetic relationships as revealed by the similarity coefficient [21].

Statically analysis

The phonological characters viz. Day to germination, Days to 50% flowering, Plant height, Total pods /plants, Seeds /pods, length of pods, width of pods, Day to maturity, Pods yield per plant, and Pod yield per hectare were analysed by Excel 2013 with IBM SPSS Statistics Ver 20. The total number of bands, number of polymorphic bands, percentage polymorphism, average number of bands per primer, average number of polymorphic bands per primer, PIC EMR, MI, and resolving power (RP) value were manually analyzed.

Results

The morphological result as shown in Table 5.0 explained that the days to germination, days to 50% flowering, plant height, number of pods per plant, number of seeds per pod, length of the pod, the width of a pod, pod yield per plant total pods per plant and pod yield quintal per hectare had significant difference among the genotypes. Days to germination of all 20 genotypes ranged from 5.80 to 7.67. The average of days to germination was recorded at 6.81 and the coefficient of variation for this observation is 2.93%. The days to 50 % flowering of all genotypes showed a wide range of variation from 36.47 to 69.07 days. The grand mean value for days to 50% flowering was recorded at 49.39 days with a coefficient of variation of 2.43%. The plant height of all genotypes ranged from 39.25 cm to 88.64 cm. The average plant height was 63.65 cm and the value of the coefficient of variation (CV) was 2.30%. The number of pods per plant had significant variation among all varieties and revealed the range between 6.80 to 16.27 pods. The average of this observation was 11.06 and the pod per plant showed a coefficient of variation of 2.91%. The number of seeds per pod also showed significant variation among all genotypes ranging from 5.73 to 7.80. A number of seeds per pod had an average value of 6.78 and the coefficient of variation was 3.50%. The data indicated significant variation with respect to the length of the pod ranging between 6.30 cm to 8.25cm. The average value for the length of the pod was recorded at 7.29 cm with a coefficient of variation of 2.48%. The width of the pod (cm) was observed from 1.12 to 1.44 cm. The average for this observation was 1.21 cm and the widths of the pod disclose a maximum value of coefficient variation of 3.17%. Days to maturity range between 56.60 days to 114.33 days. The average for this character was recorded at 82.09 days and this observation reveals a minimum coefficient of variation 1.29 %. Pod yield per plant ranged between 34.14 gm to 72.56 gm and pod yield quintal per hectare ranged to 56.48 (q/ha) to 120.66 (q/ha). Pod yield per plant (gm) and pod yield quintal per hectare the average of 50.33 gm and 89.64 (q/ha) respectively. The merit for a coefficient of variation of Pod yield per plant and Pod yield (q/ha) was 2.28% and 1.66 % respectively.

Table 5: Ten morphological traits with mean value and range of phenology .

S.N.	Character	2022		
		Mean	Range	CV (%)
1	Days to Germination	6.81	5.80-7.67	2.93
2	Days to 50 % Flowering	49.39	36.47-69.07	2.43
3	Plant height (cm)	63.65	39.25-88.64	2.30
4	Total pods/plant	11.06	6.80-16.27	2.91
5	Seeds per pods	6.78	5.73-7.80	3.50
6	Length of pods (cm)	7.29	6.30-8.25	2.48
7	Width of pods (cm)	1.21	1.12-1.44	3.17
8	Days to Maturity	82.09	56.60-114.33	1.29
9	Pod yield/plant (g)	50.33	34.14-72.56	2.28
10	Pod yield (q/ha)	89.64	56.48-120.66	1.66

The inter-character phenotypic correlation coefficient is presented in Table 6. Data showed that highly significant positive correlation for Days to germination to 50 % flowering (0.38), plant height (0.39), Days to maturity (0.48) and negatively correlated with length of pods (- 0.36), and other traits are non-significant correlated with days to germination. Days to 50 % flowering is highly correlated with plant height (0.68), number of pods per plant (0.35), Days to maturity (0.72), and pod yield per plant (0.34).
Plant height is significantly correlated with only one trait with Days to maturity (0.66). A number of pods highly correlated with the length of pods (0.40), days to maturity (0.41), Pod yield per plant (0.59), and Pod yield quintal per hectare (0.69). Seeds per pod are significantly correlated with the length of pods (0.51). The selected trait's length of pods highly correlated with pod yields per plant (0.48) days to maturity significantly correlated with pod yield per plant (0.49) and pod yields per plant are significantly correlated with pod yield q/ha (0.57). Widths of pods are not positively or negatively significantly correlated with any selected traits in this study.

Table: 6 phenotypic correlations of 10 morphological traits grown rabi season in the year 2021

Characters	Days to Germination	50% Flowering	Plant height (cm)	No. of Pods/ plant	Seeds per Pods (g)	Length of Pod (cm)	Width of Pod (cm)	Days to Maturity	Pod yield /plant (g)	Pod yield (q/ha)
Days to Germination	1.00	**S (0.38)**	**S (0.39)**	NS	NS	**S (-0.36)**	NS	**S (0.48)**	NS	NS
50% Flowering			**S (0.68)**	**S (0.35)**	NS	NS	NS	**S (0.72)**	**S (0.34)**	NS
Plant height (cm)				NS	NS	NS	NS	**S (0.66)**	NS	NS
No. of Pods/plant					NS	**S (0.40)**	NS	**S (0.41)**	**S (0.59)**	**S (0.69)**
Seeds per Pods (g)						**S (0.51)**	NS	NS	NS	NS
Length of Pod (cm)							NS	NS	NS	**S (0.48)**
Width of Pod (cm)								NS	NS	NS
Days to Maturity									**S (0.49)**	NS
Pod yield /plant (g)										**S (0.57)**
Pod yield (q/ha)										1.00

S= Significant, NS= Non-significant at 1% level respectively.

A panel of fourteen RAPD primers were used for RAPD analysis of the genome of 20 pea genotypes presented in Table 1. RAPD eleven primers out of 14 primers showed polymorphic amplification. Three primers fail to generate any amplified product in combination with one entry that count as null alleles of amplified this primer product. By observing the result given by the set of primers, showing polymorphic amplification, a total of 60 bands were obtained, with an average of 5.45 bands per primer. The various sizes of the amplified product as recognized in this study a perusal of the relevant data on the number of alleles generated by the primers. Although 60 allelic variants were detected among the 20 selected genotypes with and 5.45 alleles per locus. The number of allels per locus ranged from three in the case of OPM-9 and OPM-16 to eleven in the case of OPM -5. This panel of RAPD primers viz. OPM-4, OPM-5, OPM-12, OPM-18 and OPM-19 generate more than average allelic loci. In this study, monomorphic bands are not developed in set of selected RAPD primers.

Table-7 Primer name, Total bands, Polymorphic bands, Monomorphic bands, PIC, EMR, MI, RP, Polymorphic% of RAPD primers.

S.N	Primers	Total allels	Polymorphic bands	Monomorphic bands	PIC	EMR	MI	RP	Polymorphic %
1.	OPM-4	6	6	0	0.34	1.95	0.66	2.70	Yes
2.	OPM-5	11	11	0	0.37	5.20	1.92	6.20	Yes
3.	OPM-6	5	5	0	0.37	2.40	0.89	2.40	Yes
4.	OPM-7	5	5	0	0.32	1.35	0.43	2.70	Yes
5.	OPM-9	3	3	0	0.34	1.00	0.34	1.40	Yes
6.	OPM-10	4	4	0	0.22	3.40	0.75	1.20	Yes
7.	OPM-12	6	6	0	0.37	3.25	1.20	3.50	Yes
8.	OPM-15	4	4	0	0.32	2.90	0.93	0.80	Yes
9.	OPM-16	3	3	0	0.24	2.50	0.60	1.00	Yes
10.	OPM-18	6	6	0	0.36	3.65	1.31	3.10	Yes
11.	OPM-19	7	7	0	0.37	3.85	1.42	3.50	Yes
Total		**60.00**	**60.00**	**0**	**3.64**	**31.45**	**10.45**	**28.50**	
Mean		**5.45**	**5.45**	**0**	**0.33**	**2.86**	**0.95**	**2.59**	**100**

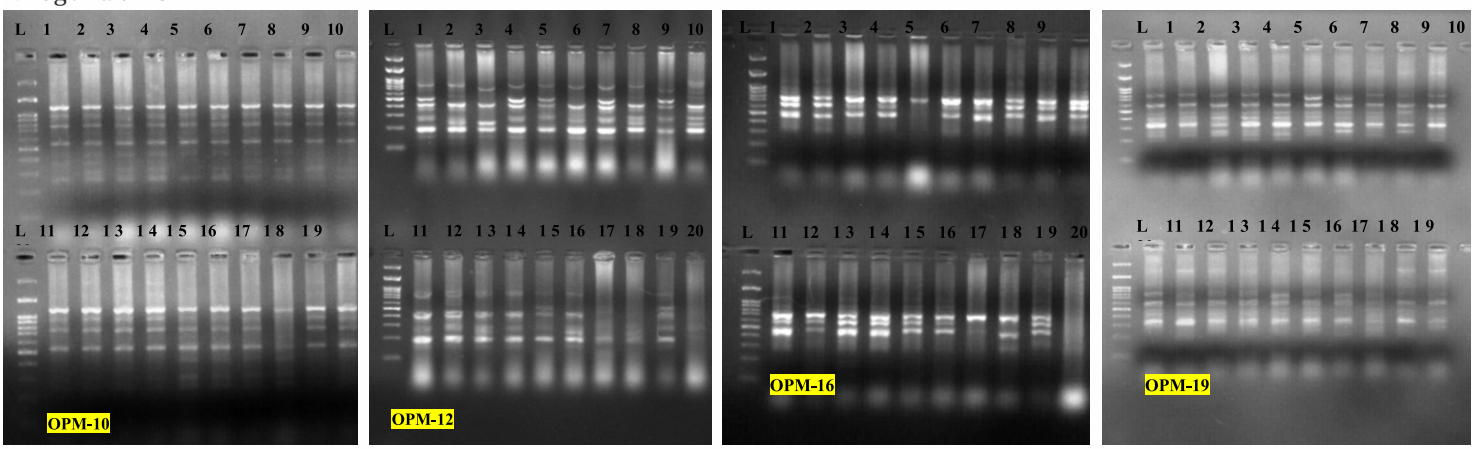
In addition, the polymorphism information content (PIC) value provides a measure of polymorphism among the entries for a marker locus inquisition and reflects allelic diversity as well as the frequency of marker among the entries under evaluation. The pertinent data clearly reflect an ample extent of variation in PIC value among all eleven primers indicating the variability in amplified product and allelic frequency among the entries. The PIC values ranged from 0.22 for OPM-10 to 0.37 for OPM-5, OPM 6, OPM 12, and OPM-19 with an average PIC value were 0.33 over the panel of RAPD primers. The EMR value ranged from 1.00 for OPM-9 to 5.20 for OPM-5 with the mean of EMR being 2.86. The MI ranged from 0.34 for OPM-9 to 1.92 for OPM-5 with a mean value of 2.59. The resolving power (RP) ranged from 0.80 for OPM-15 to 6.20 for OPM-5 with an average value of 2.59. All the 11 RAPD primers showed 100 % polymorphism in **Table 7.**

All the generated 60 bands amplified from 11 RAPD primers were further subjected to genetic similarities (GS) assessment by using Jaccard's similarities index in Table 7 significant genetic variation among total peas was examined. GS value ranged from 0.13 between genotypes VL-3 and Arka Ajit (showing the closest genetic relationship); to 0.90 between genotype AP-3 and Arka Priya (suggesting a distance genetic relationship).

Table 8 Clustering pattern of 20 pea genotypes based on genetic divergence by RAPD

Grouping of clusters	No. of sub-clusters	Clusters	No. of genotypes	Genotypes
I	3	GI-C1	7	Kashi Shakti, Kashi Samaridhi, Kashi Mukti, Arkel, Kashi Nandini, Kashi Samarath, Pant Matar-2
		GI-C2	2	Arka Ajit and Arka Sampoorna.
		GI-C3	8	Mithi Fali, Pusa Pragati, Arka Priya,Ap-3, PC-531, Kashi Ageti, Kashi Uday, Boneville
II	1	GII-C1	1	Arka Kartik
III	2	GIII-C1	2	Solan Nirog , VL-3

Cluster analysis based on a set of RAPD primers can be represented in a dendrogram to indicate the estimated relation between different genotypes. In this assessment cluster analysis is based on the unweighted paired group method of arithmetic means (UPGMA) in NTSYSpc 2.02e software. Panel of eleven RAPD primers were used for the classification of cultivars and based on clustering, 20 pea genotypes were clustered into three main groups Group I, Group II, and Group III (Table -6). Group I includes 17 genotypes and further kept into three sub-clusters GI-C1 includes seven genotypes namely Kashi Shakti, Kashi Samaridhi, Kashi Mukti, Arkel, Kashi Nandini, Kashi Samarath, Pant Matar-2; GI-C2 include 2 genotypes Arka Ajit and Arka Sampoorna and Third (GI-C3) cluster including 8 genotypes namely Mithi Fali, Pusa Pragati, Arka Priya, Azad Pea-3, PC-531, Kashi Ageti, Kashi Uday and Boneville Whereas, Group II, first cluster GII-CI includes 1 genotype namely Arka Kartik and group III include 2 genotypes viz. Solan Nirog and VL-3.



***L**-Ladder (100bp) **1.** Kashi Shakti **2.** Kashi Mukti **3.** Kashi Samrath **4.** Kashi Nandani **5.** Bonneville **6.** Pant Matar-2 **7.** Arka Ajit **8.** Arkel **9.** Arka Sampoorna **10.** Kashi Samaridhhi **11.** Mithi Fali **12.** Arka Priya **13.** AP-3 **14.** Pusa Pragati **15.** PC-531 **16.** Kashi Ageti **17.** Solan Nirog **18.** Arka Karthik **19.** Kashi Uday **20.** VL-3*

Fig. 1: RAPD profiling pattern of 20 genotypes with OPM-10, OPM-12, OPM-16 and OPM-19 Primer.

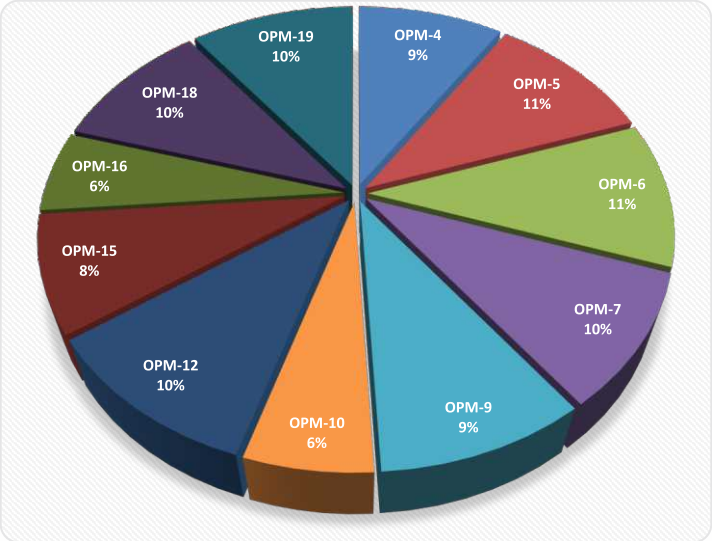


Fig.2. Pie chart depicts the PIC Value of RAPD Primers

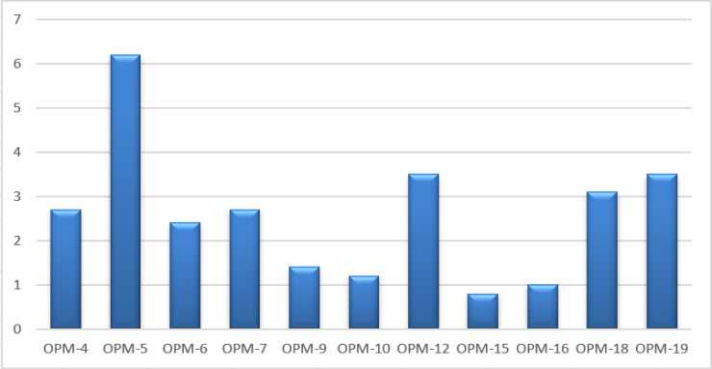


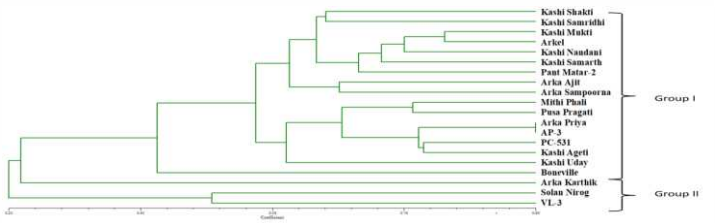
Fig. 3. Resolving power (RP) value of RAPD primers.

Discussion

Twenty genotypes of garden pea *(Pisum sativum)* collected from different regions of India were studied for morphological and molecular diversity. Crop improvement through breeding programs depends upon the genetic diversity from various genetic resources, a wide range of the genotypes of different vegetable crops were deliberate for genetic diversity analysis [22] [23]. Therefore, the estimation of genetic diversity among the genotypes has become an important aspect for identifying superior genetically different parents along with desirable traits [22] [23]. Phenotypic correlations of ten selected traits such as days to germination, 50% flowering, plant height, number of pods/plant, seed per pod, length of pods, width of pods, days to maturity, pod yield per plant, and pod yield q/ha, which are important characters for the identification, characterization and grouping of genotypes. Morphological traits discriminate the various plant species [24] [25]. However for the majority of traits interactions between genotypes and environments very complicated process. Days to germination are negatively correlated with length of pods, & days to germination (0.48), 50 % flowering (0.72), Plant height (0.66) & number of pods/ plant (0.41) are significantly correlated with days to maturity. Maturity and pod yield are highly important traits that are considered by breeders for the classification of garden pea cultivars. Morphological traits are highly affected by the environmental factors used for the estimation of genetic diversity as well as relationships among garden pea genotypes with environments. The approach depends on conventional as well as molecular studies to provide a better understanding of variation patterns among the genetic resources that can be exploited to broaden the genetic base for useful traits [26].

RAPD molecular markers are important markers for establishment of relationships and genetic diversity as they are polymorphic, dominant in nature and abundant in plant genomes [27].

The evolution of varieties in distinct agro-climatic zones demonstrates significant levels of variation in response to the selection pressure in the zones [28]. The RAPD molecular markers have been used in population genetic studies [29] [30]. Out of the total of 14 RAPD primers tried in PCR amplification, 11 primer panels indicate clear and effective amplification while the rest of the primer did not amplify. Eleven RAPD primers revealed 60 bands with an average of 5.45 bands per primer and 60 polymorphic bands that unambiguously discriminated 20 genotypes into three major groups. Out of 60 scorable bands 78.57 % band were found to be polymorphic and 21.43 % were found to be monomorphic as compared to 41.66 % & 55.70 % obtained by different studies [31]. The RAPD PCR-based technique amplifies both non-coding as well as coding sequences of the genome, but when they amplify in one region they do not amplify in another region of the genome, thus reducing the possibility of amplifying mainly polymorphic regions [32]. Results indicated the presence of wide genetic variability among different genotypes of peas. Variations in DNA sequences lead to polymorphism. Greater polymorphism is indicative of greater genetics. The results are in -diverse comparison with the findings of Ahmad *et al.* [33]. The use of parents with greater genetic diversity results in a broad genetic base of the hybrids.



PIC (Polymorphic Information Content) ranged from 0.22 to 0.37 with an average value of 0.33. PIC value is a reflection of allele diversity and frequency among the genotypes. In addition, the value of resolving power (RP) ranged from 0.80 to 6.20 with an average value of resolving power of 2.590. Based on clustering analysis using RAPD, pea cultivar Arka Kartik was completely distinct among both groups and was not clustered with any other cultivar [34] [35] [36]. Some researchers have considered RAPD markers to represent segments of DNA with noncoding regions and to be selectively neutral [37] [38] and some studies have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci [39]. Our results indicate the presence of great genetic variability among elite genotypes of peas. Both RAPD markers are useful in the assessment of pea diversity, the detection of duplicate samples in genotype collection, and the selection of a core collection to enhance the efficiency of genotype management for use in pea breeding and conservation.

RAPD markers produced 60 alleles among the 20 genotypes of pea, and the average values of the Na (number of alleles), Ne (effective number of alleles), PIC (polymorphism information content), MI (marker index), RP (resolving power) and EMR (effective marker relationship) were 5.45, 5.45, 0.33, 0.95, 2.59 and 2.86 respectively. The average value of the number of alleles per locus was 5.45 which is consistent with an earlier study [40] [41] where average number of alleles was 5.45 by using 11 RAPD-based markers a 20 selected *P. sativum* genotypes was

reported and more or less higher than the other study [42] [43]. The PIC, which represents the allele numbers and their distribution, was figured out to determine the informativeness of every marker. The lower PIC value implies a higher level of genetic similarity within the analyzed crop genotypes and *vice-versa*. The most informative RAPD marker were OPM-5, OPM-6 OPM-12, and OPM-19, with a PIC value of 0.37, while the minimum PIC was recorded for OPM-10 (PIC = 0.22). The average PIC value in the present study (0.33) was more or less similar to 0.38 for 22 cultivated (*P. sativum)* and two wild relatives (*Pisum fulvum*) reported by Yang et al. [44] and lower than the 0.4817 for 266 grass pea and 17 relative accessions estimated by Wang et al. [45] and the 0.627 for thirty-five pea genotypes estimated by Ahmad et al. [46]. The mean value of expected EMR (2.86) was comparable with 0.43 for 20 grass pea accessions reported by Shiferaw *et al*. [47] [48] [49].

Conclusions: In the current project, a multidisciplinary strategy was carried out to estimate the genetic variability among the garden pea collection. The studied agro-morphological and molecular markers detected good genetic variability among the 20 *Pisum sativum* genotypes, potentiating their use in the garden pea breeding program. RAPD markers grouped the genotypes into three major categories by cluster analysis. The genotypes pair Arka Priya & AP-3, pair Mithi phali and Pusa pragati, exhibited the greatest similarity, and VL-3 & Arka Kartik, Arka Priya exhibited the highest dissimilarity based on RAPD analysis, therefore, these pairs could be used in further pea breeding program to developing new segregants. The proposed combined approaches of morpho-agronomic characterization together with a molecular evaluation in our study can be useful in selecting diverse parental lines and widen the gene pool of garden peas for future breeding programs.

Competing interests: Authors have declared that no competing interests in this manuscripts

Table 9 Cluster analysis based on Unweighted paired group method of arithmetic mean of (UPGMA) of RAPD

	Kashi Shakti	Kashi Mukti	Kashi Samarth	Kashi Nandani	Boneville	P. Matar-2	Arka Ajit	Arkel	Arka Sampoorna	Kashi Samridhi	MithivPhali	A.Priya	AP-3	Pusa Pragati	PC-531	Kashi Ageti	Solan Nirog	Arka Karthik	Kashi Uday	VL-3
Kashi Shakti	1.00																			
Kashi Mukti	0.64	1.00																		
Kashi Samrath	0.56	0.77	1.00																	
Kashi Nandani	0.67	0.78	0.72	1.00																
Bonneville	0.50	0.45	0.47	0.53	1.00															
P. Matar-2	0.58	0.76	0.65	0.67	0.57	1.00														
ArkaAjit	0.64	0.62	0.60	0.65	0.48	0.59	1.00													
Arkel	0.67	0.79	0.64	0.69	0.45	0.63	0.70	1.00												
Arka Sampoorna	0.54	0.54	0.60	0.58	0.42	0.55	0.66	0.54	1.00											
Kashi Samridhi	0.64	0.66	0.57	0.66	0.40	0.56	0.52	0.69	0.62	1.00										
Mithi Phali	0.56	0.68	0.62	0.68	0.44	0.65	0.57	0.64	0.53	0.57	1.00									
Arka Priya	0.57	0.51	0.46	0.58	0.39	0.52	0.47	0.54	0.54	0.69	0.63	1.00								
AP-3	0.64	0.53	0.49	0.60	0.42	0.57	0.53	0.63	0.53	0.67	0.65	0.90	1.00							
Pusa Pragat	0.62	0.68	0.59	0.64	0.38	0.62	0.53	0.64	0.53	0.61	0.75	0.71	0.72	1.00						
PC-531	0.54	0.51	0.50	0.55	0.36	0.56	0.44	0.51	0.47	0.52	0.60	0.73	0.78	0.68	1.00					
Kashi Ageti	0.50	0.52	0.44	0.60	0.36	0.61	0.45	0.55	0.48	0.60	0.62	0.75	0.76	0.65	0.76	1.00				
Solan Nirog	0.22	0.35	0.30	0.31	0.30	0.36	0.27	0.31	0.27	0.27	0.30	0.25	0.26	0.34	0.35	0.32	1.00			
Arka Karthik	0.20	0.31	0.30	0.27	0.18	0.28	0.21	0.27	0.27	0.27	0.30	0.25	0.23	0.30	0.24	0.28	0.33	1.00		
Kashi Uday	0.52	0.51	0.43	0.49	0.29	0.45	0.44	0.58	0.44	0.55	0.47	0.62	0.63	0.61	0.62	0.60	0.26	0.22	1.00	
VL-3	0.14	0.23	0.22	0.20	0.16	0.23	0.13	0.19	0.16	0.20	0.19	0.18	0.16	0.22	0.22	0.23	0.50	0.17	0.21	1.00

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