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Molecular characterization in Fieldpea*(Pisumsativum*L.*vararvense)*

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Abstract

DNA-based markers provide powerful and reliable tools for discriminating variations within crop germplasm and for studying evolutionary relationships. The present study involved molecular characterization of 36 genotypes of Fieldpea*(Pisumsativum*L.*vararvense)* comprising 8 parent and 28 F_1's hybrids, using RAPD markers.Pooled RAPD analysis produced a total of 108 DNA fragments, out of which 91 were polymorphic (82.35%) average polymorphism. Parent IPFD 10-13 and KPMR 400 were least similar to each other, the cross between ADARSH X NDP-1 and DF-1 X PRAKASHrevealed lowest similarity index indicating more distance between them. A wide range of genetic similarity (0.36 to 0.89) conform that a significant genetic variation exists among the various cross combinations of fieldpea. It can be further utilized in strengthening fieldpea breeding programme for improving yield and quality characters of fieldpea.

Key words: Molecular marker, Fieldpea, RAPD

Introduction

For an effective breeding programme, information concerning the extent and nature of genetic diversity within a crop is essential. It is particularly useful for characterizing individual accessions and cultivars and as a general guide in the selection of parents for hybridization. Self pollinating crops such as *Pisumsativum* L. develop increased homozygosity due to continual self pollination (Cieslarova*et. al*., 2011). Although these factors ensure higher yields and production, they lead to unwanted genetic erosion (Akhalkatsi*et al.,* 2010).

DNA-based markers provide powerful and reliable tools for discriminating variations within crop germplasm and for studying evolutionary relationships (Gepts,1993). Random amplified polymorphic DNA (RAPD) markers offer quick screening of different regions of the genome for genetic polymorphisms. The technique of RAPD gained importance in genetic research due to its simplicity, speed (Welsh & McClelland, 1990), efficiency, relative ease to perform and non-requirement of sequence information (Karp, 1997).

In Fieldpea, the RAPD assay has been employed to molecular mapping (Chagamirza*et al*. 2002) and variety discrimination (Smykal*et al*. 2008). It has been also used assessing diversity in fieldpea cultivars (Branger*et al.* 2004). A potentially more important use of this technique would be the allocation of genotypes to specific heterotic groups which would reduce both cost and labour by eliminating intra group crossing.

Material and Methods

For molecular characterization two to three week old seedlings of 36 lines comprising 8 parent and 28 F_1's hybrids (Table 1) were grown in normal soil in greenhouse used for genomic DNA isolation for Random Amplification of Polymorphic DNA (RAPD). Genomic DNA from each pea genotype was isolated from bulked leaf samples (2g each) plucked from young seedlings of one month age. Isolation of DNA was done based on the modified protocol of Guillemant and Laurence (1992).

DNA extraction and RAPD-PCR

Genomic DNA was isolated from 2g leaf tissue using DNA Extraction Buffer(100 mM Sodium acetate, pH 4.8; 500 mMNacl; 50mM EDTA, pH 8.0; 50mM Tris, pH 8.0; 2% PVP (MW 10000);1.4% SDS). Then 0.6 volume of chilled isopropanol was mixed with the supernatant for DNA precipitation. DNA was pelleted out, washed twice with 70% ethanol and dissolve in TE

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[10 mMTris HCL (pH=8.0) and 1.0 mM EDTA (pH=8.0)]. Dissolved DNA solution was extracted with Phenol: Chloroform: Iso-amyl alcohol (P: C: I) solution in the ratio of (25: 24: 1) and RNA was removed by RNAse treatment @ 4 µl/ml of grinding buffer from stock of 50 mg/ ml of RNAse at 37°C for 1 hr. Dried pellets were dissolved into 100 µl TE buffer and stored at -20°C for further use.The quantification of DNA was carried out using nanodrop machine. The quality of DNA was checked on 0.8 % (w/v) agarose gel prepared in 0.5X TBE containing 2.0 µl of ethidium bromide (EtBr; 1 mg/ 1ml). The stocks were diluted to a final concentration of 50 ng/µl of DNA and used for further applications.

PCR reactions were performed in 25 µl total volume of TaqBuffer(2.50 µl) Sterile DDH2O (17.42 µl)MgCl2 -25mM (1.00 µl), dNTP-10mM (0.37 µl), Primer-10pmol/µ (2.50 µl), Taq DNA Polymerase -10 U (0.20 µl), Template DNA-50ng/µl (1.00 µl). Amplification was carried out in DNA thermocycler programmed for 44 cycles, after Initial Denaturation 3min at 94**°C**, Denaturation 1 min at 94**°C**,Annealing 1 min at 37**°C**, Extension 2 min at 72**°C**, Final Extension 8 min at 72**°C**, followed by hold at 4**°C.**The amplified product was collected from the thermal cycler and loaded on to 1.5 percent (w/v) agarose gel prepared in 0.5X TBE (pH 8.0) with EtBr. The band profiles were visualized and documented using Alpha innotech Flour chem. FC2 gel documentation system.

Data scoring and analysis

Data was scored for computer analysis on the basis of the presence or absence of the PCR products. The polymorphism percentage was calculated as per the method suggested by Blair *et al*., (1999). The data generated by RAPD was analyzed with the software NTSYSpc version 2.0

Result and Discussion

The genomic DNA extracted from each of the total 36 genotypes including eight parents and 28 hybrids were subjected to RAPD analysis. Total 25 primers were used, out of which 12 primers *viz.,* OPP-08, OPP-09, OPP-10, OPP-13, OPP-14, OPP-16, OPH-02, OPH-03, OPH-08, OPBA-04, OPBA-09 and OPBA-10 yielded comparatively maximum number of amplified product with high intensity and minimal smearing, good resolution and also clear bands.

The primer OPBA-09 amplified DNA fragment ranging from 152 to 1115 bp and revealed 100 per cent polymorphism with the highest PIC value (0.907) among all primers, in contrast OPH-03 produced narrow range of amplified products (149-668bp) and OPP-09 with lowest PIC value (0.727). All these 12 RAPD primers used individually revealed higher level of genetic polymorphism among the 36 Field pea lines except OPH-08 (Table 2). This conforms the RAPD analysis is a successful tool for the identification of individual genotypes in *Pisumsativum L.* RAPD markers have been used successfully for identification and finding phylogenetic relation among and within species (Agarwal *et al*.,2007).

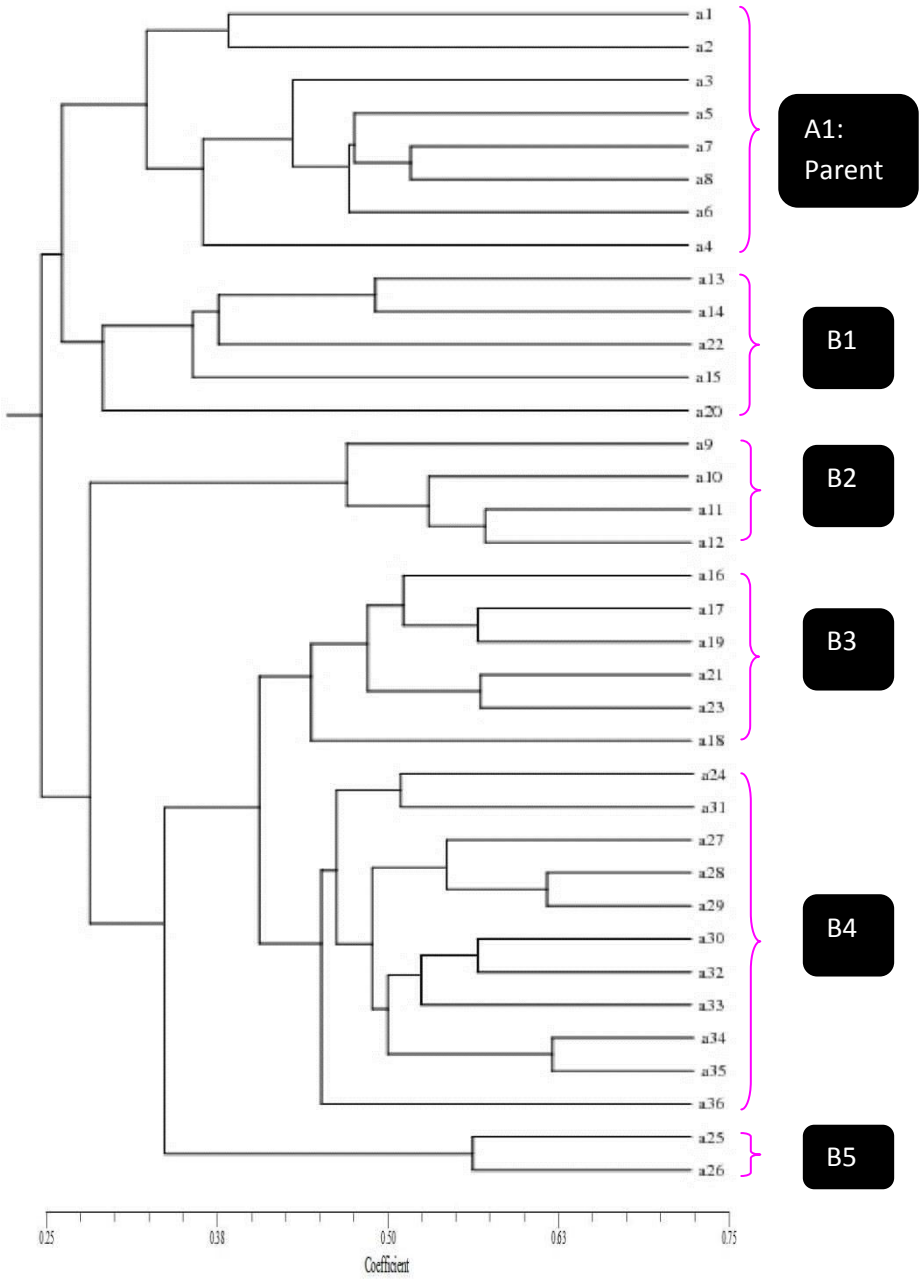
PCR amplification of DNA using 12 primers for RAPD analysis generated total of 108 DNA fragments and among the 108 DNA amplified fragments, 91 were polymorphic and gave 82.35 per cent polymorphism. The average polymorphism per band was 7.6 and per cent polymorphism ranged from 28.5 (OPH-08) to 100 (OPBA-09) *i.e*. The range of PIC value was 0.727 (OPP-09) to 0.907 (OPBA-09) (Barange*et al*., 2004).

The dendrogram constructed with the help of 12 primers subjected to 36 genotypes comprising eight parents and twenty eight F_{1s} were characterized into two major clusters. Cluster A comprising of all parents showed lower level of polymorphism among them. Simioniu*cet al.* (2002) and Samatadze*et al.,* (2008) also reported a low level of variability among the genotypes using RAPD markers. Cluster B indicated all F_1s (Fig.1) and cluster B again sub-clustered into different five groups (Kapila*et al.,* 2012).

Based on jaccard's pair wise similarity coefficient value, parents IPFD 10-13 and KPMR 400 were least similar to each other showed the lowest similarity index value (0.66).Whereas VIKAS and KPMR 400 were close to each other having the highest similarity index value (0.89). The cross between ADARSH X NDP-1 and DF-1 X PRAKASH found the lowest similarity Index (0.36) indicating more distance between them , whereas VIKAS X APARNA and VIKAS X NDP-1 has highest similarity(0.84) given in (Table 3) Similar results were found by Simioniu*cet al*. (2002).

The genetic similarity ranged from 0.36 to 0.89 which shows that a significant genetic variation exist among various combinations of fieldpea lines such a huge variability may be further utilized in strengthening fieldpea breeding programme for improving yield and quality characters of fieldpea. Similar results were found by Sedehi*et al*. (2008). Further it is evident from present data that PCR based array like RAPD can be used effectively to estimate the genetic variability and for discriminating the parents from each other. It especially suitable for breeding programmes where large numbers of lines/ accessions have to be analyzed.

Fig-1 : Dendrogram showing clustering of fieldpea parents and their F_1's constructed using UPGMA based on Jaccard's similarity

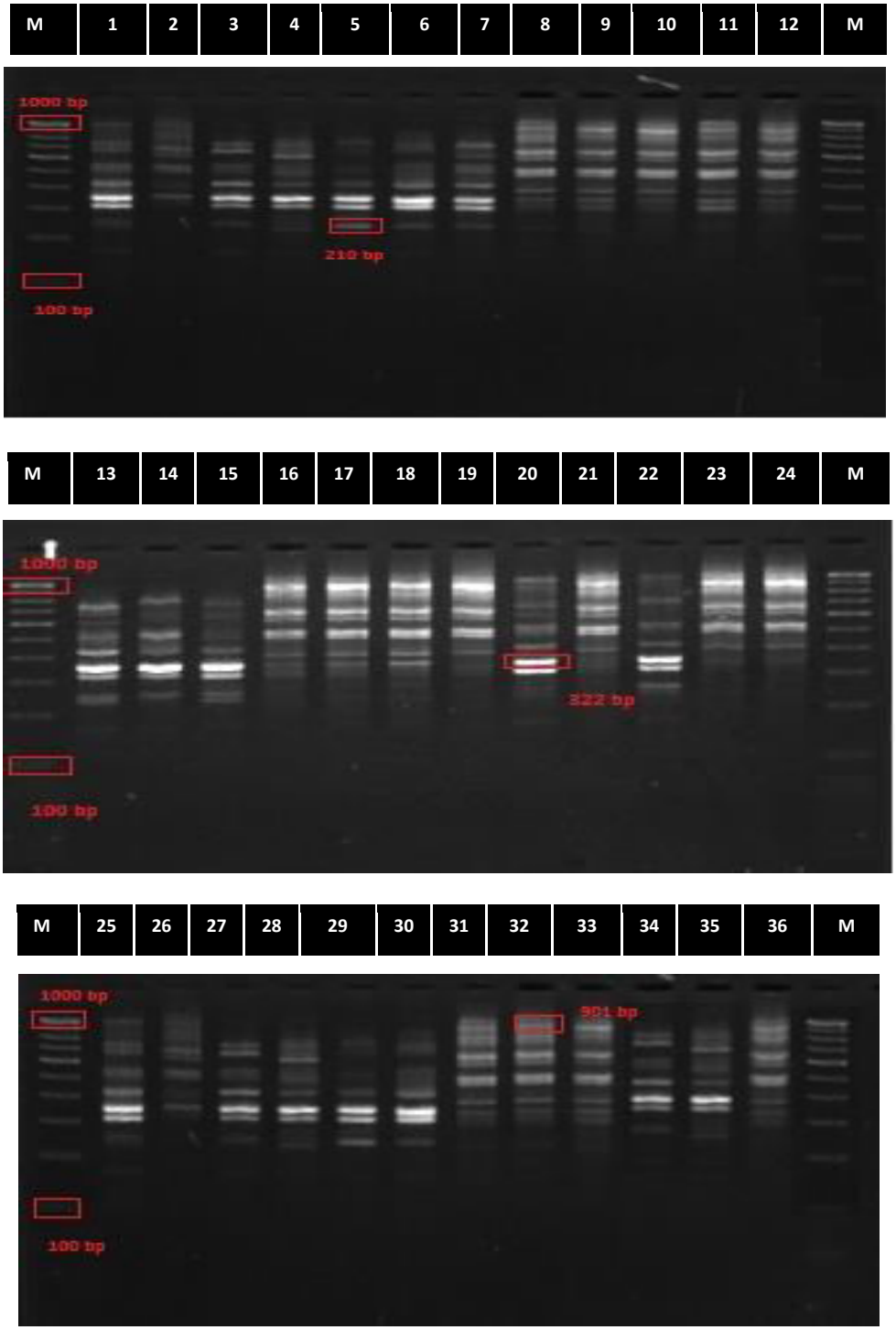


PARENTS :

1. DF 1 (Standard check) 2.KPMR 400 3.VIKAS 4.PRAHASH 5.ADARSH 6.IPFD 10-13 7.NDP-1 8. APARNA

HYBRIDS :

9. DF 1 x KPMR 400 10. DF 1 x VIKAS 11. DF 1 x PRAHASH 12.DF 1 x ADARSH 13.DF 1 x IPFD 10-13 14.DF 1 x NDP-1 15. DF 1 x APARNA 16. KPMR 400 x VIKAS 17. KPMR 400 x PRAHASH 18.KPMR 400 x ADARSH 19.KPMR 400 x IPFD 10-13 20.KPMR 400 x NDP 1 21.KPMR 400 x APARNA 22.VIKAS x PRAHASH 23.VIKAS x ADARSH 24. VIKAS x IPFD 10-13 25. VIKAS x NDP 1 26.VIKAS x APARNA 27.PRAHASH x ADARSH 28. PRAHASH x IPFD 10-13 29. PRAHASH x NDP 1 30.PRAHASH x APARNA 31. ADARSH x IPFD 10-13 32. ADARSH x NDP 1 33.ADARSH x APARNA 34.IPFD 10-13 x NDP 1 35. IPFD10-13 x APARNA 36. NDP1x APARNA



M=Ladder DNA
1. DF 1 (Standard check) **2**. KPMR 400 **3**. VIKAS **4**. PRAHASH **5**. ADARSH **6**. IPFD 10-13 **7**.NDP-1 **8**. APARNA **9**. DF 1 x KPMR 400 **10**. DF 1 x VIKAS **11**. DF 1 x PRAHASH **12.** DF 1 x ADARSH **13**. DF 1 x IPFD 10-13 **14.** DF 1 x NDP-1 **15**. DF 1 x APARNA **16**. KPMR 400 x VIKAS **17**. KPMR 400 x PRAHASH **18**. KPMR 400 x ADARSH **19**. KPMR 400 x IPFD 10-13 **20**. KPMR 400 x NDP 1 **21**. KPMR 400 x APARNA **22**. VIKAS x PRAHASH **23**. VIKAS x ADARSH **24**. VIKAS x IPFD 10-13 **25**. VIKAS x NDP 1 **26**. VIKAS x APARNA **27**. PRAHASH x ADARSH **28**. PRAHASH x IPFD 10-13 **29**. PRAHASH x NDP 1 **30**. PRAHASH x APARNA **31**. ADARSH x IPFD 10-13 **32**. ADARSH x NDP 1 **33**. ADARSH x APARNA **34.** IPFD 10-13 x NDP 1 **35**. IPFD10-13 x APARNA **36**. NDP1xAPARNA
Figure 1: RAPD patterns of different Fieldpea genotypes and their F_1'sproduced by primer OPP-10

Table 1 : The details of parents, their pedigree, source/origin :

Sr. No	Genotypes	Pedigree	Source
(1)	DF 1 (SKNP 04-09)	Selection from DDR-49	SDAU, S.K.Nagar
(2)	KPMR 400	Rachana × HFP-4	CSAU, Kanpur
(3)	VIKAS (IPFD 99-13)	HFP-4 × LFP 80	IIPR, Kanpur
(4)	PRAKASH (IPFD 1-10)	PDPD 8 x HUDP 7	IIPR, Kanpur
(5)	ADARSH (IPFD 99-25)	PDPD 8 × Pant P 5	IIPR, Kanpur
(6)	IPFD 10-13	DDRC 16 x HUDP 7	IIPR, Kanpur
(7)	NDP-1	-	NDUA&T, Faizabad
(8)	APARNA (HFP-4)	T163 × EC 10916	CCS HAU, Hisar

Hybrids:				
9.	DF 1 x KPMR 400		**23.**	VIKAS x ADARSH
10.	DF 1 x VIKAS		**24.**	VIKAS x IPFD 10-13
11.	DF 1 x PRAHASH		**25.**	VIKAS x NDP 1
12.	DF 1 x ADARSH		**26.**	VIKAS x APARNA
13.	DF 1 x IPFD 10-13		**27.**	PRAHASH x ADARSH
14.	DF 1 x NDP-1		**28.**	PRAHASH x IPFD 10-13
15.	DF 1 x APARNA		**29.**	PRAHASH x NDP 1
16.	KPMR 400 x VIKAS		**30.**	PRAHASH x APARNA
17.	KPMR 400 x PRAHASH		**31.**	ADARSH x IPFD 10-13
18.	KPMR 400 x ADARSH		**32.**	ADARSH x NDP 1
19.	KPMR 400 x IPFD 10-13		**33.**	ADARSH x APARNA
20.	KPMR 400 x NDP 1		**34.**	IPFD 10-13 x NDP 1
21.	KPMR 400 x APARNA		**35.**	IPFD10-13 x APARNA
22.	VIKAS x PRAHASH		**36.**	NDP 1 x APARNA

Table 2: Results of RAPD analysis of parents and their F_1's in Fieldpea.

Sr.No.	Primers	Primer Sequence (5'-3')	GC content (%)	Molecular weight range (bp)	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Percent polymorphism	PIC value
1	OPP-08	ACATCGCCCA	60	242-946	7	6	1	85.70	0.850
2	OPP-09	GTGGTCCGCA	70	238-1000	4	4	0	100.0	0.727
3	OPP-10	TCCCGCCTAC	70	210-901	12	12	0	100.0	0.897
4	OPP-13	GGAGTGCCTC	70	141-888	8	8	0	100.0	0.814
5	OPP-14	CCAGCCGAAC	70	100-898	6	5	1	83.30	0.788
6	OPP-16	CCAAGCTGCC	70	263-1000	9	5	4	55.50	0.843
7	OPH-02	TCGGACGTGA	60	141-839	11	10	1	90.90	0.866
8	OPH-03	AGACGTCCAC	60	149-668	8	5	3	62.50	0.804
9	OPH-08	GAAACACCCC	60	156-965	7	2	5	28.50	0.840
10	OPBA-04	TCCTAGGCTC	60	115-757	11	10	1	90.90	0.882
11	OPBA-09	GGAACTCCAC	60	152-1115	14	14	0	100.0	0.907
12	OPBA-10	GGACGTTGAG	60	186-892	11	10	1	90.90	0.876
				Total	108	91	17	988.2	10.094
				Average	9	7.6	1.4	82.35	0.84

Table 3 : Jaccard's similarity coefficient for different fieldpea genotypes based on RAPD data analysis

R/C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1.	1																																			
2.	0.704	1																																		
3.	0.735	0.891	1																																	
4.	0.768	0.736	0.768	1																																
5.	0.797	0.789	0.771	0.829	1																															
6.	0.721	0.667	0.671	0.779	0.783	1																														
7.	0.691	0.761	0.769	0.750	0.833	0.701	1																													
8.	0.691	0.788	0.797	0.776	0.779	0.727	0.867	1																												
9.	0.667	0.732	0.739	0.699	0.726	0.700	0.800	0.887	1																											
10.	0.676	0.718	0.750	0.732	0.712	0.686	0.785	0.812	0.806	1																										
11.	0.563	0.629	0.657	0.643	0.648	0.694	0.662	0.636	0.592	0.600	1																									
12.	0.554	0.595	0.620	0.608	0.592	0.583	0.623	0.623	0.648	0.611	0.831	1																								
13.	0.710	0.729	0.686	0.743	0.771	0.773	0.769	0.769	0.714	0.750	0.657	0.643	1																							
14.	0.671	0.667	0.648	0.704	0.732	0.681	0.727	0.701	0.676	0.710	0.549	0.583	0.746	1																						
15.	0.556	0.554	0.556	0.547	0.553	0.521	0.627	0.603	0.629	0.614	0.522	0.535	0.647	0.632	1																					
16.	0.653	0.649	0.632	0.662	0.688	0.640	0.681	0.635	0.615	0.667	0.581	0.592	0.699	0.836	0.662	1																				
17.	0.532	0.494	0.494	0.506	0.476	0.481	0.494	0.474	0.463	0.506	0.500	0.554	0.513	0.519	0.577	0.610	1																			
18.	0.667	0.705	0.688	0.696	0.722	0.675	0.693	0.693	0.692	0.679	0.662	0.716	0.733	0.720	0.610	0.813	0.585	1																		
19.	0.653	0.605	0.587	0.640	0.667	0.616	0.634	0.611	0.592	0.644	0.600	0.611	0.750	0.662	0.548	0.689	0.566	0.747	1																	
20.	0.701	0.696	0.679	0.753	0.713	0.646	0.662	0.707	0.684	0.671	0.590	0.600	0.724	0.757	0.603	0.756	0.598	0.833	0.737	1																
21.	0.662	0.613	0.595	0.671	0.653	0.721	0.620	0.667	0.644	0.676	0.500	0.554	0.710	0.746	0.577	0.722	0.553	0.757	0.725	0.795	1															
22.	0.403	0.500	0.479	0.436	0.462	0.427	0.479	0.500	0.507	0.493	0.403	0.419	0.459	0.529	0.619	0.541	0.500	0.519	0.493	0.494	0.543	1														
23.	0.437	0.479	0.478	0.413	0.440	0.443	0.456	0.500	0.486	0.451	0.484	0.456	0.500	0.464	0.655	0.440	0.569	0.462	0.471	0.474	0.437	0.643	1													
24.	0.493	0.493	0.473	0.449	0.474	0.459	0.472	0.493	0.480	0.486	0.457	0.452	0.493	0.588	0.609	0.575	0.627	0.532	0.528	0.564	0.535	0.571	0.755	1												
25.	0.545	0.584	0.566	0.577	0.562	0.532	0.568	0.568	0.532	0.538	0.534	0.547	0.608	0.595	0.527	0.623	0.566	0.637	0.579	0.671	0.630	0.493	0.493	0.528	1											
26.	0.595	0.633	0.615	0.646	0.630	0.582	0.618	0.597	0.542	0.568	0.526	0.500	0.658	0.623	0.558	0.671	0.556	0.683	0.628	0.738	0.636	0.526	0.486	0.500	0.841	1										
27.	0.547	0.619	0.583	0.631	0.616	0.553	0.605	0.625	0.570	0.576	0.518	0.512	0.684	0.630	0.549	0.635	0.494	0.667	0.614	0.718	0.602	0.500	0.463	0.494	0.763	0.831	1									
28.	0.532	0.551	0.532	0.544	0.512	0.581	0.494	0.513	0.500	0.526	0.423	0.456	0.573	0.539	0.493	0.570	0.513	0.625	0.608	0.637	0.616	0.459	0.457	0.473	0.750	0.775	0.705	1								
29.	0.494	0.513	0.533	0.545	0.532	0.500	0.534	0.556	0.539	0.506	0.421	0.436	0.513	0.606	0.557	0.613	0.456	0.588	0.468	0.580	0.554	0.591	0.500	0.606	0.526	0.537	0.566	0.494	1							
30.	0.487	0.506	0.487	0.464	0.524	0.475	0.506	0.468	0.512	0.463	0.400	0.415	0.469	0.532	0.527	0.582	0.487	0.560	0.481	0.535	0.487	0.557	0.515	0.642	0.500	0.549	0.523	0.526	0.785	1						
31.	0.462	0.519	0.462	0.422	0.481	0.468	0.500	0.461	0.487	0.456	0.372	0.388	0.500	0.527	0.588	0.538	0.500	0.518	0.474	0.494	0.500	0.600	0.556	0.591	0.456	0.525	0.518	0.500	0.708	0.769	1					
32.	0.474	0.457	0.456	0.469	0.458	0.481	0.493	0.474	0.519	0.506	0.367	0.418	0.456	0.562	0.580	0.551	0.456	0.530	0.432	0.506	0.494	0.567	0.478	0.536	0.415	0.464	0.494	0.474	0.750	0.731	0.734	1				
33.	0.529	0.467	0.486	0.521	0.507	0.493	0.529	0.486	0.453	0.500	0.429	0.444	0.529	0.606	0.603	0.614	0.529	0.526	0.543	0.538	0.551	0.590	0.517	0.607	0.479	0.533	0.506	0.466	0.733	0.636	0.661	0.677	1			
34.	0.440	0.476	0.476	0.471	0.477	0.463	0.475	0.494	0.519	0.470	0.425	0.405	0.476	0.538	0.554	0.549	0.424	0.547	0.452	0.540	0.494	0.521	0.522	0.556	0.470	0.518	0.581	0.458	0.710	0.671	0.696	0.686	0.571	1		
35.	0.494	0.475	0.494	0.526	0.512	0.562	0.474	0.514	0.519	0.468	0.479	0.493	0.554	0.541	0.535	0.532	0.494	0.512	0.487	0.506	0.533	0.458	0.547	0.559	0.568	0.519	0.566	0.533	0.623	0.526	0.563	0.577	0.600	0.595	1	
36.	0.430	0.450	0.430	0.427	0.451	0.418	0.429	0.447	0.438	0.407	0.432	0.429	0.487	0.474	0.551	0.506	0.468	0.506	0.462	0.482	0.449	0.471	0.540	0.552	0.443	0.440	0.542	0.468	0.549	0.541	0.557	0.549	0.522	0.657	0.642	1

Where,

1. DF 1 (Standard Check)
2. KPMR 400
3. VIKAS
4. PRAHASH
5. ADARSH
6. IPFD 10-13
7. NDP-1
8. APARNA
9. DF 1 x KPMR 400
10. DF 1 x VIKAS
11. DF 1 x PRAHASH
12. DF 1 x ADARSH
13. DF 1 x IPFD 10-13
14. DF 1 x NDP-1
15. DF 1 x APARNA
16. KPMR 400 x VIKAS
17. KPMR 400 x PRAHASH
18. KPMR 400 x ADARSH
19. KPMR 400 x IPFD 10-13
20. KPMR 400 x NDP 1
21. KPMR 400 x APARNA
22. VIKAS x PRAHASH
23. VIKAS x ADARSH
24. VIKAS x IPFD 10-13
25. VIKAS x NDP 1
26. VIKAS x APARNA
27. PRAHASH x ADARSH
28. PRAHASH x IPFD 10-13
29. PRAHASH x NDP 1
30. PRAHASH x APARNA
31. ADARSH x IPFD 10-13
32. ADARSH x NDP 1
33. ADARSH x APARNA
34. IPFD 10-13 x NDP 1
35. IPFD10-13 x APARNA
36. NDP 1 x APARNA

References

1. **Agarwal, P.K., Chaudhary, P.R., Datta, S., Singh, N.P. and Ali, M.** (2007). DNA fingerprinting of major pulses crops of India, *Tech. Bull.,* October, IIPR, Kanpur. pp. 67-82.

2. **Akhalkatsi, M., Ekhvaia, J., Mosulishvili, M., Nakhutsrishvili, G., Abdaladze, O. and Batsatsashvili, K.** (2010).Reasons and Processes Leading to the Erosion of Crop Genetic Diversity in Mountainous Regions of Georgia.Mountain Research and Development.**30** (3) :304-310.

3. **Baranger, A., Aubert, G., Arnau, G., Laine, A.L., Deniot, G., Potier, J., Weinachter, C., Lejeune-Henaut, I., Lallemand, J. and Burstin, J.** (2004). Genetic diversity within *Pisumsativum* using protein- and PCR-based markers.*Theor.and Appl. Genet.,***108** :1309-1321.

4. **Blair, M.W., Panaud, O. and McCouch, S.R.** (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and finger printing in rice (*Oryza sativa* L.). *Theor. Appl. Genet.,* **98** : 780-792.

5. **Cieslarova, J., Smykal, P., Dockalova, Z., Hanacek, P., Prochazka, S., Hybl, M. and Griga, M.** (2011). Molecular evidence of genetic diversity changes in pea (*Pisumsativum* L.) germplasm after long-term maintenance. *Genetic Resources and Crop Evolution.,***58** : 439451.

6. **Cheghamirza, K., Koveza, O., Konovalov, F. and Gostimsky, S.** (2002).Identification of RAPD markers and their use of molecular mapping in pea (*Pisumsativum* L.).*Cell Mol. Biol. Lett.* **7** (2B) : 649-655.

7. **Gepts, P.** (1993). The use of molecular and biochemical markers in crop evolution studies.In : M.K. Hecht (Ed.), *Evolutionary Biol.*, **27** : 51-94. Plenum Press, New York.

8. **Guillemant, P. and Laurence, M.D.** (1992). Isolation of Plant DNA : A fast, inexpensive and reliable method. *Pl. Mole. Biol. Reptr.*, **10** : 60-65.

9. **Kapila, R.K., Nayal, S., Dhimans, K.C., Singh, S.K. and Sharma, J.K.** (2012).Identification of field and garden pea (*Pisumsativum* L.) varieties using RAPD and ISSR markers.*Seed Res.*, **40** (1) : 1-9.

10. **Karp, A., Edwards, K., Bruford, M., Vosman, B., Morgante, M., Seberg, O., Kremer, A., Boursot, P., Arctander, P., Tautz, D. and Hewitt, G.** (1997). Newer molecular technologies for biodiversity evaluation : opportunities and challenges. *Nature Biotechnol.,***15** : 625-628.

11. **Samatadze, T.E., Zelenina, D.A., Shostak, N.G., Volkov, A.A., Popov, K.W., Rachinskaia, O.V., Borisov, A.U., Tikhonovich, I.A., Zelenin, A.V. and Muravenko, O.V.** (2008). Comparative genome analysis in pea *Pisumsativum*L. varieties and lines with chromosomal and molecular markers.*Genetika.***44** (12) : 1644-1651.

12. **Sedehi, A.A.V., Solooki, M., Arzani, A., Ghanbari, A., Lotfi, A., Imamjomeh, A.A. and Bahrami S.** (2008). Comparative analysis of genetic diversity among grass pea landraces as detected by random, semi random and morphological markers, *Asian J. Pl. Sci.,***7** : 454-460.

13. **Simioniuc, D., Uptmoor, R., Friedt, W. and Ordon, F**. (2002).Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs.*Pl. Breed.,***121** : 429-435.

14. **Smykal, P., Aubert, G., Bustin, J., Coyne, C., Ellis, N., Flavell, A. and Warkentin, T.**(2012). Pea (*Pisumsativum* L.) in the genomics era.*Agronomy*.**2** (2) : 74-115.

15. **Welsh, J. and McClelland, M.** (1990). Fingerprinting genomes using PCR with arbitrary primers.*Nucleic Acids Res.,* **18** :7213-7218.