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Short communication

Molecular marker based studies on genetic diversity in garden pea

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ABSTRACT

Genetic diversity was studied among 28 garden pea genotypes with 16 RAPD primers which generated 79 (73.15%) polymorphic markers. The highest genetic similarity was estimated between AP 3 vs. Arkel and Arkel vs. Glossy (89%) and the lowest between VP 266 vs. VP-233 and GP 6 vs. GP 207 (43%). All the accessions were grouped into 6 clusters whose size varied from 1 to 16 genotypes. The cluster I included 11 early and 5 medium maturing genotypes. Hara Bona (tall and pulse type) was grouped separately in cluster III and VP266 in cluster VI and these two were highly divergent from other genotypes.

Key words: Vegetable pea, RAPD, genetic relatedness.

Garden pea (*Pisum sativum* L. subsp. *hortense* (Neilr.) Asch. & Graebn.) is an important leguminous vegetable cultivated for green pods throughout the world. Crop improvement mainly depends on the extent of heritable diversity existing in the available germplasm. Frequent use of few parents in a breeding program led to the low genetic diversity among pea varieties (Baranger *et al.*, 1). Diverse genetic background provides desirable allelic variation among parental lines to produce new and valuable combinations (Tar'an *et al.*, 5). For the development of effective breeding programmes there is a need to evaluate the available germplasm of pea for genetic divergence. An independent set of garden pea germplasm was used to study genetic diversity based on RAPD markers.

The seeds of 28 garden pea genotypes were grown at Division of Vegetable Science, IARI, New Delhi. Genomic DNA was isolated according to the standard protocol with minor modifications. PCR reactions were run on a Biorad C 1000 thermocycler using the cycling temperature profiles for RAPD analysis. Cycling conditions used for RAPD PCR amplification were standardized. Initial denaturation at 94°C for 4 min. followed by 35 cycles of 94°C for 1 min., 34°C for 1 min., 72°C for 2 min. and a final extension step at 72°C for 10 min. The amplified PCR products was resolved on 2.5% agarose gel at 70-80 V for 3.5-4 h, using 1x TBE (Tris-boric acid-EDTA) buffer, visualized under UV light after staining with ethidium bromide and photographed under gel documentation system. DNA bands were scored '1' for its presence and '0' for its absence for each primer genotype combination. Based on the banding pattern obtained the polymorphism percentage was calculated. Genetic similarity between the genotypes/ varieties was estimated using Jaccard's similarity coefficient. Software NTSYS pc was used for clustering using UPGMA (Unweighted pair group method using arithmetic averages. Genetic diversity was calculated at each locus for allelic Polymorphism Information Content (PIC), with program CERVUS version 2.0 based on allelic frequencies among all 28 genotypes analyzed.

The polymorphism survey among all the genotypes was carried out using the bulked DNA sample in each case. A total of 72 RAPD primers were screened for amplification and only 16 were able to produce polymorphic bands, all other primers produced monomorphic bands during screening. Sixteen RAPD primers used in the present study produced scorable, unambiguous markers. These primers produced a total of 108 reproducible bands for analysis out of which 79 bands (73.15%) were found to be polymorphic and the rest were monomorphic. In terms of detection of polymorphism (73.15%), the results obtained in the present study is highly comparable with the result obtained by Choudhary *et al.* (2) and Tanveer *et al.* (4) who also observed 75% polymorphism. The degree of RAPD polymorphism was higher than that reported by Simioniuc *et al*. (3) who observed 56 % polymorphism in pea genotypes. The variation in the quantum of polymorphism might be due to the differences in genotypes and use of different RAPD primers which are randomly distributed on the genome.

The number of alleles detected per primer pair ranged from 2 to 12 with an average of 6.75 per primer. The percentage of polymorphic bands ranged from 50 to 100. The maximum number of 12 bands was observed in the profile of the primer B 474 followed by 10 in case of OPW 08, P 10 and OPO 18 (Table 1). The minimum number of amplified products (2) was observed in the primer profile OPW 01. Out of these

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16 primers, OPW08, OPN14, OPW01, P9 and V20 showed 100 per cent polymorphism. The primers OPW 04 and Y15 were found to be less polymorphic with 50 per cent polymorphism. The PIC was highest for RAPD primers OPN14 followed by HU11 and lowest for the primer Y15 (Table 1). Yadav *et al.* (8) reported average polymorphic bands of 5.3 per primer. Higher the PIC value more will be the usefulness of primer, hence, primer OPN 14 was found to be highly informative as more number of genotypes can be differentiated by using this primer.

Similarity matrix generated by Jaccard's coefficient has shown the extent of relatedness between the genotypes. Higher the dissimilarity between the genotypes better is the scope to include them in a hybridization programme for getting the transgressive segregants. Similarity coefficient was computed for all the 378 combinations of 28 genotypes based on the 16 RAPD primers that gave 108 markers and it ranged from 0.43 and to 0.89 per cent. The highest similarity was estimated between AP 3 vs. Arkel and Arkel vs. Glossy (89%). It can be supported from the fact that Arkel and AP-3 are of early maturity and Glossy is a mutant of Arkel. It was also substantiated by the dendrogram where AP 3 vs. Arkel and Arkel vs. Glossy were found to be placed very close in a single cluster (Fig. 1). The minimum similarity index 0.43 was found between VP 266 vs. VP-233 and GP 6 vs. GP 207. Other combinations showing very high genetic similarity were PSM 3 vs. AP and VL 7 vs. Glossy (0.88), PSM 3 vs. Arkel and VL 7 vs. Arkel (0.86), VL 3 vs. AP 3, AP 3 vs. PSM 4, and AP 3 vs. VL 7 and GP 18 vs. Arkel. It indicated that most of the early maturing cultivars of garden pea have been developed from very limited parental combinations and there is narrow genetic diversity among them. Most diverse relationship was obtained between the VP 266 vs. VP 233 and GP 6 vs. GP 207 (0.43). GP-6 is an afila genotype and resistant to powdery mildew, while GP207 having normal leaf and susceptible. It clearly indicated that these two genotypes are much divergent in their phenotypic expression as well which have been validated through high divergence based on molecular markers. Low genetic similarity was also evident between the genotypes VP 266/GP901 and GP55 (KN) vs. GP207 (0.46). These findings are in agreement with Tanveer *et al.* (4), while disagreement with Yadav *et al.* (8) and Thakur *et al*. (6). Moreover, range of estimated genetic similarity obtained in the present study was also high (0.43 to 0.89) because of the fact that informative primers were employed in the present study.

Table 1. Polymorphism shown by RAPD primers in garden pea genotypes.

Primer	Total No. of bands	No. of polymorphic band (s)	No. of monomorphic band (s)	Polymorphism (%)	PIC value
B474	12	7	5	58.33	0.48
BC 210	9	7	2	77.78	0.53
OPW08	10	10	0	100.00	0.58
HU11	7	5	2	71.43	0.64
OPE16	4	3	1	75.00	0.56
OPN14	6	6	0	100.00	0.74
OPO18	10	6	4	60.00	0.49
OPW01	2	2	0	100.00	0.51
OPW02	6	4	2	66.67	0.53
OPW04	2	1	1	50.00	0.22
OPW09	7	4	3	57.14	0.47
P9	4	4	0	100.00	0.54
P14	7	4	3	57.14	0.25
Y15_1050	4	2	2	50.00	0.21
P 10	10	6	4	60.00	0.57
V20	8	8	0	100.00	0.49
Total bands	108	79	29		
% of total	100	73.15	26.85		
Av.	6.75	4.94	1.81	73.15	0.48

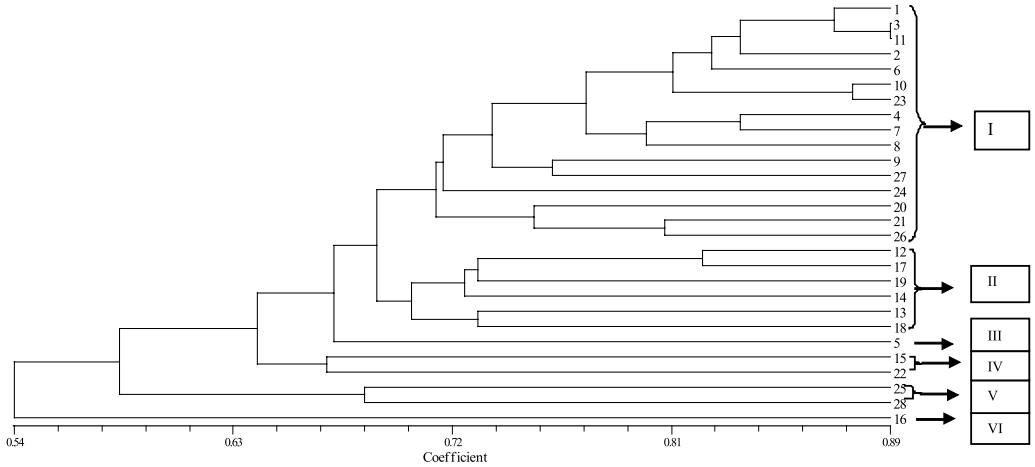

Fig. 1. UPGMA dendrogram of 28 garden pea genotypes.1: PSM-3; 2: VL 3; 3: AP 3; 4: E6; 5: Hara Bona; 6: PSM 4; 7: GP 19; 8: GP 17; 9: GP 18; 10: VL 7; 11: Arkel; 12: Pusa Pragati; 13: GP 55; 14: GP 6; 15: PM 65; 16: VP 266; 17: PM 69; 18: Tardia; 19: HUVP 4; 20: GP 901; 21: Bonneville; 22: GP 48; 23: Glossy; 24: GP 471; 25: GP 468; 26: VP233; 27: GP 473; 28: GP207.

The 28 genotypes formed 6 clusters at nearly 72% similarity level. Among the different clusters, the cluster size varied from 16 (Cluster I) to 1 (Cluster III, VI). The cluster I included mostly early genotypes (PSM 3, AP 3, PSM 4, Arkel, VL 7, Glossy, E 6, GP 17, GP 18, GP 19 and GP 901). However, some medium maturing genotypes (Bonneville, GP 473, GP 471, VL 3 and VP 233) were also grouped in cluster I. The cluster II included Pusa Pragati, PM 69, HUVP 4, GP 6, GP 55 (KN) and Tardia. Cluster IV consisted of varieties PM 65 and GP 48. Cluster V consists of GP 468 and GP 207. Hara Bona (tall and pulse type) was grouped separately in cluster III, while VP 266 was also alone in cluster VI indicating that these were developed from very different pedigrees and were diverse from other genotypes. Although, reproducibility of the result is a matter of concern in RAPD, it could be overcome by optimizing the experimental condition and can be used as marker of choice as evident by Baranger *et al.* (1) in *P. sativum*, where mean allelic frequency was found highest for RAPD than isozyme, SSR and ISSR markers.

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