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GENETIC DIVERSITY IN PEA GERMPLASM USING RANDOMLY AMPLIFIED POLYMORPHIC DNA MARKERS

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Abstract

Selection of the genotypes using plasmid assisted technology provides an efficient and useful tool for elaborating genetic relationships among genotypes. In present study, 48 Pea (*Pisum sativum* var *sativum* L.) genotypes obtained from different sources were analyzed through 20 RAPD, DNA markers for assessment of intraspecific DNA variations. Results revealed that significant variations were present in minor bands. Major bands also showed significant diversity. Considerable variations were also recorded in density of some common bands. Maximum and minimum genetic diversity i.e., 80% and 20% was found among 08 and 23 comparisons, respectively from banding profile. These variations can be used further for enhancing variability, a prerequisite for crop breeding. Phylogenetic clustering (through dendrogram analysis) of genotypes revealed that genetic diversity is independent of origin of genotypes. Forty eight genotypes of pea clustered in three main groups A, B and C comprising 23, 5 and 20 genotypes, respectively. Group A1 and C1 included the most distantly related genotypes and hence can be recommended for breeding to obtain genetically diverse segregating populations.

Introduction

Common pea [*Pisum sativum* var *sativum* L., a self pollinated annual herb is among four important cultivated legumes next to soya beans, groundnut and beans (Hulse, 1994). In Pakistan it is the second most important legume and cultivated on 0.141 million hectares with an annual production of 70800 Metric tons. The somatic chromosome number in pea is 14 with genome size of 4487 Mbp or 465 pg (1pg of genomic DNA=$9.65x10^8$bp) (Winter *et al.,* 2003). Pea is an important protein-rich crop, grown as a cash crop, a disease and weed breaker, and a nitrogen fixer for maintaining soil fertility (Ford *et al*., 2002; Ghafoor & Arshad 2008). The mature seeds of peas are rich in protein, either used fresh or in dry form (Facciola, 1990). The sulphur containing amino acids like methionine and cystine are present in limited amount in pea proteins (Muehlbauer, 1993). The roasted seeds of pea are used as coffee substitute and leaves and young shoots are used as a pot herb. The seeds are also contraceptive, fungi static and spermicidal (Facciola, 1990; Duke, 1981; Larkcom, 1991; Duke & Ayensu 1985). The pea seed oil used in traditional medicine system, and has shown the effect of preventing pregnancy by interfering in the normal functioning of progesterone in females and reduction in sperm count in males (Chopra *et al.,* 1986). Chemically, fresh seeds per 100g of pea contain 44 calories of energy, 76.5% water, 6.2 g protein, 16.9 g carbohydrate, 0.4 g fat, 2.4 g fiber and 0.9 g ash, 491.2 mg minerals and 435.19 mg vitamin (Duke, 1981).

Pea is predominantly an export crop in world trade and represents about 40% of total trade in pulses. Important production areas of world include USA, France, Russia, Ukraine, United Kingdom, Poland, Denmark, China, India, Canada, Chile, Ethiopia and Australia. Early varieties in these countries usually yield about 3000-4000 kg/ha of green pods while mid-season and late varieties give higher yields, about 600-700 kg/ha (Duke, 1981). Its average yield/hectare in Pakistan is very low as compared with its potential yield obtained in many other countries (Nisar *et al.,* 2008). Plant breeders have been evaluating genetic diversity of germplasm for numerous reasons like selection of parents, germplasm management and germplasm protection (Lee, 1995; Ali *et al.,* 2007; Nisar *et al.,* 2011). Correspondingly analysis of genetic variability within different genomes through use of molecular markers i.e. DNA and proteins are the best techniques because these macromolecules are stable under different environmental conditions. Furthermore, molecular markers are very informative and their application in studies of genetic diversity in plants has shown advantages over other markers based on description of phenotype (Liu & Furnier, 1993). In last couple of decades, molecular markers such as RFLP, RAPD, SCAR, AFLP etc. have been used to find out genetic variations at DNA level which allocate an estimation of degree of relation between individuals without influence of environmental factors (Yadav *et al.,* 2007). The legumes often suffer severe yield loss due to diseases and environmental stresses. Thus one of the most important uses of germplasm collection is its exploitation as a source for resistance and tolerance to biotic and abiotic stresses. Knowledge of genetic variability provides a base for establishing disease free populations. Previously, morphological markers were used to study genetic variations in crop species (Islam & Shepherd, 1991; Aslam *et al.,* 2010). Recent development in molecular biology especially development of unlimited number of DNA based markers has revolutionized the strategies of studying genetic variations and phylogenetic relationship among species (Kaundun & Park, 2002).

Randomly Amplified Polymorphic DNA (RAPD) technique developed in 1990 (Welsh & McClelland, 1990; Williams *et al.,* 1990). It is a relatively easier, cheaper and quicker technique with less technical complexity than other methods. Moreover this technique

does not require prior DNA sequence information (Li & Quiros, 2001). The successful application of RAPD markers is evident from a number of taxonomic and evolutionary studies documented in a variety of crop and other plant species including wheat, barley, rice, peanut, mustard and radish etc (Chicaiza *et al.,* 2006; Demeke & Adams, 1994; Helguera *et al.,* 2000). Breeding programs in peas like other crops mainly depends upon availability of genetic variability in material (Baranger *et al*., 2004; Ghafoor *et al.,* 2000; Singh 1985; Smýkal *et al.,* 2008; Barilli *et al.,* 2009). So far, very little attention has been given to varietals improvement of legumes especially peas outside peas grown regions of developed countries (Sultana *et al*., 2006; Nisar *et al.,* 2006). The present study aims to analyze the extent of genetic diversity present in Pakistani pea's genotypes for breeding and conservation purposes, using RAPD markers.

Materials and Methods

Plant material: The forty eight genotypes (populations) of pea cultivated in different regions of Pakistan were obtained from Institute of Agri-biotechnology and Genetic Resources (IABGR), NARC, Islamabad, Pakistan. The seeds were planted in pots at the Department of Botany, Hazara University Mansehra during 2008. No fungicides were used during experiment.

DNA extraction: Total genomic DNA was extracted separately from leaves of all the genotypes using simplified Kang *et al*., (1998) method. Extracted DNA samples were stored at 4°C for PCR use. After successful extraction of genomic DNA, its quality and quantity was checked on 1% agarose gel.

Thermal cycler or polymerase chain reaction (PCR) profiles: Various protocols of Polymerase Chain Reaction (PCR) were tested for obtaining the best amplification in pea *Pisum sativum* L. The protocol for PCR was optimized by varying the concentration of template DNA, Taq DNA polymerase and MgCl2. The protocol described by Devos & Gale (1992) with slight modifications was used for PCR. Twenty RAPD primers (Table 1) were used for 48 genotypes. Component of PCR reaction were 20ng/µl genomic DNA, 1 unit of *Taq* DNA polymerase, 0.2mM of each dATP, dCTP, dGTP and dTTP (dNTPs mix), 0.4 uM of each Randomly Amplified Polymorphic DNA primers (obtained from Gene Link, USA), 1X PCR buffer and 2 mM of $MgCl_2$. Thermo cycling was done using, Model ATC201, Thermo-cycler, NYXTechnik, USA. The temperature profile kept during thermo cycling was 94°C for 5 min, 34°C for 1 min and 72°C for 2 min, followed by 45 continuous cycles of 94°C for 1 min, 34°C for 1 min and 72°C for 2 min with an elongation of 72°C for 8 min. The cycling was continued for 45 cycles which amplified a particular DNA sequence up-to 10^6 copies (Devos & Gale, 1992).

Agarose gel electrophoresis: 1.5% agarose gel was prepared by melting 1.5g of agarose in 100 ml of 1X TBE buffer (Composition of 10X TBE; Tris 890mM Borate 890mM and EDTA 25mM) in a microwave oven for 2 minutes. Then 10µl of ethidium bromide (10 mg/ml) was added to gel solution and was poured in gel plate, inserted comb, and left at room temperature for 30 minutes. After solidification of gel, running buffer 1X TBE was poured into gel tank and comb was removed. PCR products were mixed with loading dye (0.25% BPB prepared in 40% sucrose solution) and loaded into wells. The samples were run at 100 volts for 45 minutes. Gels were observed under UV light using UV-tech gel documentation system (Fig. 1). Various observed DNA bands of different weights were scored and results were formulated.

Scoring of DNA bands and data analysis: The electropherograms of gel were used to score bands for RAPD based analysis. All the gels were scored twice manually. As RAPD behave as dominant markers, hence they tend to bi-state (Present-Absent) type of scoring. Each DNA fragment amplified by a given primer was considered as a unit character and RAPD fragment were scored as present (1) or absent (0) for each of the primer-genotype combinations. The molecular size of amplified product was estimated as major or minor with DNA ladder (1kb).

Statistical procedure: For statistical analysis, every Polymorphic band was considered as single allele and was either scored as present (1) or absent (0). Bivariate 1-0 data matrix was generated and genetic distance (GD) among genotypes was estimated using Un-weighted Pair Group of Arithmetic Means (UPGMA) as described by Nei & Li (1979) (Nei & Li 1979). The formula used to calculate GD is given below:

$$GD = 1-d_{xy}/d_x+d_y-d_{xy}$$

where
GD = Genetic distance
d_{xy} = Total number of common bands in two genotypes
d_x = Total number of bands in genotypes No. 1 and
dy = Total number of bands in genotype No. 2

The Bi-variate data were tabulated, using computer program Excel. The data was also used to construct a Dendrogram (phylogenetic tree) using computer program "Pop gene ver3.2".

Results

Recently developed technique of using only one arbitrary primer (RAPD) rather than 2 primers (as previously used in PCR) made PCR more user's friendly as it does not require any sequence information. During present study, genetic diversity in forty eight genotypes of common pea was studied using twenty Randomly Amplified Polymorphic DNA (RAPD) markers. Twenty, Randomly Amplified Polymorphic DNA (RAPD) primers, 10 of each series OPA and OPB amplified genomic DNA isolated from 48 pea genotypes. Results of DNA amplification using RAPD primer OPB 20 are presented in Fig. 1 (as a sample only).

Alleles were scored as present (1) or absent (0). The Bi-variate data matrix was designed to estimate further genetic diversity (GD) among genotypes through UPGMA (Un-weighted Pair Group of Arithmetic Means) procedure described by Nei & Li (1979). Estimates of average genetic diversity are presented in Table 2. It is evident that high amount of genetic diversity or distance (GD ranging from 20-80%) is present among these pea genotypes. Maximum genetic diversity (GD = 80%) was found among 08 comparisons (pop2 & 19, pop2 & 27, pop2 & 31, pop2 & 33, pop9 & 42, pop11 & 27, pop13 & 34, pop16 & 35) while 23 comparisons showed minimum genetic diversity estimated (GD = 20%). In the same way genetic diversity (GD = 30%), (GD = 40%), (GD = 50%), (GD = 60%), and (GD = 70%) was observed among 102, 303, 397, 235 and 60 comparisons, respectively. As a whole it can be easily concluded that maximum diversity i.e. 70% and 80% was found in 68 population comparisons (Table 2).

Table 1. Primer sequence used in RAPD analysis during PCR.

S. No	Oligo name	Sequence (5 -3)	S. No	Oligo name	Sequence (5 -3)
1.	OPA-01	CAGGCCCTTC	11.	OPB-11	GTAGACCCGT
2.	OPA-02	TGCCGAGCTG	12.	OPB-12	CCTTGACGCA
3.	OPA-03	AGTCAGCCAC	13.	OPB-13	TTCCCCCGCT
4.	OPA-04	AATCGGGCTG	14.	OPB-14	TCCGCTCTGG
5.	OPA-05	AGGGGTCTTG	15.	OPB-15	GGAGGGTGTT
6.	OPA-06	GGTCCCTGAC	16.	OPB-16	TTTGCCCGGA
7.	OPA-07	GAAACGGGTG	17.	OPB-17	AGGGAACGAG
8.	OPA-08	GTGACGTAGG	18.	OPB-18	CCACAGCAGT
9.	OPA-09	GGGTAACGCC	19.	OPB-19	ACCCCCGAAG
10.	OPA-10	GTGATCGCAG	20.	OPB-20	GGACCCTTAC

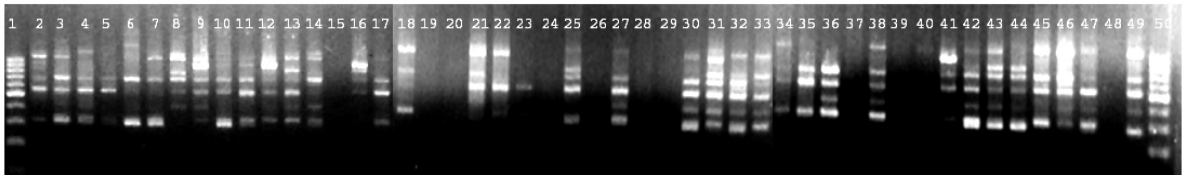

Fig. 1. PCR amplification profile of genomic DNA of forty eight genotypes (pop1-pop48) of peas through RAPD primer OPB 20. Gel picture show (left to right); 1= Molecular weight marker, 2-49 = Band profile of Pop48-pop1 and 50 = Molecular weight marker. (All other primers of both OPA and OPB series showed considerably different electropherograms in the same manner).

The Bivariate data was also used to construct a Dendrogram using computer program "pop gene" ver. 32 which showed phylogenetic relationship among 48 peas genotypes. Those genotypes were distributed in three major groups A, B, and C. Group A was the largest group comprised of five subgroups (A1, A2, A3, A4 and A5,) with 23 genotypes. Group B comprised of sub-groups B1 & B2 with Five genotypes while group C had 8 subgroups (C1, C2, C3, C4, C5, C6, C7 and C8) with 20 genotypes (Fig. 2). Four genotypes pop1, 3 17 & 18 grouped in A1 group. In contrast pop27, 28 & 29 were grouped in C8 group. Members of group A1 and C8 were most distantly related to each other. It is recommended that these genotypes can be used by breeders in order to obtain genetically diverse segregating populations with maximum genetic diversity. It was also concluded that further more studies can be carried out using variety of molecular markers that is RFLP, AFLP and SSR primers for better understanding of existing genetic diversity in pea genotypes grown in Pakistan.

Discussion

Diverse banding pattern (amplification profile) was obtained for different genotypes using same RAPD primers, which indicated presence of sufficient magnitude of genetic diversity in pea genotypes. Procedures of "Un-weighted Pair Group of Arithmetic Means (UPGMA) described by Nie & Li (1979) was used to estimate genetic distance among genotypes. Genetic Distances observed in different genotypes ranged from 20-80%. Both cluster analysis and average GD table showed the same results. These results were in close agreement with previous findings reported by Baranger *et al.*, (2004) and Yadav *et al.*, (2007) with similar range of genetic diversity in pea genotypes i.e., GD = 0-96% and 26-79%, respectively. Although high level of genetic variability was observed by Baranger *et al.*, (2004) who used both protein and DNA markers for148 accessions. Ranalli *et al*., 1997 analyzed commercial varieties of pea for yield and yield attributing traits (Ranalli *et al.*, 1997). Basavarajaiah *et al.*, (2000) and studied genetic variability among 16 quantitative characters in 81 genotypes of pigeon pea. Shinde (2000) evaluated seventy-three pea cultivars belonging to different eco-geographical regions of India for genetic variability with respect to 13 quantitative and 2 qualitative traits. He observed highly significant differences in seed yield, seed weight and pod size and plant height among genotypes (Basavarajaiah *et al.*, 2000; Shinde, 2000).

Table 2. Average genetic distance estimates among 48 peas genotypes (populations) collected from Pakistan using 20 Randomly Amplified Polymorphic DNA (RAPD) primers.

pop	Access No.	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	37	38	39	40	41	42	43	44	45	46	47	48
1	18292																																																	
2	10617	0.4																																																
3	10616	0.3	0.4																																															
4	10618	0.5	0.5	0.5																																													
5	10619	0.4	0.5	0.5	0.4																																												
6	10627	0.4	0.5	0.5	0.5	0.3																																											
7	10613	0.4	0.4	0.6	0.4	0.3	0.2																																										
8	10605	0.4	0.5	0.5	0.5	0.3	0.2	0.2																																									
9	10612	0.4	0.6	0.5	0.4	0.4	0.3	0.4	0.3																																									
10	10611	0.4	0.6	0.5	0.6	0.4	0.2	0.2	0.2	0.2																																							
11	10615	0.5	0.6	0.5	0.6	0.6	0.7	0.6	0.7	0.7	0.6																																						
12	19611	0.4	0.7	0.5	0.5	0.5	0.5	0.6	0.5	0.4	0.4	0.4																																					
13	19604	0.5	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.4	0.4																																					
14	19605	0.4	0.6	0.4	0.5	0.3	0.5	0.5	0.4	0.5	0.5	0.6	0.5	0.4																																				
15	19633	0.3	0.5	0.5	0.5	0.4	0.4	0.3	0.5	0.3	0.6	0.6	0.6	0.5	0.4																																			
16	19304	0.4	0.5	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.6	0.6	0.4	0.3	0.5																																		
17	9646	0.4	0.6	0.4	0.6	0.5	0.6	0.5	0.6	0.6	0.7	0.7	0.6	0.6	0.6	0.4	0.5																																	
18	19599	0.4	0.6	0.4	0.5	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.6	0.5	0.6	0.4	0.4	0.6	0.4																																
19	18351	0.6	0.8	0.6	0.5	0.5	0.6	0.5	0.5	0.5	0.7	0.7	0.6	0.7	0.5	0.5	0.6	0.6	0.5																																
20	19536	0.4	0.6	0.4	0.5	0.4	0.5	0.4	0.5	0.5	0.7	0.5	0.5	0.5	0.5	0.4	0.5	0.4	0.4	0.4																														
21	9055	0.4	0.5	0.4	0.5	0.2	0.4	0.4	0.4	0.5	0.5	0.6	0.5	0.6	0.4	0.4	0.5	0.5	0.4	0.5	0.2																												
22	19554	0.5	0.7	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.7	0.6	0.6	0.5	0.4	0.6	0.3	0.4	0.4	0.3	0.3																											
23	19540	0.5	0.5	0.5	0.5	0.3	0.5	0.4	0.5	0.5	0.6	0.6	0.6	0.6	0.4	0.4	0.4	0.5	0.5	0.5	0.3	0.2	0.4																											
24	19537	0.5	0.6	0.6	0.5	0.5	0.4	0.3	0.5	0.4	0.6	0.7	0.5	0.5	0.6	0.4	0.5	0.6	0.4	0.5	0.3	0.4	0.5	0.4																										
25	19579	0.4	0.5	0.4	0.5	0.4	0.4	0.4	0.4	0.5	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.3	0.3	0.4	0.4	0.4																									
26	18302	0.5	0.7	0.5	0.5	0.5	0.6	0.6	0.6	0.5	0.5	0.7	0.4	0.6	0.6	0.5	0.5	0.6	0.5	0.4	0.4	0.5	0.4	0.5	0.3	0.5																								
27	19629	0.6	0.8	0.7	0.6	0.5	0.6	0.5	0.6	0.5	0.6	0.8	0.6	0.5	0.6	0.5	0.6	0.7	0.6	0.6	0.6	0.5	0.6	0.6	0.4	0.6	0.4																							
28	19673	0.5	0.6	0.6	0.5	0.5	0.6	0.6	0.7	0.6	0.5	0.6	0.5	0.5	0.6	0.6	0.6	0.6	0.5	0.6	0.4	0.5	0.6	0.5	0.3	0.5	0.4	0.4																					
29	18300	0.5	0.6	0.7	0.5	0.4	0.5	0.5	0.6	0.6	0.5	0.6	0.6	0.6	0.4	0.5	0.5	0.6	0.5	0.6	0.5	0.4	0.6	0.5	0.4	0.4	0.4	0.3	0.3																			
30	19561	0.5	0.6	0.4	0.4	0.5	0.4	0.5	0.4	0.4	0.5	0.5	0.4	0.6	0.6	0.5	0.6	0.5	0.4	0.4	0.5	0.5	0.4	0.6	0.6	0.4	0.5	0.6	0.8	0.7																	
31	19305	0.7	0.8	0.6	0.5	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.6	0.7	0.5	0.6	0.5	0.6	0.6	0.6	0.7	0.5	0.7	0.7	0.7	0.7	0.7	0.4	0.7	0.5	0.4																
32	Climex	0.4	0.6	0.5	0.6	0.4	0.3	0.5	0.5	0.5	0.3	0.6	0.5	0.6	0.6	0.4	0.6	0.6	0.4	0.6	0.4	0.5	0.5	0.6	0.4	0.4	0.5	0.6	0.6	0.5	0.4	0.5															
33	19604	0.6	0.8	0.6	0.7	0.5	0.6	0.5	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.4	0.6	0.6	0.5	0.6	0.5	0.5	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.7	0.6	0.6	0.5													
34	19673	0.6	0.7	0.6	0.7	0.5	0.7	0.6	0.7	0.6	0.7	0.7	0.6	0.8	0.6	0.7	0.7	0.6	0.5	0.5	0.5	0.5	0.6	0.5	0.7	0.5	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.4											
35	9047	0.5	0.7	0.4	0.5	0.4	0.5	0.6	0.6	0.5	0.6	0.7	0.4	0.6	0.7	0.6	0.8	0.5	0.4	0.6	0.4	0.4	0.4	0.6	0.4	0.5	0.4	0.5	0.6	0.6	0.3	0.5	0.6	0.5	0.5										
36	19306	0.4	0.6	0.4	0.5	0.4	0.5	0.4	0.4	0.4	0.3	0.6	0.5	0.5	0.4	0.4	0.5	0.5	0.3	0.5	0.2	0.2	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.6	0.5	0.5	0.4	0.5	0.5								
37	Meteor	0.5	0.7	0.5	0.6	0.3	0.5	0.5	0.5	0.4	0.6	0.7	0.6	0.6	0.5	0.4	0.5	0.5	0.3	0.6	0.3	0.3	0.4	0.5	0.4	0.4	0.5	0.5	0.5	0.4	0.5	0.4	0.3	0.5	0.3	0.3									
38	18301	0.5	0.6	0.6	0.6	0.4	0.5	0.5	0.6	0.5	0.7	0.7	0.5	0.6	0.5	0.4	0.5	0.5	0.5	0.7	0.4	0.3	0.5	0.4	0.4	0.4	0.6	0.6	0.5	0.5	0.6	0.7	0.5	0.3	0.5	0.4	0.3	0.3							
39	19553	0.5	0.6	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.4	0.7	0.4	0.5	0.6	0.5	0.6	0.6	0.4	0.5	0.4	0.4	0.5	0.5	0.4	0.4	0.3	0.5	0.4	0.5	0.5	0.7	0.6	0.5	0.5	0.2	0.4	0.4	0.4							
40	19578	0.5	0.7	0.6	0.6	0.5	0.6	0.5	0.5	0.5	0.7	0.8	0.7	0.7	0.4	0.3	0.5	0.4	0.4	0.5	0.4	0.4	0.3	0.5	0.5	0.5	0.6	0.5	0.7	0.6	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.3	0.3	0.4						
41	DASA	0.4	0.6	0.5	0.5	0.3	0.4	0.4	0.4	0.4	0.6	0.7	0.5	0.6	0.4	0.3	0.5	0.6	0.4	0.6	0.4	0.3	0.5	0.3	0.4	0.3	0.6	0.5	0.6	0.4	0.4	0.6	0.4	0.4	0.6	0.4	0.4	0.3	0.3	0.4	0.4					
42	19566	0.4	0.6	0.5	0.5	0.4	0.4	0.4	0.5	0.5	0.8	0.6	0.5	0.5	0.5	0.3	0.5	0.5	0.4	0.5	0.3	0.3	0.5	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.6	0.4	0.4	0.6	0.5	0.3	0.3	0.4	0.5	0.4	0.3				
43	18292	0.5	0.6	0.5	0.5	0.3	0.4	0.4	0.4	0.4	0.6	0.6	0.6	0.6	0.6	0.3	0.4	0.5	0.3	0.5	0.4	0.3	0.4	0.4	0.4	0.5	0.5	0.4	0.5	0.4	0.4	0.5	0.3	0.4	0.5	0.4	0.3	0.2	0.4	0.6	0.3	0.3	0.3			
44	19599	0.6	0.7	0.6	0.6	0.4	0.5	0.5	0.5	0.5	0.7	0.6	0.6	0.6	0.5	0.4	0.5	0.5	0.4	0.5	0.4	0.3	0.4	0.4	0.5	0.5	0.6	0.6	0.6	0.5	0.5	0.5	0.4	0.3	0.5	0.6	0.3	0.2	0.4	0.5	0.2	0.3	0.3	0.2		
45	19634	0.5	0.6	0.6	0.5	0.3	0.5	0.5	0.4	0.5	0.5	0.6	0.6	0.5	0.5	0.3	0.4	0.5	0.4	0.4	0.4	0.3	0.3	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.4	0.4	0.5	0.4	0.3	0.3	0.4	0.4	0.3	0.4	0.4	0.2	0.2	
46	20478	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.4	0.6	0.6	0.5	0.5	0.6	0.5	0.5	0.4	0.3	0.5	0.4	0.4	0.4	0.4	0.5	0.5	0.4	0.6	0.4	0.4	0.5	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.5	
47	18296	0.5	0.7	0.5	0.6	0.4	0.5	0.4	0.5	0.4	0.6	0.7	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.3	0.4	0.4	0.4	0.3	0.4	0.5	0.4	0.3	0.4	0.5	0.7	0.5	0.4	0.5	0.5	0.3	0.3	0.4	0.5	0.4	0.4	0.4	0.3	0.4	0.4	0.4	
48	19309	0.5	0.8	0.6	0.5	0.3	0.4	0.4	0.4	0.4	0.5	0.7	0.6	0.6	0.6	0.4	0.5	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.3	0.5	0.5	0.5	0.5	0.3	0.6	0.4	0.4	0.5	0.5	0.4	0.3	0.4	0.5	0.3	0.2	0.4	0.2	0.2	0.2	0.3	0.3

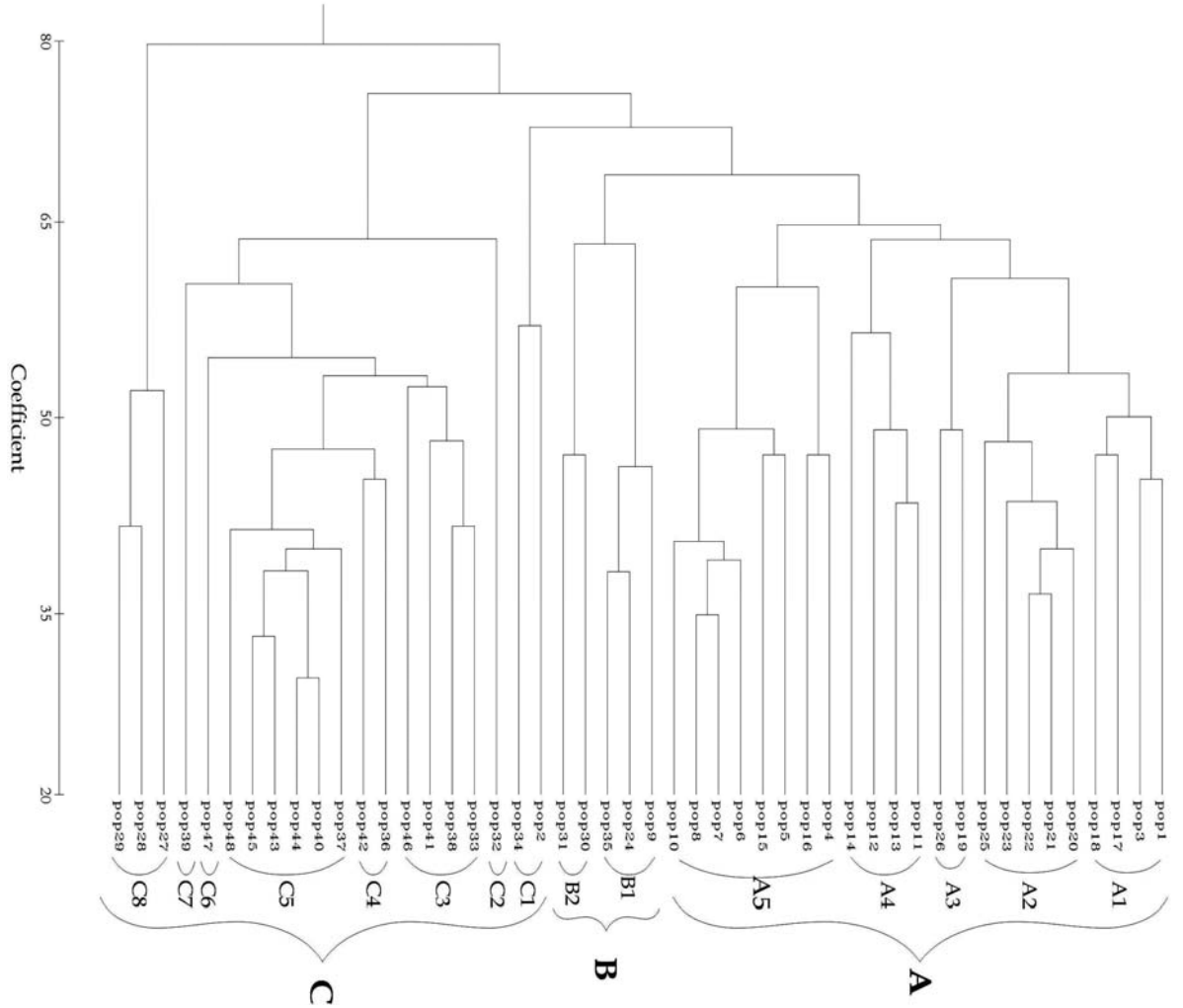

Fig. 2. Dendrogram Dendrogram illustrating coefficient similarities among 48 peas genotypes (pop1-pop48) based on data obtained from all the 20 electropherograms using 20 Randomly Polymorphic DNA (RAPD) Markers. Genotypes (populations) of group A1 and C8 are genetically most diverse and distantly related.

The data obtained was also used to find out phylogenetic relationships through Dendrogram (cluster) analysis. Forty eight genotypes of pea were grouped in 3 groups A, B and C which comprised of 23, 5, and 20 genotypes, respectively. Cluster analysis further authenticated the results of statistical analyses shown in Table 2. Similar phylogenetic relations were also designed by Saeidi *et al.,* (2008) for various lines of *Aegilops tauchii* species (Saeidi *et al.,* 2008). Results can be used for breeding purposes to obtain more potent and diverse populations of pea.

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