ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (1): 1-9, 2008
ISSN 1682-296X

Genetic Diversity in Bean Populations Based on Random Amplified Polymorphic DNA Markers

[1]Konstantinos Tertivanidis, [2]Olga Koutita, [1]Ioannis I. Papadopoulos, [3]Ioannis S. Tokatlidis, [1]Efstathios G. Tamoutsidis, [1]Vasiliki Pappa-Michailidou and [4]Metaxia Koutsika-Sotiriou
[1]Technological Educational Institute of W. Macedonia/Branch of Florina, 531 00, Greece
[2]Department of Plant Breeding, Hellenic Sugar Industry, S.A. 57400 Sindos, Thessaloniki, Greece
[3]Department of Agricultural Development, Democritus University of Thrace, 682 00, Greece
[4]Laboratory of Genetics and Plant Breeding, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece

Abstract: Genetic diversity in 19 local dry bean populations (*Phaseolus vulgaris* L.) originated from the region of Macedonia (Greece, GR and Formal Yugoslavic Republic of Macedonia, FYROM) was investigated using RAPD (Random Amplified Polymorphic DNA) markers in 10 individual plants from each population. A total of 11 random primers detected 56 polymorphic bands, with an average of 5.1 polymorphic bands/primer. The individual plants were grouped in 14 clusters on the basis of the Jaccard coefficient (Unweighted Pair Group Method and Arithmetic Average-UPGMA). The average taxonomic distances between the 19 populations were calculated on the basis of gene frequencies. These distances were used for grouping the populations, by UPGMA and Principal Coordinates Analysis (PCO), resulting in four and 10 groups, respectively. In general, there was a noticeable similarity in the grouping of the individuals and populations with the two methods. According the UPGMA and PCO procedures, the populations were clustered based on geographical origin. It was concluded that RAPD markers could be exploited as alternative or supplementary tools to already established methods for the evaluation and classification of bean genetic resources.

Key words: Genetic diversity, molecular markers, *Phaseolus vulgaris*, RAPD-PCR

INTRODUCTION

The genus Phaseolus includes five grain legumes of regional economic importance (Debouck, 1991), with dry bean (*Phaseolus vulgaris* L.) being an important source of dietary protein for many people worldwide.

Objective descriptors based on morphophysiological characters are routinely used for the evaluation of genetic resources (Hunter, 1993). However, the majority of such characters are phenotypic, being influenced both by the environmental conditions and the growth stage of the plant. Furthermore, the evaluation procedures are time consuming and relatively expensive. A more efficient procedure for germplasm evaluation has been sought by employing biochemical markers, in particular isozymes (Nielsen, 1985). Even in this case, however, isozyme expression is dependent on environment, development and tissue type (Tsaftaris, 1987). DNA-based markers such as RFLPs, RAPDs, AFLPs and SSRs have been used for germplasm identification and characterization. Such markers are phenotypically neutral, environmentally independent and not influenced by epistatic interactions (Koutita *et al*., 2005).

RAPD markers were described in 1990 (Williams *et al*., 1990) and due to their simplicity and inexpensiveness they have been widely used for a number of applications in plant breeding and genetic studies (Kumar, 1999). In phaseolus species, rapd markers have been employed to estimate intraspecific genetic relationships among genotypes (Schmit *et al*., 1993; Freyre Rios *et al*., 1996; Alvarez *et al*., 1998; Galvan *et al*., 2006; Marotti *et al*., 2007), interspecific genetic relationships between cultivars, accessions and landraces (Skröch and Nienhuis, 1995; Fofana *et al*., 1997; Briand *et al*., 1998; Duarte *et al*., 1999; Métais *et al*., 2000; Galván *et al*., 2001; Maciel *et al*., 2001; Ocampo *et al*., 2005; Tiwari *et al*., 2005) and molecular changes during a selection process (Tertivanidis *et al*., 2003).

Common bean was brought to Greece at the late of the 16th century and since then the long-term cultivation

Corresponding Author: Ioannis S. Tokatlidis, Department of Agricultural Development, Democritus University of Thrace, 682 00, Greece Tel: ++030 25520 41152 Fax: ++030 25520 41159

at distinct microenvironments, combined with the extensive genetic heterogeneity, led to various landraces with particular genetic and morphological traits (Papoutsi-Costopoulou and Gouli-Vavdinoudi, 2001). According to Beebe *et al*. (2001) most of the bean types cultivated in Europe belong to Andean genetic pool (large-seeded race Nueva Granada). In some regions of Greece and FYROM dry bean local populations are still cultivated, mainly with traditional methods (e.g., harvesting by hands). Compared to commercial varieties, these populations are less productive and more variable, but better adapted to the specific pedoclimatic conditions of these restricted areas. Moreover, their product has market desirable quality traits (i.e., easy cooking, tasteful, thin peel). Such local populations constitute valuable genetic resources that could be commercially exploited following appropriate evaluation and selection.

The present investigation was aimed at assessing the genetic diversity in 19 populations of local dry bean material in Greece and FYROM, based on RAPD molecular markers.

MATERIALS AND METHODS

Plant material: Plants from 19 local dry bean populations were used in this study (Table 1), collected mainly from the Greek Prefecture of Florina including the coastal area of the lakes Large Prespa and Small Prespa (North Greece), as well as from this coastal area inside FYROM. Additionally, two local populations from Chalkidiki (Central Macedonia, Greece) and one population from Kavala (Easter Macedonia, Greece) were included in the study. Ten plants from each population, grown in a greenhouse, were sampled (2-3 leaves from every plant) at the three to four-leaf stage and samples were stored in a deep freezer at -80°C.

DNA extraction: Total genomic DNA was isolated from each individual plant by a slightly modified CTAB method (Rogers and Bendich, 1988). Compared to the aforementioned authors' method, extraction CTAB buffer without β-mercapthoethanol and smaller amount of starting material (200 mg) was used for genomic DNA isolation. The plant material was frozen in liquid nitrogen and ground into a fine powder, to which one ml of preheated CTAB buffer was added and the mix was incubated for 30 min at 65°C. The mix was centrifuged for 10 min at 12000 g and 500 μL of supernatant was incubated with two μL of RNase A solution (10 mg mL^{-1}) for 15 min at 37°C. Chloroform extraction was carried out as follows: 200 μL chloroform/isoamylalcohol (24:1) was added and the extract was centrifuged for 10 min at 12000 g. After centrifugation, two volumes of CTAB precipitation buffer were added to the upper phase and the mixture was incubated for 60 min at room temperature. The pellet, collected by centrifugation at 12000 g for 5 min, was dissolved in 350 μL of 1.2 M NaCl solution for 15 min at room temperature. A chlorophorm/isoamylalcohol extraction step was performed and 0.6 volume of isopropanol was added to the supernatant. DNA was collected by centrifugation for 10 min at 11500 g. The DNA pellet was washed with 70% (v/v) ethanol and dissolved in 100 μL TE buffer solution. DNA concentration was estimated by agarose gel stained with ethidium bromide.

Table 1: The 19 dry bean local populations used in the study

Code	Local name	Region of origin
C1	-	Galarinos (Chalkidiki, GR)
C2	-	Galarinos (Chalkidiki, GR)
F1	Plake	Alona (Florina, GR)
F2	Plake	Alona (Florina, GR)
F3	Plake	Korestia (Florina, GR)
F4	Plake	Triantafilia (Florina, GR)
FY1	Plake	Nakolets (FYROM)
FY2	Plake	Liuboino (FYROM)
K1	Plake	Chrisoupoli (Kavala, GR)
P1	Plake	Kalithea (Prespes, GR)
P2	Plake	A. Germanos (Prespes, GR)
P3	Plake	Lefkonas (Prespes, GR)
P4	Plake	Lemos (Prespes, GR)
P5	Plake	A. Germanos (Prespes, GR)
P6	Boboni	A. Germanos (Prespes, GR)
P7	Strogilo	Lemos (Prespes, GR)
P8	Plake	A. Germanos (Prespes, GR)
P9	Plake	A. Germanos (Prespes, GR)
P10	Plake	Lemos (Prespes, GR)

RAPD analysis: For RAPD analysis random 10-mer primers (Operon Technologies Inc., Alameda, CA) were used. Amplification was carried out in 25 μL reaction mix containing about 25 ng genomic DNA, 0.2 mM of each dNTP, 0.2 μM of primer, 2 mM of $MgCl_2$ and 1 U of Taq DNA polymerase (Biotools) with supplied buffer. DNA amplification was performed in a PTC 200 thermal cycler (MJ Research) according to the following thermal profile: initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 45 sec, annealing at 35°C for 1.5 min and extension at 72°C for 1.5 min, followed by final extension at 72°C for 3 min. Amplification products were separated in 1.5-2% agarose gel and detected by staining with ethidium bromide (Sambrook *et al*., 1989). The gels were photographed under UV light. Each PCR reaction was run in duplicate and only well defined and reproducible bands were analyzed.

Data analysis: Each polymorphic band in every sample was scored as a binary character (1 for presence and 0 for

absence) and a data matrix was created. DNA bands of identical gel migration were assumed to represent the same allele at a locus. Only reproducible and clearly visible bands were scored. The bands detected in all plant samples at the same position (monomorphic) as well as bands not directly comparable between individuals because of very similar migration, were excluded from the analysis.

Genetic similarities between individual plants were calculated using the following coefficients:

- Simple matching coefficient : Sij = (a+d)/(a+b+c+d),
- Dice coefficient : Sij = 2a/(a+b+c+d),
- Jaccard coefficient : Sij = a/(a+b+c+d).

Where, Sij is the similarity between two individuals i and j, a is the number of bands scored in the both individuals, b is the number of bands detected only in the individual i, c is the number of bands detected only in the individual j and d is the number of bands absent in the both individuals (Rohlf, 2000). The pair-wise similarity matrices produced were used to construct dendrograms based on the Unweighted Pair Group Method and Arithmetic average (UPGMA) method of the software NTSYS pc version 2.1 (Rohlf, 2000) wherein modules Sahn and Tree of were used for clustering and graphical representation in a form of hierarchical dendrogram in which clusters were visually identified. Finally a number of clusters closest to the square root of the number of individuals was accepted. In order to test the goodness of fit of the cluster analysis to the data the cophenetic value was calculated by using Coph module (NTSYS pc version 2.1). POPGENE software (Yeh *et al*., 1997) was used to calculate gene frequencies. A pair-wise dissimilarity matrix was created from gene frequency data using average taxonomic distance coefficient (Simint module, NTSYS pc version 2.1). Then, this matrix was used to construct a dendrogram based on the UPGMA clustering procedure of the same software. The abovementioned matrix was also used to perform the Principal Coordinates Analysis (PCO).

The analysis of molecular variance (AMOVA) procedure was conducted to estimate the variation within and between populations, within and between groups formed by UPGMA analysis of 190 individuals and finally within and between the regions from where the populations had been collected. AMOVA was performed using GenAlEx software (Peakall and Smouse, 2001).

RESULTS

A set of 40 different random primers was tested with five individual DNA samples and 29 of them were rejected from further analysis due to poor amplification and monomorphic or non-reproducible pattern. The remaining 11 primers were used in PCR amplification reactions with all 190 individuals tested. A total of 118 bands ranging in size from 200 to 2500 bp were detected. The number of bands obtained for each primer ranged from 6 to 14, with a mean of 10.7 bands/primer. Excluding all monomorphic bands from analysis, a total of 56 polymorphic bands were finally scored. Each primer produced 1 to 9 such bands, with a mean of 5.1 polymorphic bands/primer. The percentage of polymorphic bands, averaged over all markers, was 47.5% (Table 2). Figure 1 shows a representative picture of RAPD fragments amplified by random primers OPAM11 (A) and OPAG18 (B) in individuals of the accession C1.

The three dendrograms constructed by cluster analysis, according the genetic similarity matrices produced on the basis of the three different coefficients, were very similar. The cophenetic values for each dendrogram were equal to 0.86 for Simple matching, 0.87 for Dice and 0.89 for Jaccard coefficient. Due to the highest cophenetic value, the Jaccard coefficient was accepted as more appropriate and used for further analysis. Pair-wise genetic similarity estimates for 190 individuals plants based on RAPD analysis ranged from 0.20 to 0.97 (data not shown). A dendrogram based on UPGMA analysis of the genetic similarity matrix was produced, in order to determine the extent of distinct clusters individuals from different populations form. All the 190 individuals analysed were grouped in 14 clusters based on the square root of the number of individuals criterion (Fig. 2). Clustering results are presented in Table 3. In general, individuals from the same population were grouped together. Two out of the 14 clusters, namely cluster I and cluster III, included individuals of four (P2, P5, P9, P10) and five (P1, P3, P8, F3, F4) populations,

Table 2: Polymorphism detected by the use of 11 random primers on 19 dry bean local populations

Primer	No. of amplified products*	No. of polymorphic bands**	Proportion of polymorphic bands	Products length (bp)
OPR11	14.0	7.0	0.50	600-2000
OPR12	12.0	6.0	0.50	200-2500
OPAB10	6.0	1.0	0.17	600-1400
OPAB14	8.0	6.0	0.75	300-1800
OPAG5	9.0	2.0	0.22	600-2000
OPAG18	10.0	3.0	0.30	700-2000
OPAH13	11.0	9.0	0.82	300-2500
OPAK7	9.0	5.0	0.56	300-1800
OPAL12	12.0	2.0	0.17	200-2000
OPAL19	14.0	8.0	0.57	500-2000
OPAM11	13.0	7.0	0.54	600-2500
Total	118.0	56.0		
Mean	10.7	5.1	0.46	

*: Total number of scorable bands detected,**: Total number detected as polymorphic in at least one sample

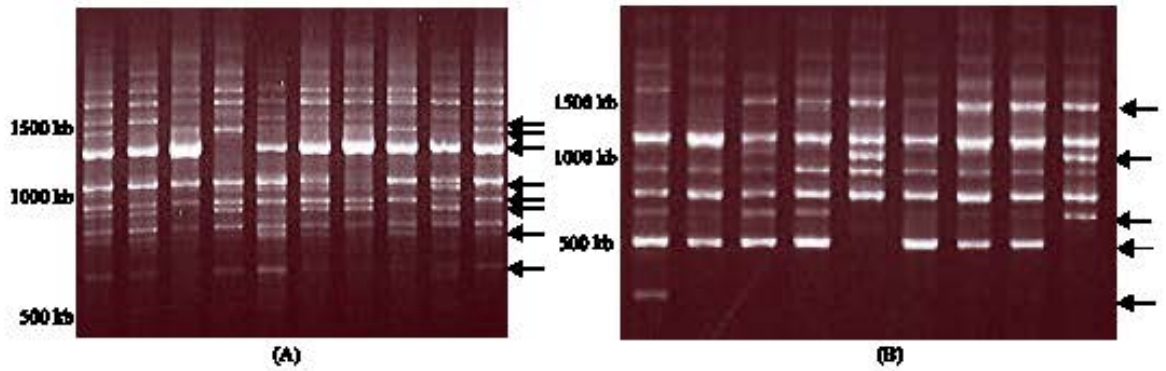

Fig. 1: RAPD profile for 10 and 9 individuals of the accession C1 amplified by primers OPAM11 (A) and OPAG18 (B). The scored polymorphic fragments are marked

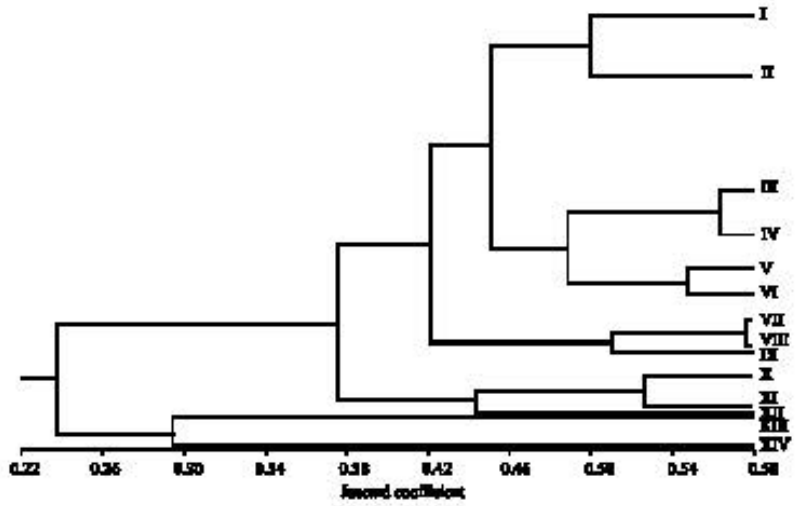

Fig. 2: Dendrogram showing the results of clustering on 190 individuals of 19 local dry bean populations (cophenetic value R = 0.89)

respectively. In case of the populations P8 and F4 nine individuals were clustered together. The cluster II consisted of nine individuals from both populations F1 and F2, while the clusters IV, V, VI, VII, VIII, X, XI and XIV included eight to 10 individuals of one population. Finally, three clusters (IX, XII and XIII) included only one to three individuals.

The 14 abovementioned clusters showed similarity in the range of 24-57% among them. All individuals from population K1 formed the most divergent cluster XIV, showing about 24% similarity with the rest clusters. Individuals of populations C1 and C2 (originated from Chalkidiki), belonging to clusters X, XI and XII, showed 37,5% similarity with the individuals of populations originated from the areas of Prespes, Florina and FYROM. Individuals of C1 and C2 (except of C1-7) were grouped in clusters X and XI, which were 52.5% similar to each other.

Individuals from the populations originated from Florina and coastal area of Prespes Lakes, including the area inside FYROM, were grouped in nine clusters, showing 43% similarity. Meaningfully, 10 individuals of population FY1 formed the cluster V, while nine individuals of population FY2 comprised the cluster VI. These two clusters, including almost all individuals from FYROM, were by 56.5% similar.

A one-way AMOVA was performed to test differentiation between and within the 19 populations (Table 4). The results revealed significant differences ($p \leq 0.010$) and 80% of total variation was found between populations. Another AMOVA was performed to estimate the differentiation among and within 12 groups formed by UPGMA analysis of 188 individuals (Table 5). Two groups (IX and XII) consisted of one individual (FY2-3 and C1-7, respectively) were excluded from this analysis. It was found that, despite of high variation

within groups (61%), these groups were significantly different ($p \leq 0.010$).

The same RAPD data on 190 individuals were analyzed by AMOVA to test the differentiation of the five different regions from which the populations were derived (Table 6). Significant differentiation ($p \leq 0.010$) was found between these regions. The within regions variation (75%) was higher compared to that between regions (25%). Gene frequencies were determined from the presence or absence of a particular amplified fragment across the populations (data not shown). Fragments of identical migration pattern on the electrophoretic gel were assumed to represent the same allele at a locus. Average taxonomic distances, calculated on the basis of gene frequencies, varied from 0.29 (among P5 and P2) to 0.79 (among P10 and K1), with an average value of 0.55 (Table 7). These distances were used to construct a dendrogram based on the UPGMA method (Fig. 3), which divided the populations into four major clusters. Populations originated from the areas of Florina and Prespes were grouped into three clusters (signed A, B, C), whereas the two populations from FYROM (FY1, FY2) were included in one of them (B). The remaining three populations that came from regions of Chalkidiki (C1, C2) and Kavala (K1) were grouped together in the most divergent cluster (D).

The same dissimilarity matrix was explored by an ordination method (PCO). The first three principal coordinates (PC1, PC2, PC3) explained together about 45.67% of the total variation. The first principal coordinate

Table 3: Description of clusters produced by UPGMA analysis of RAPD data from 190 individuals of dry bean

Group	Individuals included
I	All individuals of P2
	All individuals of P5
	P6-7, P6-10
	All individuals of P9
	All individuals of P10
	F4-2
II	F1-1, F1-2, F1-3, F1-6, F1-7, F1-8, F1-9, F1-10
	F2-1, F2-2, F2-3, F2-4, F2-5, F2-6, F2-8, F2-9, F2-10
III	All individuals of P1
	All individuals of F3
	All individuals of P3
	F4-1, F4-3, F4-4, F4-5, F4-6, F4-7, F4-8, F4-9, F4-10
	P8-1, P8-2, P8-3, P8-4, P8-6, P8-7, P8-8, P8-9, P8-10
IV	P6-1, P6-2, P6-3, P6-4, P6-5, P6-6, P6-8, P6-9
V	All individuals of FY1
VI	FY2-1, FY2-2, FY2-4, FY2-5, FY2-6, FY2-7, FY2-8, FY2-9, FY2-10
VII	All individuals of P7
VIII	P4-1, P4-2, P4-3, P4-4, P4-6, P4-7, P4-8, P4-9, P4-10
IX	FY2-3
X	C1-1, C1-2, C1-3, C1-4, C1-5, C1-6, C1-8, C1-9, C1-10
	P8-5
XI	All individuals of C2
	P4-5
XII	C1-7
XIII	F1-4, F1-5
	F2-7
XIV	All individuals of K1

Table 4: AMOVA among and between 19 local populations of dry bean

Source of variation	df	SS	Variation (%)	Φpt	p
Between populations	18	1856.46	80	0.803	≤ 0.010
Within populations	171	421.50	20		

Table 5: AMOVA among and between 12 groups formed by UPGMA analysis of 188 individuals of 19 local populations of dry bean

Source of variation	df	SS	Variation (%)	Φpt	p
Between groups	11	874.73	39	0.386	≤ 0.010
Within groups	176	1378.41	61		

Table 6: AMOVA among and between 5 regions from which 19 local populations of dry bean were originated

Source of variation	df	SS	Variation (%)	Φpt	p
Between regions	4	450.04	25	0.251	≤ 0.010
Within regions	185	1827.91	75		

Table 7: Measures of Average taxonomic distances between nineteen dry bean local populations

	C1	C2	F1	F2	F3	F4	FY1	FY2	K1	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
C1	0.00																		
C2	0.49	0.00																	
F1	0.56	0.66	0.00																
F2	0.65	0.68	0.54	0.00															
F3	0.56	0.72	0.64	0.69	0.00														
F4	0.62	0.67	0.55	0.52	0.55	0.00													
FY1	0.51	0.66	0.60	0.75	0.59	0.60	0.00												
FY2	0.51	0.69	0.60	0.62	0.63	0.51	0.52	0.00											
K1	0.55	0.69	0.63	0.60	0.69	0.65	0.70	0.61	0.00										
P1	0.57	0.72	0.65	0.67	0.37	0.53	0.59	0.59	0.72	0.00									
P2	0.56	0.72	0.60	0.70	0.62	0.61	0.66	0.64	0.77	0.54	0.00								
P3	0.59	0.76	0.64	0.66	0.41	0.46	0.54	0.59	0.73	0.44	0.61	0.00							
P4	0.63	0.67	0.61	0.63	0.69	0.68	0.59	0.65	0.66	0.66	0.64	0.69	0.00						
P5	0.56	0.73	0.54	0.58	0.57	0.57	0.63	0.61	0.74	0.55	0.29	0.59	0.62	0.00					
P6	0.51	0.66	0.55	0.60	0.48	0.50	0.55	0.53	0.64	0.52	0.58	0.56	0.53	0.52	0.00				
P7	0.68	0.74	0.63	0.47	0.65	0.54	0.66	0.64	0.64	0.60	0.62	0.60	0.50	0.57	0.56	0.00			
P8	0.54	0.69	0.63	0.59	0.38	0.55	0.56	0.56	0.61	0.53	0.66	0.50	0.65	0.57	0.47	0.54	0.00		
P9	0.58	0.67	0.57	0.65	0.62	0.53	0.69	0.63	0.71	0.64	0.46	0.58	0.70	0.44	0.56	0.68	0.64	0.00	
P10	0.63	0.75	0.58	0.63	0.60	0.56	0.68	0.63	0.79	0.60	0.43	0.54	0.65	0.40	0.58	0.61	0.63	0.46	0.00

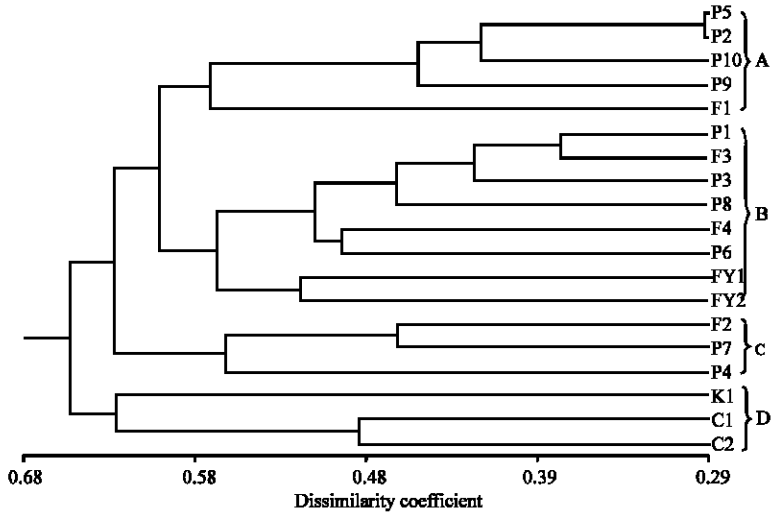

Fig. 3: Dendrogram showing the results of clustering using average taxonomic distance on nineteen local dry bean populations (cophenetic value R = 0.82)

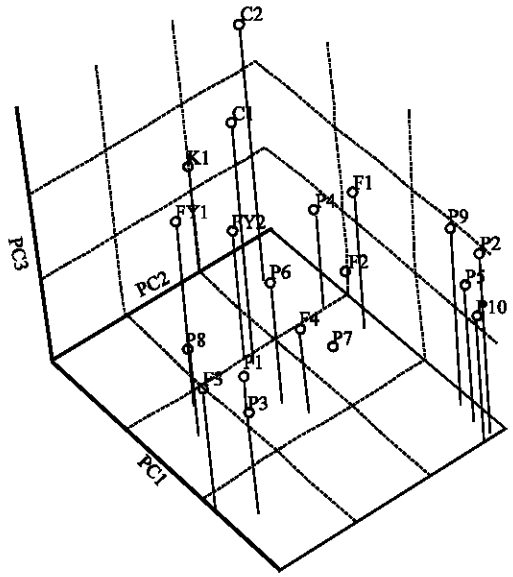

Fig. 4: Three-dimensional scatter plot of the PCO. The populations studied formed ten distinct groups, as follows: Group 1: P2, P5, P9, P10; Group 2: C1; Group 3: C2; Group 4: FY1; Group 5: P6, F4, FY2; Group 6: K1; Group 7: P1, P3, F3; Group 8: P4, F1; Group 9: P7; Group 10: P8

covered 18.09% of the variation and was mainly related to bands rapd11 and rapd42. The second principal explained 14.93% of the variation and was related to bands rapd49 and rapd54. Finally, for the third principal coordinate, which explains 12.65% of the variation, more significant bands were rapd29 and rapd40.

Since the three first principal coordinates explained 45.67% of total variation, a three-dimensional scatter plot was created (Fig. 4). Two reference lines for each axis were used and populations in the same framed cubed space were grouped together. According to the abovementioned procedure, the populations formed 10 distinct groups consisted of one to four populations (Fig. 4).

On the first principal coordinate, explained mainly by the markers rapd11 and rapd42, high negative correlation was shown by populations K1, C2, F2, P4 and C1, while the populations P2, P5, P10, P9 and P3 displayed high positive correlation. In case of the second principal coordinate, populations P1, P3, P8, F3 and FY1 were negatively correlated, whereas

populations P2, P5, P10, F1 and F2 exhibited positive correlation. Finally, on the third PC, populations P7 and F2 were negatively correlated, while C1 and C2 were positively correlated.

DISCUSSION

The local dry bean populations of the study are traditionally cultivated in certain regions of northern Greece and FYROM, where hard climatic conditions predominate. Thanks to the traditional type of cultivation they do not mutually exchange significant genetic material. Despite their low productivity and high variability compared to commercial varieties, they are cultivated almost exclusively in the above regions. Adaptability to the particular environmental conditions (climatic, territorial), as well as high quality products, renders the fact reasonable. Moreover, bean cultivation constitutes the main source of rural income in these regions. Hence, as far as commercial exploitation of these populations is concerned, certification as a product of protected designation of origin is very important. On the other hand, these populations could constitute valuable genetic sources for hereafter breeding programs. Consequently, estimation of their genetic variability via molecular approaches constitutes an important issue.

RAPD molecular markers were utilised in the study even though doubts have been reported as regards their reproducibility and suitability for genetic diversity studies (Mantzavinou *et al*., 2005). To achieve reproducibility of RAPD markers each PCR reaction was run in duplicate and only well defined and reproducible bands were analysed. Moreover, there are previous reports on the successful use of RAPD markers in bean germplasm evaluation due to their advantages (Skroch and Nienhuis, 1995; Fofana *et al*., 1997; Alvarez *et al*., 1998; Briand *et al*., 1998; Duarte *et al*., 1999; Galvan *et al*., 2001; Metais *et al*., 2000; Maciel *et al*., 2001; Tiwari *et al*., 2005; Galvan *et al*., 2006; Marotti *et al*., 2007).

In this study 11 out of the 40 random primers tested showed polymorphism (27.5%). Also, 56 polymorphic bands were scored from 118 totally detected (47.5%). The level of polymorphism detected in present study was similar to that obtained by Galvan *et al.* (2001 and 2006). Nevertheless, it was low according the so far relevant reports in bean (Skroch and Nienhuis, 1995; Alvarez *et al*., 1998; Briand *et al*., 1998; Duarte *et al*., 1999; Metais *et al*., 2000; Maciel *et al*., 2001; Ocampo *et al*., 2005; Tiwari *et al*., 2005; Marotti *et al*., 2007).

The level of intraspecies polymorphism depends on the among genotypes level of divergence. Since populations of the study are large seeded (data not shown), they can be assigned to Andean gene pool, according to definition of Gepts *et al*. (1988). Climbing habit of the 19 populations, combined with the abovementioned character, is indicative of a common ancestor (Andean origin) like Spanish bean cultivars (Ocampo *et al*., 2005). The narrow genetic base of the populations, as well as the fact that primers used for the analysis were not pre-selected, in combination with the number and quality of the amplification, may explain the relatively low level of polymorphism detected. On the contrary, authors who reported high level of polymorphism among common bean cultivars used strictly pre-selected primers (Duarte *et al*., 1999; Ocampo *et al*., 2005).

The three similarity coefficients used for UPGMA analysis (i.e., Simple matching, Dice, Jaccard) produced similar clustering results. The goodness of fit for the clustering to the similarity matrix, as determined by cophenetic value, was higher for Jaccard coefficient. Skröch *et al.* (1992) and Galvan *et al.* (2001) suggested Simple matching as the most appropriate coefficient for estimating genetic similarity at intraspecific level in common bean. However, Alvarez *et al.* (1998), Metais *et al.* (2000), Maciel *et al.* (2001) and Tiwari *et al.* (2005) used Jaccard coefficient to estimate genetic similarity in bean genetic material.

The 190 individuals studied were clustered in 14 different groups by UPGMA analysis. In most of the cases, individuals from the same population were grouped together. However, some individuals were grouped separately, like the clusters I, IX, X, XI, XII and XIII. Such type of clustering is indicative of a predominantly self-pollinating species in which cross-pollination can occur in a rate higher than 10% (Debouck and Thome, 1988).

Generally, based on the dendrograms derived from individual plants' RAPD data and gene frequencies' data, populations were clustered according to their geographical origin. Particularly, the three populations originated from the more distinct regions (Kavala and Chalkidiki) formed the most distinct group in the dendrogam based on gene frequencies data. The populations originated from FYROM were grouped together with those from Florina and Prespes, probably due to their geographical proximity. Moreover, the similarity of local bean genetic material from Florina and Prespes could be connected with the fact that the regions of Macedonia (Greece) and FYROM were liberated from Ottoman Empire just about 100 years ago and nationality migration occurred till the mid of 1960's. The obtained clustering results are consistent with the fact of a founder effect when a new crop is imported in a region and a few individuals are involved.

AMOVA analysis of RAPD data show that 20% of the variation was found within populations, whereas 61 and 75% within clusters and within regions of origin,respectively. The results could be attributed to the lack of exchange of genetic material between farmers, the traditional way of cultivation (e.g., no mechanical harvesting) and the consecutive inbreeding and selection carried out over the years by each farmer. It is suggested that the traditional way of cultivation might have led to increased genetic homogeneity within populations, which was higher than that reported for Spanish bean landraces (Alvarez *et al*., 1998) and Greek wheat landraces (Mantzavinou *et al*., 2005). In order to explore the within population diversity in a more detailed manner, two approaches could be applied: i) screening of a large number of samples from each population (either individuals or bulked) to overcome the limitation of the RAPD analysis, suggested by Mantzavinou *et al*. (2005), ii) utilization of genetically mapped markers, such as microsatellites (Metais *et al*., 2000) or semi-random PCR (Marotti *et al*., 2007).

The 19 populations formed 10 distinct groups by PCO method. The populations from Kavala and Chalkidiki were grouped again in the most distinct groups. This observation confirmed that populations fell in three major groups according to their origin.

In the future breeding programs, crosses between bean local populations from these three major groups might lead to high heterosis, despite the theory that in autogamous species that have undergone evolution under domestication the dominant and additive alleles prevail, reducing the advances of F1 heterosis (Fasoula and Fasoula, 1997). The remarkable spread of rice hybrids in China thanks to heterosis (Xiao *et al*., 1995) and high heterosis that showed crosses between okra cultivars (Koutsos *et al*., 2000), indicate that heterosis in autogamous species is not rare. Furthermore, successful hybridization among populations could be beneficially utilized in pedigree breeding schemes aiming to exploit additive gene effects via homozygosity. Galvan *et al*. (2006) speculated that genetic diversity can be exploited through breeding programs to broaden the genetic base of commercial beans and develop high yielding cultivars. Singh (1989) proposed for breeding programs in America exploitation of bean genetic resources from European countries including Greece, particularly of large seeded white races.

ACKNOWLEDGMENTS

This study is a by-product of the EPEAEK II project 2.6.1 Environment-Archimedes, A Support of Research in issues of Environment and Ecology in Technological Education Institutes financed 75% by EC and 25% by Greek government.

REFERENCES

Alvarez, M.T., L.E. Sáenz de Miera and M. Pérez de la Vega, 1998. Genetic variation in common and runner bean of the Northern Meseta in Spain. Genet. Resour. Crop E., 45: 243-251.

Beebe, S., J. Rengifo, E. Gaitan, M.C. Duque and J. Tohme, 2001. Diversity and origin of Andean landraces of common bean. Crop Sci., 41: 854-962.

Briand, L., A.E. Brown, J.M. Lenné and D.M. Teverson, 1998. Random amplified polymorphic DNA variation within and among bean landrace mixtures (*Phaseolus vulgaris* L.) from Tanzania. Euphytica, 102: 371-377.

Debouck, D.G. and J. Thome, 1988. Implicaciones Que Tienen Los Estudios Sobre Los Orígenes Del Frijol Común, *Phaseolus vulgaris* L., Para Los Mejoradores De Frijol. In: Temas Actuales En Mejoramiento Genético Del Frijol Común, Memorias Del Taller Internacional De Mejoramiento Genético De Frijol. Beebe, S. (Ed.), CIAT, Cali, Colombia, pp: 1-44.

Debouck, D.G., 1991. Systematics and Morphology. In: Common Beans: Research for Crop Improvement. van Schoonhoven, A. and O. Voysest (Eds.), CAB International, Wallingford, Oxon, UK., pp: 55-118.

Duarte, J.M., J. Bosco dos Santos and L. Cunha Melo, 1999. Genetic divergence among common bean cultivars from different races based on RAPD markers. Genet. Mol. Breed., 22: 419-426.

Fasoula, D.A. and V.A. Fasoula, 1997. Gene action and plant breeding. Plant Breed. Rev., 15: 315-370.

Fofana, B., X. Vekemans, P. du Jardin and J.P. Baudoin, 1997. Genetic diversity in Lima bean (*Phaseolus lunatus* L.) as revealed by RAPD markers. Euphytica, 95: 157-165.

Freyre Rios, R.R., L. Guzman, D.G. Debouck and P. Gepts, 1996. Ecogeographic distribution of *Phaseolus* sp. (Fabaceae) in Bolivia. Econ. Bot., 50: 195-215.

Galván, M.Z., M.B. Aulicino, S. Garciá Medina and P.A. Balatti, 2001. Genetic diversity among Northwestern Argentinian cultivars of common bean (*Phaseolus vulgaris* L.) as revealed by RAPD markers. Genet. Resour. Crop E., 48: 251-260.

Galván, M.Z., M.C. Menendez-Sevillano, A.M. DeRon, M. Santalla and P.A. Balatti, 2006. Genetic diversity among wild common beans from northwestern argentina based on morpho-agronomic and RAPD data. Genet. Resour. Crop E., 53: 891-900.

Gepts, P., K. Kmiecik, P. Pereira and F.A. Bliss, 1988. Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability: I. The Americas Econ. Bot., 42: 73-85.

Hunter, B.R., 1993. Science Based Identification of Plant Genetic Material. CSSA, Intellectual Property Rights: Protection of Plant Materials. Special Publication No. 21, pp: 93-99.

Koutita, O., K. Tertivanidis, T.V. Koutsos, M. Koutsika-Sotiriou and G.N. Scarakis, 2005. Genetic diversity in four cabbage populations based on random amplified polymorphic DNA markers. J. Agric. Sci., 143: 377-384.

Koutsos, T.V., M. Koutsika-Sotiriou, E. Gouli-Vavdinoudi and K. Tertivanidis, 2000. Study of the genetic relationship of Greek okra cultivars (*Abelomoschus esculentus* L.) by using agronomic traits, heterosis and combining ability. J. Veg. Crop Prod., 6: 25-35.

Kumar, S.L., 1999. DNA markers in plant improvement: An overview. Biotechnol. Adv., 17: 143-182.

Maciel, F.L., L.T. Gerald and S. Echeverrigaray, 2001. Random amplified polymorphic DNA (RAPD) markers variability among cultivars and landraces of common beans (*Phaseolus vulgaris* L.) of South-Brazil. Euphytica, 120: 257-263.

Mantzavinou, A., P.J. Bebeli and P.J. Kaltsikes, 2005. Estimating genetic diversity in Greek durum wheat landraces with RAPD markers. Australian J. Agric. Res., 56: 1355-1364.

Marotti, I., A. Bonetti, M. Minelli, P. Catizone and G. Dinelli, 2007. Characterization of some Italian common bean (*Phaseolus vulgaris* L.) landraces by RAPD, semi-random and ISSR molecular markers. Genet. Resour. Crop E., 54: 175-188.

Métais, I., C. Aubry, B. Hamon, R. Jalouzot and D. Peltier, 2000. Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). Theor. Applied Genet., 101: 1207-1214.

Nielsen, G., 1985. The Use of Isozymes as Probes to Identify and Label Plant Varieties and Cultivars. In: Rattazzi, M.S., J.G. Scandalios and G.S. Whitt (Eds.), Isozymes: Current Topics in Biological and Medical Research. Vol. 12, Alan R. Liss Inc., New York, pp: 1-32.

Ocampo, C.H., J.P. Martin, M.D. Sanchez-Yelamo, J.M. Ortiz and O. Toro, 2005. Tracing the origin of Spanish common bean cultivars using biochemical and molecular markers. Genet. Resour. Crop E., 52: 33-40.

Papoutsi-Costopoulou, H. and E. Gouli-Vavdinoudi, 2001. Improving a local common bean (*Phaseolus vulgaris* L.) population for yield, seed size and earliness. Plant Var. Seeds, 14: 25-34.

Peakall, R. and P.E. Smouse, 2001. GenAlEx V5: Genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Camberra, Australia. http://www.anu.edu.au/BoZo/GenAlEx. Cited 28 Feb 2006.

Rogers, S.O. and A.J. Bendich, 1988. Extraction of DNA from plant tissues. Plant Mol. Biol. Manual, A6: 1-10.

Rohlf, F.J., 2000. NTSYSpc: Numerical Taxonomy and Multivariate Analysis System. Version 2.1, Exeter Software, New York.

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning. A Laboratory Manual. 2nd Edn., New York: Cold Spring Harbor Laboratory Press.

Schmit, V., P. du Jardin, J.P. Baudoin and D.G. Debouck, 1993. Use of chloroplast DANN polymorphism for the phylogenetic study of seven Phaseolus taxa including *P. vulgaris* and *P. coccineus*. Theor. Applied Genet., 87: 506-516.

Singh, S.P., 1989. Patterns of variation in cultivated common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot., 43: 39-57.

Skröch, P., J. Tivang and J. Nienhuis, 1992. Analysis of Genetic Relationships Using RAPD Marker Data. In: Applications of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Series, Minneapolis, Minnesota, pp: 26-30.

Skröch, P.W. and J. Nienbuis, 1995. Qualitative and quantitative characterization of RAPD variation among snap bean (*Phaseolus vulgaris*) genotypes. Theor. Applied Genet., 91: 1078-1085.

Tertivanidis, K., O. Koutita, G. Skaracis, E. Traka-Mavrona and M. Koutsika-Sotiriou, 2003. The use of RAPD markers in monitoring molecular changes during a selection process in snap bean. J. New Seeds, 5: 87-96.

Tiwari, M., N.K. Singh, M. Rathore and N. Kumar, 2005. RAPD markers in the analysis of genetic diversity among common bean germplasm from Central Himalaya. Genet. Resour. Crop E., 52: 315-324.

Tsaftaris, A.S., 1987. Isozymes in Plant Breeding. In: Isozymes: Current Topics in Biological and Medical Research. Rattazzi, M.S., J.G. Scandalios and G.S. Whitt (Eds.), Vol. 12, Alan R. Liss Inc., New York, pp: 103-125.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalsky and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic. Acids Res., 18: 6531-6535.

Xiao, J., J. Li, I. Yuan and S.D. Tankley, 1995. Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics, 140: 745-754.

Yeh, F.C., R.C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao, 1997. POPGENE, the user-friendly shareware for population genetics analysis. Alberta, Canada: Molecular Biology and Biotechnology Center, University of Alberta, Canada. http://www.ualberta.ca/~fyeh. Cited 8 Sept 2003.