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Genetic diversity among common bean (*Phaseolus vulgaris* L.) Greek landraces and commercial cultivars: nutritional components, RAPD and morphological markers

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Abstract

The genetic diversity among the main local landraces and commercial cultivars of *Phaseolus vulgaris* L. cultivated in Greece, was estimated by studying the morphological, agronomical and physicochemical traits along with molecular data analysis using random amplified polymorphic DNA (RAPD markers). Cluster analysis was conducted on similarity estimates using the UPGMA algorithm. Application of cluster analysis resulted in a dendrogram representing the genetic relationship among landraces and main bean cultivars grown in Greece. A wide genetic variation was observed among collected local bean landraces in morphological characteristics such as seed color, seed size and growth habit. According to agronomic performance, significant differences were found in number and weight of pods per plant. Variation in protein and fat content among landraces and commercial cultivars was also detected. Moreover, in some landraces like Kastoria and Byzitsa M/M extremely high values for protein content (28.6% and 27.0% respectively) were recorded. Such values were greater than the average protein content previously recorded for other cultivars of this species. Genetic similarity estimated from molecular analysis with RAPDs, seemed not to be related with the seed morphological characteristics and agronomic performance. Only qualitative parameters like growth habit and occasionally geographical origin of landraces were positively correlated with the molecular classification. Local bean landraces were classified in three subgroups whereas the commercial cultivars formed another separate group underlining the narrow genetic base of cultivars.

Additional key words: dry bean; genetic relationships; organic farming; physicochemical properties.

Resumen

Diversidad genética entre variedades autóctonas de alubia (*Phaseolus vulgaris* L.) en Grecia y cultivares comerciales: componentes nutricionales, marcadores morfológicos y RAPD

Se estimó la diversidad genética entre las principales variedades autóctonas y cultivares comerciales de *Phaseolus vulgaris* L. cultivados en Grecia, mediante el estudio de caracteres morfológicos, agronómicos y fisicoquímicos, junto con el análisis de datos moleculares utilizando marcadores RAPD (amplificación al azar de DNA polimórfico). Se llevó a cabo un análisis cluster (CLA) estimando la similitud con el algoritmo UPGMA, obteniendo un dendrograma que representa las relaciones genéticas entre las principales variedades locales y comerciales de alubia cultivadas en Grecia. Se observó una amplia variación entre variedades locales de alubia en características morfológicas tales como color y tamaño de la semilla y hábitos de crecimiento. En lo que respecta al comportamiento agronómico, se encontraron diferencias significativas en el número y el peso de vainas por planta. También se observó variación en los contenidos de proteína y de grasa. Además, se encontraron valores extremadamente altos de contenido en proteína en algunas variedades como Kastoria y Byzitsa M/M (28,5% y 27,0%, respectivamente), que son mucho mayores que la media registrada previamente para cultivares de esta especie. La semejanza genética estimada mediante el análisis molecular de RAPDs no parece estar relacionada con las características morfológicas de la semilla ni el comportamiento agronómico. Sólo los parámetros cualitativos como el hábito del crecimiento y el origen geográfico de las variedades locales mostraron relación con su clasificación molecular. Las razas locales de alubia se clasificaron en tres subgrupos, mientras que los cultivares comerciales formaron otro grupo separado que refleja una estrecha relación genética entre cultivares.

Palabras clave adicionales: agricultura orgánica; alubia seca; propiedades fisicoquímicas; relaciones genéticas.

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Introduction

Common bean (*Phaseolus vulgaris* L.) is a worldwide interesting crop as both grain legume and fresh vegetable. Dry bean is an important source of protein, participating in human diet all over the world (Singh, 2001). Furthermore, the adequate nutritive composition and variable uses of beans in different culinary forms (canned or frozen grain and pod, dry seeds) make it an interesting crop both for consumers and processors (Escribano *et al.,* 1997). Since the consumption of beans in Europe is mainly based on imports (Negri and Tosti, 2002), several European countries are presently focused on products with certified origin because consumers progressively require safe and healthy products endowed with specific quality characteristics (Escribano *et al.,* 1997; Piergiovanni *et al.,* 2000). In Greece, common bean is an important crop, cultivated areas being located in Northern and Central parts of the country (Macedonia, Thrace and Thessaly). Although in some cropping areas farmers have maintained some common bean landraces, in most cases traditional cultivars have been progressively replaced with elite cultivars that ensure higher yield and income meeting the farmer's and consumer's requirements (Mavromatis *et al.,* 2004). An important factor related to bean quality is the nutritional composition of dry seeds which includes protein, fat and dietary fibber content (Arvanitoyannis and Khah, 2003). The quality of bean reaching the consumers depends on the characteristics of the seeds at harvesting time (Casañas *et al.,* 1999). Some genotypes exhibited good quality characteristics and are preferred by the consumers who would be willing to pay high prices for these specific characteristics (Negri and Tosti, 2002; Galvan *et al.,* 2006).

In order to develop such cultivars, a preliminary characterisation and evaluation of the genetic variability among and within local bean landraces with regard to widely grown commercial varieties for agronomic performance and quality traits could be useful for exploitation of this genetic material for *in situ* conservation and breeding programs. The evaluation of morphological differences is a traditional method of evolutionary and pedigree relationship determination (Skroch and Nienhuis, 1995). However, molecular analysis provides additional information that is independent of environmental effects and is appropriate for characterization of genetic material. Molecular analysis with random amplified polymorphic DNA (RAPD) fragments, is a valuable method to access genetic diversity, genetic structure and phylogenetic relationships in *Phaseolus vulgaris* L. (Beebe *et al.,* 1995, 2000; Álvarez *et al.,* 1998; Metais *et al.,* 2000).

The aim of this work was to assess the genotypic variability and relationships among local bean landraces and commercial cultivars, cultivated in Greece, based on morphological characteristics, yield components, physicochemical traits, nutritional components and RAPD molecular genetic analysis.

Material and methods

Genetic material and agronomic performance

Sixteen common bean genotypes (eight bean landraces, still cropped in several areas in Greece and eight well-known commercial cultivars) all of them originated from Mesoamerican gene pool were field evaluated in the experimental organic farm of University of Thessaly (Velestino, Greece) over the 2003-2004 cultivation period. These specific cultivars are available in the National Gene Bank (NAGREF) in Thesaloniki. Their origin and short descriptions [growth habits (I and IV)] according to CIAT (1987), flower color, and seed characteristics (color and size) are shown in Table 1. A randomised complete block (RCB) experimental design with three replications was used. All genotypes were planted in a single row plot (4.5 m). The plots spaced 1.0 m between rows and 0.3 within rows containing 15 plants per plot (33,000 plants ha^{-1}). The organic management was based on the rotation system, durum wheat/lentil, where no fertilizers were applied either on the previous or on bean culture. Appropriate culture practices (summer field ploughing, weeds removed by hand, etc.) were applied, no pest or other agrochemicals were used. Observations for the following traits: seed size, pod number and weight of pods per plant, seed weight on a plant basis and weight of 100 seeds, were collected. Fifteen plants per cultivar were used for morphological characterisation and ten plants for nutritional and molecular analysis.

Abbreviations used: CIAT (Centro International de Agriculture Tropical), DM (dry matter), kgF (kilogram force), NAGREF (National Agricultural Research Foundation), RAPD (random amplified polymorphic DNA), RCB (randomised complete block), UPGMA (unweighed pair group method with arithmetic mean).

Table 1. Name, origin, culinary use and seed morphological traits of eight local bean landraces and eight commercial cultivars

No.	Genetic material	Origin	Growth habit[1]	Seed color	Seed size[2]	Flower color	Culinary use[3]
Landraces							
1	Rodopi	Greece	IV	White	Medium	White	gs-ds
2	Xanthi	Greece	IV	White	Medium	White	gp-gs-ds
3	Kastoria	Greece	IV	White	Large	White	ds
4	Byzitsa M/M	Greece	IV	Beize/black	Large	Pearl	gp-gs-ds
5	Byzitsa A/M	Greece	IV	Black/white	Large	Pearl	gp-gs-ds
6	Byzitsa B	Greece	IV	Brown	Medium	White	gp-ds
7	Velestino	Greece	I	White	Medium	White	gp-ds
8	Byzitsa K	Greece	I	White	Large	White	gp-ds
Cultivars							
9	Zargana K	Greece	IV	Brown	Medium	White	gp-ds
10	Stara Zagorski	Poland	IV	Black/white	Medium	White	gp-ds
11	YV	France	IV	White	Medium	White	gs- ds
12	Venetto	Italy	IV	White/black	Small	Pearl	gp-gs-ds
13	Magirus	USA	I	Brown	Medium	White	gp-ds
14	Garafal	Italy	I	White/red	Medium	Pearl	gp-ds
15	Romano	Italy	I	White/red	Large	Pearl	gp-gs-ds
16	Dade	France	I	White	Medium	Pearl	gp-ds

[1] I: bushy type, IV: climbing type. [2] 100 seeds weight: small seeds, < 25 g; medium, 25-40 g; large, > 40 g. [3] ds: dry seeds, gp: green pods, gs: green seeds.

Molecular genetic analysis

Random amplified molecular markers were used to assess the level of genetic diversity among the landraces and commercial cultivars. Genomic DNA was extracted from a bulk of young leaves that came from equal amount of each of the 10 plants per accession. The CTAB DNA extraction method was used (Doyle and Doyle, 1990). Twenty single-10-mer oligonucleotide primers (Operon Technologies, USA) (Table 2) were used for molecular DNA analysis**.**

The DNA amplifications were carried out in 25 μL reaction mixture for each sample. The mix contained 50 ng template DNA, 10 mM Tris-HCl pH 9.0, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.625 μM of 10-mer primer and 1 unit *Taq* DNA polymerase (Minotec). The samples were incubated in a thermal cycler (Eppendorf Master Cycler Gradient, Germany) programmed as follows: initial denaturation at 95°C for 8 min, then 35 cycles of 94°C for 60 sec, 35°C for 60 sec, and 72°C for 90 sec followed by a step of 72°C for 8 min.

Amplification products were separated using electrophoresis in agarose gels (2%) in 0.5 TBE buffer and visualized by staining with ethidium bromide (0.004% w/v). Four replications were applied to detect the repeatability of the patterns. The molecular weight of each amplified product was estimated by using the standard DNA molecular weight marker (100 bp ladder, Sigma, USA) according to the method described by Schaffer and Sederoff (1981). All gels were scored in a binary format where the presence of a band was 1 and the absence was 0. The Jaccard (1908) and Dice (1945) similarity coefficients were estimated using the SIMQUAL command. Cluster analysis was performed using the unweighed pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973). The correlation coefficient between each similarity matrix and clustering procedure was estimated as well. The analysis was performed with the NTSYS-PC software package version 2.02 (Rolf, 1998).

Physicochemical and nutritional traits

Dry seed culinary and nutritional traits were measured two months after harvesting when seeds reached their commercial maturity. During the period from harvesting to analysis, seeds were maintained at a storage room at 4 ± 2^{o}C.

The analysis of physicochemical quality traits included protein and fat content (%), pH, seed density

Table 2. RAPD primers used in PCR reactions, number of reproducible polymorphic loci and percentage (%) of polymorphism

No.	RAPD primer	Sequence	Total amplified loci	Polymorphic loci (%)
1	OPB-7	5'-GGTGACGCAG-3'	6	4 (67)
2	OPB-10	5'-CTGCTGGGAC-3'	6	3 (50)
3	OPB -16	5'-TTTGCCCGGA-3'	5	1 (20)
4	OPB-17	5'-AGGGAACGAG-3'	6	5 (83)
5	OPC-1	5'-TTCGAGCCAG-3'	10	3 (30)
6	OPC-3	5'-GGGGGTCTTT-3'	10	2 (20)
7	OPC-4	5'-CCGCATCTAC-3'	10	5 (50)
8	OPC-5	5'-GATGACCGCC-3'	9	3 (33)
9	OPC-6	5'-GAACGGACTC-'3	10	3 (30)
10	OPC-7	5'-GTCCCGACGA-3'	10	4 (40)
11	OPC-8	5'-TGGACCGGTG-3'	9	5 (56)
12	OPC-9	5'-CTCACCGTCC-3'	10	6 (60)
13	OPC-10	5'-TGTCTGGGTG-3'	10	7 (70)
14	OPD-11	5'-AGCGCCATTG-3'	10	3 (30)
15	OPD-20	5'-ACCGGCTCAC-3'	8	4 (50)
16	OPE-14	5'-TGCGGCTGAG-3'	8	8 (100)
17	OPE-6	5'-AAGACCCCTC-3'	6	Monomorphic (0)
18	OPF-4	5'-GGTGATCAGG-3'	5	Monomorphic (0)
19	OPF-11	5'-TTGGTACCCC-3'	4	Monomorphic (0)
20	OPF- 19	5'-CCTCTAGACC-3'	8	4 (50)
Total/Mean			160	70 (49.35)

and seed hardness. A sample of 15 pods per genotype was taken and 40-50 seeds were used for the determination of physicochemical properties. The seeds were heated in distilled-deionised water, blended and the pH was measured using a pH meter (model 526 WTW MultiCal®). The protein content was recorded using the automatic micro-Kjeldahl method (Kelly and Bliss, 1975). The amount of nitrogen was quantified with a selective ammonium electrode multiplying with a factor of 6.25 to estimate the protein content. Crude fat content was determined with the diethyl ether extraction method without hydrolysis using a Soxhlet extractor for 24 hours. The values for the last two traits were expressed as a percentage dry matter of seed weight (DM %).

Mechanical hardness was measured on at least 15 seeds per cultivar using an Instron Universal Testing Machine (model 4301, USA) equipped with a Warner-Bratzler meat shear compression cell type 2830-012 (Instron Co. Canton, MA, USA).

Seed density (g mL^{-1}) was measured in seed samples of 100 g per bean cultivar placed into a cylinder with 500 mL of distilled water for 15 min (Bishnoi and Khetarpaul, 1993).

Data were treated with analysis of variance (ANOVA) and the Duncan multiple range test was used for separating means for all the physicochemical traits studied (Steel and Torrie, 1980). Significance was accepted at $p \leq 0.05$.

Results and discussion

Morphological and agronomic traits

A considerable genetic diversity was observed among the landraces and cultivars in terms of morphological traits and agronomic performance. The genetic material used belonged to two growth habits, bushy (I) and climbing (IV), according to CIAT and ICARDA classification. Among the cultivars used and within both habits, differences in seed colour, seed size, number of seeds per pod and weight of 100 seeds, were observed (Tables 1 and 3).

The dry bean yield can be expressed as the product of three components: pods per plant, seeds per pod and weight of 100 seeds (Nienhuis and Singh, 1986). According to the data obtained, some local landraces deserve particular attention since their values for the referred traits and properties were the same or better than the commercial cultivars. Data revealed that landraces are promising genetic material and have better performance than the commercial cultivars, since landraces Rodopi,

Table 3. Yield components of bean landraces and commercial cultivars. Statistical differences among cultivars for the studied traits measured according to Fisher's test

No.	Genetic material	Pods per plant		100 seeds (g)	Seeds per plant (g)
		No.	Weigth (g)		
Landraces					
1	Rodopi	48.5 ± 8.4^{a}	88.4 ± 9.2^{c}	33.37 ± 7.1^{c}	59.6 ± 12.8^{c}
2	Xanthi	51.3 ± 3.8^{a}	73.2 ± 8.7^{d}	33.87 ± 3.2^{c}	51.3 ± 5.4^{c}
3	Kastoria	39.8 ± 7.7^{b}	125.8 ± 3.7^{a}	59.82 ± 6.8^{a}	86.7 ± 12.2^{a}
4	Byzitsa M/M	21.5 ± 4.3^{d}	104.0 ± 6.6^{b}	55.53 ± 5.5^{a}	78.8 ± 6.4^{b}
5	Byzitsa A/M	28.7 ± 4.7^{c}	117.2 ± 12.4^{b}	55.90 ± 5.8^{a}	86.1 ± 8.7^{a}
6	Byzitsa B	49.5 ± 11.2^{a}	123.8 ± 12.5^{a}	28.67 ± 4.5^{d}	89.0 ± 10.2^{a}
7	Velestino	23.7 ± 4.2^{d}	54.1 ± 9.4^{e}	30.33 ± 6.9^{cd}	32.0 ± 7.4^{e}
8	Byzitsa K	29.2 ± 8.3^{c}	71.9 ± 12.2^{d}	46.87 ± 7.4^{b}	48.5 ± 4.5^{d}
Cultivars					
9	Zargana K	23.2 ± 3.2^{d}	44.9 ± 8.4^{f}	25.20 ± 3.2^{d}	24.8 ± 4.8^{e}
10	Stara Zagorski	20.5 ± 2.4^{d}	41.6 ± 9.2^{f}	36.50 ± 4.2^{c}	26.8 ± 3.6^{e}
11	YV	22.5 ± 3.2^{d}	48.2 ± 8.4^{ef}	30.60 ± 5.2^{cd}	30.6 ± 8.2^{e}
12	Venetto	28.4 ± 2.2^{c}	50.8 ± 10.4^{e}	18.77 ± 4.7^{e}	20.77 ± 5^{f}
13	Magirus	20.7 ± 2.1^{d}	40.8 ± 7.4^{f}	38.87 ± 1.2^{c}	23.4 ± 3.2^{e}
14	Garafal	22.3 ± 3.2^{d}	46.4 ± 8.5^{ef}	32.03 ± 3.1^{cd}	27.8 ± 3.4^{e}
15	Romano	24.2 ± 2.4^{d}	52.8 ± 8.2^{e}	47.17 ± 6.4^{b}	47.17 ± 6.4^{d}
16	Dade	24.5 ± 6.5^{d}	45.4 ± 2.3^{ef}	28.73 ± 3.8^{d}	28.73 ± 5.2^{e}
	Mean ± sx	29.90 ± 8.4	70.58 ± 9.8	35.5 ± 7.2	47.6 ± 8.1
	F test	**	**	***	***
	CV (%)	7.9	10.3	10.7	12.8

,*: significance for $p < 0.05$ and $p < 0.01$ respectively. Data partially published in Mavromatis *et al.* (2007).

Xanthi and Byzitsa A/M, Byzitsa M/M had a significant greater number of pods per plant, and Byzitsa A/M, Byzitsa M/M and Kastoria, were superior for weight of pods and seeds (Table 3). These observations confirmed the greater interest for landraces from Byzitsa because of their culinary use as green pods and dry seeds.

Under organic farming, the main differences observed between commercial cultivars and local landraces were in yield components (size and weight of seeds, number and weight of pods per plant). Although it could be expected the highest values to be recorded in commercial cultivars this was not the case. Such results of the landraces could be issued either on the promising genetic material either on the organic farming applied in this experiment or in their adaptation in organic farming (*genotype* × *organic farming* interaction) since landraces were cultivated in family farms with no use of agrochemicals. Whatever of the abovementioned assumptions is true this genetic material could be used either for cultivation as new released varieties or as initial germplasm for breeding varieties addressed to organic farming. The number of pods per plant of the commercial varieties ranged from 20.5 to 28.4 which is quite close to the mean value (Table 3). This seems to be an important observation since this trait is positively correlated with yield and could be indirectly used as selection criterion for yield. The weight of 100 seeds had the widest range of all the examined traits with values varying from 18.7 g up to 59.8 g. In dry beans, this trait depended on genotype and had a positive correlation with the type and size of seeds (Graham and Ranalli, 1997). For this reason, although this trait followed the pattern of other traits, the differences between landraces and cultivars were not of the same magnitude.

Physicochemical properties and nutritional value

From the nutritional point of view, the landraces seemed to be an interesting genetic material. For example, the landrace from Kastoria had a protein content as high and even higher than the best commercial cultivar Romano and much higher than the average

value (20.48 to 22.6%) reported by Álvarez *et al.* (1998) and Escribano *et al*. (1997) respectively. Concerning this trait, the commercial cultivars can be classified in increasing percentage as follows: Dade, YV, Zargana K, Magirus, Garafal, Stara Zagorski and Romano (Table 4). Concerning the fat content, it was observed that the mean value recorded was 1.15%. The landrace Byzitsa K had the highest value (1.80%), standing 62% higher from the average estimate, whereas Velestino displayed the lowest fat content (0.28%) (Table 4). It is noteworthy that, apart from Byzitsa K and Velestino, all the other landraces and cultivars were not characterised by considerable differences since their values varied from 0.93 up to 1.36% the highest values being these of three out of the four commercial cultivars (Magirus 1.36%, Romano 1.30% and Dade 1.20%). These data showed that Velestino landrace could be eventually released as a low-fat promising functional dry bean cultivar**.**

Regarding the average seed density value for commercial cultivars, this was 1.12 g mL^{-1} compared to the corresponding average of 1.07 g mL^{-1} for landraces. The highest hardness values for the landraces were recorded for Xanthi, Kastoria, and Byzitsa B (4.0 kgF, 4.25 kgF, and 3.69 kgF, respectively). The abovementioned values are comparable with the hardness of the commercial varieties Zargana, Dade and Magirus (3.91 kgF, 3.44 kgF and 3.38 kgF respectively). Since high hardness is closely related to low water content and thereby prolonged shelf life of dry beans, the abovementioned landraces could be interesting material for storability. On the other hand the Velestino, Byzitsa M/M and Byzitsa A/M landraces are less hard, with an average value of 2.6 kgF and are of the some hardness of the Garafal, Stara Zagorski, YV, Venetto commercial varieties with an average value of 2.4 kgF. Such genetic material would be more desirable for many consumers who are pleased with less hard varieties.

Molecular analysis and genetic relationships among bean landraces and commercial cultivars

Seventy (70) repeatable polymorphic bands out of a total of 160 bands were identified using 20 RAPD

Table 4. Physicochemical and nutritional traits in local bean landraces and commercial cultivars

No.	Genetic material	Seed density (g mL^{-1})	Hardness (kgF)	Protein content (%)	Fat content (%)	pH
Landraces						
1	Rodopi	1.09 ± 0.06^{bc}	3.03 ± 0.40^{b}	24.42 ± 0.39^{bc}	1.15 ± 0.01^{b}	7.24 ± 0.17^{b}
2	Xanthi	1.14 ± 0.01^{b}	4.00 ± 0.50^{a}	25.00 ± 0.92^{b}	1.02 ± 0.22^{bc}	7.32 ± 0.08^{a}
3	Kastoria	1.16 ± 0.01^{b}	4.25 ± 0.68^{a}	28.58 ± 0.79^{a}	1.32 ± 0.05^{b}	7.22 ± 0.05^{b}
4	Byzitsa M/M	1.10 ± 0.04^{bc}	2.51 ± 0.52^{c}	27.04 ± 0.54^{a}	1.03 ± 0.12^{bc}	7.20 ± 0.04^{b}
5	Byzitsa A/M	1.12 ± 0.02^{b}	2.14 ± 0.42^{c}	25.18 ± 0.40^{b}	1.12 ± 0.05^{b}	7.15 ± 0.12^{bc}
6	Byzitsa B	0.82 ± 0.03^{c}	3.69 ± 0.35^{b}	22.76 ± 0.32^{cd}	1.15 ± 0.08^{b}	7.25 ± 0.05^{b}
7	Velestino	1.05 ± 0.04^{c}	2.75 ± 0.37^{b}	24.02 ± 0.24^{c}	0.28 ± 0.09^{c}	6.99 ± 0.02^{c}
8	Byzitsa K	1.13 ± 0.01^{b}	3.01 ± 0.45^{c}	23.92 ± 0.35^{c}	1.80 ± 0.14^{a}	6.99 ± 0.03^{c}
Cultivars						
9	Zargana K	0.96 ± 0.04^{c}	3.91 ± 0.33^{b}	25.74 ± 0.24^{b}	1.13 ± 0.12^{b}	7.08 ± 0.04^{c}
10	Stara Zagorski	1.25 ± 0.02^{a}	2.95 ± 0.42^{c}	26.22 ± 0.32^{b}	1.04 ± 0.08^{bc}	7.15 ± 0.10^{bc}
11	YV	1.08 ± 0.03^{c}	2.33 ± 0.38^{c}	23.08 ± 0.30^{c}	0.94 ± 0.15^{bc}	6.78 ± 0.08^{c}
12	Venetto	1.09 ± 0.04^{c}	1.43 ± 0.65^{d}	24.60 ± 0.24^{bc}	1.08 ± 0.08^{bc}	7.45 ± 0.12^{a}
13	Magirus	1.12 ± 0.02^{b}	3.38 ± 0.35^{b}	26.14 ± 0.27^{b}	1.36 ± 0.04^{b}	7.02 ± 0.02^{c}
14	Garafal	1.13 ± 0.01^{b}	2.97 ± 0.52^{c}	26.18 ± 0.25^{b}	0.93 ± 0.12^{bc}	7.10 ± 0.03^{c}
15	Romano	1.19 ± 0.02^{b}	3.13 ± 0.54^{b}	28.06 ± 0.45^{a}	1.30 ± 0.14^{b}	6.96 ± 0.06^{c}
16	Dade	1.19 ± 0.03^{b}	3.44 ± 0.43^{b}	22.36 ± 0.56^{cd}	1.20 ± 0.18^{b}	7.03 ± 0.05^{c}
	Mean	1.099	3.06	25.25	1.15	7.09
	Sx	0.012	0.11	0.241	0.09	0.15
	F test	***	***	***	***	**
	CV (%)	2.5	14.2	2.1	10.7	2.7

,*: significance for $p < 0.05$ and $p < 0.01$ respectively.

primers. The percentage of polymorphism per primer ranged from 2 to 10 depending on the primer used. The primer OPE-14 yielded the highest number of polymorphic amplified bands whereas the primers, OPE-6, OPF-4 and OPF-19 were monomorphic (Table 2). The average Jaccard similarity index among cultivars ranged from 0.84 to 0.98, values which are higher than those reported by Marotti *et al.* (2007) for ISSRs and RAPDs for common bean and that indicate the close genetic relationship of the material used in this study. The dendrogram based on the RAPD analysis classified the genotypes in four main groups (A-D) (Fig. 1). Landraces were clearly discriminated from commercial bean cultivars. The first group (A) was subdivided into two groups including the landraces Rodopi, Byzitsa K and Kastoria and the commercial cultivar Venetto. These landraces originated from different geographical areas and their common characteristics were only the growth type and the colour of seeds (Table 1). In this group the Italian Venetto cultivar is grouped together with Greek landraces, this is not unexpected since the Mediterranean arc, including between others Italy and Greece, share common genetic material after distribution from their centre of origin (Papa *et al.,* 2006). The second group (B) included one landrace from Xanthi and two others from Byzitsa. The interesting point is that the similarity between Xanthi and Byzitsa M/M was greater in comparison to other two populations originating from the same area (Fig. 1). A concluding remark coming from the (A), (B) and (D) cluster groups, is that the four landraces from Byzitsa are well separated and distinct from each other. The third group (C) consisted mainly of commercial bean cultivars originating from Greece (Zargana, Velestino), Poland (Stara Zagorski), France (Dade, YV) and Italy (Romano, Garafal) and confirms the narrower genetic base of cultivated commercial genetic material of *P. vulgaris* in Greece in comparison with landraces. This is in agreement with Beebe *et al.* (1995), which reported that selection for resistance for developing new varieties resulted in narrowing their genetic base. The fourth group (D) was formed by the landrace Byzitsa K and the commercial cultivar Magirus (Asgrow, USA), which were still separated from the European group.

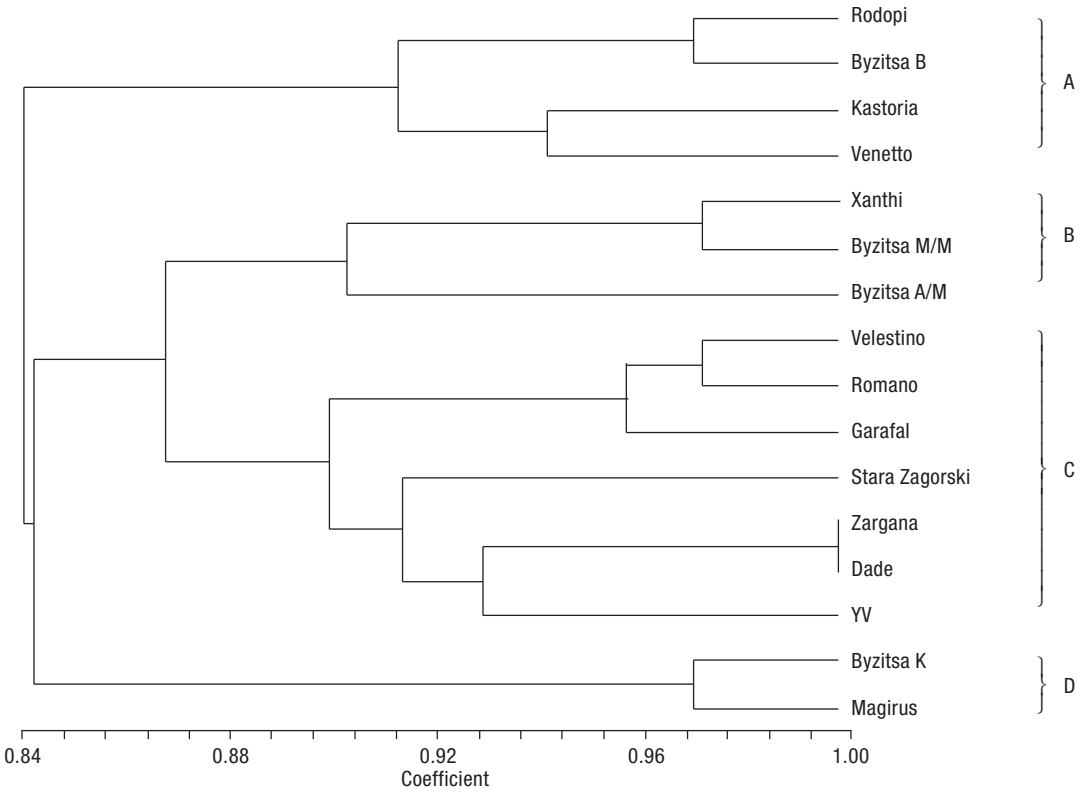

Figure 1. Genetic relationships among local bean landraces and commercial cultivars obtained by RAPD analysis using Jaccard's similarity coefficients and the UPGMA clustering method

Conclusions

The landraces used seems to be a promising genetic material either for variety release or for plant breeding projects since they had the same or better yield components performance than the commercial cultivars analyzed. The most promising landraces were Kastoria and Velestino because of their superior values of protein, fat content and yield, as compared with the commercial bean varieties.

In general, the landraces seem to be different from the commercial varieties having higher variability for the examined traits by all three types of analysis carried out. Such variability is desirable in breeding programs. Furthermore the genetic relationships found between commercial cultivars and landraces can be a matter of interest in different aspects related to intellectual properties, breeder's rights and breeding purposes.

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