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Molecular characterization of common bean (*Phaseolus vulgaris* L.) genotypes

Fasulye (*Phaseolus vulgaris* L.) genotiplerinin moleküler karakterizasyonu

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.), a member of *Leguminosae* family, is widely cultivated in the world. It is one of the most important legume species in Turkey. This study was conducted to characterize 11 common bean genotypes and a standard cultivar commonly cultivated in Burdur province. Genotypes were collected from local bean growing farmers. Highly polimorphic Amplified Fragment Lenght Polymorphism (AFLP) markers were used to determine the genetic differences among the genotypes using DNA extracted from the leaves of each genotype. The amplification products from AFLP reactions were scored and the similarity matrix for all genotypes was obtained with Dice coefficient method using NTSYS-pc program. The similarity coefficients ranged from 0.178 to 0.713. Based on these coefficient values, two main clusters were obtained. The results suggest that the collected bean genotypes truly represent the overall genetic variability of *Phaseolus vulgaris*, confirming the multiple origins of these materials, and their potential as a source of variation for breeding programs.

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ÖZ

Fasulye (*Phaseolus vulgaris* L.), Leguminosae familyası içerisinde yer almakta ve Dünyada yaygın olarak yetiştirilmektedir. Ülkemizde baklagil grubu sebzeler içerisinde önemli türlerden biridir. Bu çalışmada; Burdur sınırları içerisinde biri standart çeşit olmak üzere toplam 12 fasulye genotipinin moleküler açıdan karakterizasyonlarının yapılması amaçlanmıştır. Genotipler yerel fasulye yetiştiren çiftçilerden kış döneminde toplanmıştır. Yüksek polimorfizm üretebilen Amplified Fragment Lenght Polymorphism (AFLP) markörleri ve bireysel bitkilerden alınan DNA örnekleri kullanılarak yerel genotipler arasındaki genetik farklılıklar ortaya konulmuştur. AFLP'den elde edilen amplifikasyon ürünleri skorlanmış, ve tüm genotipler için benzerlik matriksi Dice coeficient metodu kullanılarak Numerical Taxonomy and Multivariete Analysis System (NTSYS-pc) programı yardımıyla hesaplanmıştır. Bulunan benzerlik katsayıları 0.178-0.713 arasında değişim göstermiştir. Bu koefficient değerlerine göre yapılan gruplandırmada iki ana grup oluşmuştur. Sonuçlar toplanan fasulye genotiplerinin *Phaseolus vulgaris*'in genel genetik varyabilitesini gösterdiklerini ve bu materyallerin çoklu orijinlere sahip olduklarını ve bu yüzden ıslah programlarında varyasyon kaynağı olarak önemli bir potansiyele sahip olduklarını göstermektedir.

1. Introduction

Five species among 50 *phaseolus* species (*Phaseolus vulgaris, Phaseolus lunatus, Phaseolus coccineus, Phaseolus acutifolius*, and *Phaseolus polianthus*) are grown for human consumption. It is reported that among these species *P. vulgaris* is the most cultivated species in the world and contains 75 % of all legume species grown (Broughton et al. 2003; Gepts et al. 2005). After long term breeding practices, important changes have been reported in the plant's morphology and phenotype esspecially in its characters such as growth habit, seed size, seed storage and maturity (Koinange et al. 1996; Gepts 1998). Plant genetic resources include heirloom populations and their wild relatives, unused old species, and lines that their genetic characteristics have not been fully identified. These genetic resources are important for genetic variability and contain the richness and variability of hereditary information in the gene pool of a plant species. The characterization of plant genetic resources is primarily performed with the aim of revealing the genetic variations among seed samples and populations, and the amount and dispersion of genetic variation in these samples and populations (Piergiovanni et al. 2004).

Although Turkey is not a genetic center for many plant species, it contains high level of genetic variation (Tan ve Açıkgöz 2002). It is possible to find a large genetic variation that could be used for the breeding of these species. Even in

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certain circumstances, it is reported that these variability is even larger in domesticated genotypes as compared to wild populations (Tan ve Açıkgöz 2002). It is possible to come across especially common bean heirloom populations among family members such as chickpea, pea, broad bean, kidney bean in Anatolia (Balkaya 1999; Tan ve Açıkgöz 2002). This study was performed to determine genetic relationship among 11 bean genotypes and one standard cultivar (Gina) commonly grown in Burdur province.

2. Materials and Methods

2.1. Collection of plant seeds

Common bean seeds were obtained from Yakaköy, Çatağıl (İnsuyu), Halıcılar and Günalan villages in Burdur province where common bean has been widely cultivated. Genotypes used in the study were listed in alphabetical order in Table 1. Each genotype was denoted with a number from 1 through 12.

DNA's were extracted from each genotype in order to determine the genetic differences among genotypes. For this purpose, the seeds from each genotype were germinated in plastic petri dishes, and 3-4 weeks after emergence, young leaves were collected and stored at -80 ^{o}C until DNA isolation. DNA was extracted from 100 mg leaf material using hexadecyltri-methylammonium bromide (CTAB) extraction procedure (Doyle and Doyle 1990). DNA quality and concentration were determined by agarose gel electrophoresis and reading on a spectrophotometer at 260 and 280 nm wavelengths.

2.2. AFLP analysis

AFLP markers were used to genetically characterize all genotypes. AFLP analysis was performed using DNA isolated from each genotype as described above and a commercial kit (Invitrogen Life Technologies, ABD). Briefly, 250 ng genomik DNA was cut with EcoRI and MseI restriction enzymes, and the adapters were added to the digested DNA using T4 DNA ligaz as described in the AFLP kit manual (Invitrogen). Then, preselective amplifications were performed with *EcoRI*+A and *MseI*+C primers. PCR conditions were as follows: 30 s at 90 ^{o}C, 60 s at 56 ^{o}C and 60 s at 72 ^{o}C for 20 cycles. PCR products were diluted (1:50) and used for selective amplifications using primers provided with the commercial kit. For selective amplification, 5 µl diluted PCR product, 0.5 µl *EcoR I* primer, 4.5 µl *Mse I* primer and dNTP mix, 2 µl 10X PCR buffer, 7.9 µl ultra pure water and 0.1 µl *Taq* polimerase were used, and PCR amplification was performed with touchown method. PCR conditions were as follows: in the first step, denaturation at 94 ^{o}C for 30 s, annealing starting at 65 ^{o}C for 30 s and decreasing the temperature for 0.7 ^{o}C in each step until the temperature reached to 56 ^{o}C, and extension at 72 ^{o}C for 60 s. The second step consisted of 23 cycles with denaturation at 94 ^{o}C for 30 s, annealing at 56 ^{o}C for 30 s, and extension at 72 ^{o}C for 60 s.

Before loading onto polyacrylamide gels, selective amplification products were mixed with 10 µl 3X STR dye solution (0.2 ml 5M NaOH, 5 ml % 95 formamide, 50 mg bromophenol blue, 50 mg xylene cyanol, 100 ml dH_2O) and incubated at 90 ^{o}C for 4 min. Then, the samples were loaded on to a 6 % polyacrylamide gel [19:1 acrylamide:bisacrylamide, 7.5 M urea, 1X TBE (0.1 M Tris-HCl, 0.09 M boric acide, 0.001 M EDTA)] and electrophoresed at 2000 V constant power for 3 hours in 0.5 X TBE buffer. After electrophoresis, the amplification products were visualized with silver staining method as described (Pillen et al. 2000).

2.3. Data analyses

A total of 8 primer combinations were used for the amplificatin of DNA obtained from genotypes. AFLP profiles were visually scored considering (1) as the presence of a band, and (0) its absence. Only bands that appeared consistently between two independent runs were rated. Bands that were not well defined were not included in the data set. The binary (1/0) data matrix was used to calculate Dice similarity coefficient with NTSYS-pc (version 2.2) (Rohlf 2000). Association among genotypes was revealed by cluster analysis using the UPGMA method and Principal Coordinate Analysis (Sneath and Sokal 1973). The goodness of fit of the varieties to a specific cluster in the UPGMA cluster analysis was determined by the Mantel's correlation test (Mantel 1967). Mantel's z test values were calculated using MX COMP program of NTSYS-pc.

3. Results and Discussion

Burdur province is known as possesing one of the richest common bean genetic resources in Turkey. The common bean genotypes from this region had not been genetically characterized before. In this study, 11 common bean genotypes and one standard cultivar (Gina) (Table 1) widely grown in Burdur province was characterized with AFLP markers using 8 primer combinations. All primers tested produced well-defined and scorable amplification products and showed polymorphism between 12 genotypes of *Phaseolus* analyzed. A total of 255 amplification products were produced with all primer combinations and 38 of these were found to be polymorphic among genotypes (Table 2).

The most number of bands producing primer combination was E-ACC/M-CAA and 13 of which were polymorphic. E-ACC/M-CTA primer combination produced 32 bands of which 7 were polymorphic among genotypes. The least number of amplification products and polymorphism producing primer combination was E-ACA/M-CAA. The polymorphism ratio of 8 primer combinations was 14.3 %. Polymorphism percentage varied from 8.3 (E-ACA/M-CTA) to 33.3 (E-ACC/M-CAA) with primer combinations. The results obtained were in conformity with other studies conducted on common bean genotypes which have reported the percentages of polymorphic bands ranging from 11.20 % (Maras et al. 2008) to 95 % (Maciel et al. 2003; Fabio et al. 2003). Similarity matrix for all genotypes was calculated with Dice coefficient method using NTSYS-pc program and given in Table 3. Similarity coefficients ranged from 0.178 to 0.713 suggesting a good range of genetic diversity (Kumar et al. 2008). The lowest values obtained were 0.16 between Yassi and Beyaz Oturak, 0.178 between Yassi and Akkucuk, and 0.196 between Karataneli and Akiri. The highest similarity coefficient (0.713) was determined between Şeker and Sarıkız genotypes.

The highest number of similarity coefficients were between 0.20-0.29 values (Table 3). Similarly, other studies have also reported various similarity coefficient results between bean genotypes. For example, Kumar et al. (2008) have reported similarity coefficients ranging from 0.184 to 0.762, Maras et al. (2008) from 0.73 to 0.99, Svetlava et al. (2006) from 0.840 to 1.0 and Lioi et al. (2005) from 0.408 to 0.917.

UPGMA was performed in order to reveal the genetic relationships among genotypes with Dice similarity values using NTSYS-pc program. Two main clusters were obtained.

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Table 1. Genotypes used in the study.

Genotypes	Number denoted	Origine	Certification Status
Akbağlaklı	1	Halıcılar	-
Akiri	2	Yakaköy	-
Akküçük	3	Çatağıl	-
Beyaz oturak	4	Halıcılar	-
Beyaz sırık	5	Günalan	-
Gina	6	Monsanto Gıda Tar.Tic.Ltd.Şti.	Certified
Horoz	7	Halıcılar	Certified
Karataneli	8	Çatağıl	-
Roma 2	9	Çatağıl	Certified
Sarıkız	10	Halıcılar	Certified
Şeker	11	Günalan	Certified
Yassı	12	Günalan	-

Table 2. Primer combinations used for the characterization of comon bean genotypes and the number of amplification products per combination, the number of polymorphic bands and polymorphism ratio.

Primer combination	Total number of amplification products	Number of polymorphic bands	Polymorphism ratio
E-AAC/M-CAC	34	5	14.7
E-AAC/M-CAG	28	3	10.7
E-ACA/M-CAA	15	-	-
E-ACC/M-CAA	39	13	33.3
E-ACC/M-CAC	30	-	-
E-ACA/M-CTA	24	2	8.3
E-ACC/M-CTA	32	7	21.9
E-ACT/M-CAC	25	3	12
E-ACA/M-CTG	28	5	17.9
Total	255	38	-
Average	29.3	4.2	14.3

Table 3. Similarity coefficients among genotypes calculated with Dice coefficient method.

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12
1	1.0											
2	0.408	1.0										
3	0.407	0.468	1.0									
4	0.430	0.421	0.569	1.0								
5	0.280	0.319	0.298	0.397	1.0							
6	0.338	0.263	0.267	0.333	0.500	1.0						
7	0.324	0.257	0.231	0.266	0.466	0.481	1.0					
8	0.277	0.196	0.268	0.223	0.331	0.551	0.434	1.0				
9	0.265	0.285	0.257	0.270	0.267	0.297	0.336	0.378	1.0			
10	0.333	0.245	0.266	0.253	0.351	0.415	0.342	0.485	0.336	1.0		
11	0.311	0.265	0.242	0.260	0.385	0.411	0.386	0.424	0.304	0.713	1.0	
12	0.265	0.244	0.178	0.196	0.275	0.361	0.313	0.379	0.229	0.510	0.474	1.0

Akbaglakli and Roma 2 formed the first main cluster. The Dice coefficient values for Roma 2 and Akbaglakli were 0.29 and 0.33 respectively. Both genotypes showed the least similarity to other genotypes. In the first main cluster, while Roma 2 alone formed a subcluster, Yassı, Şeker and Sarıkız formed the second subcluster and Karataneli, Gina, Horoz and Beyaz sırık formed the third subcluster. In the second main cluster, while Beyaz oturak, Akküçük and Akiri formed a subcluster, Akbağlaklı alone formed a second subcluster. Among polymorphisms produced by eight AFLP primer combinations, no polymorphism that could separate Seker and Sarikiz genotypes was produced, and both genotypes remained in the same cluster.

In order to test the goodness of fit of the clustering obtained with UPGMA to the calculated Dice similarity matrix, Mantel's z test scores were calculated with MX COMP subprogram of NTSYS-pc. The clustering pattern in the dendogram was strongly supported by high value of correlation coefficient in the Mantel's test of goodess of fit (r=0.90). The closer the obtained r value to 1, the better the goodness of fit of the grouping to the correlation matrix (Kumar et al. 2008).

The molecular data obtained from AFLP analysis were subjected to principal coordinate analysis (PCA). PCA was performed to visualize the association among genotypes in more detail. For PCA analysis, eigen values and eigen vectors were calculated using EIGEN sub-program of NTSYS-pc. Three dimensional figure in order to show the relationships among genotypes were created and given in Figure 2. The first, second and third principal coordinates explained the 56 % of the total

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variation. The results of PCA analysis largely corresponded to those obtained through cluster analysis.

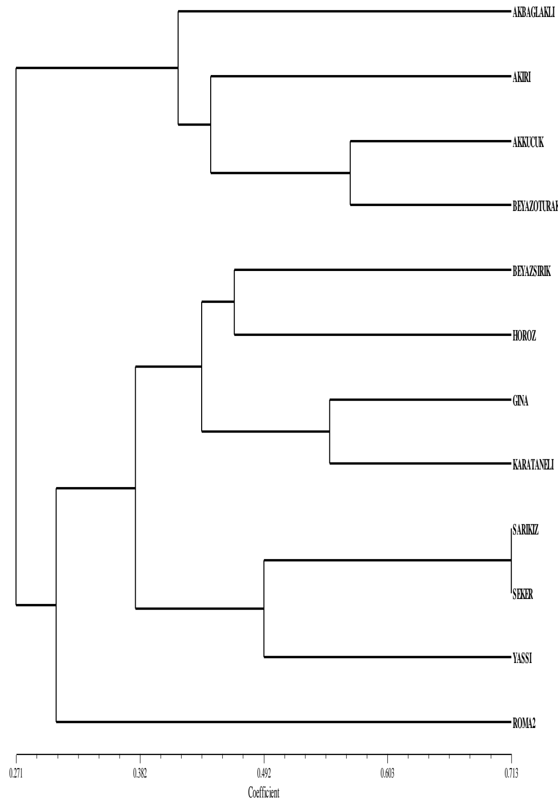

Figure 1. Clustering of common bean genotypes with UPGMA method using 8 primer combinations.

Figure 2. Patterns of relationships among 12 common bean genotypes revealed by principal coordinate analysis.

The results obtained from the study have significance in terms of determination of the genetic distance among genotypes, the control of genetic resources and genetic diversity, and the selection of genotypes for the purpose of crossings. Moreover, the common bean genotypes from the province have economically important adaptive traits that could potentially be incorporated into bean breeding studies.

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