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Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from eastern Turkey

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ABSTRACT. Green bean genotypes collected from eastern Turkey were characterized using simple sequence repeat (SSR) markers and morphological traits. Among 12 SSR markers, 10 produced successful amplifications and revealed DNA polymorphisms that were subsequently used to assess genetic relatedness of the genotypes. Based on the number of alleles generated and the probability of identity values, the most informative SSR loci were PVGLND5, PVMEIG, PV-ag001, and PV-ag004. Probably, due to the inbreeding nature of beans, the heterozygosity observed within genotypes was low at most of the SSR loci. The UPGMA dendrogram constructed based on the SSR data yielded two major clusters. The overall genetic distance was around 98%, among the genotypes. This information can be used to help select Turkish green bean lines.

Key words: Bean; Simple sequence repeat; Genetic resources; Genetic characterization

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is an important legume grown worldwide for its high nutritional and economic value. In some countries, it is the primary source of protein in the human diet. In addition, bean consumption is increasing based on perceived health benefits associated with certain vitamin and mineral contents. The evaluation of genetic diversity could greatly assist and expedite selection decisions in bean breeding. Therefore, a number of earlier studies have investigated the genetic variation within and between bean populations as well as between cultivated and wild genotypes, using isozymes (Weeden, 1984; Koenig and Gepts, 1989; Belletti and Lotito, 1996), seed protein analysis (Lioi et al., 2005), and molecular markers. For instance, among molecular markers, RFLPs were used to identify patterns of genetic diversity as well as to develop genetic linkage maps in common bean (Vallejos et al., 1992; Gepts et al., 1993; Nodari et al., 1993). Similarly, polymerase chain reaction (PCR)-based DNA markers such as RAPDs and AFLPs were also used extensively to compare the genetic structures of common bean germplasm collections (Skrock and Nienhuis, 1998; Tiwari et al., 2005; Durán et al., 2005), to assess genetic diversity within bean germplasm (Caicedo et al., 1999; Lioi et al., 2005; Sustar-Vozlic et al., 2006; Kumar et al., 2008), and to map resistance genes (Kelly et al., 2003).

More recently, simple sequence repeats (SSRs) have been used by several investigators to determine the genetic diversity within and between bean populations (Métais et al., 2002; Durán et al., 2005; Diaz and Blair, 2006; Blair et al., 2006, 2007; Benchimol et al., 2007; Hanai et al., 2007). A number of polymorphic SSR markers have been developed for beans using DNA sequence information obtained from the GenBank database (Yu et al., 2000; Blair et al., 2003) as well as sequencing of anonymous bean clones from genomic libraries (Gaitán-Solís et al., 2002; Métais et al., 2002).

With an annual production of 499,298 tons, Turkey is an important green bean producer in the world (FAO, 2007). Green bean production in Turkey is mainly in the Black Sea region. Previous genetic studies partially characterized the green bean genotypes collected from the Black Sea region (Balkaya, 1999; Balkaya and Yanmaz, 2002). The Gevaş and Erciş districts of Van province from the eastern part of Turkey are also important green bean production areas in the country. However, the green bean genotypes from this region have not been previously characterized genetically. Climatically, eastern Turkey differs greatly from the Black Sea region. Therefore, it is expected that the green bean germplasm from this region would have economically important adaptive traits that can potentially be incorporated into bean breeding programs. In an attempt to evaluate the green bean genetic resources of eastern Turkey, several green bean genotypes have been collected from this region. The aim of the present study was to assess the level of genetic diversity present in the genotypes from this region. It is hoped that the information presented here will aid selection and more efficient utilization of this germplasm in bean breeding programs.

MATERIAL AND METHODS

Plant material

The green bean genotypes used in this study were collected from the Erciş and Gevaş districts of Van province, in the eastern part of Turkey. A total of 28 green bean genotypes to-

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gether with two reference cultivars, Gina (Asgrow Seed) and Sazova, 1949 (Eskişehir), were used for morphological and SSR analysis. The locations and some of the morphological traits such as growth habit, pod and terminal leaflet characteristics of these genotypes grown and scored according to the guidelines for conducting tests for distinctness, homogeneity and stability criteria for new varieties of plants provided by the International Union for the Protection of New Varieties of Plants (UPOV) are presented as an average of two years in Table 1.

Table 1. The locations and some of the morphological traits of the genotypes used in the study.

Accession number	Location	Growth habit	Pod				Terminal leaflet		
			Ground color	Density of flecks	Degree of curvature	Texture of surface	Stringiness	Size	Shape
2	Erciş	climbing	dark green	sparse	medium	smooth	absent	medium	triangular
16	Erciş	climbing	light green	sparse	medium	smooth	absent	medium	triangular to circular
17	Erciş	climbing	light green	sparse	weak	smooth	absent	medium	triangular to circular
23	Gevaş	climbing	dark green	sparse	medium	smooth	absent	medium	triangular to circular
24	Gevaş	climbing	dark green	sparse	weak	smooth	absent	medium	triangular to circular
25	Gevaş	climbing	light green	sparse	weak	smooth	absent	medium	triangular to circular
28	Gevaş	climbing	light green	sparse	weak	smooth	absent	medium	rhombic
32	Gevaş	climbing	green	sparse	weak	smooth	absent	medium	triangular to circular
36	Gevaş	climbing	light green	sparse	medium	smooth	present	large	triangular
37	Gevaş	climbing	light green	sparse	weak	smooth	present	medium	triangular to circular
38	Gevaş	climbing	light green	sparse	weak	smooth	absent	medium	triangular
40	Gevaş	climbing	light green	sparse	weak	smooth	absent	small	triangular
45	Gevaş	climbing	light green	sparse	weak	smooth	absent	medium	triangular
47	Gevaş	climbing	light green	sparse	medium	smooth	absent	medium	triangular
52	Gevaş	climbing	light green	sparse	medium	smooth	present	medium	triangular
53	Gevaş	climbing	light green	sparse	medium	smooth	present	medium	triangular
57	Gevaş	climbing	green	sparse	weak	smooth	absent	medium	triangular
64	Gevaş	dwarf	light green	sparse	weak	smooth	absent	medium	triangular
65	Gevaş	dwarf	light green	sparse	weak	smooth	present	medium	triangular
69	Gevaş	dwarf	dark green	medium	weak	smooth	present	medium	triangular
70	Gevaş	dwarf	dark green	sparse	medium	smooth	present	medium	triangular
73	Gevaş	dwarf	light green	sparse	weak	smooth	present	medium	triangular
74	Gevaş	dwarf	dark green	sparse	medium	smooth	absent	medium	triangular
77	Gevaş	dwarf	light green	sparse	medium	smooth	absent	medium	triangular
79	Gevaş	dwarf	light green	sparse	weak	smooth	absent	medium	triangular
80	Gevaş	dwarf	dark green	sparse	weak	smooth	present	medium	triangular
84	Gevaş	climbing	light green	sparse	weak	smooth	absent	medium	triangular
87	Gevaş	climbing	dark green	dense	medium	smooth	absent	large	triangular
S.1949	Eskişehir	climbing	green	sparse	medium	smooth	absent	medium	triangular
Gina	Asgrow	dwarf	green	sparse	weak	smooth	absent	medium	triangular to circular

S.1949: Sazova 1949.

DNA extraction

Genomic DNA was extracted from young leaf tissue using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) according to instructions provided by the manufacturer. Subsequently an RNAse treatment was performed on the eluted DNA samples. Purity and concentration of the DNA were both checked on 1% (w/v) agarose gels and NanoDrop® ND-1000 Spectrophotometer.

SSR analysis

Twelve SSR primer pairs were initially selected from a list of bean SSR primers given by Yu et al. (2000) based on their reliable amplification patterns and high polymorphic information contents. The sequence information of the SSR loci, PVU70530 and PVMEIG, was obtained from Lioi et al. (2005). PCR was conducted in a volume of 10 µL and contained 15 ng genomic DNA, 5 pmol of each primer, 0.5 mM dNTP, 0.5 unit GoTaq DNA polymerase (Promega), 1.5 mM $MgCl_2$ and 2 µL 5X buffer. The forward primers were labeled with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France). Reactions without DNA were included as negative controls. PCR amplification was performed using the Biometra® PCR System. The amplification conditions consisted of an initial denaturation step of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 52-56°C and 2 min at 72°C with a final extension at 72°C for 10 min. The PCR products were first separated on a 3% (w/v) agarose gel run at 80 V for 2 h. The gel was then stained with ethidium bromide at a concentration of 10 mg/mL. A DNA ladder (100 bp) (Promega) was used for the approximate quantification of the bands. The amplification products were visualized under UV light, and their sizes were estimated relative to the DNA ladder. For further determination of polymorphisms, the PCR products were run on CEQ™ 8800 XL capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA). The analyses were repeated at least twice to ensure reproducibility of the results. Allele sizes were determined for each SSR locus using the Beckman CEQ™ fragment analysis software. In each run, Gina and Sazova (1949) cultivars were included as reference cultivars.

Genetic analysis

The genetic analysis program "IDENTITY" 1.0 (Wagner and Sefc, 1999) was used according to Paetkau et al. (1995) for the calculation of number of alleles, allele frequency, expected and observed heterozygosity, estimated frequency of null alleles, and probability of identity per locus. Genetic dissimilarity was determined by the program "MICROSAT " (version 1.5) (Minch et al., 1995) using proportion of shared alleles, which was calculated by using "ps (option 1 - (ps))", as described by Bowcock et al. (1994). The results were then converted to a similarity matrix, and a dendrogram was constructed with the unweighted pair-group method with arithmetic mean (UPGMA) method (Sneath and Sokal, 1973) using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System,version 2.0) (Rohlf, 1988).

RESULTS

The level of genetic diversity among 28 green bean genotypes collected from the eastern part of Turkey (along with two reference cultivars) were assessed using 10 SSR markers. As a result, a total of 45 alleles were detected with an average allele number of 4.5 (Table 2). The PV-at006, PV-ctt001 and PV-ag003 loci generated the lowest number of alleles (2 alleles) whereas the PVGLND5 locus generated the highest number of alleles (10 alleles) (Table 3).

Table 2. Allele sizes (bp) of 28 green bean genotypes and reference cultivars (Gina and Sazova, 1949) at 10 simple sequence repeat loci.

Accession number	Loci																			
	PV-at002		PV-ag001		PV-ag004		PV-ggc001		PV-at006		PVMEIG		PV-ctt001		PVU70530		PVGLND5		PV-ag003	
2	249	249	155	155	203	239	230	230	128	128	192	192	161	161	243	243	108	202	163	163
16	247	247	157	157	203	239	230	230	128	128	198	198	161	161	243	243	108	162	163	163
17	249	249	157	157	203	239	230	230	128	128	198	198	161	161	243	243	150	150	163	163
23	245	245	151	151	203	239	230	230	128	128	204	220	161	161	243	243	108	172	163	163
24	245	245	151	151	203	239	230	230	128	128	216	216	161	161	243	243	108	172	163	163
25	245	245	155	155	203	239	230	230	128	128	220	220	161	161	243	243	108	186	163	163
28	245	245	151	151	203	239	230	230	128	128	224	224	161	161	243	243	108	174	163	163
32	245	245	153	153	203	239	230	230	128	128	192	192	155	155	243	243	174	174	163	163
36	245	245	157	157	203	239	230	230	128	128	224	224	161	161	243	243	108	180	163	163
37	245	245	151	151	203	239	230	230	128	128	220	220	161	161	243	243	108	108	163	163
38	245	245	157	157	203	239	230	230	128	128	224	224	161	161	245	245	108	186	161	161
40	245	245	155	155	203	239	230	230	128	128	204	224	161	161	243	243	108	180	163	163
45	245	245	157	157	203	239	230	230	128	128	220	220	161	161	243	243	108	182	163	163
47	245	245	157	157	203	239	230	230	128	128	222	222	161	161	243	243	108	180	163	163
52	245	245	157	157	203	239	230	230	128	128	216	216	161	161	243	243	108	180	163	163
53	245	245	143	143	203	239	230	230	128	128	224	224	161	161	243	243	108	182	163	163
57	245	245	157	157	203	239	230	230	128	128	224	224	161	161	243	243	150	150	163	163
64	247	247	147	147	203	239	230	230	128	128	210	210	161	161	243	243	150	164	163	163
65	245	245	157	157	203	239	230	230	128	128	220	220	161	161	243	243	108	180	163	163
69	245	245	155	155	203	239	230	230	128	128	224	224	161	161	243	243	108	164	163	163
70	245	245	155	155	203	239	230	230	128	128	204	204	161	161	243	243	150	150	163	163
73	245	245	157	157	201	239	230	230	128	128	224	224	161	161	243	243	108	182	163	163
74	245	245	157	157	203	239	230	230	128	128	224	224	161	161	243	243	108	182	163	163
77	245	245	157	157	203	239	230	230	128	128	220	220	161	161	243	243	108	182	163	163
79	245	245	157	157	203	239	230	230	128	128	224	192	161	161	243	243	108	182	163	163
80	245	245	157	157	201	239	230	241	128	128	224	224	161	161	243	243	108	182	163	163
84	245	245	155	155	203	239	230	230	128	128	216	216	161	161	243	243	150	150	163	163
87	245	245	151	151	203	239	230	230	128	128	192	192	161	161	243	243	164	164	163	163
S.1949	245	245	149	149	201	239	230	230	128	128	198	198	161	161	243	243	108	164	163	163
Gina	245	245	147	147	203	239	235	235	128	128	192	192	161	161	241	241	150	150	163	163

S.1949: Sazova, 1949.

Table 3. Number of alleles, allele range (bp), expected heterozygosity, observed heterozygosity, and probability of identity values of green bean genotypes calculated at 10 simple sequence repeat loci.

Loci analyzed	Allele range (bp)	Number of alleles	Expected heterozygosity	Observed heterozygosity	Estimated frequency of null alleles	Probability of identity
PV-ag001	143-157	7	0.695	0	0.410	0.201
PV-ggc001	230-241	3	0.150	0.032	0.102	0.744
PV-at002	245-249	3	0.233	0	0.189	0.626
PV-ag004	201-241	5	0.573	1	-0.271	0.455
PV-at006	128-170	2	0.062	0	0.058	0.884
PVMEIG	192-224	8	0.805	0.096	0.392	0.110
PVGLND5	108-202	10	0.787	0.709	0.043	0.116
PV-ctt001	155-161	2	0.120	0	0.107	0.795
PV-ag003	161-163	2	0.06	0	0.058	0.884
PVU70530	241-245	3	0.122	0	0.109	0.780

The UPGMA dendrogram obtained using the SSR data generated two major clusters. A single genotype (genotype 37) clustered separately from the remaining 27 genotypes and the two reference cultivars forming a separate branch (Figure 1). The two reference cultivars together with seven genotypes, three of which were from the Erciş district clustered together within the second cluster. The remaining genotypes, all from the Gevaş district, clustered as a single branch within the second cluster.

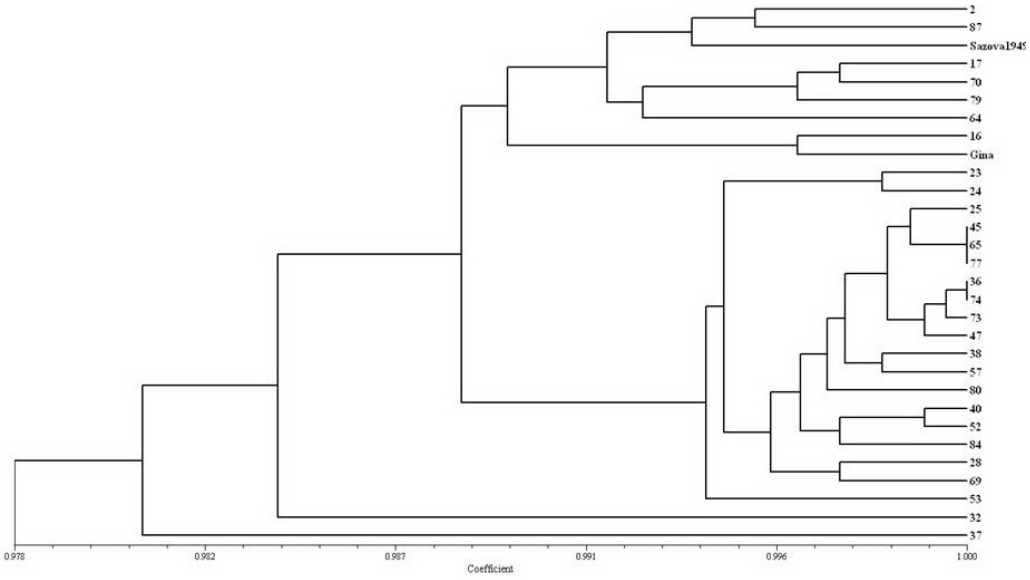


Figure 1. Dendrogram showing the relationships of 28 green bean genotypes together with two cultivars (Gina and Sazova, 1949) based on UPGMA cluster analysis of 10 simple sequence repeat marker data.

Among the green bean genotypes analyzed in the present study, two cases of identity were found (77, 45 and 65; 36 and 74). When the genetic similarity of the genotypes was analyzed, the similarity ratio of all genotypes analyzed was not below 98% (Figure 1, Table 2).

DISCUSSION

The present study, using molecular markers and morphological traits, investigated the genetic relationships of green bean genotypes from a bean growing region of Turkey. Although green bean lines originating from the Black Sea region were previously characterized (Balkaya, 1999; Balkaya and Yanmaz, 2002), the green bean genotypes from eastern Turkey had not been genetically characterized before. Therefore, these are important first steps towards better understanding and maintenance of green bean germplasm of the region.

SSR markers, widely spread throughout the nuclear genomes of eukaryotes, known to reveal high levels of polymorphisms, were used in the present study. Taken together with their

codominant nature and reproducibility, SSR markers are very useful for the analysis of genetic diversity, genomic mapping and marker-assisted selection in many plant species compared to many other marker systems. In the present study, ten of twelve SSR markers selected from the GenBank database produced successful amplification of expected sizes. For most samples tested, two of the markers, PVSBE2 and PV18791, did not produce any amplification products. Of these two primers, PVSBE2 was previously reported to reveal null alleles (Lioi et al., 2005). The SSR data generated in this study can be potentially incorporated into future studies that examine the diversity within a larger collection of bean genotypes from diverse regions.

The average number of alleles revealed in the present study is consistent with data reported in similar studies (Métais et al., 2002). Based on the number of alleles generated and probability of identity values, the most informative loci were PVGLND5, PVMEIG, PV-ag001, and PV-ag004 (Table 3). The remaining five loci, PV-at002, PV-ggc001, PV-at006, PV-ctt001, and PV-ag003, revealed a low number of alleles and high probability of identity values. Similar to our findings, PVGLND5 and PVMEIG were reported to reveal high levels of polymorphisms in some Italian common bean landraces (Métais et al., 2002; Lioi et al., 2005). Also, similar to the findings of Diaz and Blair (2006), the observed heterozygosity within each accession was low in this study for all genotypes examined, which is most likely due to the inbreeding nature of common bean. However, the observed heterozygosity was higher at the PV-ag004 locus probably due to the heterozygous state of the genotypes at this specific locus, revealed and confirmed by both gel image pictures and fragment analyzer, implying that the genotypes were not pure inbreds carrying two different alleles at this particular locus.

The close genetic distance even between the most distantly related genotypes (97.8%) indicates a very close genetic relationship among the genotypes. This low level of polymorphism observed among the genotypes could be due to the relatively close proximity of the collection sites and probably to the breeders' intense selection.

Our findings also indicated that genotypes 77, 45 and 65, and genotypes 36 and 74 were identical. However, these otherwise molecularly identical genotypes displayed some morphological differences.

Considering the environmental conditions of the region, it is expected that the green bean germplasm from the region would have economically important adaptive traits that can potentially be incorporated into bean breeding programs. Hence, it is expected that the results of this study will assist current bean breeding efforts in Turkey as well as maintain the genetic integrity of the genetic resources.

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