

## GENETIC DIVERSITY OF COMMON BEAN (*Phaseolus vulgaris* L.) LANDRACES GROWN IN NORTHEAST ANATOLIA OF TURKEY ASSESSED WITH SIMPLE SEQUENCE REPEAT MARKERS

Maya Izar KHAIDIZAR<sup>1</sup>, Kamil HALILOGLU<sup>2</sup>, Erdal ELKOCA<sup>2\*</sup>, Murat AYDIN<sup>2</sup>, Faik KANTAR<sup>3</sup>

<sup>1</sup>Strategic Resource Research Center Development Institute (MARDI), Kuala Lumpur, MALAYSIA

<sup>2</sup>Department of Agronomy, Faculty of Agriculture, Ataturk University, Erzurum, TURKEY

<sup>3</sup>Department of Agricultural Biotechnology, Faculty of Agriculture, Akdeniz University, Antalya, TURKEY

\*Corresponding author: eelkoca@atauni.edu.tr

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### ABSTRACT

Simple sequence repeat (SSR) marker system was used to analyze the genetic diversity of 38 common bean landraces grown in Northern Anatolia Region and their relationship to twelve nationally registered cultivars. A total of 72 DNA fragments across all materials were scored using 30 primers pairs. More than 97% of the amplification products showed polymorphism, indicating high variation at the DNA level among these accessions. Pair-wise genetic similarity ranged from 0.218 to 0.759, with a mean of 0.585. Based on the SSR markers, two major clusters were formed of 50 genotypes investigated. A majority of the commercial cultivars (66.6%) and landrace accessions (84.2%) were clustered into second group, whereas, small portion of commercial cultivars (33.4%) and landrace accessions (15.7%) were clustered in to first group. A Mantel's test showed significant correlation between SSR distance matrix and cophenetic matrices. Considerable variation existed within the genotypes collected from Northeast Anatolia Region and registered cultivars that can be exploited for breeding programs.

**Key words:** Genetic relationships, molecular markers, UPGMA

### INTRODUCTION

Crop plants rely on the broad genetic base of variation in order to adjust and adapt themselves for ever changing environments and pathogens. Characterization of germplasm using molecular marker techniques provides quantitative estimates of genetic diversity and the information which is essential for a rational utilization of germplasm in breeding programs. Common bean is a crucial food legume consumed worldwide. Genetic diversity in common bean have been studied using different molecular markers such as allozymes (Singh et al., 1991; Santalla et al., 2002), AFLP (Lioi et al., 2005; Svetleva et al., 2006), RAPD (Ocampo et al., 2005; Martins et al., 2006; Marotti et al., 2007), RFLP (Nodari et al., 1992), and ISSR (Svetleva et al., 2006; Marotti et al., 2007). Simple sequence repeats (SSRs), also termed microsatellites, are widely scattered at many different loci throughout the genome (Tautz and Renz, 1984; Kandemir et al., 2010). These small repetitive DNA sequences provide the basis for a PCR-based, multi-allelic, codominant genetic marker system (Saghai Maroof et al., 1994). Polymorphisms revealed by PCR amplification are due to the variation in the number of repeats in a defined region of the genome. The utility of SSR markers is due to their abundant distribution and high polymorphism in the

whole genome and power to distinguish between closely related genotypes. Simple sequence repeats (SSRs) have been used to construct a PCR-based genetic map (Blair et al., 2003), to identify genetic variability (Gomez et al., 2005; Blair et al., 2006; Buso et al., 2006; Sarikamis et al., 2009), and to develop multiplex SSR-PCR in common bean (Masi et al., 2003).

In Turkey, dry beans are an important staple food and Turkey is an important producer in the world with annual production of 212,758 tons (FAO, 2010). There are limited molecular characterization studies that partially screened the common bean genotypes collected from the Black Sea region (Balkaya, 1999; Balkaya and Yanmaz, 2002) and Ercis – Gevas district of Van province (Sarikamis et al., 2009). Northeast Anatolia of Turkey is one of main players of the common bean production in the country. However, genetic characterization of the common bean genotypes from this region has not been determined yet. Northeast Anatolia of Turkey differs significantly from the Black Sea region and Van province in terms of climate and elevation. Therefore, it is important to characterize the common bean germplasm from this region and determine the agronomically important genes that can potentially be integrated into future bean breeding programs.

The purpose of this study was to evaluate the level of genetic diversity present in the ecotypes from this region and compare them with twelve nationally registered cultivars. We believe that the information presented here will benefit in selection program and increase the efficiency of bean breeding programs.

## MATERIALS AND METHODS

### Plant material

The common bean genotypes used in this study were

collected as part of The Scientific and Technological Research Council of Turkey (TUBITAK) supported project (Collection and Evaluation of Dry Bean Gene Resources from Northeast Anatolia and Çoruh Valley TOVAG 107O400) from Northeast Anatolia of Turkey (Elkoca et al., 2010). A total of 38 common bean genotypes together with twelve nationally registered cultivars were used for SSR analysis (Table 1, 2 and Figure 1).

**Table 1.** List of common bean genotypes by collection and coordinates.

Accession No	Location	Latitude	Longitude	Altitude (m)
3	Kuruçalı Village Narman/Erzurum	40.393°	41.907°	1615
31	Yanıktaş Village Narman/Erzurum	40.274°	41.858°	1650
40	Kaleboğazi Village Oltu/Erzurum	40.523°	41.899°	1485
51	Bumaz Village Karaçoban/Erzurum	39.364°	41.994°	1580
58	Alacayar Village Narman/Erzurum	40.282°	41.895°	1690
63	Penek Village Şenkaya/Erzurum	40.662°	42.288°	1185
69	Gözalın Village Şenkaya/Erzurum	40.639°	42.262°	1489
77	Alabalık Village Narman/Erzurum	40.430°	41.936°	1560
91	Yeniköy Village Otlukbeli/Erzincan	39.999°	40.114°	1790
106	Şekerli Village Narman/Erzurum	40.313°	41.925°	1625
107	Çatalsöğüt Village Oltu/Erzurum	40.405°	42.005°	1595
115	Çatalsöğüt Village Oltu/Erzurum	40.405°	42.005°	1595
142	Kilimli Village Narman/Erzurum	40.327°	41.992°	1770
188	Petekli Village İspir/Erzurum	40.418°	40.914°	1480
240	Bumaz Village Karaçoban/Erzurum	39.364°	41.994°	1580
242	Hıms/Erzurum	39.360°	41.700°	1720
244	Ortaköy Village Hıms/Erzurum	39.403°	41.642°	1905
252	Konursu Village /Bayburt	40.403°	40.267°	1620
254	Konursu Village /Bayburt	40.403°	40.267°	1620
257	Konursu Village /Bayburt	40.403°	40.267°	1620
264	Ballıkaya Village /Bayburt	40.371°	40.389°	1480
268	Nişantaşı Village /Bayburt	40.356°	40.048°	1605
275	Cevizli Village Uzundere/Erzurum	40.627°	41.718°	1205
278	Çağlayan Village Uzundere/Erzurum	40.667°	41.689°	970
296	Anlı Village Tortum/Erzurum	40.369°	41.480°	1450
302	Doruklu Village Tortum/Erzurum	40.381°	41.443°	1749
303	Esendurak Village Tortum/Erzurum	40.326°	41.448°	1525
308	Kemerkeya Village Tortum/Erzurum	40.319°	41.442°	1715
318	Olur/Erzurum	40.820°	42.130°	1330
320	Olur/Erzurum	40.820°	42.130°	1330
328	Torul/Gümüşhane	40.550°	39.290°	933
332	Torul/Gümüşhane	40.550°	39.290°	933
335	Aşağaçayrılı Village Olur/Erzurum	40.876°	42.279°	1755
338	Aşağaçayrılı Village Olur/Erzurum	40.876°	42.279°	1755
351	Altunkaya Village Olur/Erzurum	40.877°	42.248°	1520
358	Altunkaya Village Olur/Erzurum	40.877°	42.248°	1520
361	Altunkaya Village Olur/Erzurum	40.877°	42.248°	1520
368	Çayırçukur Village Kürtün/Gümüşhane	40.665°	39.141°	1232

**Table 2.** List of nationally registered common bean cultivars used in this study.

Cultivars					
Sahin 90	Gungor	Sehirali 90	Onceler 98	Goynuk 98	Aras 98
Yakutiye 98	Tezibaba	Kantar 05	Akman 98	Elkoca 05	Eskisehir 855

### DNA Extraction

Bulk immature unifoliate leaves from each genotype were used for extracting DNA as described by Dellaporta et al. (1983).

### SSR analysis

Thirty SSR primer pairs were selected from previous studies based on their reliable amplification patterns and high polymorphic information contents (Table 3). The reactions were performed in 0.2 µl tubes in Mastercycler personal apparatus (Eppendorf, Germany) programmed to cycle 45 times under the following conditions: for the first two cycles, denaturation for 30 s at 94°C, annealing for 60 s at 37°C, and elongation for 2 min at 72°C; second two



**Figure 1.** Locations of 38 common bean genotypes collected from Northeast Anatolia.

cycles, denaturation for 30 s at 94°C, annealing for 60 s at 50°C, and elongation for 2 min at 72°C; the subsequent 41 cycles were run with the denaturation temperature reduced to 93°C, followed by a 4-min hold at 72°C. After amplification, the reaction products were separated by

electrophoresis in 3% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light with Nikon Coolpix5000. A total of 30 SSR primer pairs were used based on the band resolution and polymorphism they provided.

**Table 3.** Thirty SSR primers were used for diversity screening. The list of primer is as follows:

Marker Name	Motifs	Forward (5' – 3')	Backward (5' – 3')	Source; Year
Bmd-1	(AT) <sup>9</sup>	CAAATCGCAACACCTCACAA	GTCGGAGCCATCATCTGTGTT	Yu et al. 2000
Bmd-15		TTGCCATCGTTGCTTAATTG	TTGGAGGAAGCCATGTAATGC	Blair et al. 2003 & 2006
Bmd18		AAAGTTGGACGCACCTGTGATT	TCGTGAGGTAGGAGTTTGGTG	Blair et al. 2003 & 2006
BM053		TGCTGACCAAGGAAATTCAG	GGAGGAGGCTTAAGCACAAA	Blair et al. 2003 & 2006
BM114	(TA) <sup>8</sup> (GT) <sup>10</sup>	AGCCTGGTGAATGCTCATAG	CATGCTGTGTGCTTAACCTCT	Blair et al. 2003 & 2006
BM137	(CT) <sup>33</sup>	CGCTTACTCACTGTACGCACG	CCGTATCCGAGCACCCTAAC	Blair et al. 2003 & 2006
BM141	(GA) <sup>29</sup>	TGAGGAGGAACAAATGGTGCC	CTCACAAACCACAAACGCCACC	Blair et al. 2003 & 2006
BM143	(GA) <sup>35</sup>	GGGAAATGAACAGAGGAAA	ATGTTGGAACTTTTAGTGTG	Blair et al. 2003 & 2006
BM152	(GA) <sup>31</sup>	AAGAGGAGGTCGAAACCTTAAATCG	CCGGGACTTGCCAGAAGAAC	Blair et al. 2003 & 2006
BM153	(CA) <sup>5</sup> (TG)(CA) <sup>3</sup> CG(CA) <sup>10</sup> (TA) <sup>4</sup>	CCGTTAGGGAGTTGTGAGG	TGACAAACCATGAATATGC	Blair et al. 2003 & 2006
BM154	(CT) <sup>17</sup>	TCTTGGCAGCCAGCTTCTCC	CTGAATCTGAGGAACGATGACCAG	Blair et al. 2003 & 2006
BM156	(CT) <sup>32</sup>	CTTGTCCACCTCCCATCATAGC	TGCTTGCACTCAGCCAGAATC	Blair et al. 2003 & 2006
BM160	(GA) <sup>15</sup> (GAA) <sup>5</sup>	CGTGCTGGCGAATAGCTTTG	CGCGGTTCTGATCGTGACTTC	Blair et al. 2003 & 2006
BM161	(GA) <sup>7</sup> (GA) <sup>8</sup>	TGCAAAGGGTTGAAAGTTGAGAG	TTCCAATGCACCAGACATTCC	Blair et al. 2003 & 2006
BM167	(GA) <sup>19</sup>	TCCTCAATACCTACATCGTGTGACC	CCTGGTGTAAACCCTCGTAACAG	Blair et al. 2003 & 2006
BM175	(AT) <sup>5</sup> (GA) <sup>19</sup>	CAACAGTTAAAGGTCGCAAAATT	CCACTCTTAGCACTCACTGGA	Blair et al. 2003 & 2006
PV-CTT001	(CTT) <sup>3</sup> (T) <sup>3</sup> (CTT) <sup>6</sup>	GAGGGTGTTCACATTTGTCACTGC	TTCAATGGATGGTGGAGGAACAG	Yu et al. 1999
PV-A001	(AT) <sup>22</sup>	GGGAGGGTATGGGAAGCAGTG	GCGAACACGTTATGAATGA	Yu et al. 1999
PV-AG004	(AG) <sup>8</sup>	TTGATGACGTGGATGCAATGG	AAAGGGCTAGGGAGAGTAAGTTGG	Blair et al. 2003 & 2006
PVBR14	(AG) <sup>23</sup>	ACGCCATCCACCATCCTT	TGAGAAAGTTGATGGGATTG	Buso et al.2006
BM183	(TC) <sup>14</sup>	CTCAAATCTATTCACCTGGTCAGC	TCTTACAGCCTTGCAGACATC	Gaitan-Solis et al. 2002
BM187	(CT) <sup>10</sup> (CT) <sup>14</sup>	TTTCTCCAACCTCACTCCTTCC	TGTGTTTGTGTTCCGAATTATGA	Gaitan-Solis et al. 2002
BM188	(CA) <sup>18</sup> (TA) <sup>7</sup>	TCCGCTTGAAACTTCTTGTATC	CCCTCCAGTTAAATCAGTCG	Gaitan-Solis et al. 2002
BM199	(GA) <sup>15</sup>	AAGGAGAATCAGAGAAGCCAAAAG	TGAGGAATGGAATGTAGCTCAGG	Gaitan-Solis et al. 2002
BM200	(AG) <sup>10</sup>	TGGTGGTTGTTATGGGAGAAG	ATTGTCTCTGTTATTCCTTCCAC	Gaitan-Solis et al. 2002
BM209	(TA) <sup>4</sup> (TG) <sup>16</sup>	CAACCAATGAATGCTGACAAATG	CAATTCTTGTGATTGAAAGGCAAT	Gaitan-Solis et al. 2002
BM210	(CT) <sup>15</sup>	ACCACTGCAATCCCTCATCTTTG	CCCTCATCCTCCATTCTTATCG	Gaitan-Solis et al. 2002
BM211	(CT) <sup>16</sup>	ATACCCACATGCACAAGTTTGG	CCACCATGTGCTCATGAAGAT	Gaitan-Solis et al. 2002
Bmd-42	(AT) <sup>5</sup>	TCATAGAAAGATTTGTGGAAGCA	TGAGACACGTACGAGGCTGTAT	Blair et al. 2003 & 2006
GATS91	(GA) <sup>17</sup>	GAGTGGCGAAGCGAGTAGAG	TGTACACTCTCTCCCAAT	Blair et al. 2003 & 2006

### Data analysis

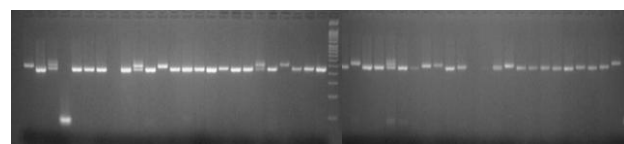
The DNA bands were scored as 0 (absence) or 1 (presence). Genetic similarity between two cultivars *i* and *j* was estimated following the formula of Nei and Li (1979). Based on the genetic similarity matrix (denoted GS), UPGMA cluster analysis were used to assess pattern of diversity among the bean entries. Dendrograms were created with the TREE program of NTSYS. All calculations were performed using the NTSYS-pc version 2.1 software (Rohlf, 2000).

### RESULTS

Simple sequence repeat (SSR) marker system was used to analyze the genetic diversity of 38 common bean landraces grown in Northern Anatolia Region and their relationship to twelve nationally registered cultivars. A total of 72 alleles were detected. The number of alleles per locus ranged from one for Bmd-15 to 6 for BM-160 with an average number of 2.4 alleles per locus. A range of alleles of expected fragment sizes were obtained by different primer pairs with strong amplifications. Figure 2 shows SSR markers detecting different alleles. More than 97% of the amplification products showed polymorphism, indicating high variation at the DNA level among these accessions.

Pairwise genetic dissimilarity estimated among 50 genotypes was presented in Table 4. Pairwise genetic similarity ranged from 0.211 to 0.796. Based on dissimilarity index, A-320 and A-268 were the closest ones showing 79.6% similarity. On the other hand, genotype pairs A-351 and A-252 were the most dissimilar ones showing only 21.1% similarity.

Based on the SSR markers, two major clusters were formed of 50 genotypes investigated (Figure 3). A majority of the commercial cultivars (66.6%) and landrace accessions (84.2%) were clustered into second group, whereas, small portion of commercial cultivars (33.4%) and landrace accessions (15.7%) were clustered in to first group.



**Figure 2.** Example of amplification products of primer BM200 detecting different alleles in 50 bean genotypes



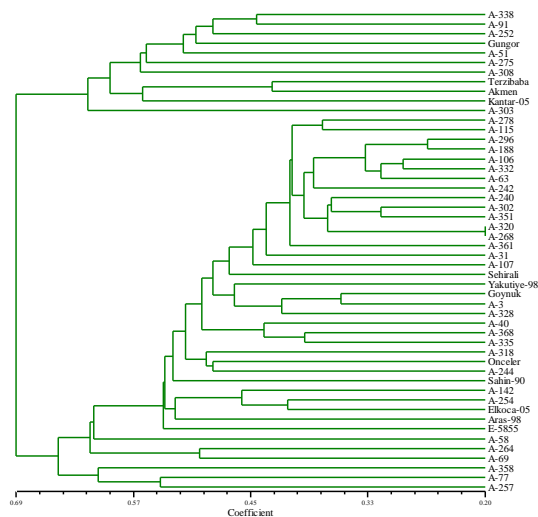


Figure 3. Phenogram constructed using the UPGMA method.

## DISCUSSION

Information about the genetic variability of genotypes helps decision making for conservation activities, which range from collecting and managing through identifying genes to adding value to genetic resources and for breeding purposes, to predict the ability to combine or to rapidly verify the breeding material (Vicente et al., 2005). Therefore, assessment of genetic variability of germplasm is a first step, named as pre-breeding, for improvement and development of superior cultivars.

A range of molecular techniques can assess crop genetic diversity, however among the most ideal for distinguishing closely related germplasm are microsatellites, which are highly informative markers that detect length polymorphisms at loci with simple sequence repeats (Powell et al., 1996). Their advantages for diversity studies include uniform genome coverage, high levels of polymorphism, codominance, and an easy-to-implement, specific PCR-based assay (Pejic et al., 1998).

In this study, genotypes collected from Northeast Anatolia of Turkey were evaluated at molecular level via SSR marker system. Though common bean genotypes collected from the Black Sea region (Balkaya, 1999; Balkaya and Yanmaz, 2002) and Van province region (Sarikamis et al., 2009) were previously characterized, the common bean ecotypes from Northeast Anatolia of Turkey had not been genetically characterized before. Therefore, these are important first steps regarding better understanding and maintenance of common bean germplasm of the region.

Genomic SSRs selected on the basis of high polymorphism information content (Blair et al. 2003, 2006; Yu et al., 1999, 2000; Gaitan-Solis et al. 2002) successfully helped discriminate genotypes in the present study. Successful amplifications of expected sizes were obtained. The results of this study indicate that SSR analysis could be successfully used for the estimation of genetic diversity among common bean genotypes and can

be potentially incorporated into future studies that examine the diversity within a larger collection of bean genotypes from diverse regions. We also hope that the results of this study will benefit current bean breeding efforts in Turkey as well as maintain the genetic integrity of the genetic resources.

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