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Genetic Diversity of Common Bean (*Phaseolus vulgaris*) Cultivars from Different Origins Revealed by Microsatellite Markers

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Genetic diversity in 20 common bean genotypes belonged to different sources were studied using with 9 microsatellite (SSR) markers. The 9 SSR loci analyzed produced 32 alleles with an average 3.5 alleles per marker. The number of alleles ranged from 2 to 5, whereas the minimum and maximum alleles were observed in BM156 and BM199, respectively. The effective allelic number (ne) ranged from 1.11 (BM156) to 3.89 (BM199) with an average of 2.3. The *He* values ranged from 0.29 to 0.61 with an average of 0.39. The PIC values of SSR primer pairs ranged from 0.37 (BM154) to 0.68 (BM199) with an average of 0.47. Cluster analysis clearly delineated the genotypes in four major clusters. Iranian cultivars showed very distinct pattern from other genotypes, while genotypes from Turkey and Iraq grouped very close to each other. Results of principle co-ordinate analysis (PCoA) analysis showed that most of the cultivars are separated by the first or second PCoA, which demonstrated distinct groups of cultivars corresponding to cluster analysis. The high diversity among common bean genotypes from diverse gene pools suggests that cross breeding among these different regions will accelerate the process of diverse germplasm creation and broaden germplasm resources in common bean.

Keywords: Genetic diversity; common bean; SSR markers.

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1. INTRODUCTION

Common bean (*Phaseolus vulgaris*) is one of the most important grain legumes crop that grown and consumed principally in America, Africa and Asia [1]. As a low cost protein source, it has been considered as major source of dietary protein, which complements carbohydrate-rich sources such as rice, maize, and cassava and play important roles in food security and wealth creation [2]. This plant is a true diploid with 11 chromosomes and a haploid complement of 588 [3] to 637 Mbp [4]. An overview of four years' data from 2011 to 2014 indicates that more than 337,000 ha were dedicated to production of 455,000 tons of common beans annually [5]. Assessment of crop gene pool genetic diversity is fundamental for germplasm conservation and crop breeding strategies in selecting the best parents in breeding hybrids [6]. Genetic diversity assessment in crops can be done by different methods such as conventional methods like as morphological traits, biochemical and DNA-based molecular markers [7,8]. Morphological traits are used to evaluate the genetic diversity in many crops, but diversity pattern obtained by morphological or phonological traits is not more reliable for discriminating of a plant variety, due to ambiguous differences and modifications of measured traits caused by environmental factors [9,10]. Genetic diversity using DNA-based molecular markers provide very powerful, robustness and reliable tools for genetic diversity analysis in crop germplasm [6]. Genetic diversity in common bean have been studied using different molecular markers such as allozymes [11], AFLP [12,13], RAPD [14,15], RFLP [16], ISSR [17,18] and SSRs [19,20,21]. 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. SSRs are co-dominant, locus specific, multi-allelic, highly reproducible and polymorphic markers [22]. In common bean, around 400 microsatellite markers have been developed and mapped [23,24,25,26,27,28,29]. In this study, we aimed to characterize genetic diversity 20 common bean genotypes from different origins using SSR molecular markers.

2. MATERIALS AND METHODS

2.1 Plant materials and DNA Extraction

A total of 20 common bean genotypes (Table 1), represented 6 countries namely Iran (6), Turkey (5), India (4), Iraq (3), Germany (1) and Netherlands (1), were collected. Pooled leaf samples (ten plants per genotypes) were grown in the greenhouse and total genomic DNA was extracted from each genotypes following a CTAB extraction protocol [30]. DNA concentrations were estimated by gel electrophoresis (0.8% agarose gel) and used for PCR analysis in final concentration of 20 ng/µl.

Table 1. List of common bean genotypes with their origines for genetic diversity analysis using SSR markers

Number	Genotype	Origin
1	Talash	Iran
2	Goli	Iran
3	Akhtar	Iran
4	Khomein	Iran
5	Polista	Netherlands
6	Milds Maxi	Germany
7	IC296363	India
8	VARUN	India
9	CONTANDER	India
10	IC121427	India
11	Akman-98	Turkey
12	Zulbiye	Turkey
13	Yunus-90	Turkey
14	Aras-98	Turkey
15	Yakutiye-98	Turkey
16	Swri	Iraq
17	Cirilla	Irag
18	Zaina	Irag
19	Landrace-Lorestan1	Iran
20	Landrace-Lorestan2	Iran

2.2 SSR Analysis

Fifteen previously published SSR primer pairs [19,24] were selected based on amplification of expected size fragments and allelic diversity reported by previous studies. These SSRs were tested for amplification in 6 genotypes (one genotype from each country) and identified 9 well-amplified SSR primer pairs (Table 2). PCRs (Termal-cycler v12.2) were performed in 20 µl total reaction volumes containing: 1× PCR buffer, 2 mM MgCl2, 0.4 mM each dNTP, 0.5 µM of each primer, 1.2 U of Taq polymerase enzyme (Cinagen, Iran) and 20 ng of DNA template. The Amplification profile followed was 94°C for 5 min (initial denaturation), followed by 32 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min, and a final extension of 10 min at 72°C. All PCR products were separated on 3% metaphor agarose gels, stained with ethidium bromide and visualized under UV.

Table 2. SSRs primer sequences and details for genetic diversity analysis in common bean

Maker	Motif	Forward (5/ to 3/)	Reverse (5/ to 3/)	Reference
BM137	(CT)33	CGCTTACTCACTGTACGCACG	CCGTATCCGAGCACCGTAAC	Blair et al. 2006
BM141	(GA)29	TGAGGAGGAACAATGGTGGC	CTCACAAACCACAACGCACC	Blair et al. 2006
BM154	(CT)17	TCTTGCGACCGAGCTTCTCC	CTGAATCTGAGGAACGATGACCAG	Blair et al. 22006
BM156	(CT)32	CTTGTTCCACCTCCCATCATAGC	TGCTTGCATCTCAGCCAGAATC	Blair et al. 2006
BM167	(GA)19	TCCTCAATACTACATCGTGTGACC	CCTGGTGTAACCCTCGTAACAG	Blair et al. 2006
BM199	(GA)15	AAGGAGAATCAGAGAAGCCAAAAG	TGAGGAATGGATGTAGCTCAGG	Gaitan-Solis et al. 2002
BM200	(AG)10	TGGTGGTTGTTATGGGAGAAG	ATTTGTCTCTGTCTATTCCTTCCAC	Gaitan-Solis et al. 2002
BM210	(CT)15	ACCACTGCAATCCTCATCTTTG	CCCTCATCCTCCATTCTTATCG	Gaitan-Solis et al. 2002
BM211	(CT)16	ATACCCACATGCACAAGTTTGG	CCACCATGTGCTCATGAAGAT	Gaitan-Solis et al. 2002

2.3 Data Analysis

Frequencies of incidence of all polymorphic alleles for each SSR markers were calculated and used for determining statistical parameters. Each bands identified as an allele and scored as 'a1', 'a2' etc., from largest to smallest sized band. Cluster analysis was conducted on the basis of unrooted NJ tree using similarity matrix was carried through DARwin 5.0.128 [31]. Node construction according to bootstrap analysis using 1000 bootstrap values was performed. Number of allele (Na), effective number of alleles (Ne), gene diversity (He), Shanon Index (I) and polymorphism information content (PIC) were calculated by GENALEX 6.1 software [32].

3. RESULTS

3.1 Allelic Variation of SSRs

DNA fingerprinting database was produced using SSR markers for genetic diversity analysis in 20 common bean genotypes. Our results showed all selected SSRs successfully amplified genotype DNAs (Fig. 1). All of the primers were able to distinguish and identify each of 20 common bean genotypes. In the present study, a total of 9 loci were analyzed. Nine SSRs in the genetic material under study were found to be highly polymorphic and produced clear polymorphic fragments among common bean genotypes. The 9 SSR loci analyzed produced 32 alleles with an average 3.5 alleles per marker. The number of alleles ranged from 2 to 5, whereas the minimum and maximum alleles were observed in BM156 and BM199, respectively (Table 3). The effective allelic number (ne) ranged from 1.11 (BM156) to 3.89 (BM199) with an average of 2.3 (Table 3). The *He* values ranged from 0.29 to 0.61 with an average of 0.39. The PIC values of SSR primer pairs ranged from 0.37 (BM154) to 0.68 (BM199) with an average of 0.47 (Table 3), indicating a high level of genetic diversity in studied common bean genotypes. Shanon diversity index (I) values ranged from 0.29 (BM156) to 1.27 (BM199) with an average of 0.77. A positive linear relationship ($p < 0.01$) was observed between Na and Ne ($r = 0.96$), between Na and Shanon Index ($r = 0.87$), between Na and He ($r = 0.62$) and between Na and PIC ($r = 0.97$). Nevertheless, a positive linear relationship ($p < 0.05$) linear relationship was observed between PIC and He ($r = 0.62$).

3.2 Genetic Similarity and Cluster Analysis

Nei similarity coefficients (GS) ranged from 0.33 (Yakutiye-98 and Cirilla vs. Polista) to 1 (Akhtar vs. Landrace-Lorestan1) (Table 4). These results indicate relatively high genetic variability among the examined common bean genotypes. The genetic similarity matrix was analyzed using un-weighted neighbor joining (UNJ) clustering algorithm by software programme DARwin 5.0 (Fig. 2). The radial branching clearly delineated the genotypes in four major clusters (Fig. 2). Group 1 contained six genotypes were all cultivars originated from Iran. In group 2, two sub-clusters contained 11 genotypes. Sub-group 2a contained 4 cultivars from India and 4 cultivars from Turkey. Cluster 2b contained two genotypes from Iraq and one cultivar from Turkey. Cultivar 'Zaina' from Iraq grouped in separately from other genotypes, while two remained cultivars from Germany and Netherlands grouped in fourth cluster (Fig 2). Iranian cultivars showed very distinct pattern from other genotypes, while genotypes from Turkey and Iraq grouped very close to each other. In order to determine the ability of SSR markers to display genetic relationships among common bean cultivars, principle co-ordinate analysis (PCoA) was carried out and cultivars were plotted in the coordinate system for the first two coordinates which accounted for 32.5% and 26.4% of the variation, respectively (Fig. 3). Most of the cultivars are separated by the first or second PCoA (Fig. 3) which demonstrated distinct groups of cultivars corresponding to cluster analysis (Fig. 2).

Table 3. Number of alleles (Na), number of effective alleles (Ne), Shanon diversity index (I), Gene diversity (He) and PIC values in common bean genotypes as revealed by SSR markers

Maker	Na	Ne	Shanon index (I)	He	PIC
BM137	4	2.68	1.15	0.41	0.57
BM141	3	1.48	0.48	0.29	0.39
BM154	3	1.39	0.44	0.61	0.37
BM156	2	1.11	0.29	0.15	0.21
BM167	4	3.10	0.77	0.49	0.59
BM199	5	3.89	1.27	0.52	0.68
BM200	4	2.98	1.21	0.46	0.58
BM210	3	1.78	0.65	0.22	0.39
BM211	4	2.69	0.72	0.35	0.50
Mean	3.5	2.3	0.77	0.39	0.47

Table 4. Similarity matrix based on jaccard coefficient showing the relationship among various common bean genotypes based on ssrs data

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.75	1.00																		
3	0.68	0.72	1.00																	
4	0.74	0.69	0.65	1.00																
5	0.36	0.38	0.43	0.16	1.00															
6	0.51	0.46	0.47	0.45	0.48	1.00														
7	0.63	0.68	0.60	0.61	0.41	0.43	1.00													
8	0.59	0.60	0.55	0.57	0.45	0.37	0.77	1.00												
9	0.53	0.61	0.57	0.65	0.45	0.37	0.72	0.77	1.00											
10	0.61	0.66	0.56	0.64	0.41	0.51	0.77	0.78	0.79	1.00										
11	0.63	0.68	0.60	0.61	0.41	0.43	0.98	0.77	0.72	0.77	1.00									
12	0.59	0.60	0.55	0.57	0.45	0.37	0.77	0.89	0.77	0.78	0.77	1.00								
13	0.53	0.61	0.57	0.65	0.45	0.37	0.72	0.77	0.99	0.79	0.72	0.77	1.00							
14	0.61	0.66	0.56	0.64	0.41	0.51	0.77	0.78	0.79	0.96	0.77	0.78	0.79	1.00						
15	0.54	0.59	0.61	0.65	0.33	0.45	0.67	0.67	0.67	0.64	0.67	0.67	0.67	0.64	1.00					
16	0.60	0.54	0.54	0.62	0.36	0.38	0.63	0.76	0.70	0.68	0.63	0.76	0.70	0.68	0.66	1.00				
17	0.62	0.65	0.59	0.63	0.33	0.49	0.63	0.67	0.63	0.71	0.63	0.67	0.63	0.71	0.75	0.74	1.00			
18	0.57	0.57	0.53	0.55	0.37	0.45	0.57	0.59	0.65	0.56	0.57	0.59	0.65	0.56	0.63	0.66	0.67	1.00		
19	0.68	0.72	1.00	0.65	0.43	0.47	0.60	0.55	0.57	0.56	0.60	0.55	0.57	0.56	0.61	0.54	0.59	0.53	1.00	
20	0.66	0.65	0.60	0.59	0.38	0.46	0.53	0.52	0.59	0.52	0.53	0.52	0.59	0.52	0.57	0.62	0.58	0.58	0.60	1.00

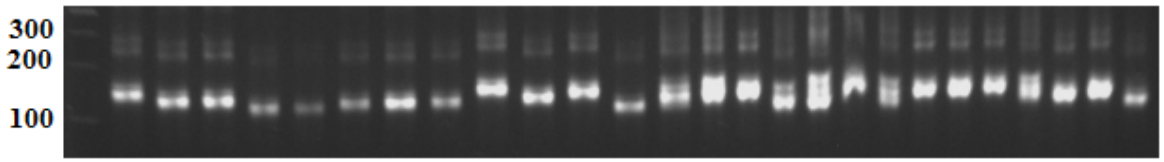

Fig. 1. SSR amplification profile for primer BM199 on common bean genotypes

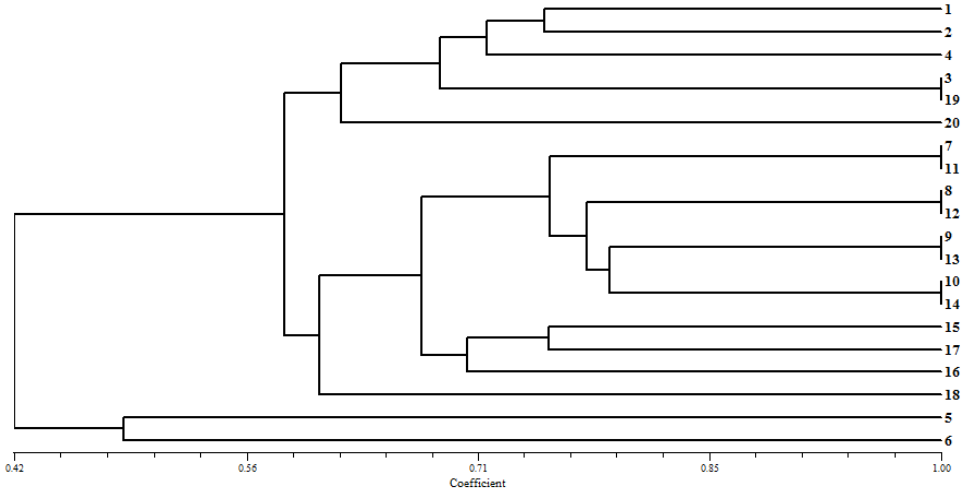

Fig. 2. Dendrogram of the 20 common bean genotypes based on the dissimilarity matrix developed using SSR markers.

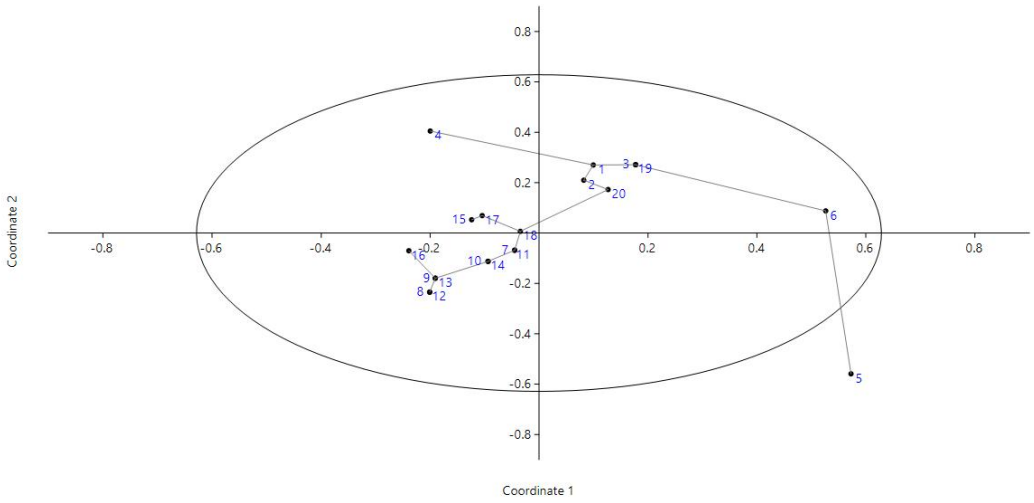

Fig. 3. Two-dimensional representation of genetic relations among 20 common bean genotypes using SSR data

4. DISCUSSION

Characterization of genetic diversity between genotypes is fundamental for applied breeding programs such as conservation of genetic resources and select appropriate parents for crossing in cultivar development [33, 34]. Different types of molecular techniques has been used for assessing the genetic diversity level in crops, 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 [35]. Microsatellite markers (SSRs) have been used successfully for the DNA fingerprinting, characterization of genetic variation and phylogenetic studies in different plant species as well as in common bean [19, 20, 21]. In this study, 18 common bean genotypes collected from Iran, Turkey, India, Iraq and two cultivars from Germany and Netherlands were evaluated at molecular level using SSR molecular markers. Nine primer pairs produced 32 polymorphic alleles among the 20 common bean genotypes, which indicate a relatively high genetic diversity in studied genotypes, even with

low number of genotypes and restricted geographical sources. Overall, 32 alleles by 9 primers were generated. Number of alleles ranged from 2 to 5 and PIC value ranged from 0.21 to 0.68 with average value of 0.47. Compared to the previous studies on genetic diversity in common bean using SSRs [21,36] our results indicated a slightly higher number of polymorphic bands and PIC values. The results of UNJ-clustering and PCoA analysis showed that the genotypes from Turkey and Iraq have close relationships, while Iranian genotypes are distinct from others. Molecular analysis of common bean genotypes from same origins have shown a similar genetic base, and also observed by other researchers [37, 38.]. The high diversity among common bean genotypes from diverse gene pools suggests that cross breeding among these different regions will accelerate the process of diverse germplasm creation and broaden germplasm resources in common bean. Accordingly, efforts are being made to collect samples from more different origins and more effective markers will be developed in order to elucidate thoroughly the genetic diversity, population structure and other details of population variability in common bean.

5. CONCLUSIONS

The current study, genetic diversity in 20 common bean genotypes belonged to different sources were studied using 9 microsatellite (SSR) markers. The results showed that the high diversity among common bean genotypes from diverse gene pools suggests that cross breeding among these different regions will accelerate the process of diverse germplasm creation and broaden germplasm resources in common bean.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Arumuganathan K, Earle ED. Nuclear DNA content of some important plant species. Plant Mol Biol Repor. 1991;3:208-218.
2. Bagheri M, Kahrizi D, Zebarjadi A. Study on genetic variation and morpho-phenologic traits in Common bean (*Phaseolus vulgaris* L.). Biharean Biol. 2017;11(1):43-47.
3. Bennett M, Leitch I. Plant DNA C-Values database. Release 4.0; 2005. Available:http://www.rbgkew.org.uk/cval/database1.html
4. Broughton WJ, Hernandez G, Blair MW, Beebe SE, Gepts P, Vanderleyden J. Beans (*Phaseolus spp*.) model food legumes. Plant Soil. 2003;252:55-128.
5. FAOSTAT. Agricultural structure (production, price, value). 2016; Available:http://apps.fao.org/faostat
6. Ahmad KMS, Talebi R. Genetic diversity assessment in chickpea using morphological characters and start codon targeted (SCoT) molecular markers. Annual Res Rev Biol. 2017;15(1):1-8.
7. Talebi R, Naji AM, Fayyaz F. Geographical patterns of genetic diversity in cultivated chickpea (*Cicer arietinum* L.) characterized by amplified fragment length polymorphism. Plant Soil Environ. 2008; 54(10):447-452.
8. Ghaffari P, Talebi R, Keshavarz F. Genetic diversity and geographical differentiation of Iranian landrace, cultivars and exotic chickpea lines as revealed by morphological and microsatellite markers. Physiol Mol Biol Plant. 2014;20(2):225-233.
9. Hosseini SA, Karami E, Talebi R. Molecular variation of Iranian local and exotic strawberry (Fragaria × ananassa Duch.) varieties using SSR markers. Biodiversity. 2013;4(1):243-252.
10. Kadir K, Talebi R, Hamidi H. Multivariate analysis and drought stress tolerance indices in chickpea (*Cicer arietinum* L.) under different irrigation regimes. J Exp Biol Agric Sci. 2017;5(1):54-60.
11. Santalla M, Rodino P, De Ron M. Allozyme evidence supporting southwestern Europe as a secondary centre of genetic diversity for the common bean. Theor Appl Genet. 2002;104:934-944.
12. Fabio LM, Sergio E, Lee TSG, Flipe GG. Genetic relationships and diversity among Brazilian cultivars and land races of common beans (*Phaseolus vulgaris* L.) revealed by AFLP markers. Genet Resour Crop Evol. 2003;50:887-893.
13. Gill-Langarica HR, Muruaga-Martínez JS, Patricia Vargas-Vázquez ML, Rosales-Serna R, Mayek-Pérez N. Genetic diversity analysis of common beans based on molecular markers. Genetic Mol Biol. 2011; 34(4):595-605.

14. Marotti I, Bonetti A, Minelli M, Catizone P, Dinelli G. Characterization of some Italian common bean (*Phaseolus vulgaris* L.) landraces by RAPD, semirandom and ISSR molecular markers. Genet Resour Crop Evol.2007;54:175-188.
15. Tahir NAR. Identification of genetic variation in some faba bean (*Vicia faba* L.) genotypes grown in Iraq estimated with RAPD and SDS-PAGE of seed proteins. Indian J Biotech. 2015;14:351-356.
16. Nodari RO, Koinange EMK, Kelly JD, Gepts P. Towards an integrated linkage map of common bean: 1. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theor Appl Genet. 1992;84:186-192.
17. Svetleva D, Pereira G, Carlier J, Cabrita L, Leitao J, Genchev D. Molecular characterization of *Phaseolus vulgaris* L genotypes included in Bulgarian collection by ISSR and AFLP analyses. Sci Hort. 2006;109:198-206.
18. Terzopoulos PJ, Bebeli PJ. Genetic diversity analysis of Mediterranean faba bean (*Vicia faba* L.) with ISSR markers. Field Crop Res. 2008;108:39-44.
19. Blair MW, Giraldo MC, Buendia HF, Tovar E, Duque MC, Beebe SE. Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet. 2006;113:100e109.
20. Sarikamis G, Yasar F, Bakir M, Kazan K, Ergul A. Genetic characterization of green bean (Phaseolus vulgaris) genotypes from eastern Turkey. Genet Mol Res. 2009; 8(3):880-887.
21. Khaidizar MI, Haliloglu K, Elkoca E, Aydin M, Kantar F. Genetic diversity of common bean (*Phaseolus vulgaris* l.) landraces grown in northeast anatolia of turkey assessed with simple sequence repeat markers. Turk J Field Crop. 2012; 17(2):145-150
22. Hajibarat Z, Saidi A, Hajibarat Z, Talebi R. Genetic diversity and population structure analysis of landrace and improved chickpea (*Cicer arietinum*) genotypes using morphological and microsatellite markers. Environ Exp Biol. 2014;12:161-166.
23. Yu K, Park SJ, Poysa V, Gepts P. Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). J Hered. 2000;91:429-434
24. Gaitán-Solís SE, Duque MC, Edwards KJ and Tohme J. Microsatellite repeats in common bean (*Phaseolus vulgaris*): Isolation, characterization, and cross-species amplification in *Phaseolus ssp*. Crop Sci. 2002;42:2128-2136.
25. Blair MW, Pedraza F, Buendia HF, Gaitan-Solıs E, Beebe SE, Gepts P, Thome J. 2003: Development of a genome-wide anchored microsatellite map of common bean (*Phaseolus vulgaris* L.). Theor Appl Genet. 2003;107:1362-1374.
26. Masi P, Spagnoletti Zeuli PL, Donini P. Development and analysis of multiplex microsatellite markers sets in common bean (*Phaseolus vulgaris* L.). Mol Breed. 2003;11:303-313.
27. Yaish MWF, Pérez de la Vega M. Isolation of (GA)n microsatellite sequences and description of a predicted MADS-box sequence isolated from common bean (*Phaseolus vulgaris* L.). Genetics Mol Biol. 2003;26:337–342
28. Guerra-Sanz JM. New SSR markers of *Phaseolus vulgaris* from sequence databases. Plant Breed. 2004;123:87-89.
29. Buso GSC, Amaral ZPS, Brondani RPV, Ferreira ME. Primer note: Microsatellite markers for the common bean *Phaseolus vulgaris*. Mol Ecol Notes. 2006;6:252-254.
30. Lassner MW, Peterson P, Yoder JI. Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. Plant Mol Biol Rep. 1989;7:116-128.
31. Perrier X, Flori A, Bonnot F. Data analysis methods. In: Hamon P., Seguin M., Perrier X., laszmann J.C. (eds) Genetic diversity of cultivated tropical plants. Science Publishers, Enfield, Montpellier. 2003;43–76.
32. Peakall R, Smouse PE. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006;6:288-295.
33. Hajibarat Z, Saidi A, Hajibarat Z, Talebi R. Characterization of genetic diversity in chickpea using SSR markers, Start Codon Targeted Polymorphism (SCoT) and Conserved DNA-Derived Polymorphism (CDDP). Physiol Mol Biol Plant. 2015; 21:365-373.
34. Amirmoradi B, Talebi R, Karami E. Comparative of genetic variation and differentiation among annual *Cicer* species

using start codon targeted (SCoT) polymorphism, DAMD-PCR and ISSR markers. Plant Syst Evol. 2012;298:1679-1688.

35. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed. 1996; 2:225-238.
36. Sicard D, Nanni L, Porfiri O, Bulfon D, Papa R. Genetic diversity of *Phaseolus vulgaris* L. and *P. coccineus* L. landraces in central Italy. Plant Breed. 2005; 124:464-472
37. Ocampo CH, Martin JP, Sanchez-Yelamo MD, Ortiz JM, Toro O. Tracing the origin of spanish common bean cultivars using biochemical and molecular markers. Genet Resour Crop Evol. 2005;52:33-40.
38. Sustar-Vozlic J, Maras M. Genetic diversity and origin of Slovene common bean (*Phaseolus vulgaris* L.) germplasm as revealed by AFLP markers and phaseolin analysis. J Amer Soc Hort Sci. 2006; 131(2):242-249.

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