

**STUDY OF GENETIC DIVERSITY AND SEARCH FOR ANTHRACNOSE
RESISTANCE ALLELES IN COMMON BEAN (*Phaseolus vulgaris* L.) GENOTYPES
CULTIVATED IN AZERBAIJAN**

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Common bean has become very popular and widespread among the population since its introduction to Azerbaijan in the second half of the 18th century. The paper represents for the first time the genetic diversity and anthracnose resistance of 37 common bean accessions cultivated in Azerbaijan. ISSR marker characterization in the present study revealed a total of 47 bands, with 33.6% of average polymorphism. The polymorphism information content (PIC) and genetic diversity index (GDI) for each primer were in the range of 0.25–0.48 (mean 0.35) and 0.45–0.73 (mean 0.59), respectively, indicating a moderate level of genetic diversity in the current collection. UNJ tree showed that the common bean accessions tended to cluster according to the local and introduced gene pools, indicating the same original sources of these accessions, which is also supported by PCo analysis. Screening with linked SCAR markers revealed the existence of common bean genotypes with single or multiple Co resistance alleles. Among studied genes *Co-4* locus and its alleles were found in all samples, followed by *Co-6* (40.5%) and *Co-3⁴* (16%). Three genotypes had all studied resistance loci, while 12 had *Co-4* and *Co-6*, and 3 had *Co-4* and *Co-3⁴*. The results could provide valuable information for future common bean breeding activities and conservation. The use of genotypes with two or more

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resistance genes as donor parents can accelerate the development of new common bean cultivars with durable resistance to anthracnose.

Keywords: common bean; genetic diversity; ISSR; anthracnose; SCAR

INTRODUCTION

The common bean (*Phaseolus vulgaris* L., $2n=22$) is the most important cultivar of the genus *Phaseolus* providing a food staple to millions of people worldwide. It is consumed as dry grains, and fresh pods that are rich in protein, cellulose and carbohydrate content (GONÇALVES-VIDIGAL *et al.*, 2013). It was first domesticated over 7000 years ago independently in the Andean region and in Mesoamerica (Mexico and Central America), which also relate to the two major gene pools (KAPLAN, 1981; GEPTS *et al.*, 1986). In the early decades of the sixteenth century, the common beans were introduced into Europe, which can be regarded as a secondary diversification center of this crop (LIOI and PIERGIOVANNI, 2013). The introduction of *Ph. vulgaris* to post-Soviet countries occurred only in the 18th and early 19th centuries, where each country selected its own set of landraces and constituted smaller collections, tailored to the needs of local farmers and consumers (HEGAY *et al.*, 2012; ASADOVA *et al.*, 2016). Characterization of these collections and identifying useful variants are crucial for global conservation strategies, and genetic improvement programs. Different types of molecular markers have already been used for the estimation of genetic diversity in common beans (KUMAR *et al.*, 2008; JOSE *et al.*, 2009; HEGAY *et al.*, 2012; CABRAL *et al.*, 2018). Among them, ISSR markers are universal, highly polymorphic, and have high reproducibility of results, which make them suitable for genetic diversity studies (SILVA *et al.*, 2016).

Several pathogens significantly reduce both grain yield and the quality of common beans. Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Lams.- Scrib is one of the major constraints of common bean production. The seed-borne disease can attack all aerial parts of the plant and are stored in seeds for up to 5 years (PASTOR-CORRALES and TU, 1989). Preventive measures such as crop rotation, as well as the treatment with fungicides, are less effective in combating the disease, whereas using resistant varieties is considered the most cost-effective and environmentally friendly method. The existence of more than 100 variants and races is the main difficulty in breeding (PEREIRA *et al.*, 2010). Thus, pyramiding different resistance genes in a single genotype can ensure long-term sustainability. In common beans, up to 20 genes (Co) conferring resistance to anthracnose have been described. The use of molecular markers linked to these genes can accelerate the selection and breeding of genotypes with one or more resistance loci. Several DNA markers linked to anthracnose resistance genes in common beans have been reported previously (ADAM-BLONDON *et al.*, 1994; YOUNG and KELLY, 1997). Due to accuracy and repeatability problems associated with RAPD techniques these markers have been used to obtain more specific and accurate SCAR markers (de QUEIROZ *et al.*, 2004).

Ph. vulgaris, was first introduced to Azerbaijan during the second half of the XVIII century (ASADOVA *et al.*, 2016). After the independence and the collapse of the collective farms, common bean accessions were mainly grown on peasant farms and small farmers' fields. As a result of folk selection, a lot of landraces were created during the last century, such as Gabiliyyet, Yerli Piyada, Girmizi hindi, AzNİİZ-352 and Sevinj (ASADOVA *et al.*, 2016). For decades systematic studies on the common bean accessions in the country have been carried out

only by recording morphological and agronomical traits. So far, no analysis has been conducted to assess genetic diversity in common bean accessions cultivated in Azerbaijan. Also limited information is available on anthracnose resistance which causes serious damage to seed material and leads to a partial yield loss in the country every year (MAMMADOVA and SHIKHLINSKI, 2015).

In this context, this study aimed to evaluate the genetic diversity of 37 common bean accessions cultivated in Azerbaijan using ISSR markers and to screen them for the *Co* resistance genes with linked SCAR markers.

MATERIALS AND METHODS

Plant materials and DNA extraction

Thirty-seven local and introduced common bean accessions of the Mesoamerican gene pool obtained from the National Gene bank of Genetic Resources Institute (GRI) of the Ministry of Science and Education of Azerbaijan were used as research material (Table 1). Leaf tissue samples from each tested common bean genotype were collected and DNA was extracted using the CTAB method described by ROGERS and BENDICH (1985).

Table 1. The common bean genotypes used in the study

№	Accessions	Country	Region	№	Accessions	Country	Region
1	Yerli piyada	Azerbaijan	Absheron	20	K-13036	Russia	VIPI
2	K-14044	Russia	VIPI	21	AzePHA-34	Ukraine	
3	AzePHA-6	Russia	Moscow	22	AzePHA-210	Azerbaijan	Pirshagi
4	AzePHA-18	Azerbaijan	Lankaran	23	AzePHA-20	Azerbaijan	Lankaran
5	AzePHA-36/2	Azerbaijan	Agdash	24	K-3498	Russia	VIPI
6	AzePHA-33	Russia	Stavropol	25	AzePHA-14	Azerbaijan	Masalli
7	AzePHA-209 t	Azerbaijan	Absheron	26	Azeqri/69	Azerbaijan	Nakhchivan
8	K-15274	Russia	VIPI	27	AzePHA-27	Azerbaijan	Nukha
9	AzePHA-23	Azerbaijan	Gusar	28	AzePHA-29	Azerbaijan	Gusar
10	AzePHA-t/1	Iran	Tabriz	29	AG-3307	Azerbaijan	
11	AzePHA-t/10	Russia	Moscow	30	K-13037	Russia	VIPI
12	AG-1894	Azerbaijan	Absheron	31	AzePHA-36	Azerbaijan	Balaken
13	AzePHA-t/6	Russia	Stavropol	32	AzePHA-t/9	Russia	Dagestan
14	Sekunda	Russia	Moscow	33	AzePHA-t/29		Turkiye
15	K-13044	Russia	VIPI	34	AzePHA-t/5	Russia	Stavropol
16	K-13038	Russia	VIPI	35	AzePHAV-213t	Azerbaijan	Khachmaz
17	AzePHA-t/5-N16	Russia	Stavropol	36	AzePHA-13/1	Azerbaijan	Barda
18	AG-1891	Azerbaijan	Zagatala	37	Sonesta	Russia	Moscow
19	AzePHA-15	Azerbaijan	Agdash				

Marker analysis

PCR reactions for six ISSR primers were performed in a 20 μ l, containing 2 μ l 10x PCR buffer; 2 μ l mixture dNTP (5 mM); 1.5 μ l MgCl₂ (50mM); 2 μ l of each primer (15 pmol/ μ l); 0.1 μ l of Taq-polymerase enzyme (5 U/ μ l L) and 2 μ l of extracted DNA (50 ng/ μ l). The PCR program consisted of pre-denaturation at 94°C for 5 minutes; 35 cycles of – denaturation at 94°C for 1 min, annealing for 45 seconds (temperature depended on the primer used, Table 2.), elongation for 5 minutes at 72°C; the final elongation at 72°C for 10 minutes.

Common bean cultivars were also screened with five SCAR markers. The PCR reaction mix of 20 μ l consisted of 2 μ l 10x PCR buffer; 0.2 μ l mixture dNTP (20 mM); 1.2 μ l MgCl₂ (25 mM); 0.4 μ l of F and R primers (10 μ M); 0.2 μ l of Taq-polymerase enzyme (5 U/ μ l) and 30 ng DNA. PCR conditions were performed according to EKINCIALP and ŞENSOY (2018) for SAS 13, SF10, SZ04 and according to VIEIRA *et al.* (2018) for SY20 and SZ20. Amplification products were analyzed in 1.5-2% agarose gel in 1x TBE buffer at 90 V, stained with ethidium bromide and visualized under UV light using the gel documentation system BioRad. The band size was determined by using Photo-Capt version 12.4 with reference to the standard 100 bp ladder.

Table 2. Marker parameters of genetic variation calculated for ISSR markers

Primer name	Primer sequence, 5'~3'	T _a , °C	Total number of bands	Number of polymorphic bands	Polymorphism, %	Genetic diversity index	PIC	EMR	MRP	RP	MI
UBC 812	(GA) ₈ A	41	8	2	25	0.52	0.31	0.50	0.63	0.80	0.16
UBC 818	(CA) ₈ G	46.5	8	3	38	0.45	0.25	1.13	0.31	1.09	0.28
UBC 823	(TC) ₈ C	45	7	2	29	0.49	0.48	0.57	0.31	1.62	0.27
UBC 828	(TG) ₈ C	51	10	4	40	0.70	0.41	1.60	0.09	2.69	0.66
UBC 834	(AG) ₈ YT	46.5	6	2	33	0.62	0.37	0.67	0.42	1.20	0.25
UBC 885	BHB(GA) ₇	46	8	3	38	0.73	0.30	1.13	0.18	1.87	0.34
Total			47	16							
Mean			7.8	2.6	33.6	0.59	0.35	0.93	0.32	1.55	0.33

Y=C, T; H= A, C, T; B=C, G, T

Data analysis

ISSR bands were presented in a matrix of binary data. The cluster analysis for the creation of an unweighted NJ tree and Principal Coordinate Analysis were performed using the DarWin 6.0 software. The genetic diversity index (GDI) (WEIR, 1990), polymorphism information content (PIC) (ROLDAN-RUIZ *et al.*, 2000), effective multiplex ratio (EMR), marker index (MI) (POWELL *et al.*, 1996), resolution power (RP) and mean resolution power (MRP) (PREVOST and WILKINSON, 1999) for each ISSR primer were calculated based on molecular profiles.

The presence of specific resistance-linked bands reported by earlier researchers (EKINCIALP and ŞENSOY, 2018; VIEIRA *et al.*, 2018) was used to screen common bean genotypes against *C. lindemuthianum* resistance.

RESULTS

Genetic diversity

Six ISSR primers were applied to evaluate the genetic diversity among local and introduced *Ph. vulgaris* accessions. The ISSR markers allowed detection of a total of 47 bands for 37 genotypes, of which 16 were polymorphic. The number of polymorphic bands per primer varied from 2 to 4, the average was 2.6. The overall measurement results are summarized in Table 2. The highest number of both total and polymorphic bands was generated by the primer UBC 828. The polymorphism percentage at each locus varied from 25% for UBC 812 to 40% for UBC 828, with a mean of 33.6%. The genetic diversity indices ranged from 0.45 to 0.73, and the mean value was 0.59. The mean value of PIC for all loci was 0.35, varying from 0.25 for UBC 818 to 0.48 for UBC 823. Among the 6 ISSRs used, the mean EMR was 0.93, with values ranging from 0.50 to 1.60. The RP and MRP per primer were in the range of 0.8-2.69 (mean 1.55) and 0.09-0.63 (mean 0.32), respectively. MI ranged from 0.16 for UBC 812 to 0.66 for UBC 828. Overall, UBC 828 was the best marker for the discrimination of the common bean accessions.

Cluster and principal coordinate analysis

The Jaccard's genetic distance coefficients between pairs varied from 0 to 1, the average was 0.58. Maximum genetic distance was presented between AzePHA-23 and AzePHA-29 accessions, while genotypes AG-1891 and AzePHA-20 could not be discriminated based on six ISSR loci. Three major clusters were observed in the UPGMA cluster analysis (Figure 1). Cluster I comprised 19 common bean accessions, mainly from Azerbaijan, while Cluster II consisted of 17 genotypes with a prevalence of the introduced material. As a result of further diversification of Cluster I, local genotypes formed a more compact, nearly homogenous group within subclusters. The only accession from Iran AzePHA-t/1 clustered very closely to local AzePHA-36, while the only Turkish accession had a similar genetic background with another local genotype AzePHA-15. Genotype AzePHA-209t was the most distant from all other accessions and formed a separate Cluster III, indicating its potential to contribute to hybridization programs through heterosis. The distance indices between local AzePHA-209t and the remaining accessions were in the range of 0.6-0.94. PCoA analysis plotted accessions in two distinct groups, which corresponded to the first two clusters (Figure 2). The first two axes explained 47.8% of the total variation. Genotypes belonging to clusters I and II were located in the left, and right quadrants, respectively, whereas genotype number 7, which formed an independent cluster, was located at the very end of the negative PCo-Y axis, far from other genotypes. As in the dendrogram, further separation of local material from introduced ones within Group I was observed.

While comparing results from morphological and molecular data, a correlation between ISSR clustering and pod number per plant in common bean accessions was observed. Thus, pods per plant in 18 out of 19 genotypes comprised Cluster I were less than 10, while in 65% of

accessions of Cluster II the indices for this trait ranged from 10 to 23. The averages for Clusters I and II were 6.9 and 13, respectively, with a mean of 9.7 for the whole collection.

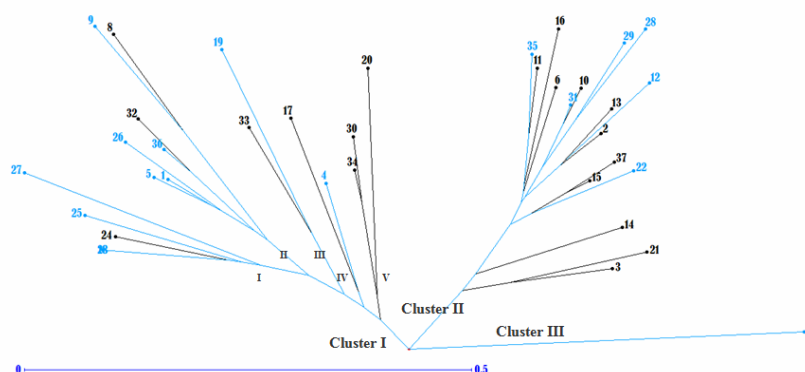


Fig. 1. NJ tree for 37 common bean genotypes based on ISSR data. Blue-local genotypes; black-introduced genotypes

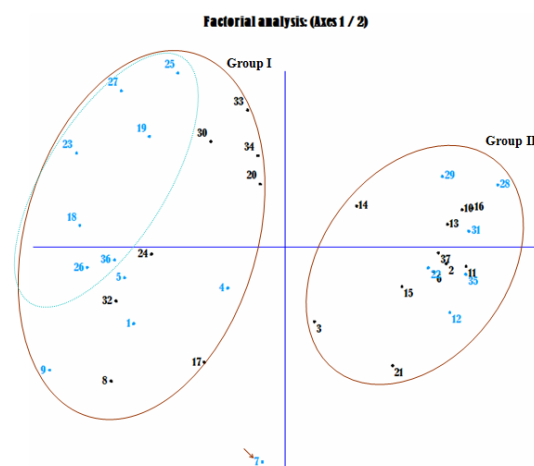


Fig. 2. Scatter plot of common bean genotypes using principle coordinate analysis based on ISSR data. Blue-local genotypes; black-introduced genotypes

Screening for anthracnose resistance genes

Studied common bean accessions were evaluated for many traits, including fungal disease resistance (data not given). During the 2020-2022 sowing years no symptoms of anthracnose in the studied collection were recorded. Genotypes were screened with five SCAR markers

associated with *Co-4*, *Co-4²*, *Co-6*, and *Co-10* (*Co-3⁴*) resistance genes, conferring independently or combined resistance. All SCAR primers yielded amplification products of the expected size from common bean accessions. The first marker SAS13 targeted the *Co-4²* gene provided a single polymorphic band (950 bp) for all the genotypes, except AzePHA-t/1 from Tabriz (Iran) (Figure 3). The SY20₈₃₀ a dominant marker linked to the *Co-4* allele produced specific amplicons of 830 bp from only ten genotypes, of which 5 are local, including a highly productive Yerli Piyada variety. Two primers (SZ04 and SZ20) associated with the *Co-6* gene were used in the study. The amplification by SZ04 showed that two different bands were produced. Diagnostic bands of 567 bp were detected for eleven genotypes, whereas all the other 26 accessions showed 527 bp bands. The second SZ20 primer showed the presence of a target amplicon of 845 bp only in four (AzePHA-6, AzePHA-18, AG-1891, and AzePHA-34) out of 37 common bean genotypes. Another marker SF10, located at a distance of 12.3 cM from *Co-3⁴*, generated a target 1072 bp band from six genotypes, that is, three local and three introduced accessions (Figure 4).

Table 3 summarizes the data on Co genes found in the studied *Ph. vulgaris* collection. In general, four diagnostic marker bands were identified in one local AG-1894 genotype, three markers in 4, and two markers in 19 genotypes. The resistance to *C. lindemuthianum* in 13 accessions was controlled only by the *Co-4* locus.

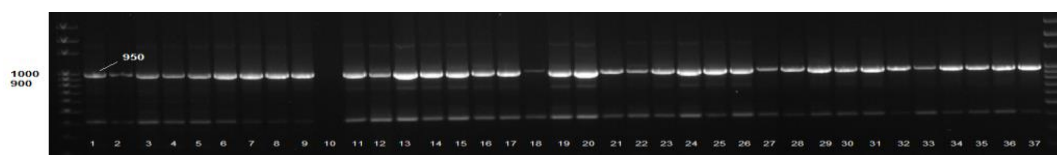


Fig. 3. DNA amplification patterns of 37 common bean genotypes using the SAS13 SCAR marker



Fig. 4. Diagnostic bands of 1072 bp amplified in common bean genotypes using the SF10 SCAR marker

Table 3. Amplification patterns of used SCAR markers in 37 common bean genotypes

№	Genotypes	SAS13 950 bp	SY20 830 bp	SZ04 567 bp	SZ20 845 bp	SF10 1072 bp
1	Yerli piyada	+	+			
2	K-14044	+	+			
3	AzePHA-6	+			+	
4	AzePHA-18	+			+	
5	AzePHA-36/2	+				
6	AzePHA-33	+		+		
7	AzePHA-209 t	+				
8	K-15274	+				
9	AzePHA-23	+				
10	AzePHA-t/1		+	+		
11	AzePHA-t/10	+				
12	AG-1894	+	+	+		+
13	AzePHA-t/6	+	+	+		
14	Sekunda	+		+		
15	K-13044	+		+		
16	K-13038	+		+		
17	AzePHA-t/5-N16	+				
18	AG-1891	+			+	
19	AzePHA-15	+	+			
20	K-13036	+	+			
21	AzePHA-34	+			+	+
22	AzePHA-210	+		+		+
23	AzePHA-20	+				
24	K-3498	+	+			+
25	AzePHA-14	+				
26	Azeqri/69	+	+			
27	AzePHA-27	+				
28	AzePHA-29	+				
29	AG-3307	+				+
30	K-13037	+				
31	AzePHA-36	+		+		
32	AzePHA-t/9	+				
33	AzePHA-t/29	+				+
34	AzePHA-t/5	+				
35	AzePHAV-213t	+		+		
36	AzePHA-13/1	+	+			
37	Sonesta	+		+		

DISCUSSION

Even though the beans were brought to Azerbaijan only in the 18th century, this plant has become very popular and widespread among the population, thanks to which dozens of varieties and forms highly adapted to the local climatic conditions of the country have been created. Some part of these local accessions together with introduced ones is now being conserved at the National Genebank of GRI. This paper presents estimations of genetic variation and anthracnose resistance among 37 common bean accessions that also include, for the first time, 18 local Azerbaijani genotypes and introduced material obtained from the National Genebank. A dominant ISSR marker characterization of common beans in the present study revealed 47 bands, with 33.6% of average polymorphism (Table 2). The polymorphism obtained is compatible with GALVAN *et al.* (2003) who studied 13 common bean genotypes using 23 ISSRs, but lower than the diversity noted by CABRAL *et al.* (2018) and BEHAILU *et al.*, (2018). MAROTTI *et al.* (2007) observed high polymorphism in 16 common bean genotypes from Italia using 20 ISSR primers. Other studies also showed similar high polymorphism of ISSR markers and its suitability for DNA fingerprinting in different crops (IZZATULLAYEVA *et al.*, 2014; HASANOVA *et al.*, 2017; BABAYEVA *et al.*, 2018; HAJIYEVA *et al.*, 2018; ASADOVA *et al.*, 2020). The studied ISSR markers exhibited a moderate level of genetic diversity in the current study, with the mean GDI equal to 0.59. PIC values were also high to moderate (range: 0.25-0.48), taking into account the biallelic nature of dominant markers. Among a set of used ISSR primers UBC 828 and UBC 834 were proved to be the most informative for the study of genetic diversity in common beans. CABRAL *et al.* (2018) also noted high performance for several ISSR markers in 57 bean genotypes, including UBC 834.

In general, common bean accessions in our research had moderate genetic diversity. SORTE (2016) argues that introduced species can be subjected to selective pressures and bottlenecks, leading to loss of genetic diversity as compared to their native populations, which are also observed in common beans. New environmental conditions in which bean was introduced, and the preference for the selection of high-yielding, disease-resistant forms that meet the expectations of the local population, has led to the reduction of polymorphism and loss of the part of the germplasm carried from America (LIOI and PIERGIOVANNI, 2013). Thus, taking into account only two centuries of cultivation history of beans in Azerbaijan, low to moderate diversity observed in the current collection is expected. HEGAY *et al.* (2012) also found low allelic diversity for microsatellite loci in 28 common bean accessions, including local Kyrgyz cultivars.

Another point to be noted is that all accessions in the current study represent the Mesoamerican center of origin. ALZATE-MARIN *et al.* (2003) have concluded that the use of parents from the Mesoamerican genetic base with the same set of genes with limited variability reduces the effectiveness of the breeding and diversity of common bean worldwide. The use of only local and introduced material from low-diversity countries, which is neither the origin nor the diversification center for common bean, can also contribute to relatively low polymorphism in the present study.

In the present study, common bean accessions showed a partial tendency to cluster according to the local and introduced gene pools, rather than the geographical region/collection site. Thus, Cluster I included 11 of 18 local accessions and Cluster II consisted of 11 of 19

introduced accessions. Moreover, further branching of Cluster I revealed tighter grouping of local accessions; 75% of accessions in the first two subclusters out of five were local genotypes (Figure 1). The results differ from CABRAL *et al.* (2018) who found that local common bean genotypes from the Fortaleza community did not cluster together and were widely distributed in different groups. Common beans were initially introduced to Azerbaijan most probably from neighboring countries such as Turkey, Georgia, and Iran, and mainly from Russia by merchants and grown by local farmers. As a consequence, over time landraces well adapted to certain areas of the country were produced, spread, and exchanged among farmers. The fact that some local common bean accessions in the current study are grouped together with a small diversity index possibly indicates the same original sources of these accessions. LIOI and PIERGIOVANNI (2013) have concluded that local collections well adapted to certain areas represent a substantial source of variation and constitute an important resource for breeders for identifying and recombining useful alleles between different gene pools.

A comparison of dendrograms based on morphological traits (data not given) and DNA marker data shows no concordance between the clusters in general. However, when comparing individual traits, a certain relationship between the number of pods per plant and the ISSR clustering of the genotypes was observed. So, while in 95% of the accessions grouped in cluster I, the number of beans per plant was lower than 10, in 65% of the samples in cluster II, this indicator ranged from 10 to 23. In a paper by CABRAL *et al.* (2018) common bean genotypes clustered according to their growth habit, while JOSE *et al.* (2009) and MAROTTI *et al.* (2007) revealed a correlation between seed weight of common bean landraces and RAPD clustering. The obtained result in the present study opens new opportunities for further association studies involving more loci and statistical analyses.

Results of screening with markers linked to alleles for resistance to anthracnose in 37 common bean accessions revealed the existence of genotypes with single or multiple resistance alleles. The first marker SAS13 targeted the *Co-4²* gene. According to the results, 36 out of 37 tested genotypes had *Co-4²* which provides resistance to races 7, 23, 64, 73, 89, 521, 1545 and 2047 (BALARDIN and KELLY, 1998). Despite the tight linkage (0.4 cM), several studies have shown that the SAS13 marker presents in cultivars with other alleles at the *Co-4* locus (AWALE and KELLY, 2001; ALZATE-MARIN *et al.*, 2000). Thus the fact that the majority of accessions had target amplicons could also be due to the presence of different alleles of the *Co-4* locus (*Co-4*, *Co-4²*, *Co-4³*) in the collection.

The dominant marker SY20₈₃₀ (BERALDO *et al.*, 2009) was found to be present in ten out of 37 genotypes. Several genotypes showed unspecific bands close to the locus or very weak bands. Several studies have shown that the primer SY20 is also not specific and could not distinguish between different alleles at this locus (VIEIRA *et al.*, 2018). However, these primers are still very effective in screening for *Co-4* resistance locus in common bean accessions.

The primers used for the screening of the *Co-6* gene in linkage group 7 were SZ04 and SZ20. The SZ04, located at a distance of 2.9 cM from the *Co-6* locus, gave two fragments, of 527 and 567 bp, of which diagnostic bands of 567 bp were found only in 11 genotypes. The SZ20 primer linked to the *Co-6* locus at a distance of 7.1 cM produced a target of 845 bp in two local and two introduced common bean genotypes. In other genotypes, weak unspecific bands were observed. This is in agreement with KAMIRI *et al.* (2021), who noted the presence of many

unspecific bands close to the target locus using SZ20 in differential varieties without the *Co-6* locus. The incompatibility of the results with two primers linked to the same locus can be explained by the different recombination frequencies due to the different distances from the target locus.

The *Co-10* gene was first described as an independent locus. However, it was later determined that *Co-10* was an allele of the *Co-3* locus (*Co-3^d*) located in LG 4 (COELHO, 2013). Molecular marker analysis showed that the SF10 resulted in a target 1072 bp band from six genotypes.

As a result of the molecular screening, it was also possible to determine the gene frequency in the studied collection. Thus, among the 37 common bean genotypes the *Co-4* locus (and its alleles) was found in all samples, followed by the *Co-6* locus (40.5%) and *Co-3^d* (16%). It is known that the *Co-4* locus is the most utilized in the common bean for anthracnose resistance (KAMIRI *et al.*, 2021). In this context, our research confirms the importance of the current common bean germplasm as a valuable source of anthracnose resistance.

Of the 37 accessions, three genotypes (AG-1894, AzePHA-34, AzePHA-210) had all studies resistance loci, while 15 and 19 genotypes possessed two and one anthracnose resistance loci, respectively (Table 3). These loci provide resistance to a total of about 30 races, including the highly virulent 2047 race.

In conclusion ISSR primers used in the study enabled the determination of the molecular identity of 95% of genotypes, which provides important information and guidelines for future common bean breeding programs, conservation, and management strategies both in Azerbaijan and all over the world. Moreover, this paper revealed the existence of important genetic sources with different Co resistance genes in common bean accessions cultivated in Azerbaijan. The use of genotypes will be beneficial in MAS programs in order to accelerate the development of new common bean cultivars through gene introgression and pyramiding which is essential for effective and long-term sustainability.

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REFERENCES

- ADAM-BLONDON, A.F., M., SEVIGNAC, M., DRON, H., BANNEROT (1994): A genetic map of common bean to localize specific resistance genes against anthracnose. *Genome*, 37:915-924.
- ALZATE-MARIN, A.L., M.R., COSTA, A., SARTORATO, M.J., DEL PELOSO, E.G.D., BARROS, *et al.* (2003): Genetic variability and pedigree analysis of Brazilian common bean elite genotypes. *Science Agriculture*, 60:283-290.
- ALZATE-MARIN, A.L., H., MENARIM, J.M., CHAGAS, E.G.D., BARROS, M.A., MOREIRA (2000): Identification of a RAPD marker linked to the Co-6 anthracnose resistant gene in common bean cultivar AB 136. *Genetics and Molecular Biology*, 23(3):633-637.
- ASADOVA, A., L., AMIROV, M., ABBASOV (2016): Biodiversity of some legume plants of Azerbaijan. Shikhliniski H.M. (editor(s). Muellim Press, Baku, Azerbaijan, pp. 30-36.
- ASADOVA, A., S., BABAYEVA, V., IZZATULLAYEVA, S., AKBAROVA, G., AGHAZADE, *et al.* (2020): Molecular characterization of *L. sativus* L. collection based on ISSR markers. *Genetika*, 52(2):777-786.
- AWALE, H.E., J.D., KELLY (2001): Development of SCAR markers linked to Co-4² gene in common bean. Annual Report of the Bean Improvement Cooperative, 44:119-120.

- BABAYEVA, S., Z., AKPAROV, L., AMIROV, K., SHIKHALIYEVA, S., HASANOVA, *et al.* (2018): Genetic relationship among introduced lentil germplasm using agronomic traits and ISSR markers. *Genetika*, 50(2):575-590.
- BALARDIN, R.S., J.D., KELLY (1998): Interaction between *Colletotrichum lindemuthianum* races and gene pool diversity in *Phaseolus vulgaris*. *Journal of the American Society for Horticultural Science*, 123(6):1038-1047.
- BEHAILU, M.A., D., KIFLE, K.W., GEMECHU, *et al.* (2018): Genetic diversity study of Ethiopian Faba bean (*V. faba* L.) varieties based on phenotypic traits and inter simple sequence repeat (ISSR) markers. *Af. J Biotec*, 433-446.
- BERALDO, A.L.A., C.A., COLOMBO, A.F., CHIORATO, M.F., ITO, S.A.M., CARBONELL (2009): Use of SCAR markers in common bean breeding for anthracnose resistance. *Bragantia*, 68(1):53-61.
- CABRAL, P., L., DE SOUZA, G., DA COSTA, F., SILVA, T., SOARES (2018): Investigation of the genetic diversity of common bean (*Phaseolus vulgaris*) cultivars using molecular markers. *Genetics and Molecular Research*, 17(4):1-11.
- COELHO, R., M., GONÇALVES-VIDIGAL, P., VIDIGAL FILHO, G., LACANALLO, L., DARBEN, *et al.* (2013): Characterization of the anthracnose resistance gene in the Mesoamerican common bean cultivar Crioulo 159. *Annual Report of the Bean Improvement Cooperative*, 56:43-44.
- DE QUEIROZ, V., C., DE SOUSA, M., COSTA, D., SANGIAD, K., ARRUDA, T.L.P.O., DE SOUZA, V.A., RAGAGNIN, E.G., DE BARROS, M.A., MOREIRA (2004): Development of SCAR markers linked to common bean anthracnose resistance genes *Co-4* and *Co-6*. *Annual Report of the Bean Improvement Cooperative*, 47:249-250.
- EKINCIALP, A., S., ŞENSOY (2018): Phenotypic and molecular determination of anthracnose disease resistance in Lake Van Basin's bean genotypes (*Phaseolus vulgaris* L.). *Legume Research*, 41(1): 135-42.
- GALVAN, M., B., BORNE, P., BALATTI, M., BRANCHARD (2003): Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). *Euphytica*, 132:297-301.
- GEPTS, P., T., OSBORNE, K., RASHKA, F., BLISS (1986): Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris* L.): Evidence for multiple centers of domestication. *Economic Botany*, 40:451-468.
- GONÇALVES-VIDIGAL, M., A., CRUZ, G., LACANALLO, P., VIDIGAL FILHO, L., SOUSA, *et al.* (2013): Co-segregation analysis and mapping of the anthracnose *Co-10* and angular leaf spot Phg-ON disease-resistance genes in the common bean cultivar Ouro Negro. *TAG*, 126: 2245-2255.
- HAIJIYEVA, S., Z., AKPAROV, N., HASANOV, Z., MUSTAFAYEVA, E., HAIJIYEV, A.T., MAMMADOV, V.I., IZZATULLAYEVA, S.M., BABAYEVA, S.S., SHARIFOVA, A.M., MAMMADOV, M.A., ABBASOV (2018): ISSR analysis of variability of cultivated form and varieties of pomegranate (*Punica granatum* L.) from Azerbaijan. *Russian journal of genetics*, 54(2): 188-197.
- HASANOVA, S., Z., AKPAROV, A., MAMMADOV, L., AMIROV, S., BABAYEVA, J., NASIBOVA, Z., MUKHTAROVA, K., SHIKHALIYEVA, V., IZZATULLAYEVA, M., ABBASOV (2017): Genetic diversity of chickpea genotypes as revealed by ISSR and RAPD markers. *Genetika*, 49(2): 415-423.
- HEGAY, S., M., GELETA, T., BRYNGELSSON, L., GUSTAVSSON, H., HOVMALM, R., ORTIZ (2012): Comparing genetic diversity and population structure of common beans grown in Kyrgyzstan using microsatellites. *Scientific Journal of Crop Science*, 1(4): 63-75.
- IZZATULLAYEVA, V., Z., AKPAROV, S., BABAYEVA, J., OJAGHI, M., ABBASOV (2014): Efficiency of using RAPD and ISSR markers in evaluation of genetic diversity in sugar beet. *Turkish Journal of Biology*, 38 (4): 429-438.
- JOSE, F., M., MOHAMMED, G., THOMAS, G., VARGHESE, N., SELVARAJ, *et al.* (2009): Genetic diversity and conservation of common bean (*Phaseolus vulgaris* L., Fabaceae) landraces in Nilgiris. *Current science*, 227-235.

- KAMIRI, A., E., ARUNGA, F., ROTICH, R., OTSYULA (2021): Response of French bean genotypes to *Colletotrichum lindemuthianum* and evaluation of their resistance using SCAR markers. *African Journal of Biotechnology*, 20(2): 51-65.
- KAPLAN, L. (1981): What is the origin of the common bean? *Economic Botany*, 35:240–254.
- KUMAR, V., S., SHARMA, S., KERO, S., SHARMA, A., SHARMA, M., KUMAR, K.V., BHAT (2008): Assessment of genetic diversity in common bean (*Phaseolus vulgaris* L.) germplasm using amplified fragment length polymorphism (AFLP). *Scientia Horticulturae*, 116:138–143.
- LIOLI, L., A., PIERGIOVANNI (2013): European common bean. In: Genetic and genomic resources of grain legume improvement. Elsevier, pp.11-40.
- MAROTTI, I., A., BONETTI, M., MINELLI, P., CATIZONE, G., DINELLI (2007): Characterization of some Italian common bean (*Phaseolus vulgaris* L.) landraces by RAPD, semi-random and ISSR molecular markers. *Genetic Resources and Crop Evolution*, 54(1): 175-188.
- MAMMADOVA, S., H., SHIKHLINSKI (2015): Affect of fungal diseases to biochemical traits of lentil and common bean genotypes. *Scientific works of Scientific Research Institute of Crop Husbandry*, 24:169-171.
- PASTOR-CORRALES, M.A., J., TU (1989): Anthracnose. In: Bean production problems in the tropics. Schwartz, H.F., Pastor-Corrales, M.A. (editors). CIAT, Cali, Colombia, pp 77-104.
- PEREIRA, R., F., ISHIKAWA, J., PINTO, E., SOUZA (2010): Occurrence of anthracnose in common bean cultivars collected in the state of Minas Gerais-Brazil. *Annual Report of the Bean Improvement Cooperative*, 53:224-225.
- POWELL, W., M., MORGANTE, C., ANDRE, M., HANAFEY, J., VOGEL, S., TINGEY, A., RAFALSKI (1996): The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, 2:225-238.
- PREVOST, A., M.J., WILKINSON (1999): A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *TAG*, 98: 107-112.
- ROGERS, S.O., A.J., BENDICH (1985): Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol. Biol.*, 5: 69-76.
- ROLDAN-RUIZ, I., J., DENDAUIW, E., VANBOCKSTAELE, A., DEPICKER, M., DE LOOSE (2000): AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding*, 6:125–134.
- SILVA, B., A., ROSSI, J., DARDENGO, V., ARAUJO, F., ROSSI, L.O., DE OLIVERA, W.R., CLARINDO (2016): Genetic diversity estimated using inter-simple sequence repeat markers in commercial crops of cupuassu tree. *Ciência Rural*, 46:108-113.
- SORTE, C. (2016): Invasive Species, Evolution and. In: Kliman RM (editor(s)). *Encyclopedia of Evolutionary Biology*. Academic Press, pp. 282-287.
- VIEIRA, A., L., ALMEIDA, L., RODRIGUES (2018): Selection of resistance sources to common bean anthracnose by field phenotyping and DNA marker-assisted screening. *Genetics and Molecular Research*, 17(3): gmr18066.
- WEIR, B. (1990): *Genetic Data Analysis. Methods for Discrete Population Genetic Data*. Sinauer Associates, Sunderland.
- YOUNG, R., J., KELLY (1997): RAPD markers linked to three major anthracnose resistance genes in common bean. *Crop Sci.*, 37:940–946.

**PROUČAVANJE GENETIČKOG DIVERZITETA I IDENTIFIKACIJA ALELA
ZA OTPORNOST NA ANTRAKNOZU KOD GENOTIPOVA PASULJA
(*Phaseolus vulgaris* L.) KOJI SE GAJE U AZERBEJDŽANU**

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Izvod

Pasulj je postao veoma popularan i rasprostranjen među stanovništvom od njegovog introdukovanja u Azerbejdžan u drugoj polovini 18. veka. U radu je po prvi put predstavljena genetska raznovrsnost i otpornost na antraknozu 37 vrsta pasulja uzgajanih u Azerbejdžanu. Karakterizacija ISSR markera u ovoj studiji otkrila je ukupno 47 traka, sa 33,6% prosečnog polimorfizma. Sadržaj informacija o polimorfizmu (PIC) i indeks genetičke raznovrsnosti (GDI) za svaki prajmer bili su u rasponu od 0,25–0,48 (srednja vrednost 0,35) i 0,45–0,73 (srednja vrednost 0,59), respektivno, što ukazuje na umeren nivo genetske raznolikosti u ispitivanoj kolekciji. UNJ stablo je pokazalo da su uzorci običnog pasulja imali tendenciju grupisanja prema lokalnim i introdukovanim genotipovima, što ukazuje na iste originalne izvore ovih uzoraka, što je takođe potvrđeno analizom PCo. Skrining sa povezanim SCAR markerima je otkrio postojanje zajedničkih genotipova pasulja sa jednim ili više alela otpornosti na *Co*. Među ispitivanim genima u svim uzorcima je pronađen *Co-4* lokus i njegovi aleli, zatim *Co-6* (40,5%) i *Co-34* (16%). Tri genotipa su imala sve ispitivane lokuse otpornosti, dok je 12 imalo *Co-4* i *Co-6*, a 3 *Co-4* i *Co-34*. Rezultati bi mogli pružiti vredne informacije za buduće aktivnosti uzgoja i očuvanja običnih pasulja. Upotreba genotipova sa dva ili više gena otpornosti kao roditelja donatora može ubrzati razvoj novih sorti pasulja sa trajnom otpornošću na antraknozu.

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