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Detection of Ten Resistance Genes Against *P. syringae* pv. *phaseolicola* and *X. axonopodis* pv. *phaseoli* in Twelve Local Bean Varieties Using SCAR Markers

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ABSTRACT: Bean (*Phaseolus vulgaris*) is an important food and source of vitamin worldwide. It is preferred in different cooking forms in different cultures. Some bacterial and fungal diseases cause serious loss in bean production and impend nutrition and economy. In this study, we aimed to investigate the presence of some resistance genes against two plant diseases (*Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *phaseoli*) in twelve local bean varieties (Aslan, Elinda, İstanbul, Bursa, Flash, Belluga, White cordinal, Beryl, Yunus-90, Göynük-98, Önceler-98, Eskişehir-855). We scanned four resistance genes (*SR13, ST8, SH11* and *SB10*) for *P. syringae* pv. *phaseolicola* and six resistance genes (*SAP6, BAC6, SU91, BC420, R7313* and *R4864*) for *X. axonopodis* pv. *phaseoli*. PCR amplifications were performed with specific SCAR markers for each resistance gene. The obtained DNA bands were scored as present or absent for the detection of resistance genes. For comparison, the virulence rates of two plant diseases against twelve bean varieties were obtained from field results. We especially observed that two bean varieties (Aslan and Beryl) including four resistance genes against *P. syringae* pv. *phaseolicola* are more resistant to this disease in field. We revealed analysis results for all resistance genes in twelve bean varieties and compared with resistance rates in field. The data obtained from this study will seriously be conduce to the improvement of resistant varieties against pathogens in bean production.

Keywords: PCR, *phaseolus vulgaris*, plant diseases, resistance gene markers

SCAR Markörler Kullanarak On İki Yerel Fasulye Çeşidinde *P. Syringae* Pv. *Phaseolicola* ve *X. Axonopodis* Pv. *Phaseoli*'ye Karşı On Direnç Geninin Tespit Edilmesi

Ö**ZET:** Fasulye (*Phaseolus vulgaris*), dünya genelinde önemli bir besin ve vitamin kaynağıdır. Farklı kültürlerde farklı yemek formları olarak tercih edilmektedir. Bazı bakteriyel ve fungal hastalıklar, fasulye üretiminde ciddi kayıplara neden olmakta ve beslenme ve ekonomiyi tehdit etmektedir. Bu çalışmada, on iki yerel fasulye çeşidinde (Aslan, Elinda, İstanbul, Bursa, Flash, Belluga, White cordinal, Beryl, Yunus-90, Göynük-98, Önceler-98, Eskişehir-855), iki bitki hastalığına (*Pseudomonas syrin*gae pv. phaseolicola and *Xanthomonas axonopodis* pv. *phaseoli*) karşı bazı direnç genleri belirlenmesini amaçlanmıştır. *P. syringae* pv. *phaseolicola* için dört direnç geni (*SR13*, *ST8*, *SH11* ve *SB10*) ve *X. axonopodis* pv. *phaseoli* için ise altı (*SAP6*, *BAC6*, *SU91*, *BC420*, *R7313* ve *R4864*) direnç geni taranmıştır. Her bir direnç geni için polimeraz zincir reaksiyonu (PCR)-çoğaltımları spesifik SCAR belirteçler ile gerçekleştirilmiştir. Direnç genlerinin tespiti için elde edilen DNA bantları, var veya yok olarak kaydedilmiştir. Karşılaştırma yapmak için, iki bitki hastalığına karşı hastalık yapma oranları tarla verilerinden elde edilmiştir. Özellikle, *P. syringae pv. phaseolicola*'ya karşı dört direnç geni içeren iki fasulye türünün (Aslan ve Beryl), tarlada bu hastalığa karşı daha dirençli olduğunu gözlenmiştir. On iki fasulye çeşidindeki tüm direnç genleri için analiz sonuçlarını ortaya konulmuş ve tarladaki direnç oranları ile karşılaştırılmıştır. Bu çalışmadan elde edilen veriler, fasulye üretiminde patojenlere karşı dirençli çeşitlerin geliştirilmesine ciddi bir katkı sağlayacaktır.

Anahtar Kelimeler: Bitki Hastalıkları, direnç gen belirteçleri, pcr, *phaseolus vulgaris*

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INTRODUCTION

Bean (*Phaseolus vulgaris*) is popular legume worldwide (Raggi et al., 2014; Scarano et al., 2014; Meziadi et al., 2016) and have many specialties as food source in human nutrition (Osdaghi and Rak, 2015; Darkwa et al., 2016). It includes A, B1, B2, C vitamins and has a high protein level, assisting the neutralization of acids accumulated in human body (FAO, 2014).

Dry bean is one of the most important major crop in Turkey and its annual production is about 200.000 tones (Ceylan et al., 2014). *Pseudomonas syringae* pv. *phaseolicola* is a very dangerous bacterial pathogen causing greenish or grayish angular blots in bean cotyledons. This pathogen infects all bean varieties and cause serious economic lose worldwide (Taylor et al., 1996). *Xanthomonas axonopodis* pv. *phaseoli* is a pathogen of a plant disease decreasing efficiency and quality of bean.

This pathogen also causes same and pale blots in cotyledons (KFFABS, 2016). Fighting against these diseases is very difficult and expensive. The pesticides including copper are generally used for fighting against bacterial diseases (Madakbaş and Ellialtıoğlu, 2012).

However, these pesticides are very costly chemicals and not usually preferred by bean farmers. The environmental pollution, cost of bean production and marketing problems increased due to expensive chemical pesticides, which rise negative attitudes of farmers against bean farming (Çalışkan, 2014).

Plant genetic resources are very important for progress in plant breeding, genetics and molecular biology (Martins et al., 2006). The higher genetic diversity in plants provides a strong base for selecting superior genotypes for plant breeding (Ceylan et al., 2014).

Breeding of resistant bean cultivars against bacterial and fungal pathogens is an alternative method in fighting the diseases (Turunç, 2010; Meziadi et al., 2016; Zhu et al., 2016). Four resistance genes have been identified (*SR13, ST8, SH11* and *SB10*) for *P. syringae* pv. *phaseolicola* and six resistance genes (*SAP6, BAC6, SU91, BC420, R7313* and *R4864*) for *X. axonopodis* pv. *phaseoli* (Carlos et al., 2009; Perry et al., 2013; Miklas et al., 2014).

The use of pathogen resistant cultivars provides important economic and environmental advantages (Broughton et al., 2003). In this study, we scanned ten resistance genes for these diseases using SCAR (Sequence characterized amplified region) markers in order to reveal the presence of these genes in twelve local bean varieties.

MATERIAL AND METHODS

Materials

Twelve bean varieties and seed samples were provided from Geçitkuşağı Agricultural Research Institute (GARI) (Eskişehir, Turkey) (Table 1.). Resistance levels for bean varieties were obtained from the field data by GARI. Bean seeds were germinated in sterile petri dishes using filter paper wetted with sterile water at room temperature. The fresh leaves from the germinated beans were stored at -20^{o}C. For the DNA isolation, leaf samples were ground to fine powder and transferred to eppendorf tubes.

DNA isolation

DNA extraction from bean leaves was performed using the CTAB (cetyl trimethylammonium bromide) procedure with minor modifications (Doyle and Doyle 1987). Approx. 100 mg powdered materials for each bean varieties was transferred to a 2 ml Eppendorf tubes and 1 ml freshly prepared extraction buffer was added. The quantity and quality of DNA samples were determined by using a Nanodrop Spectrophotometer (Shimadzu, Kyoto, Japan). DNA samples were diluted with sterile distilled water to 2 ng µl.

Table 1. Bean varieties and resistance levels in field

Used Wheat Varieties	**Resistance to P. syringae pv. phaseolicola**	**Resistance to X. axonopodis pv. phaseoli**
Aslan	Resistant	Resistant
Elinda	Semi Resistant	Semi Resistant
İstanbul	Semi Resistant	Semi Resistant
Bursa	Sensitive	Sensitive
Flash	Sensitive	Sensitive
Belluga	Resistant	Resistant
White cordinal	Resistant	Resistant
Beryl	Resistant	Resistant
Yunus-90	Semi Resistant	Semi Resistant
Göynük- 98	Semi Resistant	Semi Resistant
Önceler-98	Semi Resistant	Semi Resistant
Eskişehir-855	Sensitive	Sensitive

Table 2. SCAR Markers used in PCR amplifications

SCAR Markers	Pathogen	Forword ve Reverse Primers	Referance
SR13	*P. syringae* pv. *phaseolicola*	F: 5'GGACGACAAGGAACATATTCA 3' R: 5'GGACGACAAGGCTGCAAGAACCAT 3'	Miklas et al., 2000
ST8	*P. syringae* pv. *phaseolicola*	F: 5'AACGGCGACATCAGTGTAAAGG 3' R: 5'AACGGCGACAACCGACCATGTTTTAC 3'	Miklas et al., 2000
SH11	*P. syringae* pv. *phaseolicola*	F: 5'CTTCCGCAGTCGAGAGAT 3' R: 5'CTTCCGCAGTAGCACC 3'	Miklas et al., 2000
SB10	*P. syringae* pv. *phaseolicola*	F: 5'CTGCTGGGACAATCACCAAGTC 3' R: 5'CTGCTGGGACTCTCTTAC3'	Fourie et al., 2004
SAP6	*X. axonopodis* pv. *phaseoli*	F: 5'GTCACGTCTCCTTAATAGTA 3' R: 5'GTCACGTCTCAATAGGCAAA 3'	Miklas et al., 2000
BAC6	*X. axonopodis* pv. *phaseoli*	F: 5'TAGGCGGCGGCGCACGTTTTG 3' R: 5'TAGGCGGCGGAAGTGGCGGTG 3'	Jung et al., 1999
SU91	*X. axonopodis* pv. *phaseoli*	F: 5'CCACATCGGTTAACATGAGT 3' R: 5'CCACATCGGTGTCAACGTGA 3'	Pedraza et al., 1997
BC420	*X. axonopodis* pv. *phaseoli*	F: 5'GCAGGGTTCGAAGACACACTGG 3' R: 5'GCAGGGTTCGCCCAATAACG 3'	Yu et al., 2000
R7313	*X. axonopodis* pv. *phaseoli*	F: 5'ATTGTTATCGTCGACACG 3' R: 5'AATATTTCTGATCACACGAG 3'	Bai et al., 1997 Beattie et al., 1998
R4865	*X. axonopodis* pv. *phaseoli*	F: 5'TCCAAAGCCATTCTAGTT 3' R: 5'CAGCTACTTTCAAAC 3'	Bai et al., 1997 Beattie et al., 1998

PCR amplifications

PCR amplification for SCAR marker primers (Table 2) from genomic DNA was performed in a total reaction volume of 25 μl containing 10 ng of template bean DNA, 1X *Taq* polymerase reaction buffer, 2 mm $MgCl_2$, 0.1 mm of each dNTPs (dATP, dCTP, dGTP, and dTTP), 0.2 mM primers and 1 U of *Taq* DNA polymerase (Fermentas). Amplifications were performed in a Techne TC Plus thermocycler (Techne Inc.) programmed as follows: 4 min denaturation at 94 ^{o}C, 35 cycles of 45 sec. denaturation at 94 ^{o}C, 50 sec annealing at 45-60 ^{o}C for PCR amplification, and a 1.5 min extension at 72 ^{o}C, followed by a final extension at 72 ^{o}C for 7 min.

SCAR marker primers were used in PCR amplifications the detection of resistance genes. Amplification products were separated on 1.3% agarose gel containing ethidium bromide (0.5 μg/ml). Gels were visualized under UV light and digitally photographed with Gel Logic 212Pro imaging system (Carestream, USA). Molecular weights of ISSR-PCR products were estimated using a 100 bp Plus DNA Ladder (Fermentas).

RESULTS AND DISCUSSION

Bean is a premier plant on account of sowing area and production among other edible grain legumes (FAO, 2014). It is also extensively consumed as fresh vegetable aside from dried beans (TUIK, 2013). Bean, lentil and chick pea farming comprises 60% of the world legume production. The production of legumes in world is 58,7 million tons in 63 million ha sowing area. These numbers for bean production in Turkey is respectively 200.000 tons in 93.174 ha (TUIK, 2013; Ceylan et al., 2014; GTHB, 2016). Additionally, because of its economical and nutritional importance, the self-pollinating trait and small genome, bean is an excellent species for genetic analysis (Xu et al., 2014; El-Garhy et al., 2016). Despite being a premier plant in the world legume production, bean varieties are adapted to some farming regions and the use of certificated seed is very inadequate. In addition to this, bacterial and fungal diseases have especially increased in some farming regions (Friesen et al., 2014). For example, the hemibiotrophic fungus *Colletotrichum lindemuthianum* caused anthracnose disease and it is one of the most important diseases of bean causing serious crop loss worldwide. Anthracnose is a difficult disease to control because the pathogen shows high genetic variability, survives in cultural debris and can be efficiently transmitted through seeds (Freitas and Stadnik, 2012). Freitas and Stadnik (2012) aimed to monitor and compare race-specific and ulvan-induced defense responses to race 73 of *C. lindemuthianum* in resistant and susceptible bean plants (Freitas and Stadnik, 2012). Similarly, common bacterial blight (*X. axonopodis* pv. phaseoli) is easily spread from infected seed and is the number one foliar disease of dry bean (Friesen et al., 2014; Zhu et al., 2016). Friesen et al. (2014) investigated effects of microwave radiation on dry bean seed infected with *X. axonopodis* pv. phaseoli with and without the use of chemical seed treatment (Friesen et al., 2014). Additionally, Zhu et al. (2016) showed that QTL (quantitative trait loci) and candidate genes were associated with common bacterial blight resistance in the common bean cultivar Longyundou 5 from China (Zhu et al., 2016). The development of wilt-resistant common bean cultivars is the most cost-effective and long lasting method for controlling the disease.

Osdaghi and Rak (2015) aimed to evaluate the common bean cultivars and lines in Iran for their resistance to bacterial wilt. (Osdaghi and Rak, 2015). Molecular markers are very important for the detection of resistance against plant diseases. Meziadi et al. (2016) performed some research on the development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence (Meziadi et al., 2016). In this study, we performed PCR amplifications for the detection of *resistance* genes, using SCAR marker primers. We scanned twelve local bean varieties for four resistance genes (*SR13, ST8, SH11* and *SB10*) against *P. syringae* pv. *phaseolicola* and six resistance genes (*SAP6, BAC6, SU91, BC420, R7313* and *R4864*) against *X. axonopodis* pv. *phaseoli*. Our results revealed the presence or absence of these resistance genes in twelve bean varieties against *P. syringae* pv. *phaseolicola* and *X. axonopodis* pv. *phaseoli* bacterial pathogens (Table 3.).

Table 3. Resistance genes in twelve bean varieties (✔: Present, ✪: Mutant form)

Bean Variety	***For P. syringae* pv. *phaseolicola***				***For X. axonopodis* pv. *phaseoli***					
	SR13	ST8	SH11	SB10	SAP6	BAC6	SU91	BC420	R7313	R4865
Aslan	✔	✔	✔	✔	o	✔	o	o	o	o
Elinda	o	o	✔	✔	o	o	o	o	o	o
İstanbul	o	o	✔	✔	o	o	o	o	o	o
Bursa	o	o	✔	✪	o	o	o	o	o	o
Flash	o	o	✔	✪	o	o	o	o	o	o
Belluga	o	o	✔	✪	o	o	o	o	o	o
White cordinal	o	o	✔	✪	o	o	o	o	o	o
Beryl	✔	✔	✔	✔	✔	✔	o	o	o	o
Yunus 90	o	o	✔	✔	✔	✔	o	o	o	o
Göynük-98	o	o	✔	✪	o	o	o	o	o	o
Önceler-98	o	o	✔	✔	o	o	o	o	o	o
Eskişehir 855	o	o	✔	✔	o	o	o	o	o	o

We detected the presence of some resistance genes in twelve bean varieties. We obtained 800 bp band yield for *SH1*1, 820 bp band yield for *SAP6* (Figure 1), 525 bp band yield *SB10*, 1350 bp band yield *ST8* (Figure 2) and 1250 bp band yield *BAC6*, 1150 bp band yield *SR13* (Figure 3) with PCR amplification.

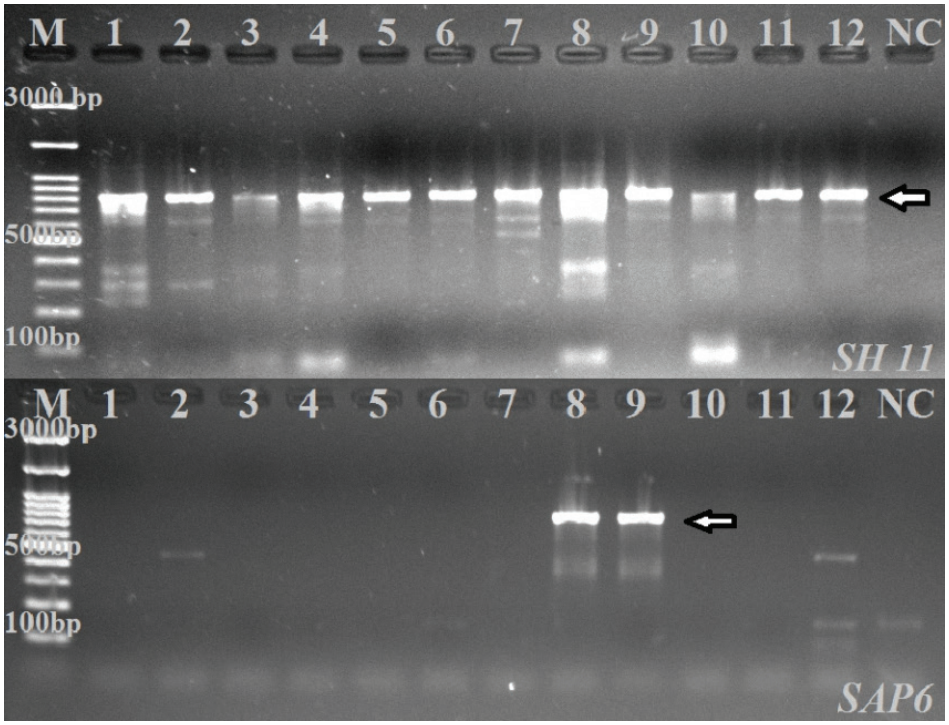

Figure 1. Agarose gel image of PCR amplification with *SH11* (800 bp yield) and *SAP6* (820 bp yield) primers. M: 100 bp DNA marker, 1-12: Bean variesties, NC: Negative control

We observed that all bean varieties include *SH11* and *SB10* genes. Aslan and Beryl varieties are more resistant to *P. syringae* pv. *phaseolicola*. These two varieties also include *BAC6* gene (Figure 3) and verify to field data against two diseases. We determined Eskişehir-855 variety as sensitive consistent with the literature.

On the contrary, while Belluga was notified resistant in the field data, this variety includes only SH11 and mutant SB10 genes.

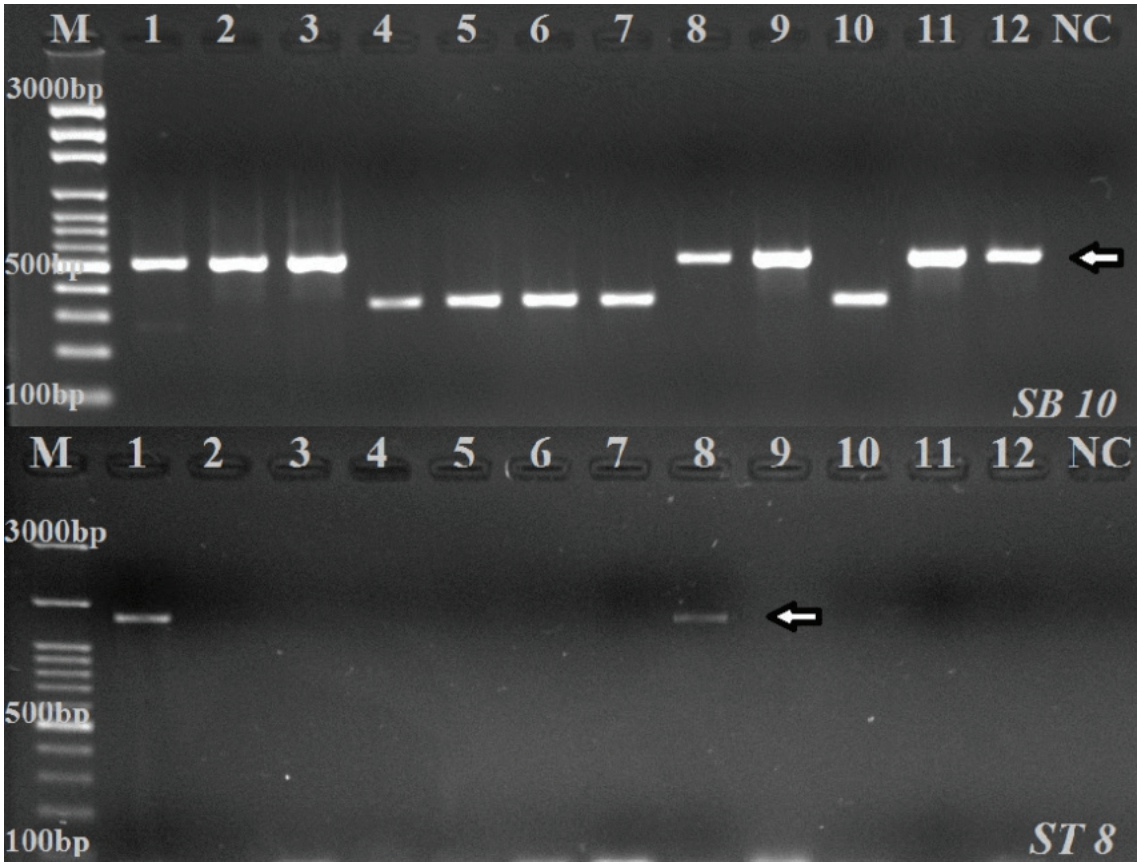

Figure 2. Agarose gel image of PCR amplification with *SB10* (525 bp yield) and *ST8* (1350 bp yield) primers. M: 100 bp DNA marker, 1-12: Bean variesties, NC: Negative control

Despite Yunus-90 is semi-sensitive, it is interesting that we determined four resistance genes in Yunus-90 against both diseases. We didn't observe any result for *SU91*, *BC420*, *R7313* and *R4864* resistance genes. Today, crop improvement programs based on plant disease resistance genes are being optimized by incorporating molecular marker techniques and biotechnology.

Therefore, plant resistance genes need to be studied extensively to decrease the existing problem of pest and diseases apart from the abiotic challenges (Gururani et al 2012).

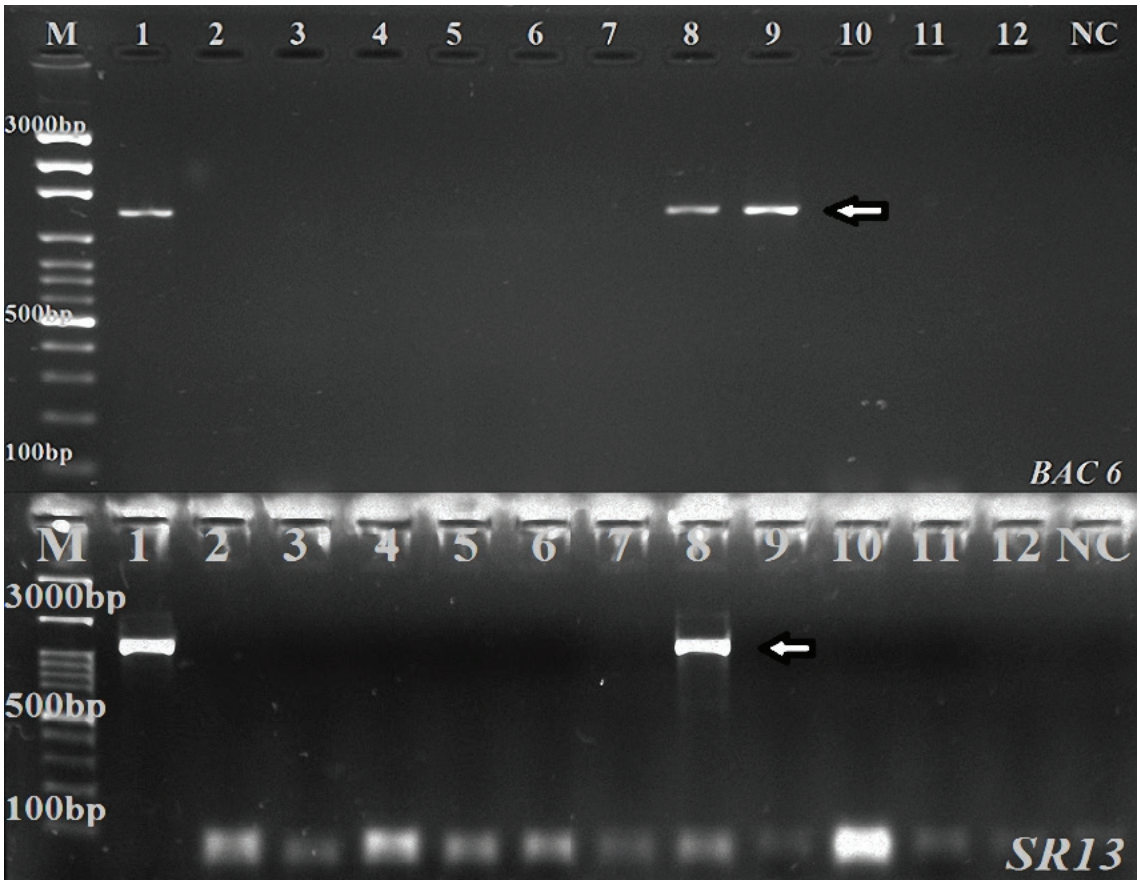

Figure 3. Agarose gel image of PCR amplification with *BAC6* (1250 bp yield) and *SR13* (1150 bp yield) primers. M: 100 bp DNA marker, 1-12: Bean variesties, NC: Negative control

CONCLUSION

The genetic research for resistance gene sources in cultivation plants has an important role for the plant breeding studies in agriculture. The genetic potential of many local bean varieties in Turkey haven't been identified yet. We observed that the SCAR markers developed to detect resistance genes against bean diseases are very useful and sensitive for breeding studies. In this study, we determined that some bean varieties as Beryl and Aslan have resistance genes against both *P. syringae* pv. *phaseolicola* and *X. axonopodis* pv. *phaseoli* pathogens.

These results are compatible with the field data of GARI. These bean varieties may be used as parental resource in bean breeding studies. Consequently, we assert that the results of this study will seriously conduce to the improvement of resistant varieties against pathogens in bean production.

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