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Screening of snap and dry bean (*Phaseolus vulgaris* L.) genotypes for resistance to Bean common mosaic virus and Bean common mosaic necrosis virus

Taze ve kuru fasulye (*Phaseolus vulgaris* L.) genotiplerinin Bean common mosaic virus ve Bean common mosaic necrosis virus'a dayanıklılık durumlarının araştırılması

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ABSTRACT

The most effective control of Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) is achieved by using the seeds of resistant cultivars. During conventional breeding, resistance against BCMV and BCMNV in common bean can be developed by pyramiding the strain-nonspecific dominant *I* gene with strain-specific recessive (*bc*-) resistance genes for long-term virus control. In this study, a total of 58 bean genotypes involving registered green and dry bean cultivars, local genotypes, and breeding lines were tested for the presence of known resistance genes. First of all, each genotype was inoculated with the NL-3 strain of BCMNV and the NL-4 strain of BCMV separately, and the plants were evaluated for the symptom appearance and tested by DAS-ELISA to confirm the presence or absence of the virus after three weeks of sap-inoculation. In the last part of the study, the resistance genes in bean genotypes were investigated by SCAR markers of SW-13 linked with the *I* gene and SBD-5 linked to *bc-1*^{2}. According to the phenotypic and molecular tests, out of 58 common bean genotypes tested, 37 involved the *I* gene, and seven and three genotypes contained *bc-2*^{2} and *bc-1*^{2} genes, respectively.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important leguminous crop in human consumption worldwide. Common bean crops can be affected by several viral agents, and Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) are among the most important viral pathogens limiting common bean production. Both viruses belong to the *Potyvirus* genus in the *Potyviridae* family, which is the largest plant virus family (Kyle and Provvidenti 1993). BCMV and BCMNV are transmitted by aphids in a non-persistent manner, seed and pollen (Galvez and Morales 1989, Silbernagel et al. 2001). In susceptible cultivars, the seed transmission ratio may reach up to 39.7-54.4% (Morales and Castano 1987). Combining the use of healthy seeds and resistant cultivars

is the most effective management method for these viruses (Drijfhout 1978, Worrall et al. 2015). BCMV and BCMNV strains are classified into eight pathogroups (PG) based on interactions of resistance genes in differential bean cultivars with pathogenicity genes (Drijfhout et al. 1978, Feng et al. 2015).

Resistance to BCMV and BCMNV in common bean are conferred by the *I* gene (Ali 1950) and six recessive alleles (*bc-1, bc-1^2, bc-2, bc-2^2, bc-3*, and *bc-u*) distributed across four loci (Drijfhout 1978). Dominant *I* gene is widely used in breeding new bean cultivars, and it is associated with either immunity or systemic vascular necrosis in the infected common bean plants (Kelly 1997). Vascular necrosis occurs as a result of hypersensitive reaction of the *I* gene-bearing plant, which is also termed "black root syndrome" or "top necrosis," which may subsequently be followed by plant death, especially in situations without protection by recessive genes (Kelly 1997, Silbernagel et al. 2001). BCMV strains are classified as necrotic and non-necrotic strains according to reactions of the *I* gene-carrying bean cultivars. The necrotic strains usually produce vascular necrosis at higher temperatures (>30 °C) in the dominant *I* gene-carrying cultivars, and this condition is called temperature-dependent necrosis (TDN). However, recently, a strain that induces top necrosis in common bean cv. Jubila carrying *I+bc-1* (Arli-Sokmen et al. 2016, Feng et al. 2014) and cvs. Amanda and Isabella having *I+bc-1^2* (Arli-Sokmen et al. 2016) at lower temperatures were identified. The *I* gene-carrying plants normally show extreme resistance against non-necrotic strains of BCMV. These plants do not exhibit any symptoms, and no virus is recoverable from inoculated leaves at typical growing temperatures and higher temperatures (McKern et al. 1992). On the other hand, when the *I* gene-carrying plants are challenged with BCMNV, vascular top necrosis occurs regardless of temperature, called temperature-independent necrosis (TIN) (Kelly 1997, Worrall et al. 2015). The dominant *I* gene provides broad-spectrum resistance; namely, it gives resistance to BCMV and some other BCMV-related potyviruses such as Watermelon mosaic virus, Cowpea aphid borne mosaic virus, Passionfruit woodiness virus-K (Fisher and Kyle 1994). Apart from the *I* gene, several recessive *bc* genes (*bc-1, bc-1^2, bc-2, bc-2^2, bc-3*, and *bc-u*) have been shown to protect bean plants against both BCMV and BCMNV strains. The *bc-u* is the strain-nonspecific helper gene and is necessary if the *I* gene is absent and for the other recessive *bc* genes to express; the rest are strain-specific genes (Drijfhout 1978, Kelly 1997). The *bc-3* gene has been shown to be translation initiation factors, *eIF4E* and *eIF*(*iso*)*4E* (Naderpour et al. 2010).

The dominant *I* gene and recessive *bc*-genes have been used to obtain virus-resistant common bean cultivars in breeding programs. Although gene pyramiding studies mostly require intensive and challenging long-term efforts, common bean genotypes with *I+bc-3* or *I+bc-2^2* gene combinations, which are known to confer resistance to most of BCMV and BCMNV strains, have been used in breeding new common bean cultivars (Drijfhout 1994, Kelly 1997). For instance, common bean genotypes carrying the *I+bc-2^2* genes respond to BCMNV and necrotic strains of BCMV by giving local necrotic lesions or limited vein necrosis (Deligoz and Sokmen 2013, Kelly 1997).

Combining the dominant *I* gene with recessive genes offers long-lasting resistance since the two types of genes have different mechanisms (Tryphone et al. 2013). Molecular markers have been used in breeding studies to incorporate monogenic resistance genes during gene pyramiding strategy for more durable resistance. Virus resistance studies based on screening by a combination of phenotypic evaluations after biological test and marker-assisted selection (MAS) have been used in plant breeding to improve new common bean cultivars (Basavaraja et al. 2020, Kelly et al. 2003, Miklas et al. 2000, Mondo et al. 2019, Ruhimbana and Mutlu 2019). Molecular markers such as Sequence Characterized Amplified Region (SCAR), Simple Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD), and Amplified Fragment Length Polymorphism (AFLP) increase the efficiency of breeding programs. Gene-specific markers have been developed and used for the *I* gene on chromosome Pv02 (Bello et al. 2014, Haley et al. 1994, Melotto et al. 1996), *bc-3* on chromosome Pv06 (Johnson and Gepts 1994, Johnson et al. 1997, Miklas et al. 1996, Mukeshimana et al. 2005, Naderpour et al. 2010) and *bc-1^2* on chromosome Pv03 (Miklas et al. 2000, Myers et al. 1996).

BCMV was detected in common bean crops in Türkiye more than thirty-five years ago (Acikgoz 1984), and it is more prevalent than BCMNV (Arli Sokmen et al. 2016, Kilic et al. 2020). There are limited studies on screening bean genotypes for resistance genes to BCMV and BCMNV in Türkiye (Cetin et al. 2021, Deligoz et al. 2013, Deligoz et al. 2021, Palacioglu et al. 2020, Yeken et al. 2018). The majority of bean genotypes grown in Türkiye have been poorly characterized for the presence of virus resistance genes.

In this study, 58 bean genotypes including dry and snap bean cultivars, local populations, and breeding lines were tested to evaluate their responses to the NL-4 strain of BCMV and the NL-3 strain of BCMNV under controlled room conditions by using a mechanical sap-inoculation method and molecular markers linked to the resistance genes, *I* and *bc-1^2*.

MATERIALS AND METHODS

Bean seed materials

In the present study, 58 bean genotypes consisting of registered bean cultivars, local populations, and breeding lines were tested. The seeds of registered snap and dry bean cultivars were supplied from private companies and research institutes of the Ministry of Agriculture and Forestry of Türkiye. The seeds of local bean populations and breeding lines were obtained from the Black Sea Agricultural Research Institute, Samsun, Türkiye. Differential varieties Sutter Pink, Redlands Greenleaf B (*bc-1*^{2}), Monroe (*bc-2*^{2}), IVT-7214 (*bc-3*), Widusa (*I*), Amanda (*I, bc-1*^{2}), IVT-7233 (*I, bc-2*^{2}), BRB-195 (*I, bc-3*) were included in the study as a control. The seeds of resistant and susceptible bean controls were supplied by USDA-ARS (United States Department of Agriculture- Agricultural Research Service).

Virus inoculum

Seeds of common bean plants infected with the NL-4 strain [Pathogroup (PG)-VII] of BCMV or the NL-3 strain (PG-VI) of BCMNV, which has been maintained since our previous study (Deligoz and Arli Sokmen 2013) were used as an inoculum source. The seeds were germinated in a plastic tray with an organic substrate and transferred into plastic pots of 7 cm in diameter. Symptomatic seedlings were tested to confirm the presence of both viruses by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), and the infected seedlings were used to inoculate ten days-old plants of susceptible bean cv. Sutter Pink for virus propagation and maintenance.

Phenotypic evaluations and serological testing

The seeds of each bean genotype to be tested were planted into plastic pot trays containing sterile compost and placed in a controlled growth room. The inoculums was prepared by grinding 1 g of leaf tissue from systemically infected plants in 10 ml phosphate buffer (1% K_2HPO_4, 0.1% Na_2SO_3, pH: 7.5) and used to inoculate Carborundum 400 mesh-dusted primary leaves of bean seedlings. At least five seedlings of each bean genotype were individually inoculated with NL-3 and NL-4 strains on different occasions to prevent strain contamination. One seedling of each genotype was also mock-inoculated as a control. Inoculated plants were kept at 25 °C (light) and 20 °C (dark) for 14 hr photoperiod. All bean genotypes tested were assessed periodically for virus symptoms and vigour up to 28 days after inoculation. Non-inoculated leaves of the plants were tested by DAS-ELISA to evaluate the presence of BCMV or BCMNV using a commercial kit (Bioreba, Switzerland) according to Clark and Adams (1977) and manufacturer's instructions. Samples were considered positive if absorbance readings (405 nm) were greater than two times those of healthy control plants using an ELISA microplate reader (Tecan Spectra II, Austria).

According to symptomatic reactions of plants challenged by NL-3 (PG VI) and NL-4 (PG VII) strains (Drijfhout et al. 1978, Kelly 1997), the presence of the dominant *I* gene and bc genes, except the *bc-3* gene, were predicted (Table 1). The plants with the obvious systemic symptom or no symptom but reacting positively in ELISA four weeks after inoculation were evaluated as susceptible, otherwise resistant.

Molecular evaluations

The presence of dominant *I* and recessive *bc-1*^{2} genes in common bean genotypes was screened by multiplex polymerase chain reaction (PCR) using SCAR markers SW-13 (Melotto et al. 1996) and SBD-5 (Miklas et al. 2000), respectively (Table 2).

Table 1. Evaluation of different common bean genotypes regarding to resistance genes after inoculation with NL-4 (BCMV) and NL-3 (BCMNV) strains (Drijfhout et al. 1978, Kelly 1997)

Genotype*	BCMNV/ NL-3	BCMV/NL-4
i	Susceptible – mosaic	Susceptible - mosaic
I	Susceptible – systemic necrosis	Resistant - no reaction
i+bc-1^2*+bc-u*	Susceptible – mild mosaic	Susceptible - mosaic
I+bc-1^2	Resistant- vein necrosis	Resistant - no reaction
i+bc-2^2*+ bc-u*	Resistant - no reaction	Susceptible – mosaic
I+ bc-2^2*+ bc-u*	Resistant- necrotic local lesion	Resistant - no reaction
i+bc-3+ bc-u	Resistant- no reaction	Resistant - no reaction
I+ bc-3	Resistant – no reaction	Resistant – no reaction

Table 2. SCAR markers used in this study

Marker	Gene	Primer Sequence (5'…3')	Size (bp)
SW-13	*I*	Forward: CACAGCGACATTAATTTTCCTTTC Reverse: CACAGCGACGAGGAGCTTATTA	690
SBD-5	*bc-1*^{2}	Forward: GTGCGGGAGAGGCCATCCATTGGTG Reverse: GTGCGGAGAGTTTCAGTGTTGACA	1300

Total genomic DNAs were extracted from bean leaves according to the protocol of DNeasy Plant Mini Kit (Qiagen, USA). The constituents of PCR reagents included 5 µl 5xPCR buffer, 2 µl 10 mM dNTPs, 0.25 µl (25 µM) each primer, 0.12 µl Taq DNA polymerase (5 u/µl), 5 µl 25 mM MgCl2 and 0.5 µl DNA (50 ng). The total reaction volume was completed to 25 µl with nuclease-free sterile water. Amplification conditions for SBD-5 and SW-13 primers were 2 min at 94 °C, 34 cycles of 10 s at 94 °C, 40 s at 66 °C, 2 min at 72 °C, and the reaction was completed with one cycle of 5 min at 72 °C (Strausbaugh et al. 2003). PCR products were visualized and photographed under the GelDoc XR system (Biorad) after running in 1% agarose gel prepared with TBE buffer at 80 mA for 90 min.

RESULTS AND DISCUSSION

Reactions of bean genotypes

Seventeen dry bean cultivars, 12 dry bean local genotypes, 16 dry bean breeding lines, and 13 green (snap) bean cultivars were analyzed for resistance to BCMV and BCMNV (Table 3). Reactions of different bean genotypes against to the NL-4 strain of BCMV and the NL-3 strain of BCMNV are recorded periodically after inoculation until the fourth week. Some bean genotypes started to show symptoms in 3-4 days on inoculated leaves and 12-16 days on non-inoculated trifoliate leaves after inoculation, depending on virus strain and bean genotypes. Systemic mosaic was the only symptom in susceptible plants after inoculation with the NL-4 strain (Figure 1). In contrast, two types of symptoms occurred in susceptible plants inoculated with NL-3; one is necrotic lesions and vein necrosis which is spread out to other parts of the plant resulting in premature death within 3-5 days (Figure 2), and the other is systemic mosaic (Figure 3). These reactions had consistency with the results of our previous studies (Deligoz and Sokmen 2013, Deligoz et al. 2021).

Figure 1. Mosaic symptom on trifoliate leaf of dry bean breeding line "TB 773" inoculated with Bean common mosaic virus NL-4 strain

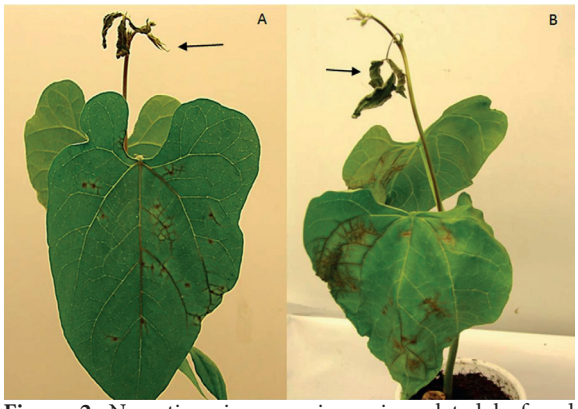

Figure 2. Necrotic vein necrosis on inoculated leaf and systemic vascular necrosis in dry bean cv."Yunus-90" (A) and breeding line "TB- 543" (B) challenged with NL-3 strain of Bean common mosaic necrosis virus. Arrow shows top necrosis

Figure 3. Mosaic symptom on trifoliate leaf of dry bean local genotype "Beyaz Bodur 25" inoculated with Bean common mosaic necrosis virus NL-3 strain

Out of the 58 dry bean cultivars tested, 36 of them showed systemic necrosis to the NL-3 strain of BCMNV, and they were found to be susceptible (Table 3). Most of the genotypes died within a couple of days after inoculation with the NL-3 strain of BCMNV due to systemic top-necrosis. However, top-necrosis progressed slowly in some bean genotypes like Noyanbey, Aras-98, Önceler-98, and Yakutiye, especially in some replicates of these cultivars that relatively stayed alive for a longer time. These genotypes did not show any visible symptoms either on inoculated or non-inoculated leaves after inoculation with NL-4 and were found to be resistant to NL-4 strain. The resistance studies showed that a total of 36 bean genotypes carried the *I* gene. Similarly, NL-3 strain caused systemic necrosis in genotypes with the unprotected *I* gene, while NL-4 strain did not infect these genotypes as obtained similarly in the studies by Kelly (1997) and Strausbaugh et al. (2003).

Table 3. Resistance genes in dry bean genotypes determined by a combination of phenotypic scoring, ELISA and molecular markers

	Phenotypic evaluation						Molecular evaluation		Proposed
	NL-3			NL-4					
	Symptom	ELISA	Reaction	Symptom	ELISA	Reaction	*I*	*bc-1²*	Genotype
Dry Bean Cultivars									
Akman 98	SN	*	S	AS	N	R	+	-	*I*
Aras 98	SN	P	S	AS	N	R	+	+	*I*
Elkoca-05	M	P	S	M	P	S	-	+	*i*
Eskişehir 855	M	P	S	M	P	S	-	+	*i*
Göynük	SN	*	S	AS	N	R	+	+	*I*
Güngör	AS	N	R	M	P	S	-	+	*i+bc-2²*
Kantar-05	AS	P	S	MM	P	S	-	+	*i+bc-1²*
Karacaşehir 90	SN	*	S	AS	N	R	-	-	*I*
Nihatbey	M	P	S	M	P	S	-	+	*i*
Noyanbey 98	SN	P	S	AS	N	R*	+	+	*I*
Önceler 98	SN	P	S	AS	N	R	+	+	*I*
Şahin 90	M	P	S	M	P	S	-	+	*i*
Şehirali 90	SN	*	S	AS	N	R	-	-	*I*
Şeker	M	P	S	M	P	S	-	+	*i*
Terzibaba	AS	P	S	MM	P	S	-	+	*i+bc-1²*
Yakutiye 98	SN	P	S	AS	N	R	+	+	*I*
Yunus 90	SN	*	S	AS	N	R	+	+	*I*
Dry Bean Local Genotypes									
Arda Şeker	SN	P	S	AS	N	R	+	+	*I*
İspir Şeker	R	N	R	M	P	S	-	+	*i+bc-2²*
Kelkit Şeker	SN	*	S	AS	N	R	+	+	*I*
Kara Yaprak	SN	P	S	AS	N	R	+	+	*I*
Ladik Şekeri	M	P	S	M	P	S	-	+	*i*
Beyaz Bodur 20	AS	N	R	M	P	S	-	+	*i+bc-2²*
Beyaz Bodur 24	M	P	S	M		S	-	+	*i*
Beyaz Bodur 25	AS	N	R	M	P	S	-	+	*i+bc-2²*
Beyaz Bodur 28	SN	N	S	AS	N	R	+	+	*I*
Beyaz Bodur 78	NL,VN,SN	P	S	AS, M	P/N	V	+	+	*I*
Beyaz Bodur 82	SN	N	S	AS	N	R	+	-	*I*
Beyaz Bodur 84	M	P	S	M	P	S	-	+	*i*
Dry Bean Breeding Lines									
TB 543	SN	*	S	AS	N	R	+	+	*I*
TB 773	AS	N	R	M	P	S	-	+	*i+bc-2²*
TB 542	SN	*	S	AS	N	R	+	+	*I*
TB 112	SN	*	S	AS	N	R	+	+	*I*
TB 198	SN	*	S	AS	N	R	+	+	*I*
TB 138	SN	*	S	AS	N	R	+	+	*I*
TB 146	SN	*	S	AS	N	R	+	+	*I*
TB 277	SN	*	S	AS	N	R	+	+	*I*
TB 280	SN	*	S	AS	N	R	+	+	*I*
TB 125	SN	*	S	AS	N	R	+	+	*I*
TB 174	SN	*	S	AS	N	R	+	+	*I*
Cranberry	SN	*	S	AS	N	R	-	+	*I*
Arjantin Şeker	SN	*	S	AS	N	R	+	+	*I*
Pinto 1	SN	*	S	AS	N	R	+	+	*I*
Pinto 2	SN	*	S	AS	N	R	+	+	*I*
CB686	SN	*	S	AS	N	R	+	+	*I*
Snap Bean Cultivars									
Nadide	SN	*	S	AS	N	R	+	+	*I*
Sofia	SN	*	S	AS	N	R	-	+	*I*
Gina	SN	P	S	AS	N	R	+	+	*I*
Öz Ayşe	M	P	S	M	P	S	-	+	*i*
Limka	SN	*	S	AS	N	R	-	+	*I*
Ferasetsiz	AS	P	S	AS	P	S	-	+	*i+bc-1²*
Helda	SN	*	S	AS	N	R	-	+	*I*
Volare	SN	*	S	AS	N	R	+	+	*I*
Yalova 5	AS	N	R	M	P	S	-	+	*i+bc-2²*
Yalova 17	AS	N	R	M	P	S	-	+	*i+bc-2²*
Kara Ayşe	M	P	S	M	P	S	-	+	*i*
Y-39 Şekerpare	M	P	S	M	P	S	-	+	*i*
Magnum	SN	*	S	AS	N	R	+	+	*I*
Control Varieties									
Sutter Pink	M	P	S	M	P	S	*	*	*i*
RGB	MM	P	S	MM	P	S	*	*	*i+bc-1²*
Monroe	AS	N	R	M	P	S	*	*	*i+bc-2²*
IVT 7214	AS	N	R	AS	N	R	*	*	*bc-2+bc-3*
Widusa	SN	*	S	AS	N	R	*	*	*I*
Amanda	VN	N	R	AS	N	R	+	+	*I+bc-1²*
IVT 7233	NL	N	R	AS	N	R	*	*	*I+bc1²+bc-2²*
BRB-195	AS	N	R	AS	N	R	*	*	*I+bc-3*

E: ELISA, R: Resistant, S: Susceptible, P: positive, N: negative, AS: Asymptomatic, M: Mosaic, MM: Mild mosaic, SN: systemic necrosis, VN: Vein necrosis, Mo: Mosaic, V: variable (reactions ranged from susceptible to resistant), +: presence of the marker, -: absence of marker, *: No data

Interestingly, the other genotype Beyaz Bodur 78 appeared to have phenotypically two different situations. Out of five plants, two died due to top necrosis; the rest showed necrotic lesions and limited vein necrosis when tested with the NL-3 strain of BCMNV. On the other hand, when Beyaz Bodur 78 was tested with NL-4 strain, two plants occurred to have mosaic and positivity in ELISA for BCMV. The rest was negative and did not show any symptoms. These results indicated that Beyaz Bodur 78 seeds had genetic heterogeneity and some plants of this genotype have the dominant *I* gene (Table 3). On the other hand, when five dry bean genotypes (Güngör, İspir Şekeri, Beyaz Bodur 20, Beyaz Bodur 25, TB-773) and two snap bean genotypes (Yalova-5 and Yalova-17) were challenged with NL-3 strain, none of them showed any symptom, and they gave negative result against BCMNV antiserum in DAS-ELISA (Table 3). These genotypes showed systemic mosaic symptoms after inoculation with NL-4 and positive result in ELISA for BCMV. These seven genotypes were evaluated to be resistant to NL-3 strain but susceptible to NL-4, similar to the control variety Monroe (Table 3). These results indicated that seven common bean genotypes are more likely to carry *bc-2²*, as shown by the studies of Drijfhout et al. (1978) and Kelly (1997) (Table 1). In our previous studies, one dry bean cultivar (Deligoz et al. 2013) and five breeding lines (Deligoz et al. 2021) were also found to be resistant to NL-3 but susceptible to NL-4.

Two dry bean cultivars (Kantar-05 and Terzibaba) did not show any visible symptoms either on inoculated or non-inoculated leaves after inoculation with NL-3, but their ELISA results were positive. Mild mosaic symptom after inoculation with NL-4 and positive ELISA results indicated that these cultivars might contain the *bc-1²* gene (Table 3). Interestingly, snap bean cultivar "Ferasetsiz" did not show any symptoms after verified by inoculation with both virus strains, but ELISA showed the presence of BCMV and BCMNV in non-inoculated leaves. These results showed that Ferasetsiz also might carry the *bc-1²* gene (Table 3). Kelly (1997) reported that varieties possessing the *bc-1²* gene may exhibit mild mosaic symptoms to NL-3 and NL4 and show delayed development of the NL-3 strain of BCMNV, with mild mosaic symptoms appearing within four to six weeks after inoculation. The absence of virus symptoms in these cultivars after inoculation with the NL-3 strain could be related to the early symptom assessment time of the fourth week after inoculation in the current study. Eleven common bean genotypes reacted positively to NL-3 and NL-4 strains in phenotypic and serological tests. They are verified as susceptible to both BCMV and BCMNV (Table 3).

According to the result of phenotypic tests, out of 58 bean genotypes tested, 37 involved the *I* gene, and seven and three genotypes contain *bc-2²* and *bc-1²* genes, respectively (Table 3). However, none of the tested bean genotypes was found to carry the *bc-3* gene. In the phenotypic evaluations completed in Türkiye so far, any common bean genotype identified to be resistant against both NL-3 and NL-4 strains are not present. However, Palacioglu et al. (2020) recently identified *bc-3* gene in three snap bean cultivars (4F-89 Fransız, 40 Günlük ve Karabacak) by using ROC-11 and eIFE4 markers.

Analysis with SCAR markers

Screening of 58 common bean genotypes for resistance to BCMV and BCMNV was conducted using SCAR markers tightly linked to the genes of resistance to these viruses. The dominant *I* gene and *bc-1²* genes were analyzed with SCAR markers, SBD-5, and SW-13, respectively. Total DNAs were extracted from all bean genotypes tested, and the presence of the resistance gene was investigated by multiplex RT-PCR. Amanda (*I+bc-1²*) was used as a control. Out of the 58 bean genotypes tested, 31 gave the expected product of 690 bp with SW-13 marker, which is linked to the *I* gene, while 25 genotypes gave the only a 1300 bp product specific for SBD-5 marker known to be linked to the *bc-1²* (Tablo 3, Figure 4). On the other hand, both the dominant *I* gene and *bc-1²*-specific products were determined in 29 genotypes, as similar to Amanda control (Figure 4) whereas none of the gene-specific products was obtained in two common bean genotypes (Şehirali 90 and Karacaşehir 90).

The results of phenotypic evaluation indicated that 37 common

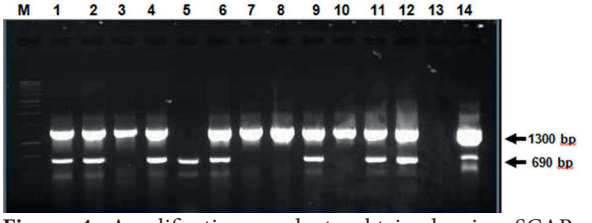

Figure 4. Amplification products obtained using SCAR markers of SW-13 and SBD-5 linked with the dominant *I* and *bc-1²* genes, respectively. M: 1 kb Ladder (Promega), 1: Göynük, 2: Aras 98, 3: Erzurum Şeker, 4: Arda Şeker, 5: Akman, 6: Önceler 98, 7: Terzibaba, 8: Şahin 98, 9: Volare, 10: Erkoca, 11: Yakutiye, 12: Amerikan Kara Yaprak, 13: Negative control, 14: Positive control (Amanda: *I+ bc-1²*)

bean genotypes had the dominant *I* gene, whereas molecular marker-based tests revealed that 31 out 37 genotypes had (84%). Deligoz et al. (2021) had similar observation with the SW-13 marker, and the success rate in identifying the *I* gene was 87% (133 out of 153) when compared to the phenotypic test (153). Palacioglu et al. (2020) investigated resistance genes in 39 common bean cultivars using DNA markers (SW-13, SBD-5, ROC11, eIFE4) and identified the *I* and *bc-1²*-related sequences in most of the cultivars, and the *b-3* gene in three cultivars. Similar to our findings, they revealed

that cvs Gina, Magnum, Önceler, Karacaşehir 90, Göynük 98, Yakutiye 98, and Aras 98 involved the *I* gene and *bc-1²* genes. However, the same SCAR marker (SW-13) failed to identify the *I* gene in common bean cv. Sofia in their study, while both phenotypic test and SW-13 marker detected the *I* gene in the present study. On the other hand, when Yeken et al. (2018) evaluated common bean cvs. Gina, Göynük, Aras 98, Akman 98, Önceler 98, and Yakutiye 98 with SW-13 marker to determine the effectiveness of it in these cultivars, they found that SW-13 marker worked well as similar to the results of the current study. However, in cvs. Güngör, Elkoca 05, Kantar 05, and Karacaşehir 90, the *I* gene was not molecularly detected by SW-13 marker in both studies. In the present study, phenotypic tests based on symptoms indicated that Karacasehir 90 might have the *I* gene (Table 3), as it was molecularly detected in this dry bean cultivar by Palacioglu et al. (2020). Other controversial results belong to snap bean cv. Helda and dry bean cv. Terzibaba. In the current study, screening resistance genes with phenotypic observations and molecular marker (SW-13) suggested that the *I* gene was not present in cv. Terzibaba, but present in cv. Helda. When Yeken et al. (2018) tested these cvs. with the same marker, they identified the *I* gene in Terzibaba, but did not in Helda. These conflicting results could be due to the low efficiency of the SW-13 marker in detecting the *I* gene indicating limitations of the use of marker. Alternatively, an SNP marker used for MAS of the *I* gene (Bello et al. 2014) could be tested for these genotypes in the future.

Phenotypic test results revealed that only three genotypes involved the *bc-1²* gene, whereas the SBD-5 marker resulted in *bc-1²* in 44 genotypes in the present study (Table 3). Yeken et al. (2018) and Palacioglu et al. (2020) reported that the SBD-5 marker gave high positive results through a polymerase chain reaction. Previous studies also reported that the results of SBD-5 marker deviated from phenotypic observations significantly (Deligoz et al. 2021, Miklas et al. 2000, Pasev et al. 2014, Strausbaugh et al. 2003). This could be attributed to the factors involving the genetic background of genotype. Therefore, phenotypic tests are necessary to confirm the results of molecular analysis.

Their rapid dispersal by aphid species and a high percentage of seed transmission make the control of BCMV and BCMNV difficult. The use of resistant plants is known to be the most economical and efficient way of virus control. In this study, 58 common bean genotypes were screened by phenotypic evaluations and molecular markers. More than half the genotypes (37) tested were found to carry the dominant I gene, while seven and three genotypes were likely to have *bc-2²* and *bc-1²*, respectively. Resistant cultivars are recommended to be grown in common bean areas where BCMV and BCMNV are problematic. Also, these resistant genotypes could be used in plant breeding as a parental source. The SBD-5 marker gave inconsistent results in some common bean genotypes to determine *bc-1²* in the present study. The use of this marker in selecting resistant plants during breeding studies seems to be not suitable. Although the SW-13 marker was found to be reasonably accurate in identifying the *I* gene, testing plants for observable traits by biological methods is recommended.

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ÖZET

Fasulyede Bean common mosaic virus (BCMV) ve Bean common mosaic necrosis virus (BCMNV)'a karşı mücadelede en etkili yol dayanıklı çeşit kullanmaktır. Fasulye ıslah çalışmalarında, dominant *I* geni ve ırka-spesifik resesif genlerin (*bc*-) kombine edilmesi ile fasulyede BCMV ve BCMNV'ye karşı uzun süreli dayanıklılık sağlanabilmektedir. Bu çalışmada kuru ve taze fasulye çeşitlerini, yerel genotipleri ve ıslah hatlarını içeren toplam 58 fasulye genotipi, BCMV ve BCMNV'ye karşı test edilmiş ve genotiplerin sahip oldukları dayanıklılık genleri araştırılmıştır. Öncelikle her bir genotip, BCMV'nin NL-4 ve BCMNV'nin NL-3 ırkı ile ayrı ayrı inokule edilmiş; inokulasyondan üç hafta sonra genotipler, ortaya çıkan simptomlara ve DAS-ELISA sonuçlarına göre değerlendirilmiştir. Çalışmanın son bölümünde; fasulye genotiplerinin içerdiği dayanıklılık genleri, *I* genine spesifik SCAR markör SW-13 ve *bc-1²* geni ile ilişkili SCAR markör SBD-5 kullanılarak araştırılmıştır. Fenotipik ve moleküler test sonuçlarına göre test edilen 58 genotipin 37'sinin *I* geni, yedi tanesinin *bc-2²* geni ve üç tanesinin ise bc-12 genine sahip olduğu ortaya konulmuştur.

Anahtar kelimeler: ELISA, mekanik inokulasyon, moleküler markör, *I* geni, *bc* genleri

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