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ORIGINAL PAPER

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PHENOTYPIC AND MOLECULAR SCREENING OF DRY BEAN (*Phaseolus vulgaris* L.) BREEDING LINES FOR RESISTANCE TO *bean common mosaic virus* AND *bean common mosaic necrosis virus*

Ilyas Deligoz✉[1], Miray Arli-Sokmen[2], Mucella Tekeoglu[3]

[1] Department of Plant Health, Black Sea Agricultural Research Institute, Samsun, Turkey

[2] Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey

[3] Department of Plant Biotechnology, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey (retired)

ABSTRACT

Bean common mosaic virus (BCMV) and *bean common mosaic necrosis* virus (BCMNV) are among the most economically important virus species infecting common bean. The use of resistant plant cultivars is the most effective way to control these viruses. In this study, 204 breeding lines were tested for resistance levels to BCMV and BCMNV. Initially, BCMNV NL-3 and BCMV NL-4 strains were individually sap-inoculated onto the leaves of bean plants belonging to each breeding lines with 10 replications, and the reactions of plants were evaluated for symptomatic appearance of virus infection 21 days after inoculation. Two sets of plants from each line were inoculated with NL-3 and NL4 respectively. Additionally, molecular markers linked to resistance genes confirmed phenotypic evaluation. As a result, 153 breeding lines were found to carry the dominant *I* gene whereas four and five of the tested lines had the recessive genes *bc-1²* and *bc-2²*, respectively. In conclusion, these resistant breeding lines could be proposed to be registered as new improved cultivars after evaluating them in terms of yield and grain quality. In addition, seeds of the resistant breeding lines can be used as the source of virus-resistant germplasm in breeding studies and be maintained at the national genebank facility in Turkey.

Key words: *Phaseolus vulgaris*, BCMV, BCMNV, dominant *I* gene, recessive genes

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is attacked by around twenty major viruses worldwide [Loebenstein and Lecoq 2012]. *Bean common mosaic virus* (BCMV) and *bean common mosaic necrosis virus* (BCMNV) are considered among the main limiting factor in terms of yield reductions in common bean. Both viruses belong to the genus *Potyvirus* and are transmitted by aphids non-persistently, also by seed and pollen [Morales and Bos 1988]. Yield reductions may reach up to 100% when susceptible cultivars are grown [Asensio et al. 2006, Verma and Gupta 2010]. Various strains of BCMV and BCMNV have been identified in bean growing areas worldwide. NL-1 (US-1), NL-2, NL-4 (US-6), NL-6 (US-4), NL-7, US-2 and US-5 are classified as strains of BCMV whereas NL-3, NL-5 and NL-8 are of BCMNV [Drijfhout et al. 1978]. The strains of BCMV and BCMNV are classified into eight pathogenicity groups (PG) in terms of their interactions with resistance genes in differential bean genotypes [Drijfhout et al. 1978, Feng et al. 2015]. Most BCMV strains were placed into PGs I, II, IV, V and VII, while BCMNV strains were placed into PGs III and VI [Drifhout et al 1978, Drijfhout 1994]. BCMV RU-1 strain has

✉ ilyasdeligoz@yahoo.com

firstly been considered as a strain of BCMNV in PG VI, but then it has been characterized as BCMV strain [Silbernagel et al. 2001]. So far, NL-1, NL-4, NL-6, NL-7, US-5 and RU-1 like strains of BCMV, NL-3 and NL-5 like strains of BCMNV were identified in Turkey [Deligoz and Arli-Sokmen 2008, Arli-Sokmen et al. 2016]. In addition, a BCMV isolate (TR-180) with different phenotype and interacting with five recessive alleles (*bc-u*, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}) was identified [Arli-Sokmen et al. 2016].

The most efficient control of these viruses is achieved through the use of resistant cultivars [Kelly et al. 1995]. Resistance is conferred by both dominant and recessive genes in common bean [Kelly 1997]. The dominant *I* gene, identified by Ali [1950], is effective against most BCMV strains. Some BCMV strains, named necrosis inducing strains, cause local or systemic necrosis (also known as top necrosis or black root) on common bean plants carrying the dominant *I* gene as a result of hypersensitive reaction generally at higher temperatures (≥30°C), which is referred to as temperature-sensitive necrosis (TSN); whereas the BCMNV causes top necrosis at room temperature conditions which is named temperature-insensitive necrosis (TIN). A new BCMV isolate (RU1-M) has been identified causing TIN below 30°C in the Jubila bean cultivar, that carries the *I* gene and a recessive gene *bc-1* [Feng et al. 2014]. The dominant *I* gene also confers hypersensitive resistance to other potyviruses (*Soybean mosaic virus* and *Watermelon mosaic virus*) [Kyle and Provvidenti 1993]. Bean breeders have made attempts to combine the dominant *I* gene and recessive resistance genes to prevent severe systemic necrosis and plant death caused by BCMNV and the necrosis inducing strains of BCMV, the resulting situation called "protected *I* gene" [Drijfhout 1994, Coyne et al. 2003]. Two of recessive genes (*bc-u*, *bc-1*, *bc-2*, *bc-3*) have two alleles, *bc-1* vs. *bc-1*^{2} and *bc-2* vs. *bc-2*^{2}. When the *I* gene is absent, the *bc-u* gene is required for the other recessive genes to be effective [Drijfhout 1978, Kelly 1997].

Until recently, the *bc-3* gene has been known to provide resistance to all known BCMV and BCMNV strains, however, a new BCMV isolate (1755a) was identified to overcome the *bc-3* gene [Feng et al. 2015]. Naderpour et al. [2010] cloned and sequenced *P. vulgaris* homologues of genes encoding the eIF proteins eIF4E, eIF(iso)4E and nCBP. Bean genotype involving *bc-3* resistance were found to carry non-silent mutations at codons 53, 65, 76 and 111 in mutated form of transcription initiation factor 4E (eIF4E) [Naderpour et al. 2010]. A combination of the dominant *I* gene with the *bc-u*+*bc-1*^{2}+*bc-2*^{2} and *bc-3* confer more stable resistance to wider spectrum of virus strains.

Molecular markers that are linked to the resistance genes have significant use to select gene combinations desired in breeding programs. They are less time consuming and less expensive comparing to greenhouse screening. Haley et al. [1994] developed a RAPD (*Random Amplified Polymorphic DNA*) marker, the OW13, linked to the *I* gene, then this marker was converted to a more reliable SW-13 SCAR (Sequence-characterized Amplified Regions) marker and adapted to breeding studies [Melotto et al. 1996]. Markers specific to *bc-3* gene were developed by Johnson et al. [1997] and Naderpour et al. [2010]. Miklas et al. [2000] used SCAR marker "SBD-5" specific for the *bc-1*^{2} gene, but, it was reported that SBD-5 was not suitable for kidney and cranberry beans.

Dry bean is the third most grown legume species after chickpea and lentil in Turkey. A total of 230,000 tons of dry bean were produced in a 848,000 ha area [TUIK 2019]. So far, about 34 dry bean cultivars have been developed in Turkey, 17 of which were screened by biological and molecular methods and nine cultivars were found to have the *I* gene, two of them carry the *bc-1*^{2} gene and one the *bc-2*^{2} gene. Out of 20 dry bean breeding lines developed by the Black Sea Agricultural Institute, seventeen and three were found to have the *I* gene and the *bc-2*^{2}, respectively [Arli-Sokmen et al. 2012]. Up to now, there is no detailed study regarding BCMV and BCMNV resistance in common bean at the seven research institutes conducting dry bean breeding studies in Turkey. In the current research, a total of 204 dry bean breeding lines selected in terms of yield and quality characters were screened and characterized for BCMV and BCMNV resistance through conventional and molecular approaches.

MATERIAL AND METHODS

Dry bean breeding lines. Seven different governmental agricultural research institutes in Turkey have

conducted national dry bean breeding studies, and advanced breeding lines have been developed by using the selected local dry bean populations and crossing studies. In this study, 204 advanced dry bean lines previously selected based on the results of field trials conducted by seven different research institutes in terms of yield and quality traits were used. Differantial varieties Widusa (*I*), UI-129 (*bc-1²*), Amanda (*I+bc-1²*), Monroe (*bc-2²*), IVT-7233 (*I+bc-1²+bc-2²*), IVT-7214 (*bc-2+bc-3*), BRB-195 (*I+bc-3*) and Long Tom (*i*) were included in the study as controls. The seeds of breeding lines were obtained from The Black Sea Agricultural Research Institute, Eastern Agricultural Research Institute, Bahri Dagdas International Agricultural Research Institute, Transitional Zone Agricultural Research Institute, Eastern Mediterranean Transitional Zone Agricultural Research Institute, Horticultural Research Institute and Maize Research Institute. The seeds of resistant and susceptible bean controls were supplied by USDA-ARS (United States Department of Agriculture-Agricultural Research Service).

BCMV and BCMNV strains. For the phenotypic evaluation of the dry bean breeding lines, NL-3 strain of BCMNV and NL-4 strain of BCMV were used. NL-4 and NL-3 strains were maintained in infected seeds of Dubbele Witte or Sutter Pink at 4°C since our previous study [Deligoz and Arli-Sokmen 2013].

Biological testing. The seeds of the dry bean breeding lines were sown in pots of 10 cm diameter containing a sterile mixture of soil and peat (1 : 1) with 10 replicates for each set of inoculation with NL3 and NL4 strains. Primary leaves of bean seedlings were mechanically sap-inoculated with NL-4 or NL-3 strains when the leaf size reached to ½–¾ of whole size. The inoculum was prepared by homogenizing infected leaf tissues in 1% K_2HPO_4 phosphate buffer containing 0.1% Na_2SO_3 (pH: 7.5) (1 g/10 ml) [Deligoz and Arli-Sokmen 2013]. Inoculated plants were maintained for 21 days in a climatized room at 20°C/25°C (light/dark) with 14 hrs photoperiod. Plants exhibiting systemic symptoms were considered as susceptible, the plants with no systemic symptom were accepted as resistant [Drijfhout 1978]. Plant reactions were recorded and evaluated according to Kelly [1997] and Drijfhout et al. [1978] based on the differential host reactions and symptom interactions of virus genetic determinants with resistance genes (Tab. 1).

Screening resistance genes by molecular markers. The presence of the dominant *I* and recessive *bc-1²* genes in dry bean lines were investigated by multiplex polymerase chain reaction (PCR) using SCAR markers. SCAR markers SW-13 and SBD-5, which were linked to the dominant *I* and *bc-1²* genes, respectively; the sequences of primers used for genotyping BCMV and BCMNV resistance are given in Table 2. DNAs were isolated from trifoliate leaves of two week-old bean plants according to the protocols of Qiagen's DNeasy DNA extraction kit and used in Multiplex PCR. Reaction mix composed of 5 µl of 5× reaction buffer, 2 µl of 10 mM dNTPs, 0.25 µM of each primer, 0.12 µl of Taq DNA polymerase (5 u/µl), 5 µl of 25 mM MgCl2, 0.5 µl DNA (50 ng) and sterile nuclease-free water to a volume of 25 µl. After a first denaturation step of 2 min at 94°C, PCR conditions consisted of 34 cycles of 10 s at 94°C, 40 s at 66°C and 2 min at 72°C. The reaction was completed by a single cycle of 5 min at 72°C in a thermal cycler (BioRad). PCR products were separated on 1% agarose gel in 1X TBE buffer and visualized with the UVP GelDoc-It 310 Imaging System. The expected lengths of amplified DNA fragments are shown in Table 2.

RESULTS AND DISCUSSION

Reactions of breeding lines to BCMNV NL-3 and BCMV NL-4 strains. A total of 204 dry bean lines were tested for resistance upon mechanical inoculation with NL-4 strain of BCMV and NL-3 strain of BCMNV. After inoculation of these lines with NL4, some lines did not produce any symptoms at temperature below 30°C, similar to the control variety Widusa (unprotected *I* gene), suggesting resistant reaction (Tab. 3). When the same lines challanged with NL3, they developed systemic necrosis and most of individuals died due to top necrosis within a week after inoculation at temperatures below 30°C (Fig. 1, Tab. 3). Similarly, in previous studies, bean genotypes carrying the unprotected *I* gene, namely without any support of *bc-1²*, *bc-2²* and/or *bc-3* resistance recessive genes, showed susceptibility profile against BCMNV NL-3 strain, but resistance to BCMV NL-4 [Drijfhout 1978, Kelly 1997, Deligoz and Arli-Sokmen 2013]. On the other hand, five breeding lines showed no disease reaction upon inoculation with NL-3 and were found to be

Table 1. The differential host reactions and symptoms of eight genotypes of common bean inoculated with the NL3 strain of BCMNV and NL-4 strain of BCMV

Plant genotype*	BCMNV-NL-3		BCMV-NL-4	
	reaction	symptom	reaction	symptom
i	susceptible	SM	susceptible	SM
I	susceptible	SN	resistant	NS
i+*bc-1*^{2}	susceptible	MM	susceptible	SM
I+*bc-1*^{2}	resistant	VN	resistant	NS
i+*bc-2*^{2}	resistant	NS	susceptible	SM
I+*bc-2*^{2}	resistant	NL	resistant	NS
i+*bc-3*	resistant	NS	resistant	NS
I+*bc-3*	resistant	NS	resistant	NS

*: the presence of the *bc-u* gene is assumed in those genotypes with additional *bc* genes but is not shown,
SM: systemic mosaic, SN: systemic necrosis, NS: no symptom, MM: mild mosaic, VN: vein necrosis, NL: necrotic local lesion

Table 2. Sequences of primers used for genotyping BCMV and BCMNV resistance

Marker	Gene	Primer sequence (5' → 3')	Size (bp)	Reference
SW-13	*I*	CACAGCGACATTAATTTTCCTTTC (F) CACAGCGACAGGAGGAGCTTATTA (R)	690	Melotto et al. 1996
SBD-5	*bc-1*^{2}	GTGCGGGAGAGGCCATCCATTGGTG (F) GTGCGGAGAGTTTCAGTGTTGACA (R)	1300	Miklas et al. 2000

resistant, however, they produced typical mosaic symptoms on non-inoculated leaves 2–4 weeks after inoculation with NL-4 strain (Fig. 2), similar to the control cultivar Monroe, suggesting the presence of *bc-2*^{2} allele [Drijfhout 1978, Kelly 1997].

Out of the 204 breeding lines, four of them displayed mild mosaic with the NL-3 strain, but developed apparent mosaic symptoms with NL-4 similar to the control differential cultivar UI-129 (possessing *bc-1*^{2} allele), which could be linked to the presence of *bc-1*^{2}. This gene (*bc-1*^{2}) might also mitigate symptom appearance following inoculation with NL-3 [Larsen et al. 2005]. Based on the biological tests (phenotypic analysis), 153 breeding lines were found to be resistant to NL-4 and other 5 to NL-3 strains, whereas 46 breeding lines were susceptible to both strains (Tab. 3). Results showed that most of breeding lines (153) had the *I* gene and other five and four breeding lines had *bc-2*^{2} and *bc-1*^{2}, respectively. Similarly, our previous studies revealed that while most of dry bean cultivars and breeding lines assessed had the dominant *I* gene just a number of them had the *bc-1*^{2} or *bc-2*^{2} gene in Turkey [Arli-Sokmen et al. 2012, Deligoz and Sokmen 2013]. So far, the recessive *bc-3* gene phenotype (resistant to NL3 and NL-4 strain) was not identified in the aforementioned studies and also in the current study conducted in Turkey. Dry bean lines evaluated in this study were previously selected by breeders based only on yield and quality traits. In Turkey, the dry bean genotypes developed by the International Center for Tropical Agriculture (CIAT) are mostly used as parents in dry bean breeding programmes. High frequency of the dominant resistance gene in dry bean lines tested in this study could be due to parental CIAT materials carrying resistance genes.

Table 3. Phenotypic and molecular evaluations of dry bean breeding lines for resistance to BCMV and BCMNV

Line	Type	BCMNV-NL3		BCMV-NL-4		Phenotypic analysis	Marker analysis	
		symptom	reaction	symptom	reaction		SW13 (*I*)	SBD5 (*bc-1*[2])
1	2	3	4	5	6	7	8	9
ESF-719	w. kidney	SN (8/10[1])	S	NS (0/10)	**R**	*I*	–	+
ESF-747	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	–
ESF-752	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
ESF-585	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
ESF-706	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	–	–
2004ITAŞ-5	g. northern	SN (7/10)	S	NS (0/10)	**R**	*I*	+	–
2004ITAŞ-10	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	–	+
09ÜKFAÇM3	g. northern	SN (8/10)	S	NS (0/10)	**R**	*I*	+	–
ESF-510	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	–	–
ESF-632	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	–	+
ESF-492	g. northern	SN (8/10)	S	NS (0/10)	**R**	*I*	–	–
2004İTAŞ-7	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
ESF-582	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
ESF-615	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
ESF-616	g. northern	SN (7/10)	S	NS (0/10)	**R**	*I*	–	+
ESF-622	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
ESF-325	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
ESF-242	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
ESF-531	pinto	SM (7/10)	S	M (8/10)	S	–	–	+
11ESKFMB/17	w. kidney	M (8/10)	S	M (7/10)	S	–	–	+
12-ESKFMB/1	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	–	–
12-ESKFMB/2	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
12-ESKFMB/6	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
12-ESKFMB/7	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
12-ESKFMB/8	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
12-ESKFMB/9	w. kidney	M (8/10)	S	M (7/10)	S	–	–	+
12ESKFMB/10	w. kidney	M (8/10)	S	M (9/10)	S	–	–	+
12ESKFMB/11	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/12	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/13	w. kidney	M (8/10)	S	M (9/10)	S	–	–	+
12ESKFMB/16	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/17	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	–	+
12ESKFMB/20	w. kidney	M (7/10)	S	M (8/10)	S	–	–	–
12ESKFMB/21	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/22	w. kidney	M (8/10)	S	M (7/10)	S	–	–	+
12ESKFMB/23	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/24	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/25	w. kidney	M (7/10)	S	M (9/10)	S	–	–	+
12ESKFMB/26	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/27	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+

1	2	3	4	5	6	7	8	9
12ESKFMB/28	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/30	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/3	w. kidney	M (9/10)	S	M (8/10)	S	–	–	+
12ESKFMB/4	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
12ESKFMB/5	w. kidney	M (8/10)	S	M (7/10)	S	–	–	+
12ESKFMB/15	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	–	+
12ESKFMB/29	w. kidney	M (7/10)	S	M (9/10)	S	–	–	+
2006 Ada1/4	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
2006 Ada1/6	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
2006 Ada1/12	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
2008 Ada1/1	w. kidney	SN (1010)	S	NS (0/10)	**R**	*I*	–	–
2008 Ada1/2	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	–	–
2008 Ada1/3	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	–	–
2008 Ada1/4	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	–	–
2009 Ada/7-4	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	–
2010 Ada/13-6	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
2010 Ada/16-1	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
2010 Ada/16-2	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
2010 Ada/16-5	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
1-Kmf-05-02	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
1-Kmf-05-08	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
8-Kmf-05-45	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
9-Kmf-07-42	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
13-Kmf-07-76	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
2-Kmf-08-16	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
5-Kmf-08-28	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
5-Kmf-08-35	w. kidney	SN (6/10)	S	NS (0/10)	**R**	*I*	+	+
Erzincan 6	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
Can 7	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
E.16	pinto	M (9/10)	S	M (8/10)	S	–	–	+
E.17	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
V18	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
EV.22	navy	NS (0/10)	**R**	M (7/10)	S	*bc-2²*	–	+
E.7	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
E.1	pinto	NS (0/10)	**R**	M (8/10)	S	*bc-2²*	–	+
E.12	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
E.24	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
EV.13	navy	M (8/10)	S	M (7/10)	S	–	–	+
E.10	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
E.11	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
E.19	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
E.3	g. northern	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
E.6	navy	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
E.23	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+

1	2	3	4	5	6	7	8	9
E.9	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
EV.17	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
E.20	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
E.8	pinto	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
E.26	g. northern	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
E.5	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
EV.16	g. northern	SN (8/10)	S	NS (0/10)	**R**	*I*	–	+
TB.166	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
TB.183	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	–
TB.179	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
TB.180	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
TB.145	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	–	–
TB.162	pinto	SN (6/10)	S	NS (0/10)	**R**	*I*	+	+
TB.168	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
TB.174	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
TB.200	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
TB.224	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
TB.158	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
TB.130	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
TB.116	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
TB.155	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	–
TB.114	g. northern	SN (8/10)	S	NS (0/10)	**R**	*I*	–	–
TB.117	g. northern	SN (8/10)	S	NS (0/10)	**R**	*I*	–	–
TB.156	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	–	–
TB.199	g. northern	SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
TB.107	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
TB.113	g. northern	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
TB.115	g. northern	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
SM.108	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
SM.107	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
SM.109	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
SM.103	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
SM.110	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
FÇ.250	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
KTAE.113	g. northern	M (8/10)	S	M (7/10)	S	–	–	+
BD.105	w. kidney	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
BD.107	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.110	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
BD.111	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
BD.112	pinto	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
BD.113	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.114	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
BD.115	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.116	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+

1	2	3	4	5	6	7	8	9
BD.117	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.118	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
BD.119	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	–	+
BD.120	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
BD.122	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.123	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
BD.124	pinto	SN (10/10)	S	NS (0/10)	**R**	*I*	–	–
BD.125	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
BD.126	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.128	pinto	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
BD.131	w. kidney	M (7/10)	S	M (9/10)	S	–	–	+
BD.133	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.134	w. kidney	SN (6/10)	S	NS (0/10)	**R**	*I*	+	+
BD.135	pinto	SN (6/10)	S	NS (0/10)	**R**	*I*	+	+
BD.136	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
BD.137	navy	SN (7/10)	S	M (10/10)	S	–	–	+
BD.138	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.139	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.142	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
BD.143	navy	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
BD.144	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
BD.147	navy	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
BD.148	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
BD.151	pinto	SN (6/10)	S	NS (0/10)	**R**	*I*	+	+
BD.152	pinto	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
BD.153	pinto	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
BD.154	pinto	SN (9/10)	S	NS (0/10)	**R**	*I*	+	+
EP.4	navy	M (8/10)	S	M (7/10)	S	–	–	+
EP.7	navy	M (8/10)	S	M (9/10)	S	–	–	+
EP.12	navy	M (7/10)	S	M (10/10)	S	–	–	+
EP.13	navy	M (8/10)	S	M (7/10)	S	–	–	+
EP.14	navy	M (10/10)	S	M (8/10)	S	–	–	+
EH.44	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
EH.42	navy	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
EH.38	navy	SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
ESD 10-2	g. northern	SN (7/10)	S	NS (0/10)	**R**	*I*	+	–
ESD 35-1	g. northern	M (7/10)	S	M (8/10)	S	–	–	+
A.13	w. kidney	M (8/10)	S	M (9/10)	S	–	–	+
A.14	w. kidney	M (7/10)	S	M (9/10)	S	–	–	+
A.20	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
A.26	w. kidney	M (8/10)	S	M (7/10)	S	–	–	–
A.27	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
A.40	w. kidney	MM (8/10)	S	M (8/10)	S	*bc-1*[2]	–	+
A.130	w. kidney	SY (0/10)	**R**	M (7/10)	S	*bc-2*[2]	–	+

1	2	3	4	5	6	7	8	9
A.341	w. kidney	M (8/10)	S	M (9/10)	S	–	+	+
A.349	w. kidney	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
A.367	w. kidney	M (8/10)	S	M (8/10)	S	–	–	+
A.378	w. kidney	M (9/10)	S	M (7/10)	S	–	–	+
K.1248	navy	M (6/10)	S	M (9/10)	S	–	–	+
K.1250	navy	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
K.1012	navy	M (9/10)	S	M (9/10)	S	–	–	+
K.1032	navy	M (7/10)	S	M (5/10)	S	–	–	+
K.1033	navy	M (8/10)	S	M (7/10)	S	–	–	+
K.1039	navy	M (6/10)	S	M (8/10)	S	–	–	+
K.1044	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
K.1046	navy	M (8/10)	S	M (9/10)	S	–	–	+
K.1047	navy	M (7/10)	S	M (8/10)	S	–	–	+
K.1048	navy	M (9/10)	S	M (8/10)	S	–	–	+
K.1083	navy	M (8/10)	S	M (9/10)	S	–	–	+
K.1084	navy	NS (0/10)	**R**	M (7/10)	S	*bc-2²*	-	+
K.1121	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
K.1128	navy	M (8/10)	S	M (8/10)	S	–	–	+
K.1133	navy	M (7/10)	S	M (10/10)	S	–	–	+
K.1154	navy	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
K.1163	navy	SN (10/10)	S	NS (0/10)	**R**	*I*	+	+
GK.322	w. kidney	SN (8/10)	S	NS (0/10)	**R**	*I*	+	+
GK.401	w. kidney	M (8/10)	S	M (9/10)	S	–	–	+
GK.314	w. kidney	NS (0/10)	**R**	M (9/10)	S	*bc-2²*	–	+
GK.315	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
GK.341	w. kidney	MM (7/10)	S	M (9/10)	S	*bc-1²*	–	+
GK.359	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	+
Suşehri Şekeri	navy	MM (8/10)	S	M (8/10)	S	*bc-1²*	–	+
Seyfe Şekeri	navy	MM (7/10)	S	M (8/10)	S	*bc-1²*	–	+
Kepsut Fasulyesi	w. kidney	M (7/10)	S	M (8/10)	S	–	–	+
Horoz Fasulye	w. kidney	SN (7/10)	S	NS (0/10)	**R**	*I*	+	–
Check Varieties								
Long Tom		M (9/10)	S	M (8/10)	S	*i*	–	–
Widusa		SN (9/10)	S	NS (0/10)	**R**	*I*	+	–
UI-129		MM (8/10)	S	M (8/10)	S	*bc-1²*	–	+
Amanda		VN (9/10)	**R**	NS (0/10)	**R**	*I+bc-1²*	*	*
Monroe		NS (0/10)	**R**	M (9/10)	S	*bc-2²*	*	*
IVT-7233		NL (8/10)	**R**	NS (0/10)	**R**	*I+bc-2²*	*	*
IVT-7214		NS (0/10)	**R**	NS (0/10)	**R**	*bc-3*	*	*
BRB-195		NS (0/10)	**R**	NS (0/10)	**R**	*I+bc-3*	*	*

[1]: number of plants with symptom/ number of plants tested, SN: systemic necrosis, M: mosaic, MM: mild mosaic, VN: vein necrosis, NL: necrotic local lesion, NS: no symptom, S: susceptible, R: resistance, +: presence of gene, – : absence of gene, *: no data

Fig. 1. Systemic top necrosis on dry bean breeding line ESF-747 carrying the dominant *I* gene after inoculation with BCMNV NL-3 strain

Fig. 2. Systemic mosaic symptoms on dry bean breeding line EV-22 carrying the recessive *bc-2*^{2} gene after inoculation with BCMV NL-4 strain

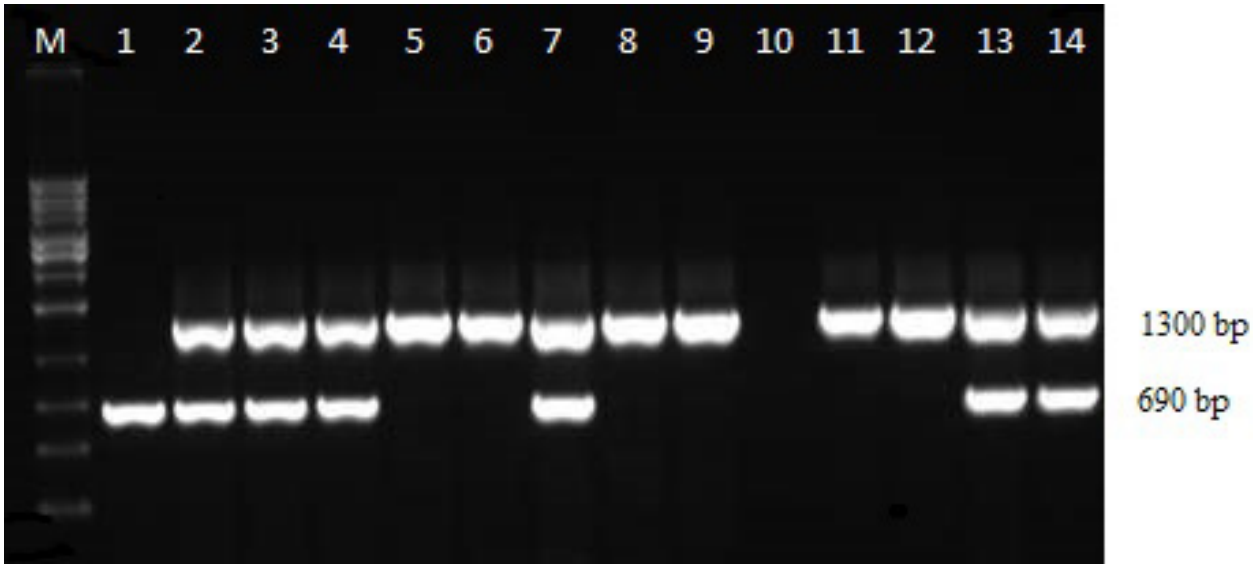

Fig. 3. Amplification products for markers SW-13 linked to *I* gene and SBD-5 linked to *bc-1*^{2} gene. M: 1 kb Ladder (Solis Biodyne); Lane 1: bean lines possessing the only *I* gene (ESF-622); Lanes 2, 3, 4, 7 and 13: bean lines possessing the *I* and the *bc-1*^{2} gene (ESF-752 ESF-585, E.17, GK-322 and K.1250); Lanes 5, 6, 8, 9, 11 and 12: bean lines possessing the only *bc-1*^{2} gene (A.40, Sefye Şekeri, EP.7, EP.12; EP.13 and A.367); Lane 10: bean line lacking resistance gene (A.26); Lane 14: positive control for *I* and *bc-1*^{2} gene (cv. Amanda)

Evaluation of resistance genes by molecular markers. PCR-based method was used to investigate resistance genes in 204 dry bean breeding lines using SCAR markers associated with the dominant *I* and recessive *bc-1*^{2} genes. The DNA fragments of the expected sizes of 690 bp and 1300 bp were obtained by SW-13 and SBD-5 markers, respectively similar to Miklas et al. [2000] and Pasev et al. [2014] (Fig. 3). When compared to phenotypic test (153 positive), the success rate of SCAR marker SW-13 in identifying the *I* gene was 87% (133 out of 153). Arli-Sokmen et al. [2012] had similar observation with SW-13 marker in

34 bean cultivars and breeding lines with the *I* gene phenotyping, only 88% of which had marker-specific product. Due to a linkage distance of ~5 cM between the *I* gene and SW-13 marker, a recombination between the *I* gene and linked SW-13 occurs [Vandemark and Miklas 2005], leading to erroneous genotyping. For this reason, a pathogen testing was recommended by Miklas et al. [2006] to confirm presence of the *I* gene.

When we screened dry bean breeding lines, by phenotyping upon mechanical inoculation with BCMNV NL-3 and BCMV NL-4 strains, results were consistently similar to what was found using the SCAR marker SBD-5, thus the *bc-1²* gene was determined only in four bean breeding lines; however, 169 dry bean breeding lines were erroneously scored as positive with SBD-5, giving a band of 1300 bp (Tab. 3). It was stated that the implementation of screening procedure with the SBD-5 marker might give limited utility [Strausbaugh et al. 2003, Pasev et al. 2014], especially with kidney and cranberry beans [Miklas et al. 2000]. In addition, our study indicated that SBD-5 marker gave erroneous results with great northern, pinto, kidney and navy beans.

CONCLUSIONS

In this study, a total of 204 advanced dry bean lines were screened biologically and molecularly to verify the presence or absence of their resistance genes to BCMV and BCMNV strains. Our study revealed that most of the breeding lines (153) carried the *I* gene, while only a group of five and four breeding lines had *bc-2²* and *bc-1²* genes, respectively. These dry bean breeding lines could be an important resistance source for breeding programmes in Turkey. The *I* gene, protects bean plants against most BCMV strains, except necrotic strains and BCMNV that display symptoms ranging from necrotic lesions and vein necrosis on inoculated leaves to systemic necrosis and plant death at temperatures generally above 30°C. The *bc-2²* gene is associated with resistance to BCMNV and most of BCMV strains, apart from the strains in pathogroup VII. A combination of resistance genes in bean cultivars is desirable for a more efficient control against to BCMV and BCMNV. Dry bean breeding lines investigated in this study have the potential to be registered and used in the future to supply the source of virus-resistant seed. Selection of parental lines carrying desirable resistance genes for breeding programs will allow breeders to develop bean genotypes involving more favourable gene combinations (*I+bc-2²* or *I+bc-3*) in the future.

PCR-based tests with SCAR markers, SW-13 and SBD-5 did not always support phenotypic assessment in our study. Our results show that, combination of molecular tests with virus inoculation studies are necessary to confirm the peresence of resistance genes.

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