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EXPLORING *I*, *bc-1²* AND *bc-3* GENE LOCUS IN PROMISING COMMON BEAN LINES

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Abstract: Common beans (*Phaseolus vulgaris* L.), known as the "poor man's meat", is an internationally important legume crop that appeals to farmers as well as consumers. Many biotic stressors such as bean common mosaic virus (BCMV), bean common mosaic necrosis virus (BCMNV) cause significant yield and quality losses in common bean. The most efficient and cost-effective way to lessen of these factors is to develop resistant cultivars. Local genotypes have been cultivated in many areas for years and have varied distinguishing characteristics as a result of spontaneous mutations. Identifying bean germplasm harboring gene sources is critical for developing resistant cultivars against BCMV and BCMNV. For this purpose, a total of 43 promising common bean lines selected from local genotypes cultivated across various regions of Türkiye were subjected to screening using diverse molecular markers (ROC11, SBD-5 and SW-13) to investigate gene sources associated with BCMV and BCMNV. The findings revealed that 21 lines had both *I* and *bc-1²* gene locus. In addition, the *bc-1²+bc-3* gene loci were discovered to be present in the 8 common bean lines. The combination of *I+bc-3* resistance genes, which guarantees immune reaction to BCMV and BCMNV, was found in only one line; YLV-32. These gene sources can be evaluated in marker-assisted breeding to develop modern cultivars resistant to BCMV and BCMNV by breeders.

Keywords: Common bean, Marker, Viral pathogens

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a diploid plant (2n = 22) and is known to be the most widely grown crop in the grain legume crops (Nadeem et al., 2021). It originates from Latin America and is domesticated independently in the Andean and Mesoamerican centers, which are characterized by large seeds and small seeds (Sperotto and Ricachenevsky, 2017). The seeds of this crop are an important source of protein, minerals and vitamins, playing a vital role in the human diet for lower-income families of the least developed countries of Africa such as Burundi, Democratic Republic of Congo, Rwanda, Uganda and Latin America such as Guatemala, Nicaragua, and El Salvador (Blair, 2013). Türkiye's historical position at the crossroads of the Palearctic region, both culturally and geographically, makes it potentially influential in the dissemination of common bean genotypes from Asia to Europe and Africa. Although Türkiye is not recognized as the primary origin or center of domestication for the common bean, the previous studies indicated a wide diversity in common bean germplasm from Türkiye (Nadeem et al., 2018; Haliloğlu et al., 2022; Özkan et al., 2022; Yeken et al., 2022). These studies revealed that the common landrace collection in Türkiye harbored a substantial amount of genetic diversity. Different local genotypes have been growing by smallholder-farmers in Türkiye for years. The wide genetic diversity of these genotypes constitutes a significant potential source of resistance to biotic stresses such as BCMV/BCMNV.

Viruses belonging to the Potyvirus genus, which is part of the *Potyviridae* family (Çelik et al., 2023a), known as the largest family of plant viruses, pose a significant threat as viral pathogens that severely impact common bean production (Kyle and Provvidenti, 1993). Aphids, seeds, and pollen all play a role in the non-persistent transmission of BCMV/BCMNV (Galvez and Morales, 1989; Silbernagel et al., 2001; El-Sawy et al., 2014; Çelik et al., 2023b). The most influential management technique for these viruses is to combine the use of healthy seeds with resistant cultivars (Drijfhout, 1978; Worrall et al., 2015). *I* gene which is the dominant (Ali, 1950) and six recessive alleles (*bc-u*, *bc-1*, *bc-1²*, *bc-2*, *bc-2²* and *bc-3*) spreaded throughout four loci, providing resistance to BCMV/BCMNV (Drijfhout, 1978). Hence, the *I* gene and *bc*-genes have been performed to obtain virus-resistant common bean cultivars in breeding programs. Containing *I+bc-3* or *I+bc-2²* gene combinations, which are known to give resistance to the majority of BCMV/BCMNV strains, have been employed in the

development of novel common bean cultivars (Drijfhout, 1994; Kelly, 1997).

In breeding programs, molecular markers linked to resistance genes for BCMV/BCMNV are commonly utilized to efficiently select desired gene combinations. This approach is more cost-effective and time-efficient compared to greenhouse screening methods (Deligoz et al., 2021). In literature, various specific markers associated with resistance genes were reported such as SW-13 SCAR (Sequence-characterized Amplified Regions) marker for *I* gene, ROC11 SCAR marker for *bc-3* gene, and SBD-5 SCAR marker for the *bc-1²* gene by Melotto et al. (1996), Miklas et al. (2000) and Johnson et al. (1997), respectively. These markers were used to discover desired genes to develop common bean varieties resistant to BCMV/BCMNV in diverse investigations (Deligöz ve Sökmen, 2013; Pasev et al., 2014; Yeken et al., 2018; Palacıoğlu et al., 2020; Deligöz et al., 2022). While previous studies have identified desirable genes for BCMV/BCMNV in various genetic resources within Türkiye, conducting a comprehensive investigation across different germplasm collections is of utmost importance. Thus, the objective of this study was to examine the presence of *I*, *bc-1²*, and *bc-3* genes in promising common bean lines selected from local genotypes in different provinces of Türkiye. The findings will support further breeding studies for developing new common bean cultivars resistant to BCMV/BCMNV.

2. Materials and Methods

2.1. Plant Material

The forty-three common bean lines selected as promising according to yield and yield components from local genotypes which were collected from different provinces of Türkiye in projects previously conducted projects supported by TUBITAK (Project ID: 115R042 and 109O163) were used as material in the study. The Cornell 49–424, Perry Merrow, (MDRK) Michigan Dark Red Kidney, BMN-RMR-13 (Reg. Number: GP-252, PI 642019, USDA-ARS Beltsville Agricultural Research Center, Beltsville, MD; Pastor-Corrales et al., 2007) were included in the study as control. These genotypes obtained from the United States Department of Agriculture Research Service (USDA-ARS). The information of the common bean lines used in the study was presented in Table 1.

Table 1. The passport data of common bean lines used in the study

No	Lines	Collection Site	District	Altitude(m)	Coordinates
1	Balıkesir-3	Balıkesir	Manyas	428	40° 5'58.61"N/27°56'16.65"E
2	Balıkesir-4	Balıkesir	Manyas	30	40° 7'16.68"N/27°51'15.26"E
3	Balıkesir-19	Balıkesir	Sındırgı	1051	39°18'52,7"N/28°32'53,1"E
4	Bilecik-7	Bilecik	Pazaryeri	876	39°59'12.52"N/ 29°51'7.17"E
5	Bingöl-8	Bingöl	Merkez	1154	39°02'06.1"N/ 40°27'14.4"E
6	Bingöl-12	Bingöl	Merkez	1542	39°02'25.2"N/ 40°29'08.1"E
7	Bingöl-23	Bingöl	Kiğı	1489	39°17'11.8"N/ 40°20'04.0"E
8	Bingöl-25	Bingöl	Solhan	1176	38°54'29.4"N/ 40°56'39.9"E
9	Bingöl-50	Bingöl	Yedisu	-	-
10	Bitlis-5	Bitlis	Hizan	1629	38°13'35.5"N/ 42°25'14.4"E
11	Bitlis-68	Bitlis	Mutki	1303	38°29'08.1"N/ 41°48'17.4"E
12	Bitlis-123	Bitlis	Güroymak	1615	38°30'09.8"N/ 42°07'10.5"E
13	Bitlis-124	Bitlis	Güroymak	1615	38°30'09.8"N/ 42°07'10.5"E
14	Bursa-3	Bursa	Yenişehir	377	40°10'18.45"N/29°37'15.12"E
15	Bursa-4	Bursa	İnegöl	327	40°4'18.83''N/29°26'47.76''E
16	Bursa-21	Bursa	Kestel	435	40° 7'39.19"N/ 29°21'9.43"E
17	Bursa-22	Bursa	Kestel	360	40°10'2.02"N/ 29°18'58.01"E
18	Bursa-23	Bursa	Kestel	360	40°10'2.02"N/ 29°18'58.01"E
19	Bursa-24	Bursa	Orhaneli	487	39° 48' 8.96''N/29° 2' 7.45''E
20	Çanakkale-2	Çanakkale	Yenice	320	39°57'6.22"N/ 27°10'54.75"E
21	Çanakkale-4	Çanakkale	Biga	25	40°14'38.70"N/ 27°22'17.65"E
22	Çanakkale-6	Çanakkale	Biga	25	40°17'26.36"N/ 27°25'14.56"E
23	Çanakkale-8	Çanakkale	Bayramiç	100	39°44'15.48"N/ 26°41'34.82"E
24	Düzce-2	Düzce	Merkez	859	40°42´28.42"N/31°13´33.16"E
25	Düzce-3	Düzce	Merkez	859	40°42´28.42"N/31°13´33.16"E
26	Elazığ-11	Elazığ	Maden	1266	38°30'43.2"N/ 39°33'06.4"E
27	Hakkâri-8	Hakkâri	Merkez	2096	37°36'02.0"N/ 43°41'24.1"E
28	Hakkâri-18	Hakkâri	Merkez	1135	37°29'45.6"N/ 43°34'58.7"E
29	Hakkâri-51	Hakkâri	Merkez	1601	37°25'15.7"N/ 43°33'45.9"E
30	Hakkâri-76	Hakkâri	Merkez	1135	37°29'45.6"N/ 43°34'58.7"E

Table 1. The passport data of common bean lines used in the study (continue)

No	Lines	Collection Site	District	Altitude(m)	Coordinates
31	Malatya-20	Malatya	Doğanşehir	1410	38°03'22.1"N/ 37°44'60.0"E
32	Malatya-25	Malatya	Doğanşehir	1235	38°06'54.4"N/ 37°54'54.7"E
33	Malatya-44	Malatya	Akçadağ	1158	38°15'20.6"N/ 37°55'37.5"E
34	Muş-24	Muş	Hasköy	1350	38°41'04.4"N/ 41°40'25.8"E
35	Muş-48	Muş	Bulanık	1550	39°03'41.7"N/ 42°19'04.2"E
36	Tunceli-8	Tunceli	Pertek	-	-
37	Van-17	Van	Çatak	1702	38°05'51.7"N/ 43°15'37.3"E
38	Van-19	Van	Çatak	1629	38°01'34.2"N/ 43°09'10.9"E
39	Van-49	Van	Gürpınar	1748	38°19'09.1"N/ 43°24'09.6"E
40	Van-64	Van	Bahçesaray	1702	38°06'15.3"N/ 42°51'00.7"E
41	Yalavo-14	Yalova	Çiftlikköy	125	40°39´33.41"N/29°24´36.19"E
42	Yalova-28	Yalova	Merkez	362	40°33'12.70"N/29°12'52.17"E
43	Yalova-32	Yalova	Merkez	428	40°33'38.32"N/29°19'34.07"E

2.2. DNA Extraction

Total DNA was extracted by employing a buffer solution consisting of 125 mM, Tris-HCl pH 8.0, 0.8 M NaCl, 25 mM EDTA pH 8.0, 1% sarcosyl, 1% CTAB, 0.5% sodium disulphite, and 2% PVP-40 (K29-32) following the DArT DNA isolation methodology effectively isolating the genetic material (http://www.diversityarrays.com). After diluting the total DNA to a concentration of 20 ng/L, its quality and concentration were evaluated using a DS-11 FX+ spectrophotometer (DeNovix Inc., USA).

2.3. The Analysis of SCAR Markers

PCR amplifications were carried out in a 20 µL reaction volume, combining 10x PCR buffer, 0.3 µM primer 0.2 µM dNTPs, 1.5 mM $MgCl_2$, 10-20 ng DNA, and 1U Taq DNA polymerase. Amplification reactions were conducted utilizing a T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The temperature profile for PCR amplification in SCAR markers can be found in Table 2. The amplified fragments were resolved on a 1.4% (w/v) agarose gel in 1x TAE buffer. Subsequently, the gel was stained with ethidium bromide, and the fragments were visualized under UV light and documented using the G:BOX F3 gel documentation system (Syngene, Synoptics Ltd., Cambridge, UK). The sizes of the PCR products were determined by using the GeneRuler 100 bp Plus DNA ladders (Thermo Scientific) as reference standards. The presence (+) or absence (-) of amplification products associated with resistance genes was determined in this study.

3. Results and Discussion

Using SCAR markers tightly linked to the resistance genes to BCMV/BCMNV, the forty-three common bean lines were screened for resistance to these viruses. The dominant *I* gene in common bean lines was analyzed using SCAR marker, SW-13. Out of the 43 common bean lines tested, 22 gave the expected product of 690 bp with SW-13 marker, which is related to the *I* gene, as similar to BMN-RMR-13 control (Figure 1). The gene-specific products were not determined in 21 common bean lines and MDRK control (Table 3). The results of the PCR test with SBD-5 marker SBD-5 gave a positive product with 1250 bp for the *bc-1²* gene in all lines (except for Yalova-32). The positive and negative products were also obtained from Perry Marrow and Cornell 49–424, respectively. On the other hand, SCAR marker ROC11 known to be related to the recessive *bc-3* gene was also analyzed in all lines. Findings revealed that 34 common bean lines gave the 350 bp amplified product with this marker, as similar to Perry Marrow (Figure 1; Table 3).

Negative signals were also determined in 9 common bean lines (Balıkesir-19; Bingöl-8; Bingöl-23, Bingöl-50; Bursa-22, Bursa-23, Hakkari-76, Malatya-44 and Yalova-32) and Cornell 49–424 control. The absence of signal reveals the presence of *bc-3* in this marker. Thus, these lines had the recessive *bc-3* gene. The same DNA fragments of the expected size of SW-13, SBD-5 and ROC11 markers were obtained in previous studies. For instance, Yeken et al. (2018) investigated *bc-1²*, *I* and *bc-3* genes using molecular markers in 43 common bean cultivars. Findings revealed that 32 cultivars had the dominant *I* gene, while 40 cultivars contained the *bc-1²* gene. In another study, Palacıoğlu et al. (2020) investigated resistance genes in 39 common bean cultivars using SW-13, SBD-5, ROC11 and eIFE4 markers. They determined the *I+bc-1²* genes in most of the cultivars, and the *bc-3* gene in three cultivars.

SCAR markers were utilized to investigate the presence of the dominant *I* and recessive *bc-1²* genes in a set of 204 dry bean breeding lines (Deligöz et al., 2021). Very recently, Deligöz et al. (2022) screened *I+bc-1²* genes in 58 common bean genotypes using SBD-5 and SW-13 markers, respectively. Out of the tested genotypes, 31 had both the dominant *I* gene and *bc-1²* specific products. In the current study, the combinations of *I+bc-1²* and *bc-1²+bc-3* resistance genes were detected in screened 21 and 8 lines, respectively. However, it was observed that no common bean landrace possessed all three resistance genes.

Table 2. The sequences and PCR conditions of primers associated with resistance genes

Primers	Gene Locus	Marker	Primer sequences (5′–3′)	Band Length	PCR Conditions	References
SW-13	*I*	SCAR	CACAGCGACATTAATTTTCCTTTC CACAGCGACAGGAGGAGTTTA	690	95°C 4 min, 94°C 10 s, 60°C 40 s, 72°C 2 min 35 cycles, 72°C 5 min	Melotto et al. (1996)
SBD-5	*bc-1²*	SCAR	GTGCGGAGAGGCCATCCATTGGTG GTGCGGAGAGTTTCAGTGTTGACA	1250	95°C 4 min, 94°C 10 s, 65°C 40 s, 72°C 2 min 35 cycles, 72°C 5 min	Miklas et al. (2000)
ROC11	*bc-3*	SCAR	CCAATTCTCTTTCACTTGTAACC GCATGTTCCAGCAAACC	350/420	95°C 4 min, 94°C 10 s, 65°C 10 s, 72°C 30 s 35 cycles 72°C 5 min	Johnson et al. (1997)

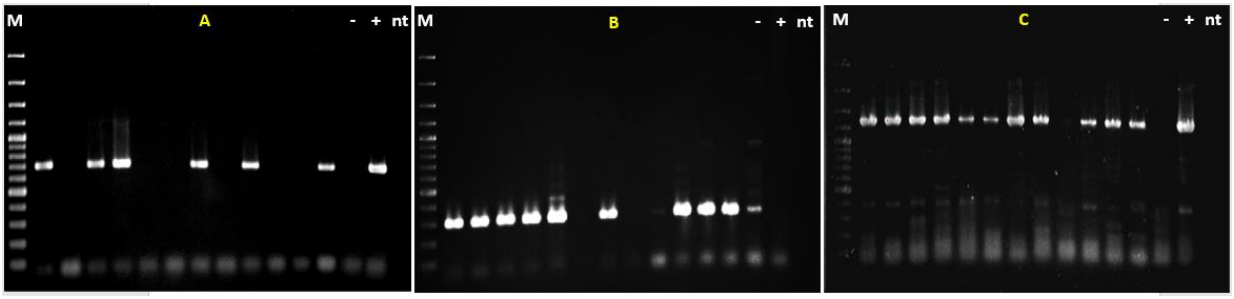

Figure 1. Band profiles obtained as a result of amplification of genes linked to resistance to BCMV/BCMNV in common bean with specific primers. (A: specific PCR product obtained as a result of amplification of dominant *I* gene with SW-13/690 bp marker, MDRK (-), BMN-RMR-13 (+), B: specific PCR product observed by amplification of *bc-3* gene with ROC11/350 bp marker, Perry Marrow (-), Cornell 49–424 (+), C: amplification of *bc-1²* gene with SBD-5/1250 bp marker the resulting specific PCR product, Cornell 49–424 (-), Perry Marrow (+), M: 100 bp DNA ladder).

Table 3. Resistance sources of common bean lines to BCMV and BCMNV

Lines	*I* (SW-13/690 bp)	*bc-3* (ROC11/350-420 bp)	*bc-1²* (SBD-5/1250 bp)	*I+bc-1²*	*bc-3+bc-1²*
Balıkesir-3	+	-	+	*	
Balıkesir-4	+	-	+	*	
Balıkesir-19	-	+	+		*
Bilecik-7	+	-	+	*	
Bingöl-8	-	+	+		*
Bingöl-12	-	-	+		
Bingöl-23	-	+	+		*
Bingöl-25	-	-	+		
Bingöl-50	-	+	+		*
Bitlis-5	+	-	+	*	
Bitlis-68	+	-	+	*	
Bitlis-123	+	-	+	*	
Bitlis-124	+	-	+	*	
Bursa-3	-	-	+		
Bursa-4	+	-	+	*	
Bursa-21	+	-	+	*	
Bursa-22	-	+	+		*
Bursa-23	-	+	+		*
Bursa-24	+	-	+	*	
Çanakkale-2	-	-	+		
Çanakkale-4	-	-	+		
Çanakkale-6	+	-	+	*	
Çanakkale-8	-	-	+		
Düzce-2	+	-	+	*	
Düzce-3	-	-	+		

Table 3. Resistance sources of common bean lines to BCMV and BCMNV (continue)

Lines	*I*	*bc-3*	*bc-1²*	*I+bc-1²*	*bc-3+bc-1²*
	(SW-13/690 bp)	(ROC11/350-420 bp)	(SBD-5/1250 bp)		
Elazığ-11	-	-	+		
Hakkâri-8	+	-	+	*	
Hakkâri-18	-	-	+		
Hakkâri-51	-	-	+		
Hakkâri-76	-	+	+		*
Malatya-20	+	-	+	*	
Malatya-25	+	-	+	*	
Malatya-44	-	+	+		*
Muş-24	+	-	+	*	
Muş-48	-	-	+		
Tunceli-8	+	-	+	*	
Van-17	-	-	+		
Van-19	+	-	+	*	
Van-49	+	-	+	*	
Van-64	+	-	+	*	
Yalavo-14	+	-	+	*	
Yalova-28	-	-	+		
Yalova-32	+	+	-		

In contrast, previous studies conducted by different researchers have consistently demonstrated that the combination of the dominant *I* gene and the recessive *bc-3* gene, each offering distinct mechanisms of resistance and provides comprehensive and potentially durable resistance to strains of BCMV and BCMNV (Melotto et al., 1996; Pastor-Corrales et al., 2007; Pasev et al., 2014). In the current investigation, only one landrace, YLV-32, exhibited the combination of the recessive *bc-3* gene and the dominant *I* gene, providing an immune response to BCMV and BCMNV. Therefore, YLV-32 has potential as a parental source in marker-assisted selection for plant breeding purposes. In a previous study, Palacıoğlu et al. (2020) noted that no common bean cultivars have both *I* and *bc-3* resistance genes in tested cultivars. This finding revealed that local genetic resources provide an excellent source of various genes and could be played a key role in plant breeding to develop new common bean cultivars resistant to BCMV and BCMNV. In this context, more in-depth investigations in different common bean germplasm of Türkiye will reveal the genetic richness and provide significant contributions to developing modern resistant cultivars to BCMV and BCMNV.

4. Conclusion

BCMV and BCMNV are the most important viral pathogens that significantly mitigate common bean production. The most economical and efficient way of virus control is to use resistant plants. To develop resistant plants for these viral pathogens, local genetic resources have potential reservoirs of novel alleles. In this study, 43 common bean lines derived from local genotypes in different provinces of Türkiye were evaluated for gene sources associated with BCMV and BCMNV by using molecular markers (ROC11, SBD-5, and SW-13). The *I*, *bc-3* and *bc-1²* resistant genes were found in the numbers of 22, 9, and 42 common bean lines, respectively. The combination of *I+bc-3* resistance genes, which guarantees immune reaction to BCMV and BCMNV, was found in only one line; YLV-32. This unique material could be used as a parent to develop new common bean cultivars in breeding studies.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	O.E.	M.Z.Y.	A.Ç.	V.Ç.
C	25	25	25	25
D	25	25	25	25
S				100
DCP	40	30	30	
DAI		50	50	
L	20	40	40	
W		60	40	
CR		50	50	
SR		50	50	
PM	25	25	25	25
FA	25	25	25	25

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this

study because of there was no study on animals or humans.

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