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Research Article

Screening of Bean Genotypes Against Bean Common Mosaic Virus (BCMV) by Artificial Inoculation and Molecular Confirmation

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Abstract: Bean mosaic virus (BCMV) is a widespread plant pathogen that causes significant bean yield losses in several bean-growing regions worldwide. The use of resistant common bean varieties to BCMV is considered the most efficient and feasible approach to control its effects. Numerous genes and molecular markers associated with resistance to these pathogens have been discovered and used extensively in breeding studies around the world. Screening bean genotypes for resistance to these viruses is a critical step in developing resistant varieties. The goals of the study are to identify virus sources in the region and artificially inoculate Lake Van basin bean genotypes with BCMV. The recovered BCMV strain NL-4 was inoculated with 45 bean cultivars, most of which originated from the Lake Van basin in Turkey. Differentiation between resistant and susceptible was based on visual symptoms, and of the 45 genotypes, 29 were found to be resistant to NL-4, while 16 genotypes were susceptible (8 of them moderately susceptible and 8 of them highly susceptible).

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a vital staple crop cultivated in both developed and developing countries. It holds great significance as the most prominent grain legume in the human diet worldwide, primarily intended for human consumption. In terms of calorie contribution, beans rank second after maize, making them a substantial source of nutrition (CGIAR, 2015). Common beans, which are widely distributed in Türkiye, have a broad range of variations, and Türkiye is an important bean-producer country (Erdinç et al., 2017a). Common bean is attacked by several abiotic and biotic stress factors (Erdinç et al., 2017b; Ekincialp and Şensoy, 2018; Bilge et al., 2019; Kıpçak et al., 2019; Tunçtürk et al., 2019). The application of molecular markers in plant breeding programs facilitates the improvement resistant many different viruses (Ibrahim and Erdinç, 2020). Bean common mosaic virus (BCMV), a

member of the genus Potyvirus in the family Potyviridae, is one of the main viruses that damage common beans (Tang and Feng, 2023). A bean seed-borne and aphid-transmitted virus, BCMV causes devastating losses in crop yields and quality. The spread of the virus around the world can occur in nonpersistent ways via the spread of seeds, pollen, and certain aphid species (Morales and Bos, 1988). Genetic resistance is the most successful and long-term management strategy for combating BCMV (Kelly et al., 1995). Bean is also produced in Lake Van Basin which compromises rich bean genotypes which might contain resistance to BCMV. Molecular and morphological (phenotypic evaluation) markers have been increasingly used in plant genetics and breeding research in recent years (Deligöz and Sökmen, 2013; Mangeni et al., 2014; Erdinç et al., 2017b; Ekincialp and Şensoy, 2018; Hatipoğlu and Şensoy, 2022; Uçar and Şensoy, 2022; Usta et al., 2023).

The geographical diversity of bean production, such as the Lake Van Basin in Turkey, presents a unique opportunity to discover natural resistance traits within bean genotypes. In light of these challenges and opportunities, the primary aim of our study is to comprehensively investigate the reactions of the Lake Van Basin's bean genotypes to a selection of BCMV strains. We endeavor to employ a multifaceted approach, combining molecular and classical methods, to assess the responses of these genotypes. Through rigorous artificial inoculation with BCMV, we intend to scrutinize their resistance and susceptibility patterns. By delving into the genetic and phenotypic makeup of these genotypes, we aim to uncover potential resistance mechanisms and markers that could be harnessed to bolster BCMV resistance in common bean cultivation. This study seeks to bridge the gap between theory and practice by elucidating the intricate interactions between BCMV strains and the diverse bean genotypes of the Lake Van Basin. Through an in-depth exploration of their responses, we aspire to contribute valuable insights and tangible solutions to the persistent challenge of BCMV in common bean cultivation, ultimately safeguarding this crucial global food resource.

2. Materials and Methods

A total of 45 bean genotypes were used in the present study (Table 1).

2.1. Culture of BCMV strains and mechanic inoculation of bean genotypes

The Ru-1 strain and NL-4 strain were obtained Directorate of Plant Protection Central Research Institute (Ankara) and artificially inoculated on the 20 plants of the susceptible Stringless Green Refuges (SGR) cultivar in March 2021. The climate chamber room was prepared at a temperature of 26°C with a 16/8 h day/night photoperiod with 60% moisture.

Forty-five bean genotypes were grown in a climate chamber. The seeds of the bean cultivars used in the study were divided into two groups the first group inoculation by NL-4 strain of BCMV included three replications in a randomly replicated experimental design each included 10 seeds per variety and the second group (control) included one replication that included two seeds per variety. Phosphate buffer containing 1% K_2HPO_4 and 0.1% Na_2SO_3 (pH: 7.2) was used (Sengooba et al., 1997). Ten days after planting, the first true leaves were transmitted by mechanical inoculation.

The cultivars were inoculated in 3 replications each replication consisted of 10 plants per genotype and each plant was inoculated with two primary leaves. Four control plants were used for each replication.

Inoculated leaves were washed under tap water and placed in a climate room set at 22 °C ±1 and 12 hours photoperiod against the possibility of the presence of strains that can cause temperature-induced necrosis in the samples.

2.2. Total RNA extraction and cDNA synthesis

Total RNA extractions were conducted using approximately 0.1 g of frozen leaf tissues following the protocol described by Foissac et al. (2001) with minor adjustments. Specific forward and reverse primer sets were adopted from Bhadramurthy and Bhat (2009) which are designed within the coat protein gene (CP), to detect BCMV in the common bean leaf tissues and synthesized to the relevant company (Oligomer/Turkey). In all cDNA processing, a random primer (9mer) was also utilized (Table 2).

Table 1. Passport data of bean genotypes used in the study

Genotype No	Name of Genotype	Provided Place/cultivar name	Growth habit
G.1	Stringless Green Refuges	USDA	Bush
G..2	U1-36 Red mex	USDA	Pole
G.3	Redland green leaf	USDA	Bush
G.4	U1-111	USDA	Pole
G.5	Pinto 114-8	USDA	Pole
G.6	Jubila	USDA	Bush
G.7	Monroe	USDA	Pole
G.8	TR 66342 (Afyon)	Cukurova Univ.	Pole
G.9	TR 68587 (Eskişehir)	Cukurova Univ.	Pole
G.10	Line 10 (USA)	Cukurova Univ.	Pole
G.11	Sarıkız Fasulyesi	Cukurova Univ.	Pole
G.12	Gülnar-II (Barbunya)	Cukurova Univ.	Pole
G.13	Gülnar-VI	Cukurova Univ.	Pole
G.14	France-Gandiyam (Sarı meyve)	Cukurova Univ.	Pole
G.15	F1 103 950	Anadolu Agr. Res.Inst.	Bush
G.16	Van-Merkez	University of Van YYU	Pole
G.17	Van-Merkez	University of Van YYU	Pole
G.18	Van-Merkez	University of Van YYU	Pole
G.19	Bitlis-Tatvan	University of Van YYU	Pole
G.20	Bitlis-Tatvan-Gevar	University of Van YYU	Pole
G.21	Bitlis-Hizan	University of Van YYU	Bush
G.22	Bitlis-Tatvan	University of Van YYU	Pole
G.23	Bitlis-Tatvan	University of Van YYU	Pole
G.24	Van-Erciş-Purmak	University of Van YYU	Pole
G.25	Van-Erciş-Çelebibağı	University of Van YYU	Pole
G.26	Van-Erciş	University of Van YYU	Pole
G.27	Van-Gevaş-G.konak	University of Van YYU	Pole
G.28	Van-Gevaş-G.konak	University of Van YYU	Bush
G.29	Van-Gevaş	University of Van YYU	Pole
G.30	Van-Gevaş	University of Van YYU	Bush
G.31	Bitlis-A.cevaz	University of Van YYU	Pole
G.32	Bitlis-Adilcevaz	University of Van YYU	Pole
G.33	Melisa	University of Van YYU	Pole
G.34	Aysel	University of Van YYU	Bush
G.35	Alman Ayşe	University of Van YYU	Pole
G.36	Karacaşehir-90	University of Van YYU	Pole
G.37	Terzibaba	University of Van YYU	Bush
G.38	Şehirali-90	University of Van YYU	Bush
G.39	Şeker fasulye	University of Van YYU	Pole
G.40	Önceler 98	University of Van YYU	Bush
G.41	Efsane	University of Van YYU	Bush
G.42	Magnum	University of Van YYU	Bush
G.43	Van-Gevaş	University of Van YYU	Pole
G.44	Van-Edremit	University of Van YYU	Pole
G.45	Van-Bahçesaray	University of Van YYU	Bush

Table 2. Primer Information for RT-PCR Detection of BCMV.

Virus		Sequence (5'-3')	Length base	Reference
BCMV	BCMV-F	5'-GGATGCGGAGAATCTGTG-3'	850 bp	Bhadramurthy and Bhat, 2009
	BCMV-R	5'-GATTGACGTCCCTTGCAG-3'		

For the first-strand cDNA synthesis, the total RNAs that were extracted were utilized. To summarize the procedure, the following steps were followed:

1. In a nuclease-free microfuge tube, 1 µl of random primer (20 pmol/µl) was added as a template.

2. 5 µl of total RNA and 1 µl of dNTP (10 mM) were added to the tube.
3. The volume was completed to 12 µl with nuclease-free water.
4. The mixture was incubated at 65 °C for 5 minutes.
5. Subsequently, the mixture was chilled on ice.

To prepare the reaction mixture, 4 µl of 5X RT Reaction buffer, 2 µl of 0.1M DTT, 1 µl of RNAse inhibitor, and 1 µl of Reverse Transcriptase enzyme (Thermo Scientific, USA) were added, bringing the total volume to 20 µl. The reverse transcription (RT) step was carried out at 42 °C for 60 minutes. Subsequently, to deactivate the RT enzyme, the mixture was incubated at 70 °C for 15 minutes. The resulting cDNAs were stored at -20 °C until further processing.

2.3. Detection of BCMV by RT-PCR assays

The cDNA products obtained from total RNAs served as templates for the RT-PCR assay. To detect BCMV, the reagents were adjusted empirically as follows in a total volume of 50 µl: 36.6 µl sterile distilled water, 2 µl cDNA as the template, 3 µl MgCl2 (25 mM), 1 µl dNTPs (10 mM), 1 µl of forward and reverse primers (20 pmol each), 5 µl of 10X Taq buffer, and 0.4 µl Taq DNA polymerase (5 U/µl) (Thermo Scientific, USA).

The temperature cycles for the RT-PCR reaction were optimized as follows: an initial denaturation step at 94 °C for 2 minutes, followed by 37 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 45 seconds, and elongation at 72 °C for 1 minute. A final elongation step was performed at 72 °C for 5 minutes. (Modified from Bhadramurthy and Bhat, 2009).

After electrophoresis on a 1.5% agarose gel containing Ethidium bromide (EtBr), the amplified fragments (15 µl) were visualized under UV light. A negative control consisting of healthy plants was included in the analysis. Additionally, positive control was incorporated using a confirmed BCMV isolate, verified through sequence analysis.

3. Results and Discussions

BCMV strains have been identified in many industrialized countries where bean varieties are grown, and the development of resistant varieties has significantly reduced the rate of damage from this virus. Bean varieties are selected for disease resistance based on yield factors and their adaptation to the region. Currently, many growers use BCMV-infected seed, resulting in crop losses due to infection. The most prevalent potyvirus affecting legumes is the bean-specific bean common mosaic virus (BCMV), which has been recognized for its impact on the geographic distribution of beans. Beans were introduced to Anatolia approximately 250 years ago and subsequently dispersed throughout the region (Sehirali, 1988). The bean crop is susceptible to various bacterial, fungal, and viral diseases, as well as abiotic factors, posing a significant threat. Multiple genera have been identified to harbor at least 30 virus diseases, all of which contribute to substantial yield losses in bean cultivation areas (Loebenstein and Thottappilly, 2004). Different scientists observed bean symptoms, such as longer, narrower leaves and leaf distortion, along with vein banding and a typical mosaic pattern, in virus-introduced beans (Melgarejo et al., 2007; Deligoz and Sokmen, 2013; Mangeni et al., 2014). In the present study, data were obtained that may be useful for the development of resistant cultivars. Information was obtained on which plants with which gene combination the virus studies can be started.

The NL-4 and RU-1 strains were inoculated on the 20 plants of the susceptible Stringless Green Refuges (SGR) cultivar that were grown in the climate chamber room Plant Protection Department of Van Yuzuncu Yil University in April 2021. Three bands positive for NL-4 and one band positive for RU-1 were obtained for BCMV. However, strain RU-1 was lost during the increase; therefore the genotypes were artificially inoculated with the NL-4 strain.

3.1. Symptoms NL-4 and RU-1

After four days of inoculated Stringless Green Refuges (SGR) cultivar by NL-4 and RU-1, the symptoms of each other of the strains appeared (Figure 1).

Figure 1. Symptoms of RU-1 and NL-4.

3.2. Replicated artificial inoculation trial results with NL-4 strain of BCMV

The 45 bean genotypes were artificially inoculated at 3 replications each consisting of 10 plants per genotype. According to symptoms, BCMV was detected in about 1/3 of the studied bean genotypes in three replications (Table 3-5). The total number of plants infected with BCMV in sampling locations was summarized in Tables 3, 4, and 5.

The results study scored for each plant were inoculated by NL-4 strain for the first replication. 92 plants were susceptible and 358 plants were resistant among 450 plants. According to the number of susceptible plants, the highest susceptible genotypes were G7, G10, and G32 had ten plants that were susceptible after inoculation. Based on the mean of plant susceptibility for the BCMV-NL-4, the highest value (4) was from G1 Stringless Green Refuge, and G29-Van-Gevaş had the lowest value (0.2). According to the scoring, fifteen genotypes (19%) were susceptible and thirty genotypes (79%) were resistant (Table 3).

The second replication inoculation was scored for all bean genotype plants. The result 77 plants were susceptible and 373 plants were resistant among the 450 plants. According to the number of susceptible plants, the highest susceptible genotypes were G1, G8, G10, and G22 had ten plants that were susceptible after inoculation. Based on the mean of plant susceptibility for the BCMV-NL-4, the highest value (4.5) was from G8, and G28 had the lowest value (0.4). According to the scoring, eleven genotypes (24.4%) were susceptible and thirty-four genotypes (75.6%) were resistant (Table 4).

The results study scored for each plant were inoculated by NL-4 strain for the third replication. As a result, 82 plants were susceptible and 368 plants were resistant among 450.

According to the number of susceptible plants, the highest susceptible genotypes were G1, G8, and G22, while ten plants were susceptible after inoculation. Based on the mean of plant susceptibility for the BCMV-NL-4, the highest value (3) was obtained from G8, while G2 had the lowest value (0.1). According to the scoring, twelve genotypes (26.6%) were susceptible and thirty-three genotypes (73.3%) were resistant (Table 5).

Table 3. The first replication group bean genotypes were inoculated by BCMV-NL-4

Genotype	Symptoms BCMV-NL-4										
	R1.P1	R1.P2	R1.P3	R1.P4	R1.P5	R1.P6	R1.P7	R1.P8	R1.P9	R1.P10	Mean
G.1	5	5	4	4	5	3	0	5	5	4	4
G.2	0	1	0	1	0	1	0	0	2	1	0.6
G.3	2	1	5	0	1	1	3	1	0	0	1.4
G.4	0	0	0	0	0	0	0	0	0	0	0
G.5	0	0	0	0	0	0	0	0	0	0	0
G.6	0	0	0	0	0	0	0	0	0	0	0
G.7	2	1	1	2	2	1	3	2	2	2	1.8
G.8	2	4	0	1	5	4	2	1	0	0	1.9
G.9	0	0	0	0	0	0	0	0	0	0	0
G.10	5	4	5	3	4	2	5	4	3	3	3.8
G.11	0	0	0	0	0	0	0	0	0	0	0
G.12	0	0	0	0	0	0	0	0	0	0	0
G.13	0	0	0	0	0	0	0	0	0	0	0
G.14	0	0	0	0	0	0	0	0	0	0	0
G.15	0	0	0	0	0	0	0	0	0	0	0
G.16	0	0	0	0	0	0	0	0	0	0	0
G.17	0	0	0	0	0	0	0	0	0	0	0
G.18	0	0	0	0	0	0	0	0	0	0	0
G.19	0	0	0	0	0	0	0	0	0	0	0
G.20	0	0	0	0	0	0	0	0	0	0	0
G.21	0	0	0	0	0	0	0	0	0	0	0
G.22	3	3	3	2	5	5	0	1	2	1	2.5
G.23	0	0	0	0	5	4	5	4	0	3	2.1
G.24	0	0	0	0	0	0	0	0	0	0	0
G.25	0	0	0	1	2	2	5	4	5	5	2.4
G.26	0	0	0	0	0	0	0	0	0	0	0
G.27	0	0	0	0	0	0	0	0	0	0	0
G.28	3	2	0	0	0	0	0	0	0	0	0.5
G.29	0	0	0	0	0	0	0	0	0	2	0.2
G.30	0	0	0	0	0	0	0	0	0	0	0
G.31	3	3	2	1	0	0	5	2	3	4	2.3
G.32	3	2	2	2	3	3	2	3	5	5	3
G.33	0	0	0	0	0	0	0	0	0	0	0
G.34	0	0	0	0	0	0	0	0	0	0	0
G.35	0	0	0	0	0	0	0	0	0	0	0
G.36	0	0	0	0	0	0	0	0	0	0	0
G.37	0	0	0	0	0	0	0	0	0	0	0
G.38	0	0	0	0	0	0	0	0	0	0	0
G.39	0	0	0	0	0	0	0	0	4	0	0.4
G.40	0	0	0	0	0	0	0	0	0	0	0
G.41	0	0	0	0	0	0	0	0	0	0	0
G.42	0	0	0	0	0	0	0	0	0	0	0
G.43	0	0	0	0	0	0	0	0	0	0	0
G.44	0	0	0	0	0	0	0	0	0	4	0.4
G.45	0	0	0	0	0	0	0	0	0	0	0

R: replication, P: plant, 0: Resistance (non-symptom), 1-5: Susceptible (symptom scoring).

Table 4. The second replication group bean genotypes were inoculated by BCMV-NL-4

	Symptoms BCMV-NL-4										
Genotype	R2.P1	R2.P2	R2.P3	R2.P4	R2.P5	R2.P6	R2.P7	R2.P8	R2.P9	R2.P10	Mean
G.1	4	4	5	3	2	4	3	4	3	5	3.7
G.2	0	0	0	0	0	0	0	0	0	0	0
G.3	1	0	3	0	0	3	0	1	0	0	0.8
G.4	0	0	0	0	0	0	0	0	0	0	0
G.5	0	0	0	0	0	0	0	0	0	0	0
G.6	0	0	0	0	0	0	0	0	0	0	0
G.7	2	0	2	2	4	3	1	1	3	2	2
G.8	5	5	4	5	5	5	5	5	3	3	4.5
G.9	0	0	0	0	0	0	0	0	0	0	0
G.10	5	4	2	2	5	3	3	2	2	2	3
G.11	0	0	0	0	0	0	0	0	0	0	0
G.12	0	0	0	0	0	0	0	0	0	0	0
G.13	0	0	0	0	0	0	0	0	0	0	0
G.14	0	0	0	0	0	0	0	0	0	0	0
G.15	0	0	0	0	0	0	0	0	0	0	0
G.16	0	0	0	0	0	0	0	0	0	0	0
G.17	0	0	0	0	0	0	0	0	0	0	0
G.18	0	0	0	0	0	0	0	0	0	0	0
G.19	0	0	0	0	0	0	0	0	0	0	0
G.20	0	0	0	0	0	0	0	0	0	0	0
G.21	0	0	0	0	0	0	0	0	0	0	0
G.22	4	4	1	1	4	5	5	4	5	4	3.7
G.23	0	0	0	0	0	0	0	0	0	0	0
G.24	0	0	0	0	0	0	0	0	0	0	0
G.25	0	0	0	0	0	0	0	0	4	5	0.9
G.26	0	0	0	0	0	0	0	0	0	0	0
G.27	0	0	0	0	0	0	0	0	0	0	0
G.28	0	0	0	0	0	0	2	2	0	0	0.4
G.29	0	0	0	0	0	0	0	0	0	0	0
G.30	0	0	0	0	0	0	0	0	0	0	0
G.31	0	0	0	0	3	2	1	1	2	3	1.2
G.32	3	2	3	1	2	0	0	0	3	3	1.7
G.33	0	0	0	0	0	0	0	0	0	0	0
G.34	0	0	0	0	0	0	0	0	0	0	0
G.35	0	0	0	0	0	0	0	0	0	0	0
G.36	0	0	0	0	0	0	0	0	0	0	0
G.37	0	0	0	0	0	0	0	0	0	0	0
G.38	0	0	0	0	0	0	0	0	0	0	0
G.39	0	0	3	2	2	0	1	2	2	1	1.3
G.40	0	0	0	0	0	0	0	0	0	0	0
G.41	0	0	0	0	0	0	0	0	0	0	0
G.42	0	0	0	0	0	0	0	0	0	0	0
G.43	0	0	0	0	0	0	0	0	0	0	0
G.44	0	0	0	0	0	0	0	0	0	0	0
G.45	0	0	0	0	0	0	0	0	0	0	0

R: replication, P: plant, 0: Resistance (non-symptom), 1-5: Susceptible (symptom scoring).

Table 5. The third replication group bean genotypes were inoculated by BCMV-NL-4

	Symptoms BCMV-NL-4										
Genotype	R3.P1	R3.P2	R3.P3	R3.P4	R3.P5	R3.P6	R3.P7	R3.P8	R3.P9	R3.P10	Mean
G.1	4	1	1	4	4	4	5	2	5	5	3.5
G.2	1	0	0	0	0	0	0	0	0	0	0.1
G.3	0	1	2	1	0	0	2	1	0	0	0.7
G.4	0	0	0	0	0	0	0	0	0	0	0
G.5	0	0	0	0	0	0	0	0	0	0	0
G.6	0	0	0	0	0	0	0	0	0	0	0
G.7	3	2	4	4	4	5	2	2	2	2	3
G.8	5	5	5	4	3	2	5	5	4	3	4.1
G.9	0	0	0	0	0	0	0	0	0	0	0
G.10	1	2	5	5	4	0	5	5	0	1	2.8
G.11	0	0	0	0	0	0	0	0	0	0	0
G.12	0	0	0	0	0	0	0	0	0	0	0
G.13	0	0	0	0	0	0	0	0	0	0	0
G.14	0	0	0	0	0	0	0	0	0	0	0
G.15	0	0	0	0	0	0	0	0	0	0	0
G.16	0	0	0	0	0	0	0	0	0	0	0
G.17	0	0	0	0	0	0	0	0	0	0	0
G.18	0	0	0	0	0	0	0	0	0	0	0
G.19	0	0	0	0	0	0	0	0	0	0	0
G.20	0	0	0	0	0	0	0	0	0	0	0
G.21	0	0	0	0	0	0	0	0	0	0	0
G.22	2	3	2	1	2	2	1	2	1	3	1.9
G.23	0	0	0	0	4	5	0	0	0	0	0.9
G.24	0	0	0	0	4	4	0	0	4	4	1.6
G.25	0	0	0	0	0	0	0	0	0	0	0
G.26	0	0	0	0	0	0	0	0	0	0	0
G.27	0	0	0	0	0	0	0	0	0	0	0
G.28	0	0	0	0	0	0	0	0	0	0	0
G.29	0	0	0	0	0	0	0	0	0	0	0
G.30	0	0	0	0	0	0	0	0	0	0	0
G.31	0	0	0	0	2	1	1	1	4	4	1.3
G.32	3	2	3	3	1	2	0	0	4	4	2.2
G.33	0	0	0	0	0	0	0	0	0	0	0
G.34	0	0	0	0	0	0	0	0	0	0	0
G.35	0	0	0	0	0	0	0	0	0	0	0
G.36	0	0	0	0	0	0	0	0	0	0	0
G.37	0	0	0	0	0	0	0	0	0	0	0
G.38	0	0	0	0	0	0	0	0	0	0	0
G.39	1	0	2	2	1	0	1	1	3	3	1.4
G.40	0	0	0	0	0	0	0	0	0	0	0
G.41	0	0	0	0	0	0	0	0	0	0	0
G.42	0	0	0	0	0	0	0	0	0	0	0
G.43	0	0	0	0	0	0	0	0	0	0	0
G.44	0	0	0	0	0	0	0	0	0	0	0
G.45	0	0	0	0	0	0	0	0	0	0	0

R: replication, P: plant, 0: Resistance (non-symptom), 1-5: Susceptible (symptom scoring).

3.3. Comparison of three replication symptoms with the control group

Plants in the control group were compared with plants inoculated with the BCMV-NL4 strain based on the symptoms observed on the leaves of the bean plants. The results in Table 6 show that sixteen bean genotypes were susceptible, while twenty-nine bean genotypes out of forty-five inoculated with NL -4 were resistant. According to the symptoms, the leaves of some bean genotypes were highly infected with the virus, while the leaves of some bean genotype leaves were not infected with the virus.

Table 6. Overall evaluation of the bean genotypes/cultivar reaction and symptoms with BCMV-NL-4

Genotype No	Phenotypic Reaction BCMV-NL-4	Symptom
G.1	S	+
G.2	S	-/+
G.3	S	-/+
G.4	R	-
G.5	R	-
G.6	R	-
G.7	S	+
G.8	S	+
G.9	R	-
G.10	S	+
G.11	R	-
G.12	R	-
G.13	R	-
G.14	R	-
G.15	R	-
G.16	R	-
G.17	R	-
G.18	R	-
G.19	R	-
G.20	R	-
G.21	R	-
G.22	S	+
G.23	S	-/+
G.24	S	-/+
G.25	S	-/+
G.26	R	-
G.27	R	-
G.28	S	-/+
G.29	S	-/+
G.30	R	-
G.31	S	+
G.32	S	+
G.33	R	-
G.34	R	-
G.35	R	-
G.36	R	-
G.37	R	-
G.38	R	-
G.39	S	+
G.40	R	-
G.41	R	-
G.42	R	-
G.43	R	-
G.44	S	-/+
G.45	R	-

R: Resistance, S: Susceptible, +: Have symptom, -: No symptom, -/+: Weak symptom.

Depending on the different bean genotypes, leaves showed different shapes (curled, short, and tall) after inoculation and exhibited symptoms of NL -4. As a comparison control group with three replicate groups, four weeks after inoculation, the number of plants of bean genotypes was more resistant than those of susceptible bean genotypes. For further identification, the data numbers susceptible and resistant bean genotypes, each bean genotype plant leaves after 4 weeks after inoculation compared with the control group by two forms the first compared after the triple leaves and the second compared after the single leaves for all plants were inoculated. The prevalence of common bean infection in Türkiye has been estimated to be around 30%, while the transmission rate of BCMV through seeds has been

approximated at 25-40% (Klein et al., 1988; Acikgoz and Citri, 1986; Bashir et al., 2000). In Iran, several researchers have reported the presence of viruses affecting pulse crops, including BYMV, BCMV, and CMV on French beans from the Zanjan province (Kaiser et al., 1967, 1971; Shahraeen, 1993, 2002; Mehraban et al., 2002; Makkouk et al., 2003).

Virus movement within a plant can occur locally, where the virus spreads slowly from cell to cell, or systemically, where it rapidly moves throughout different parts of the infected plant. Host plant resistance to BCMV is conferred by a single dominant *I* gene, which is associated with hypersensitivity (Hegay, 2013).

Although common beans may exhibit similar phenotypes such as seed color, flower color, and molecular markers (e.g., microsatellites), these traits can be utilized to differentiate genotypes within morphologically homogeneous germplasm. These findings are consistent with a previous study by Singh et al. (1991b), which also observed variations in growth habits among common beans (Figure 2).

Figure 2. Plant reactions to the infection of BCMV- NL-4 strain in a climate growth.

3.4. Confirmation of BCMV NL-4 by PCR from molecular methods

According to the gel electrophoreses, the results of susceptible bean genotypes were 31% while due to the score symptoms, the results of 35% of the bean genotypes were recorded to be positive after 21 days from the inoculation. The occurrence of virus infections was investigated by inoculating 2700 leaves of different bean genotypes. It was found that 502 leaves (19% of the total) displayed mixed infections by multiple viruses, highlighting the complex nature of disease resistance in common bean breeding programs. Specifically, the results revealed that 19% of the bean genotypes tested were infected by NL-4 strain viruses. The percentage of BCMV-NL-4 infected samples (19%) was relatively low. After 21 days from inoculated bean genotype plants by BCMV-NL-4 were determined fourteen bean genotypes positive band and thirty-one bean genotypes negative band among forty-five bean genotypes (Figure 3).

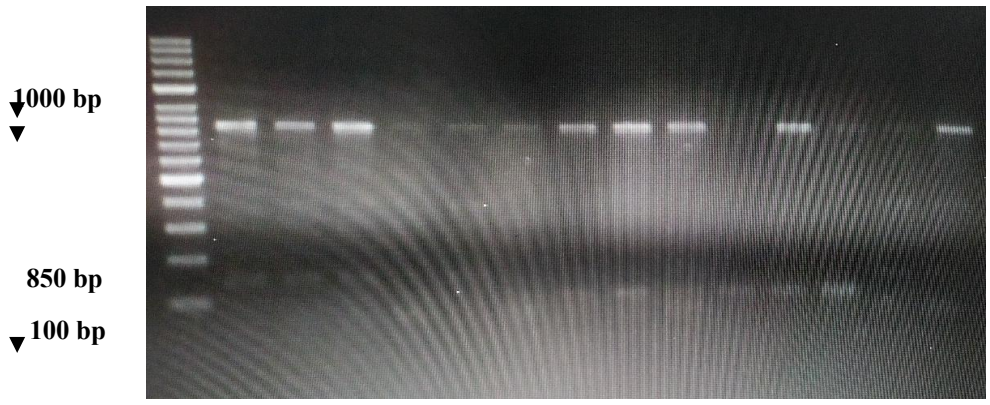

Figure 3. Determination of BCMV-NL-4 virus by the primer BCMV-PCR for G1,3,4,8,10,17,18,19,20, 22, 23, 31, 32, and 34.

The common bean is known to be susceptible to various viruses that have been documented to infect common beans on a global scale. Among them, bean common mosaic virus (BCMV) is recognized as the most prevalent virus worldwide (Hall, 1991). In West Asia, common bean fields in Iraq have reported the presence of BCMV, CMV, and BYMV (Makkouk and Kumari, 1996) (Figure 4). In a study conducted by Deligöz and Sökmen (2013), the resistance of various common bean genotypes against BCMV and BCMNV was assessed using multiple approaches, including symptomatology, enzyme-linked immunosorbent assay (ELISA), and molecular methods. The researchers observed that certain genotypes exhibited a notable level of resistance to both viruses.

In a separate study by Hegay (2013), marker-aided breeding techniques were employed to develop resistance against BCMV and anthracnose in beans cultivated in Kyrgyzstan. The objective of this study was to utilize molecular markers to aid in the breeding process, specifically targeting resistance traits against BCMV and anthracnose. The author used molecular markers linked to the resistance genes and found that marker-aided selection could significantly increase the efficiency of breeding for resistance to BCMV. Mangeni et al. (2014) investigated the distribution and pathogenic characterization of BCMV and BCMNV in western Kenya. They found that both viruses were present in the region and that the prevalence of BCMNV was higher than BCMV. The authors suggested that screening for resistance to both viruses is necessary to develop effective management strategies in the region.

4. Conclusion and Recommendations

In the present study, the responses of 45 bean cultivars to BCMV were determined by artificial inoculation, and the presence of the virus was confirmed at the molecular level. Considering the molecular data obtained, the results of the study were confirmed by measuring the responses of cultivars with resistance genes supported by virus inoculation. The evaluation of the results obtained by inoculation together with the molecular data phenotypically confirmed the success of the markers. The results of the field experiment allow us to conclude: based on the symptoms observed on the leaves of the bean genotypes, the sixteen bean genotypes showed typical mosaic symptoms; however, the remaining twenty-nine bean genotypes had no visible symptoms. Based on the previously mentioned conclusions the following recommendations can be given: The BCMV-NL-4 could be used for the resistance level of the bean genotypes. For the certain determination of bean genotypes other BCMV strains could also be used. The identification of resistant genotypes through these studies holds promise for the development of common bean cultivars with enhanced resistance to BCMV, BCMNV, and anthracnose. These resistant genotypes potentially possess specific genes that confer resistance to these pathogens. By incorporating these resistant genes into breeding programs, it is possible to develop new varieties with improved agronomic traits, ultimately leading to higher yields in field bean production. Screening bean genotypes for BCMV resistance and applying qualitative, quantitative, and molecular methods are critical for developing resistant varieties. Several methods can be used to evaluate the level of resistance in bean genotypes, including symptomatology, ELISA, and molecular methods. Marker-assisted breeding can significantly increase the efficiency of resistance breeding against BCMV. In regions where both viruses are present, screening for resistance to both viruses is necessary to develop effective management strategies.

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