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Determination of Genetic Diversity and Screening of BCMV and BCMNV Resistance in Some Bean Genotypes Using Molecular Markers

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ABSTRACT

Fresh bean (*Phaseolus vulgaris* L.) is an important legume crop in Turkey used as a major source of protein in the human diet. The total fresh bean production of Turkey is estimated at 651,094 tons. Bean production shows a decreasing factor due to various abiotic and biotic factors in Turkey. Among the viruses that infect beans, Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) are the most widespread destructive agents. The main objective of this study was to carry out a diagnostic survey for Bean common mosaic disease (BCMD) in beans, characterize its causal agent, and evaluate host resistance to BCMV and BCMNV. In addition to this, genotypes were tested for the levels of genetic diversity by 58 SSR markers. A total of 123 alleles with a PIC value of ≥0.2 were obtained from 75 bean genotypes. The unweighted NJ dendrogram was created using the scoring data, which demonstrated the molecular genetic relationships among the bean genotypes. SCAR (Sequence Characterized Amplified Region) markers were used to determine the resistant genotypes to BCMV and BCMNV. According to the result, 21 out of 75 genotypes have resistance alleles to both pathogens.

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INTRODUCTION

Common bean (P. *vulgaris*) belongs to the Leguminosae genus, which originated from Middle American and South American countries (Şalk *et al*., 2008). It has two gene sources; Mesoamerican and Andean (Duran *et al*., 2005; Erdinç, 2012). The researchers reported that the introduction of beans to Anatolia was about 250–300 years ago (Şalk *et al*., 2008). While the world's total fresh bean production is 23,595,714 tons, the total fresh bean production is 651,094 tons in Turkey (Faostat, 2017). One of the most important vegetables for healthy nutrition, the fresh bean is a rich dietary source of calcium, iron, phosphorus elements, and B1, B2, A, E vitamins. These data prove that the bean is essential for human nutrition and significant for its agricultural potential (Madakbaş, 2007).

Even though the bean genus originated from America, both natural selection and breeders' selection led to many regional varieties over time in many regions in Anatolia (Ergün, 2005). Due to the potential of integration into the breeding program, local populations should be well defined and need to be protected for their valuable gene

pools (Ulutaş, 2016). In this regard, it is critical to determine the genetic diversity among the local bean populations.

Biodiversity can be defined in detail in genetic materials using biochemical and molecular techniques. DNA samples from inanimate materials are essential for the identification of genetic diversity. RFLP and PCR (ISSR, SSR, RAPD, etc.), which are commonly employed techniques, can be utilized for this purpose. Thanks to these methods, it is possible to protect and define the diversity of the samples at the gene level in a short time (Özgen *et al*., 2000; Balkaya and Yanmaz, 2001). In this respect, numerous researchers have carried out studies for molecular description using this marker system to determine the genetic diversity of the population (Ergün, 2005; Duran *et al*., 2005; Blair *et al*., 2006; Sarıkamış *et al*., 2009; Ulukapı, 2009; Erdinç, 2012; Ulutaş, 2016).

In addition to the identification and protection of genetic resources, studies should be conducted that increase yield and quality properties. In Turkey, susceptible varieties, biotic and abiotic factors, disease, and pathogen problems cause severe yield losses in bean production (Ulutaş, 2016). Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) are among the most critical pathogen problems causing yield loss in beans (Kumar *et al*., 1994). BCMV and BCMNV are seed-borne and aphid-borne viruses that infect bean plants (Hongying et al., 2002). Depending on the common bean cultivar and the stage of development, the seed transmission rate varies from less than 1% to 50% (Hong-Soo Choi et al., 2006). In susceptible common bean genotypes, symptoms can appear at typical growing temperatures (26–28℃) such as a severe mosaic, curling of the leaves, vein banding, and mottled and malformed pods (A. Muute et al., 2021). The systemic necrosis appears in beans at elevated temperatures (above 30℃). The emerging symptoms depend on the type of infection (either seed-borne or vector transmitted), cultivar type, the strain of the virus, and the age of the plant at infection. Symptoms of BCMV are mosaic, systemic necrosis (black root) or local lesions, or leaf malformation. It is extremely difficult to control the spread of the disease and to provide effective protection against these viruses. The use of resistant cultivars, clean production materials, and control against vectors are essential in the struggle against these diseases. The lack of resistant cultivars and an effective chemical control increases the significance of the disease (Kılıç *et al*., 2013; Kılıç and Yardımcı, 2014). To find a solution, a number of studies have been conducted with regard to the molecular description to determine the resistance against the two diseases (Kumar *et al*., 1994; Strausbaugh *et al*., 1999; Madakbaş, 2007; Deligöz, 2007; Petrović *et al.*, 2010; Deligöz and Sökmen, 2013; Kılıç *et al*., 2013; Johary *et al.*, 2016; Martin *et al*., 2016; Wani *et al*., 2017).

This study aims to screen for resistance to BCMV and BCMNV and determine the genetic diversity among bean varieties/lines using DNA SCAR and SSR markers.

MATERIALS and METHODS

Plant Samples

The experimental plants were grown in a greenhouse in Antalya. Molecular studies were carried out on 75 common beans (*P. vulgaris* L.) genotypes. The leaf samples for DNA extraction were taken from five plants per genotype. DNA extraction was performed according to the EURX (Gene Matrix Plant-Fungi DNA Kit) procedure.

Research Design

SSR Analyses for Molecular Diversity

As shown in Table 1, 58 SSR markers (Müller *et al*., 2015) were used for molecular genetic diversity analysis among the common bean genotypes. A PCR reaction mixture with a total volume of 20 µL was prepared using 2 µL AmplitaqGold® PCR buffer, 1.5 µL MgCl2, 0.5 µL dNTP (Promega), 1 µL Primer F and Primer R, 0,25 µL AmplitaqGold® polymerase, 1 µL DNA, and 12.75 µL distilled deionized water. The following heat protocol was applied on the bean samples to amplify SSR: 10 min at 95°C for the initial denaturation, then 35 cycles at 95°C (30 sec), 30 sec at 60°C for annealing, and 30 sec at 72°C for extension, and the final extension at 72°C (10 min).

Table 1: *The SSR Markers Used for Molecular Diversity*

Locus	Forward Primer (5'>3')	Reverse Primer (5'>3')
PV25	GAGCTTCTCCGTCCTGTGT	CGAACTGAATCAGAAAGGAA
BM114	AGCCTGGTGAAAGCTCATAG	CAGCTTGTTGCCTACTCTCT
PV35	TCTACGCGTTCCCTCTGTCT	AGTGGATGTGGGGAAAAGC
BM202	AGCGAAAGAGGAACATCG	CTTTACCCACACGCCTTC
PV272	CAGAACAGAAGAAGAAACAGAAAATG	GCGTGTTCCTCTGTGTGTGT
BM154	TCTTGCGACCGAGCTTCTCC	CTGAATCTGAGGAACGATGACCAG
PV13	TGAGAAAGTTGATGGGATTG	ACGCTGTTGAAGGCTCTAC
BM183	CTCAAATCTATTCACTGGTCAGC	TCTTACAGCCTTGCAGACATC
PV163	TGAGAGTGGAGAAGGAGAGAGA	TGACAACACTGCAAACACCA
BM143	GGGAAATGAACAGAGGAAA	ATGTTGGGAACTTTTAGTGTG
PV169	TGGAAAGTCGGAGGAGAAGA	AAAAGGGTCCCAACCAAAAC
PV5*	ATTAGACGCTGATGACAGAG	AGCAGAATCCTTTGAGTGTG
BM155*	GTTCATGTTTGTTTGACAGTTCA	CAGAAGTTAGTGTTGGTTTGATACA
BM187	TTTCTCCAACTCACTCCTTTCC	TGTGTTTGTGTTCCGAATTATGA
PV11*	AAACTCAAAGTCGTTGTTCC	CCACTGACTCTAGCTCCTCC
PV251*	TGAAGTTGCAGCTAGGTTGG	GGTTGTGCTTGTGTTGTTGG
BM164*	CCACCACAAGGAGAAGCAAC	ACCATTCAGGCCGATACTCC
BM138	TGTCCCTAAGAACGAATATGGAATC	GAATCAAGCAACCTTGGATCATAAC
BM189	CTCCCACTCTCACCCTCACT	GCGCCAAGTGAAACTAAGTAGA
BM210*	ACCACTGCAATCCTCATCTTTG	CCCTCATCCTCCATTCTTATCG
PV113	TGCATTCTTCCTCCCATCTT	TTGATTTGATTTGATCAGTGGTG
PV87	CTCATTGCGTCTACCAGTGC	CCTAGGTTCCGCAGCATGT
BM181*	ATGCTGCGAGTTAATGATCG	TGAGGAGCAAACAGATGAGG
BM201*	TGGTGCTACAGACTTGATGG	TGTCACCTCTCTCCTCCAAT
PvComp4	TCTTCACCACTTTGAAAACACG	TCAGATAAATGTTGGTATTGGCA
PvPenta4	AGCAACTTTCGGTCTGGTAAGT	TGAATCATTGCTCCTAACCCTT
PvPenta5	AACTGTTCCTTGTGGGTTCAAT	GCCCAAGGAGTAATTAACAATAAAA
PvComp9	TGAATGCAACATCTAATACTAACTCAC	TGTTCTAGGTCTAAAGGCCACA
PvPenta8	AAGAGCATGTTTACTTCACTCATTTT	TGGGGATGGTGTTTGTTTT
PvTetra47*	TGGAATGGAGAAGAGACATCCT	CAAACCATGTTTCCAGCATCTA
PvPenta14*	GAAACTATTCACGGAACAAGCC	AGGAGTGGTGGAGGCAGTATAA
PvTetra65*	TCTGAATCAAACATGTCCTCAGA	GGCCAAACTTGTTTAAGGTGAG
PvComp2	CTCCGAATCAGAAACCCTATTG	GTGGATGAAGAGAAAGGCAAAG
PvTetra25	CACTTTCCTTATGCCTTCACAG	TAATTTGACCAATGCCAAACAC
PvTri6*	CGAATGGGAGAAGAAGGTTATG	GACATTCTTGTGTCGTTTCCAA
PvHexa20*	GTGTCTTCTATAGGTGTCCCCG	GAGTATTTCAAAGCTTGGCCTT
PvTri5	AGAAAATCATGCAGGTTGAGGT	TCCAGCTAAATAGATGATACGTAATTG
PvPenta10	GTTCTTCTATTTTCCATCTATC	AATATACATAAGAGTCACTTCCT
PvTetra50	TAACATGGTTAGGCCTTTTGAA	TCGTACGGATCCAAGTATTAATTT
PvComp21*	ACGAGTTATTGTTCCAGATGGG	TTATCCTTCTTATGCGGAGACC
PvHexa39	TTAATGCCTCCACTTGTTGTTG	CATGAGGCCCAAGTCAAAA
PvPenta19	TTAGGTCTTCAAAGAGATTTGG	TGTGGTAGTAGATGTTAAAGTCATTT
PvHexa10	AACTTGTTTATCGCATCCAGAA	ATGCAATCAAGGAATGCTCATA
PvTri8	CAATGTGGAACAAACTGAGGTG	AAGCAAAGTGTCTGAATTGCTG
PvTetra32	ATTCCTGCCACTAACGAAGTGT	CTAAAGGCCTAGCAGATTGCAT
PvComp10	AAATTCTATGATCAACCCGTGG	TGATCCCTTGTAGAGGAATCTCA
PvTetra49	TGGGTAGAGCTTGGTCTTCATT	AGTTTGTGAGTGATGTGATGGG
PvHexa36	TCACTTTGGCACCTCCTTATTT	AGGATTGTTTGCCTAAACCAGA
PvPenta16	ACATTTGGTTTTGGTTTTGGTC	TCTAAAATGGTCTCGAATTTATTCAC
PvComp27*	CAAGATGAACATCACCATTCCT	AAAAGAACATTTGTCACGTCCA
PvHexa12	TGCTAAATAGCCAAAGCAACAA	CATCACCACAGCACCAAGTATT
PvHexa15	CCAAACGAACCGACTATTTCTC	TTTGCACCTCCCTTTATGTTCT
PvHexa19	TGCTTCTCTGTCGTACTTGGAA	AATTACAAGCCTGAAGCTGCTC
PvComp8	TCACTATGTGAAATTGAACCCA	TTCCTACCTAACTTACTTGTACCACTT
PvPenta13*	ACTGAAGAAAGTACTAGAAACCTTACA	CCCCTTTTAATCAGAGAATTTTA
PvTetra57	ATCAACTATGGCGGATTGACTT	GAAAACAAATCCTTTTGACCCA
PvTetra73	TGGTATCGAAGCATTAGGTTCA	GTACTGGGTAACGGGTGTGAAT
PvTetra76	TACTCAAGCTTCTTCTGCAC	TGAAATATATGTTGCGGAAT

Genetic Diversity Analyses

In order to determine the similarity coefficient, the polymorphic SSR marker bands scored as absence/presence (0–1).

This data was used to calculate Nei's genetic similarity index (Nei and Li, 1979). DARwin (6) was employed to explore genetic diversity among the studied beans. The principal component analysis (PCA) based on genetic diversity was performed to identify the patterns of the common bean variation.

Determination of BCMV and BCMNV-Resistant Common Bean Genotypes Using DNA Markers

SCAR (Sequence Characterized Amplified Region) markers (SW13, SBD5, ROC11) were used to determine the genotypes resistant to BCMV and BCMNV (Miklas et al., 2000; Haley et al., 1994; Johnson et al., 1997). Table 2 presents the details of these markers.

Table 2: *The Markers Used to Determine Resistant Genotypes*

Gene Name	Marker	Forward Primer (5'>3')	Reverse Primer (5'>3')	Allel length
I	SW13	CACAGCGACTAATTTTCCTTTC	CACAGCGACAGGAGGAGTTTA	690 bp
bc-1²	SBD5	GTGCGGAGAGGCCATCCATTGTG	GTGCGGAGAGTTTCAGTGTTAA	1300 bp
bc-3	ROC11	CCAATTCTCTTTCACTTGTAACC	GCATGTTCCAGCAAACC	420 bp

The markers given in Table 2 were performed under appropriate PCR conditions, and these marker bands were visualized by Qiaxcel Fragment Analyzer (Qiagen Sample & Assay Technologies) capillary electrophoresis system. The data were evaluated manually.

RESULTS AND DISCUSSION

The study started with seed sowing in the vegetation period of 2017. The samples were taken from young and healthy plant leaves for molecular studies. The present study performed the molecular diversity analysis and the screening of resistance against two diseases (BCMV and BCMNV) among bean genotypes.

Molecular Characterization by SSR Method

In common beans, gene-based markers are particularly effective for the separation of diversity among genotypes. Therefore, Blair et al. (2006) reported that it would be useful for diversity analysis and comparative and transcript mapping. The polymorphism information content (PIC) value of each microsatellite marker is a measure of marker diversity. The PIC value provides an estimation of the discriminatory power of a locus by taking into account not only the number of alleles expressed but also the relative frequency of those alleles (Wani *et al*., 2017). In this study, a total of 123 SSR alleles with a PIC value ≥0.2 were obtained from 75 common bean genotypes. Another study was conducted to investigate the genetic relationships among 28 fresh bean genotypes collected from Erciş and Gevaş, two districts of the province of Van in Turkey. The study revealed 45 polymorphic bands using 10 SSR markers out of 12 SSR primers (Sarıkamış *et al.*, 2009). The highest PIC value was calculated as 0.5 at pv25 and pv13 from polymorphic SSR markers, while the minimum value was calculated as 0.254 at bm201 and bm181 primers. The polymorphic information content (PIC) scores ranged from 0.254 to 0.5. The polymorphism observed in SSR markers among common bean genotypes in the present study demonstrated that this method could effectively identify the genetic variation. All the SSR markers were highly informative in revealing the genetic diversity. Our results are parallel with the results of previous studies (Burle *et al*., 2010; Wani *et al*., 2017). The unweight NJ dendrogram (Figure 1) and the PCA graphic (Figure 2) were created based on the scoring data.

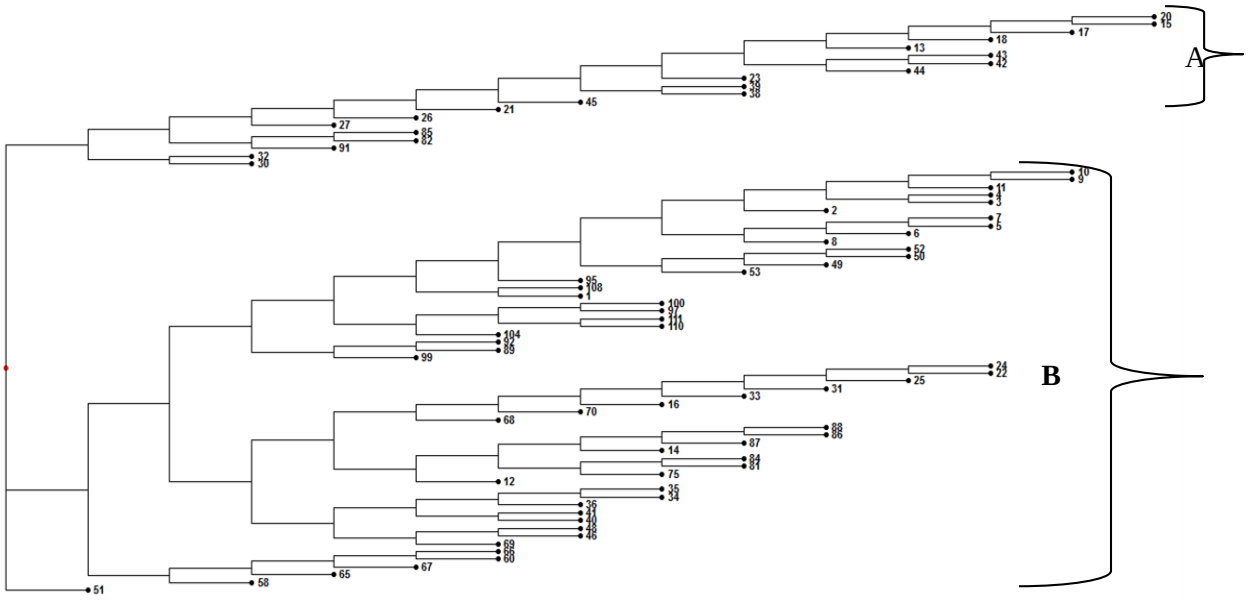

Figure 1: The unweight NJ dendrogram

The unweighted neighbor-joining (NJ) dendrogram shows the molecular genetic relationships among bean genotypes. As a result of the diversity analysis, the bean genotypes were separated into three main groups and clustered into two large groups. Most of the genotypes were clustered in the B group (49 genotypes). As a result of the mantel test, the R-value was found 0.9773, which is an indication that the genotypes analyzed are highly similar to each other.

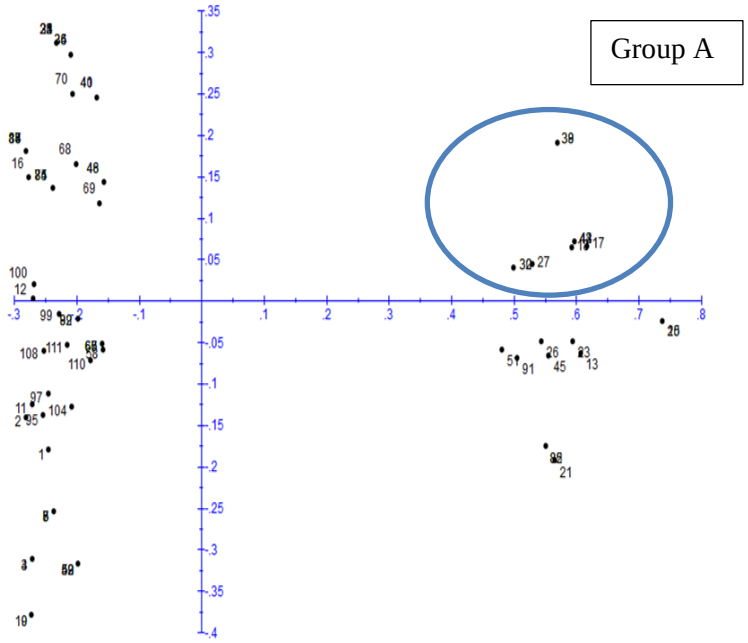

Figure 2: The Principal Component Analysis graphic

In a PCA analysis, when genotypes in the coordinate plane are all in one place or close to each other, there is less genetic variation among the lines. According to Figure 2, 38 genotypes are located differently from Group A, which suggests that these genotypes are genetically distant from each other. Our results are parallel with the results of previous studies (Ulukapı, 2009).

Determination of Resistance to BCMV and BCMNV Diseases

Disease-resistant genotypes were determined according to the resistance allel of SW13 SBD5 and ROC11 markers and a sample electropherogram displays for resistant allel for ROC11 SCAR marker (Figure 3).

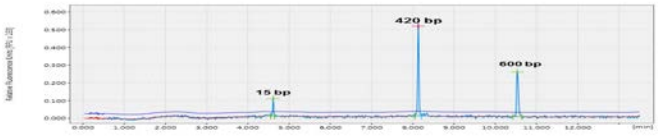

Figure 3: *Electropherogram appearance of genotypes showing resistance to Roc11 marker*

The determination of disease-resistant genotypes showed that 21 out of 75 genotypes possessed resistance alleles against both pathogens (both BCMV and BCMNV).

Deligöz (2007) reported that no study was conducted in Turkey on viral pathogens that cause disease in bean cultivation until 2007. Therefore, no bean genotypes resistant to these two viral diseases have been found. In a study conducted in Turkey, 41 breeding lines and 31 commercial cultivars were tested for resistance levels against BCMV. The study results showed that 29 breeding lines and 24 commercial cultivars were resistant to BCMV. This study suggested that the resistant breeding lines can be registered as cultivars after evaluating their yield and quality criteria. Also, the resistant commercial cultivars might be used as breeding material (Deligöz *et al.*, 2015). An evaluation of the results of our study indicates that our genotypes can be used as genetic material in breeding studies and the field of infection. The results of the present study show parallelism with previous studies (Strausbaugh *et al*., 1999; Madakbaş, 2007; Deligöz, 2007; Burle *et. al*., 2010; Petrović *et al*., 2010; Deligöz and Sökmen, 2013; Deligöz *et al*., 2015; Kılıç *et al.*, 2013; Johary *et al.* 2016; Martin *et al*. 2016; Wani et al. 2017).

In addition, the results of the diversity analysis show that 21 genotypes with resistance to more than one pathogen are clustered together in the same group in the dendrogram. Therefore, the results of this study demonstrated a strong correlation between allelic composition and resistance/susceptibility to the two viral diseases.

RECOMMENDATIONS

Studies on resistance to these two pathogens in beans are limited in Turkey, and bean cultivation areas should be screened as a priority. As a result of these studies, resistant gene sources can be determined in the number of genotypes. Thus, it is thought that I and bc-3 genes, which provide resistance to BCMV and BCMNV, can be transferred to green bean genotypes. Additionally, it is thought that genetic relationships calculated based on molecular genetic data will provide valuable information for future breeding programs by determining the genetic diversity within the population, and it will be beneficial for breeding programs to be planned.

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