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Detection of *Bean Common Mosaic Virus* in Bean Seeds by Immunocapture RT-PCR and DAS-ELISA Methods

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Research Article

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Abstract

This study was carried out in bean seeds in Isparta and Antalya provinces to determine *Bean common mosaic virus* (BCMV) in 2016. For this purpose, 106 seed samples were collected from the research area. Seed samples were used for biological, serological, and molecular methods. The virus incidence, as a percentage of seeds samples for BCMV, was found 87.73% by DAS-ELISA and 90.56% by Immunocapture Reverse Transcriptase-Polymerase Chain Reaction (IC-RT-PCR) methods. As a result of the study, BCMV was detected in a total of 96 (90.56%) seed samples. 93 samples were detected positive with the DAS-ELISA method, while 96 samples were found to be infected with BCMV using the IC-RT-PCR method. In the IC-RT-PCR method, approximately 850 bp of the coat protein gene was amplified with specific primers and BCMV specific bands were obtained at the expected levels.

Keywords: BCMV, bean, seed, molecular detection, serological method

Fasulye Tohumlarında Fasulye Adi Mozayik Virüsü'nün Immunocapture RT-PCR ve DAS-ELISA Yöntemleriyle Teşhisi

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Öz

Bu çalışmada, 2016 yılında Isparta ve Antalya illerinde fasulye tohumlarında fasulye adi mozayik virüsü (*Bean common mosaic virus:* BCMV*)'*nün belirlenmesi amacıyla yapılmıştır. Bu amaçla araştırma alanından 106 tohum örneği toplanmıştır. Tohum örnekleri biyolojik, serolojik ve moleküler çalışmalar için kullanılmıştır. DAS-ELISA testi sonucunda tohum örneklerinde BCMV'nün hastalık oranı %87.73 olarak belirlenirken, Immunocapture ters transkripsiyon polimeraz zincir reaksiyonu (IC-RT-PCR) yönteminde örneklerdeki hastalık oranı %90.56 olarak belirlenmiştir. Yürütülen çalışmalar sonucunda toplam 96 tohum örneğinde (%90.56) BCMV belirlenmiştir. DAS-ELISA yöntemi ile 93 örnek BCMV ile pozitif belirlenirken; IC-RT-PCR yöntemi ile 96 örnek BCMV ile enfekteli bulunmuştur. IC-RT-PCR yönteminde spesifik primerler ile kılıf protein geninin yaklaşık 850 bp'lik bir kısmı amplifiye edilmiş ve BCMV'ne özgü beklenen seviyede bant elde edilmiştir.

Anahtar Kelimeler: BCMV, fasulye, tohum, moleküler teşhis, serolojik metot

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Introduction

Bean (*Phaseolus vulgaris* L.) is one of the most important crops with a very high nutritional value. Edible legumes, of which bean is a member, due to their 18-37% protein (2-3 times those of cereals), vitamins A, B, and D, and rich mineral contents and its cheaper price compared to animal-based proteins, are very important food sources. Beans can be consumed in various ways including fresh vegetables, dry beans, and canned food. Bean plant is a Central America Originated crop, coming to Anatolia 250 years ago and has covered a very widespread area [1]. The world's green bean production is 4.310.733 tons in 26 million hectares. According to 2016 data, in 528.931 ha of a total of 799.379 (ha) legume fields, 614.948 tons of green bean and 200.673 tons of dry beans were produced in Turkey [2]. Legumes, due to their high protein content which constitutes a perfect nutritional condition, are susceptible to being affected by pests and diseases. In vegetable farming, due to many diseases, economic losses occur in bean production areas which constitute important production values and income sources. There are large numbers of fungal, bacterial, and viral agents which limit production in the bean-producing areas in the world [3]. A minimum of 30 viral diseases cause significant yield losses in the bean-producing areas [4]. Among them, *Bean common mosaic* virus (BCMV) is one of the common seed-borne viruses [5-7]. This virus can be transmitted via seeds, aphids, and mechanically. It was reported that BCMV was carried via bean seeds and the rate of transmission in seeds obtained from infected plants can increase up to 83% [8]. Drijfhout and Morales [9] reported the rate of BCMV transmission as 35%, while Fidan and Yorganci [10] reported the rate of transmission for this virus as 56%. Studies for maintaining a higher production in the unit area, and increase the quality and the yield comprise the arrangement of environmental conditions, proper management of farming activities, and protection of plants and products from diseases and pests. The absence of chemical control methods for pathological viruses increases the importance of these diseases. Using virus-free production materials in the control of these viruses which cause problems in production areas is very important [11]. This study was conducted for the detection of BCMV in bean seeds of the Isparta and Antalya provinces. For this purpose, biological, serological, and molecular methods were used.

Material and Methods

In this study, 106 seed samples were collected from bean production areas in Antalya (51 samples) and Isparta Provinces (42 samples) in 2016. Seeds were kept at +4ºC until the conduction of tests. For the determination of BCMV presence in seed samples; bean seeds were planted in small pots in the growth cabins and left to germinate. The Cotyledonary leaf of germinated bean seeds was used in mechanical inoculation, DAS-ELISA, and IC-RT-PCR studies. Leaf samples taken from infected bean seedlings that tested positive in the DAS-ELISA test were used in mechanical inoculation studies as an inoculum source. *Phaseolus vulgaris* L., *Chenopodium amaranticolor, C. quinoa,* and *Nicotiana benthemiana* plants were used in mechanical inoculation studies as a test plan. Phosphate buffer (0.01 M; pH 7.2)

containing 0.01% 2-Mercaptoethanol at 1:1 (W/V) was inoculated to the leaves of the test plants. For symptom emergence, the test plants were placed in plant growth cabins at 20-24°C (Figure 1).

Figure 1. *Cotyledon leaves germinated from bean seeds*

For the serological test method, BCMV DAS-ELISA (BIOREBA AG, Switzerland) kit was used in the study. The application was performed according to the procedure prepared by the related commercial company [12]. For molecular detection, microtubes were coated with antibodies (100 µl) for BCMV specific antibodies at concentrations of 1 µg/ml. The tubes were incubated overnight at 4°C. After washing with PBS−Tween buffer, plant tissue extract, as prepared above for ELISA, was loaded into the antibody-coated tubes and incubated. After thorough washing with PBS−Tween and a final rinse with sterile water, the treated PCR tubes were ready for RT-PCR. In IC-RT-PCR studies, an 850 bp of coat protein gene was amplified with BCMV specific primer pairs. These primer sequences were F-5-GGATGCGGAGAATCTGTG-3; R-5-GATTGACGTCCCTTGCAG-3 obtained from by Bhadramurthy and Bhat article [13]. In molecular studies, plant samples that were found to be infected with BCMV in DAS-ELISA studies were used as the positive control, and the leaves of a healthy bean plant were used as the negative control. RT-PCR studies were conducted in a single step according to Primescript One-step RT-PCR kit (Takara Bio Inc., Japan) protocol in 50 µL volume. Amplification stages were conducted as 50°C 30 minutes, 94°C 2 minutes, 94°C 30 seconds, 55°C 30 seconds, 30 cycles, 72°C 1 minutes and +4°C ∞. The amplified RT-PCR products were electrophoresed in 1% agarose gel (Bio-Rad, France) and stained with ethidium bromide and Doc-It (UVP, UK) was used for imaging.

Results and Discussion

In seeds collected from the survey areas, symptoms were observed including deformities, wrinkling, changes in shell color, mottling, and grain size reduction. For the determination of BCMV presence in seed samples; bean seeds that were left to germination in plant growth cabins were harvested after 4-10 days. Leaves developed from the seeds, regardless of showing any symptomatological signs, and were used in biological, serological, and molecular studies. In mechanical inoculation studies, systemic

mosaic and leaf deformations and vascular retractions in *Phaseolus vulgaris* L. (Figure 2) and deformation, mosaic in *N. benthamiana* were observed (Figure 3). Local lesions were observed in *Chenopodium quinoa*. No symptoms developed on *C. amaranticolor.* The symptoms obtained in this study also show similarities with other studies [14-16].

Figure 2. *Deformation and mosaic symptoms observed on inoculated leaves of Phaseolus vulgaris L.*

Figure 3. *Mosaic and deformation symptoms in N. benthamiana*

DAS-ELISA and IC-RT-PCR methods were applied to a total of 106 seed samples (42 samples from Isparta + 51 samples from Antalya). According to DAS-ELISA test results, 93 of the collected samples were found to be infected with BCMV. Additionally, in the colorimetric evaluations, a yellow color formation was observed in the plates containing these samples. Also, in the studies carried out in the different regions of Turkey, BCMV was detected in seed beans. In the macroscopic examination performed on the bean seed samples collected from Tokat province and neighboring districts, symptoms

including wrinkling, shrinking, cracking, splitting, color changes, and yellowing were observed. In the tests performed using DAS-ELISA, it was detected that 59% of the bean seed samples were infected with BCMV [11]. Guzel and Arli-Sokmen [6] in their study conducted in Samsun province, reported that 18.9% of 53 different bean seed samples collected from producers and seed retailers were infected with BCMV [17, 18]. In IC-RT-PCR studies 96 of 106 samples were found to be infected with BCMV. Specific primers which amplify approximately 850 bp of the coat protein gene were used in the study. With IC-RT-PCR studies, it has been also verified that 96 samples and positive controls gave band at the expected levels and were infected with BCMV. Also, *Phaseolus vulgaris* L. which showed mosaic symptoms as a result of mechanical inoculation was used in the molecular studies and the expected 850 bp bands were obtained (Figure 4).

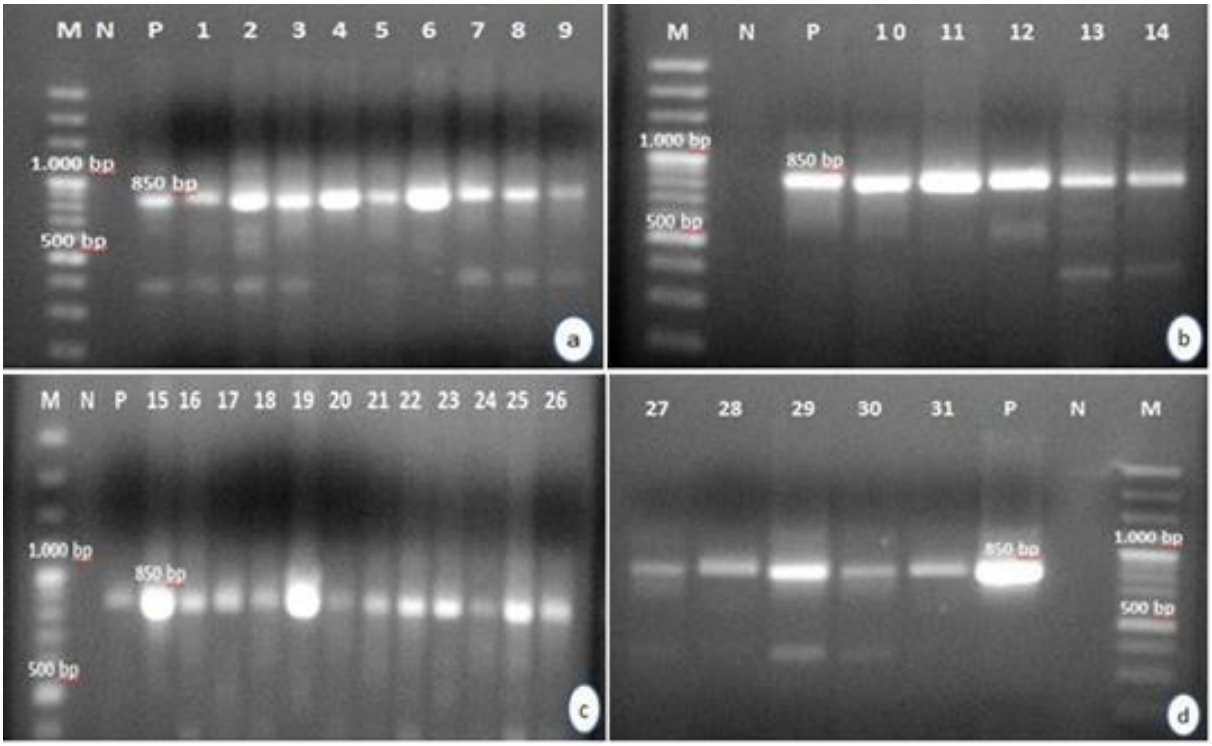

Figure 4. *Agarose gel electrophoresis of IC-RT-PCR products obtained from BCMV, M: Marker (100 bp DNA ladder; P: Positive control; N: Negative control; Lane 1-26 infected samples, Lane 27-31: Inoculated-Phaseolus vulgaris L.*

BCMV was detected in 93 bean seeds collected from the investigation area using DAS-ELISA whereas 96 samples were found to be infected using IC-RT-PCR. In the light of these findings, it was shown that the IC-RT-PCR method can be more successfully used, compared to DAS-ELISA. In the IC-RT-PCR method, plant extracts are directly used without nucleic acid isolation and thus some challenges faced during isolation are eliminated. Different researchers have used this advantage of the IC-RT-PCR method for the identification of virus diseases in beans [19, 20]. There are a limited number of studies in Turkey on the determination of viruses in bean seeds [11]. The results obtained in this study are the first results obtained from Isparta and Antalya provinces regarding BCMV. With this study, the existence of BCMV have been found out using biological, serological, and molecular methods in bean

seeds in Isparta and Antalya provinces. No study has been observed previously in the region to detect BCMV in bean seeds.

Conclusions

BCMV detected as a result of the studies is a virus that is carried with the seed and transmitted to long distances via aphids. In 106 of the tested bean seeds, 96 seeds were found to be infected with BCMV and this indicates that BCMV is a serious threat in the bean production areas in this area. A vast majority of the producers in the region use the seeds obtained from the plants of the previous years and therefore BCMV constitutes a threat to bean farming in Antalya and Isparta provinces in terms of the epidemiology of viral diseases. For the prevention of disease caused by BCMV, primarily virus-free certified seeds should be used and procedures such as vector control and improvement of resistant varieties should be disseminated. This study will form the basis of future epidemiological studies and endurance studies.

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References

[1] Loebenstein, G., & Thottappilly, G. (2004). *Virus and virus-like diseases of major crops in developing countries*. Dordrecht, Kluwer Academic Publishing. 840.

[2] TUIK (2016, October). *Crop Production Statistics.* http://tuikrapor.tuik.gov.tr/reports.

[3] Nyvall, R. F. (1989). *Field Crop Diseases Handbook*. New York, Van Nostrand Reinhold, 791.

[4] Matthews, R. E. F. (1991). *Plant Virology*. Academic Press Publishing.

[5] Kumar, C. A., Khetarpal, R. K., Parakh, D. B., Singh, S., & Nath, R. (1994). *In Check list on seed transmitted viruses, Leguminous hosts.* National Bureau of Plant Genetic Resources, New Delhi.

[6] Guzel, O., & Arli-Sökmen, M. (2003). Determination of some viruses infecting common bean (*Phaseolus vulgaris* L.) and their incidences in seed lots in Samsun Province. *Journal of Turkish Phytopathology*, *32*(2), 99-106.

[7] Ghorbani, S. G. M., Shahraeena, N., & Elahinia, S. A. (2010) Distribution and impact of virus associated diseases of common bean (*Phaseolus vulgaris* L.) in Northern Iran, *Archives of Phytopathology and Plant Protection*, *43*(12), 1183–1189. https://doi.org/: 10.1080/03235400802366834

[8] Omar, R. A, Mehiar, F. F., Zayed, E., & Deit, A. A. (1985). Biological studies on some seed-borne viruses and their effect on vegetative growth and yield component of the host plant, *Acta Phytopathologica Academiae Scientiarum Hungaricae*, *20*(3), 80-85.

[9] Drijfhout, E., & Morales, F. J. (2005). Compendium of bean diseases. In H. Schwartz, J.R. Steadman, R. Hall & R.L. Foster, (Ed), *Bean mosaic virus*, (pp. 63–64).

[10] Fidan, U., & Yorganci, U. (1990). Investigation on the detection and seed transmission of the virus diseases occuring on the pulse crops in Aegean Region. Seed transmission of virus diseases by grower seeds and seeds of artificial infected pulse crops, *Journal of Turkish Phytopathology, 19*(1), 1-6.

[11] Kutluk-Yilmaz, N. D., Gumus, M., & Erkan, S. (2002). Studies on determination of virus diseases in the seeds of bean from Tokat Province. *Ege University, Journal of Agriculture Faculty, 38*(3), 49-55.

[12] Naghavi, A., Habibi M. K., & Firouzabadi, F. N. (2008). Detection and identification of some soybean viral mosaic viruses, using molecular techniques in Lorestan Province, Southwest of Iran, *Asian Journal of Plant Science, 7*(6), 557-562. https://doi.org/ 10.3923/ajps.2008.557.562

[13] Bhadramurthy, V., & Bhat, A. I. (2009). Biological and molecular characterization of *Bean common mosaic virus* associated with vanilla in India. *Indian Journal of Virology, 20*(2), 70-77.

[14] Udayashankar, A. C., Chandra-Nayaka, S., Niranjana, S. R., Mortensen, C. N., & Prakash, H. S. (2012). Immunocapture RT-PCR detection of *Bean common mosaic virus* and strain blackeye cowpe a mosaic in common bean and black gram in India. *Archives of Phytopathology and Plant Protection, 45*(13), 1509–1518. https://doi.org/10.1080/03235408.2012.679123

[15] Hosseini, A., & Hosseini, S. (2014). Occurrence and distribution of *Bean common mosaic virus* and *Bean yellow mosaic virus* from common bean fields of Kerman province, Iran, *Indian Journal of Fundamental Applied Life Sciences, 4*(2), 528-535.

[16] Johary, T., Dizadji, A., & Naderpour, M. (2016). Biological and molecular characteristics of *Bean common mosaic virus* isolates circulating in common bean in Iran. *Journal of Plant Pathology, 98*(2), 301-310. https://dx.doi.org/10.4454/JPP.V98I2.027

[17] Hao, N. B., Albrechtsen, S. E., & Nicolaisen, M. (2003). Detection and identification of the blackeye cowpea mosaic strain of *Bean common mosaic virus* in seeds of *Vigna unguiculata* spp. from North Vietnam, *Australas Plant Pathology, 32*, 505-509. https://doi.org/10.1071/AP03052

[18] Vinayarani, G., Madhusudhan, K. N., Deepak, S. A., Niranjana, S. R., & Prakash, H. S. (2011). Detection of mixed infection of tobamoviruses in tomato and bell pepper by using RT-PCR and duplex RT-PCR. *International journal of Plant Pathology, 2*, 89-95. https://doi.org//10.3923/ijpp.2011.89.95

[19] Abtahi, F. S., Hbibi, M. K., & Motlagh, M. K. (2009). Some biological and molecular characterization of *Bean common mosaic necrosis virus* isolated from soybean in Tehran Province, Iran. *World Academy Science, Engineering and Technology, 49*, 126-129.

[20] Petrovic D, Ignjatov, M., Nikolic, Z., Vujakovic, M., Vasic, M., Milosevic, M., & Taski-Ajdukovic, K. (2010). Occurrence and Distribution of viruses infecting the bean in Serbia. *Archives of Biological Science, 62*(3), 595-601. https://doi.org/doi. 10.2298/ABS1003595P