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Research Article

Bean Common Mosaic Virus and *Bean Common Mosaic Necrosis Virus* Infections in Bean Production Areas in The Lakes Region of Turkey

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Abstract

Common bean, belonging to family *Leguminosae* (*Fabaceae*), has an important place in people nutrition for thousands of years. Although many viruses infect bean plants, economically most important ones are potyviruses. Among them, BCMV (*Bean common mosaic virus*) and BCMNV (*Bean common mosaic necrosis virus*) are the most common viruses in common bean production areas all over the world. During 2014-2015 growing season, total of 361 bean samples (275 leaf and 86 seed) were collected from several localities in the Lakes Region. According to the results of DAS-ELISA (Double Antibody Sandwich Enzyme Linked Immunusorbent Assay) test, percentages of the infections of BCMV and BCMNV in the leaf samples were 26.18% and 15.27%, respectively. In seed samples, BCMV and BCMNV infection rates were 2.32% and 6.97%, respectively. Percentages of the mixed infections of the viruses in the leaf samples were 25.45%, while that in the seed samples were. 55.81%. In the mechanically inoculation studies where two isolates (BCMV-I and BCMN-BT) were used, typical symptoms of the viruses were observed. In molecular studies, a 850 bp fragment of the CP (Coat Protein) gene was amplified by IC-RT-PCR (Immunocapture Reverse Transcription Polimerase Chain Reaction) using BCMV specific primer pairs, but amplification of the CP region of RNA genome of BCMNV could not be succeeded. This is first report of biological and serological methods in Lakes region.

Keywords: Bean, BCMV, BCMNV, Detection

Göller Bölgesi Fasulye Üretim Alanlarında *Fasulye Adi Mozayik* ve *Fasulye Adi Mozayik Nekroz Virüs'*ü *E*nfeksiyonları

Öz

Fasulye binlerce yıldır insan beslenmesinde önemli bir yeri olan *Leguminosae (Fabaceae)* familyası bitkisidir. Fasulye bitkisi çok sayıda virüs hastalığından önemli miktarda etkilense de ekonomik olarak en önemli zararları Potyvirüsler yapmaktadır. Bunların içerisinde en önemlileri *Fasulye adi mozayik virüs* (BCMV) ve *Fasulye adi nekroz mozayik virüsü* (BCMNV)'dür. Bu çalışma da, 2014-2015 yıllarında Göller Bölgesi fasulye üretim alanlarından 275 yaprak ve 86 tohum örneği olmak üzere toplam 361 bitki örneği toplanmıştır. DAS-ELISA metoduna göre; alınan yaprak örneklerinde BCMV'nün enfeksiyon yüzdesi, %26.18 iken; BCMNV'nün %15.27'dir. Tohum örneklerinde ise BCMV'nün enfeksiyon yüzdesi %2.32; BCMNV'nün %6.97'dir.Karışık enfeksiyon yüzdesi yaprak örneklerinde %25.45 iken tohumlarda bu oran %55.81 olarak belirlenmiştir. Mekanik inokulasyon çalışmalarında (BCMV-I ve BCMN-BT) iki izolat kullanılmıştır. Çalışmaların sonucunda virüslere özgü tipik simptomlar gözlenmiştir. Moleküler çalışmalarda;

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BCMV'ne özgü primer çiftleri ile kılıf protein geninin 850 bp'lik bir kısmı IC-RT-PCR (Immunocapture Reverse Transcription Polimerase Chain Reaction) metoduyla çoğaltılabilirken, BCMNV'nün amplifikasyonunda başarı elde edilememiştir.

Anahtar Kelimeler: Fasulye, BCMV, BCMNV, Teşhis

1. Introduction

Legumes that constitute a major source of vegetable protein, are important agricultural products in the world and also in Turkey. Nowadays, big amount of beans are produced all over the World, especially in the subtropical zones. Common Bean (*Phaseolus vulgaris* L.) is an agricultural product in Turkey and is producted in almost all parts of the country.

Turkey has a 3% share in the world's fresh bean production. According to the 2015 statistics of the Ministry of Food, Agriculture and Livestock, fresh bean production is estimated as 640.836 tons, while that of dry bean is 235.000 tons in Turkey. There are various abiotic and biotic factors that have negative effects on bean production. Among those, viruses are the most important ones causing crop losses in bean production areas (Babovic, 2003).

There are many viruses that infect bean plants, but economically the most important ones are Potyviruses (Kumar et al., 1994). Among potyviruses, BCMV and BCMNV are the most important viruses and can cause severe damage on bean plants. Disease incidence as high as 100% with yield losses of about 35–98% was reported to be caused by BCMV and BCMNV (Hampton, 1975; Galvez and Morales, 1989; Wortmann et al., 1998; Albrechtsen, 2006). These viruses are transmitted with aphids as non-persistent and seed –borne (Hongying et al., 2002).

Although BCMV and BCMNV have been reported in various regions of Turkey, no detailed study was conducted in the Lakes region where about 22% of Turkey's bean production has been performed (Açıkgöz, 1984; Kutluk Yılmaz et al., 2002; Deligöz and Sökmen, 2008). In this study, detection of BCMV and BCMNV in bean plants collected in Lakes Region were investigated. Mechanical inoculation, DAS-ELISA, RT-PCR and IC-RT-PCR methods were used in the study.

2. Materials and Methods

2.1. Samples

Surveys were conducted in common bean fields during 2014-2015 growing seasons and samples were taken. During the surveys, 275 bean leaf samples showing mosaic, mottling, curling, yellowing, chlorotic and necrotic lesions and deformation symptoms were collected (Figure 1.). Besides, 86 seed samples were also obtained from the growers. Samples were labeled and maintained at 4°C until they are used in the future studies. Studies were carried out with totally 361 samples.

Figure 1. Mosaic symptoms on bean leaves and deformations on bean seeds.

2.2. DAS-ELISA Method

BCMV and BCMNV ELISA kits were used in serological studies. DAS-ELISA was applied following the manufactures'instructions of antisera producer. Kits, negative and positive controls used in the study were obtained from BIOREBA Company, Switzerland. Plates were evaluated using Versamax reader at 405 nm. The absorbance values was measured positive reaction if the mean absorbance value was greater than twice from negative control (Çulal-Kılıç et al., 2015).

2.3. Test Plants

DAS ELISA positive BCMV-I leaf and BCMN-BT seed samples were used for mechanical inoculation studies. The inoculum was prepared with 1 grams of plant tissue and 10 ml phosphate buffer (pH, 7.2) and inoculated on test plants. *Nicotiana rustica*, *Nicotiana tabacum* L. "Xanthii", *Phaseolus vulgaris*, *Lupinus* sp, *Glycine max*, *Chenopodium amaranticolor*, *C. quinoa* were used as test plants. Test plants were held in a greenhouse at room temperature.

2.4.Total RNA Extraction, RT-PCR and IC- RT-PCR

Total RNA was extracted from 100 mg DAS-ELISA negative leaves applied the RNeasy® Plant Kit according to the manufacture's protocol (Bio Basic, Canada Inc). The samples were analyzed for the presence of BCMV and BCMNV using RT-PCR and IC-RT- PCR. Primers used in this study are given below:

BCMV; F-5-GGATGCGGAGAATCTGTG and

R-5-GATTGACGTCCCTTGCAG (Bhadramurthy and Bhat, 2009)

BCMNV;NL-3D -CCATTGCTGCTGAGATTC and

NL-3-AGTTCACCGTGAGATGTC (Larsen et al., 2005)

IC-RT-PCR mehod was performed according to the procedure described by Rowhani et al (1995). PCR tubes were coated with BCMV and BCMNV- specific antibodies and incubated at 4° C overnight. Following the incubation, the tubes were washed two times with washing buffer (PBS-Tween Buffer) and kept in the freezer until the molecular studies.

PCR reaction was performed in a 50 µl reaction mixture containing, 21µl H2O, 25 µl 2x1 PrimeScript One Step RT-PCR buffer (containing dNTP mixture, One step Enhancer solution), 2 µl Prime Script 1 step enzyme mix, 1 µl 20 Mm primers. Thermocycling was carried out as follows: 50ºC for 30 min., 94ºC for 2 min., then 30 cycles of 94ºC for 30 second, 55ºC for 30 second for BCMV, 48ºC for 30 second for BCMNV and 72ºC for 1 min., followed by 72 ºC for 3 min. PCR products were separated on a 1% agarose gel and illuminated under UV light after staining with ethidum bromide

3. Results and Discussion

3.1. DAS-ELISA

According to DAS-ELISA, BCMV and BCMNV were detected in bean leaf and seed samples from various areas of Lakes Region of Turkey. Total numbers of collected leaf and seed samples and plants infected with BCMV and BCMNV in sampling locations were summarised in Table 1 and 2. Among 361 leaf and seed samples, 223 (61.77%) were found to be infected with at least one of the two examined viruses.

DAS ELISA revealed the presence of 70 BCMV and 42 BCMNV positive among 275 leaf samples, and 2 BCMV and 6 BCMNV positive among 86 seed samples. Mixed infections of two viruses were also determined in 33 leaf samples and 70 seed samples. Considering the results of ELISA tests, it is observed that mixed infections are more intense from single infections of these viruses.

Table 1. The Results of Serological Assays of Leaf Samples

Locations	Number of samples	BCMV	BCMNV	BCMV+BCMNV	Rate (%)
Akdere	117	33	9	2	37.60
Insuyu	100	12	28	15	55.00
Çallıca	31	14	3	6	74.19
Kemer	16	8	1	5	87.50
Mürseller	11	3	1	5	81.81
Total	275	70	42	33	52.72

Table 2. The Results of Serological Assays of Seed Samples

Locations	Number of samples	BCMV	BCMNV	BCMV+BCMNV	Rate (%)
Akdere	26	1	2	22	92.30
Insuyu	23	1	4	17	95.65
Çallıca	15	-	-	13	86.66
Kemer	12	-	-	10	83.33
Mürseller	10	-	-	8	80.00
Total	86	2	6	70	90.69

3.2. Assays of Test Plants

The characteristic disease symptoms associated with BCMV and BCMNV infections have been shown on test plants included mosaic, leaf distortion and necrosis (Figure 2.) and developing symptoms are summarised in Table 3.

Figure 2. Symptoms on bean leaves after mechanical inoculations.

Table 3. Symptoms on Test Plants After Mechanical Inoculations.

Test plants	BCMV	BCMNV
Nicotiana rustica	Mo	SCS
Nicotiana tabacum L. "Xanthii"	-	-
Phaseolus vulgaris	Mo, Def, LT, S	N, Def
Lupinus sp	VP, Mo, Def	Mo, C
Glycine max	Mo, Def, B	SM
Chenopodium amaranticolor	-	-
C.quinoa	-	-

Mo:Mosaic, SCS:Systemic chlorotic spot, Def:Deformations, LT:Leaf tortion, S:Stunting, C:Chlorosis, N:Necrosis, B: Blistering, SM: Systemic mosaic, VP: Vein puckering.

3.3. RT-PCR and IC-RT-PCR Assays

Total RNAs extracted from thirteen leaf and seed samples were used in PCR studies. Virus-specific bands could not be obtained in RT-PCR studies. DNA fragments of the expected size (850 bp) were amplified from CP region of RNA genome of BCMV leaf and seed samples by IC-RT-PCR methods. However, amplification of the CP region of RNA genome of BCMNV could not be succeeded by RT-PCR and IC-RT-PCR methods (Figure 3 ,4.).

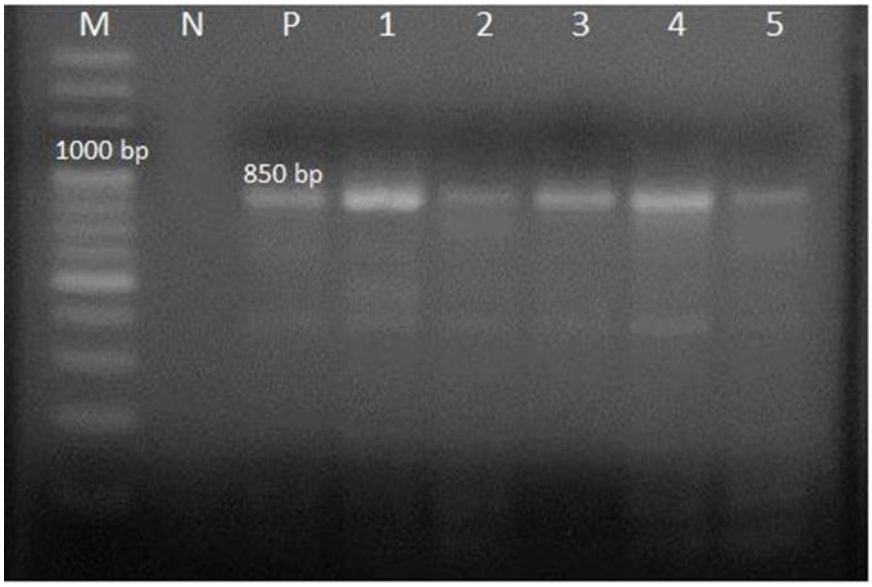

Figure 3. IC-RT-PCR products of BCMV from leaf samples. M: Marker (100 bp DNA ladder); P: Positive control; N: Negative control

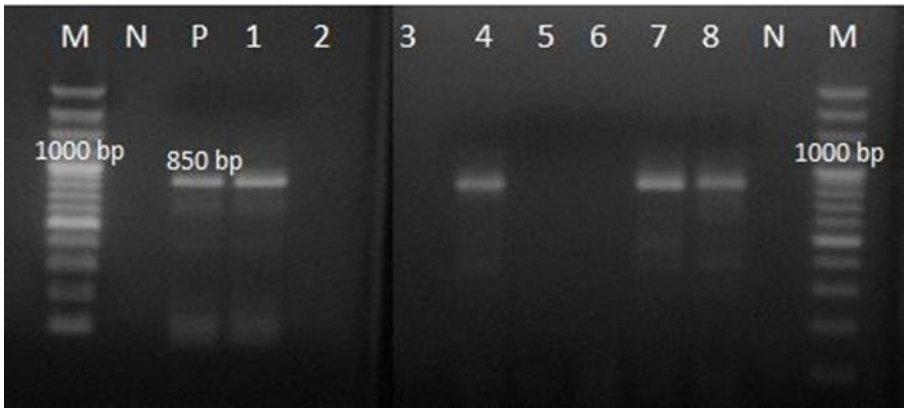

Figure 4. IC-RT-PCR products of BCMV from seed samples. M: Marker (100 bp DNA ladder); P: Positive control; N: Negative control

BCMV and BCMNV are most worldwide (Galvez and Morales, 1989; Gilbertson et al., 2001). These potyviruses can be transmitted and spread by both Aphididae vectors and infected seeds (Spence and Walkey, 1995; Galvez and Morales, 1989).

These viruses constitute a potential problem for Turkey and also for this region which is an important bean production part of the country. DAS ELISA results confirmed the presence of both BCMV and BCMNV in Lakes Region.. BCMV was found in 25.45% and BCMNV was found in 15.27% of the total 275 leaf samples. Although the rate of single infections were too low in seed samples, mixed infections were remarkable (81.39%).

These results are similar with those of previous findings. Most of the earlier studies reported higher rates of BCMV than those of BCMNV (Sáiz et al., 1995 Melgarejo et al., 2007; Mangeni et al., 2014; Mandour et al., 2013; Dizadji et al., 2011; Petrovic et al., 2010). This study showed that BCMV and BCMNV are widespread in this region and often present together.

In this study, the presence of BCMV was confirmed using specific IC- RT-PCR. We used the primers designed by Bhadramurthy and Bhat (2009) and the same conclusion was reached. Nine samples (five leaf samples, four seed samples) were detected as positive for BCMV at the leaves.

4. Conclusion and Recommendations

BCMV and BCMNV have previously been detected in various regions of Turkey (Açıkgöz, 1984; Güzel and Arlı Sökmen, 2003; Kutluk Yılmaz et al., 2002; Çulal Kılıç et al., 2015; Arlı Sökmen et al., 2016; Çolak Ateş et al., 2017). In this study, the occurrence of BCMNV in the collected seed samples in this region determined and DAS-ELISA data indicated that this virus is widely distributed in the selected seed samples of Lakes region. But probably due to the problems arising from the primers or samples, BCMNV could not be determined by IC-RT-PCR and RT-PCR. This is first report of BCMNV with biological and serological methods in the Lakes region.

References

Açıkgöz, S. (1984). The Identification of viruses on Phaseolus vulgaris L. and their distribution and damages in Erzincan and Erzurum Regions. Atatürk University, Agriculture Faculty, Erzurum, PhD Diss. Abst. 75p.

Albrechtsen, S. E. (2006). Testing methods for seed transmitted viruses: principles and protocols. Oxfordshire (UK), CABI Publishing.

Arlı-Sökmen, M., Deligöz, I., & Kutluk-Yılmaz, N. D. 2016. Characterization of Bean common mosaic virus and Bean common mosaic necrosis virus isolates in common bean growing areas in Turkey. *European Journal of Plant Patholog,* 146, 1-16. DOI: 10.1007/s10658-016-0886-x.

Babović, M. (2003). Osnovi patologije biljaka. Poljoprivredni fakultet Univerziteta u Beogradu.

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

Çolak-Ateş, A., Fidan, H., Yılmaz, N., & Konuksal, A. (2017). Botrytis fabae and Bean common mosaic virus (BCMV) are the most common diseases of Faba bean (Vicia faba L.) in TRNC. Akademik Ziraat Dergisi 6(2), 115-122.

Çulal –Kılıç, H., Yardımcı, N., Açıkyürek, S., & Uzal, A. (2015). Detection of BCMV AMV and CMV by DAS ELISA and Immunocapture RT PCR in Bean Growing Areas in The West Mediterranean Region Turkey. *Fresenius Environmental Bulletin,* 24(5), 1752-1756.

Deligöz, I., & Sökmen, M. A. (2008). Differention of *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) strains infecting common bean in Samsun Province. *The Journal of Turkish Phytopathology,* 37(1-3), 1-14.

Dizadji, A., & Shahraeen, N. (2011). Occurrence, distribution and seasonal changes of viruses infecting common bean in northwestern Iran. *Archives of Phytopathology and Plant Prot*ection, 44 (17), 1647–1654.

Galvez, G. E., & Morales, F. J. (1989). Aphid-transmitted viruses. Bean production problems in the Tropics. Shawartz H. F., Pastor-Corrales M. A. (Ed.). (333-361pp). 2nd. Edition, Cent.Int. Agric.Trop. (CIAT): Cali, Colombia.

Gilbertson, R. L., Guzman, P., Rojas, M., Crnov, R., & Mkandawire, A. (2001). Detection of bean-infecting viruses in California with an emphasis on the CRSP facilitated work. Bean/Cowpea Collaborative Research Support Program in East Africa Proceedings: January 12-14, Bean Seed Workshop, Arusha, Tanzania.

Güzel Ö., & Arlı-Sökmen, M. (2003). Determination of some viruses infecting common bean (Phaseoulus vulgaris L.) and their incidences in seed lots in Samsun Province. *The Journal of Turkish Phytopathology,* 32(2), 99-106.

Hampton, R. O. (1975). The nature of bean yield reduction by Bean yellow and Bean common mosaic viruses. *Phytopathology*, 65, 1342-1346.

Hongying, Z., Jiong, C., Jianping, C., Michael, J. A., & Mingsheng, L. T. (2002). BCMV isolates causing different symptoms in Asparagus bean in China differ greatly in the 5 parts of their genomes. *Archives of Virology,* 147, 1257-1262.

Kumar, C. A., Khetarpal R. K., Parakh, D. B., Singh, S., & Nath, R. (1994). Check list on seed transmitted viruses: Leguminous hosts. National Bureau of Plant Genetic Resources, New Delhi, 14p.

Kutluk Yılmaz, N. D., Gümüş, M., & Erkan, S.(2002). Studies on determination of virus diseases in the seeds of bean from Tokat Province. Ege University, Journal of Agricultural Faculty, 38(3), 49-55.

Larsen, R. C., Miklas P. N., Ruffel, K. L., & Wyatt, Ds. D. (2005). NL-3 K strain is a stable and naturally occurring interspecific recombinant derived from Bean common mosaic necrotic virus and Bean common mosaic virus. *Phytopathology*, 95, 1037–1042.

Mandour, A. M., Abdelmaksoud, H. M. M., & El-Tahlawey, M. A. (2013). Seed transmission of Bean common mosaic virus. *Egyptian Journal of Agricultural Research,* 91(2), 403.

Mangeni, B., Abang, M. M., Awale, H., Omuseleitch, C. N., Arinaitwe, R., Mukoye, W., Kelly, B., & Were, J. D. (2014). Distribution and pathogenic Characterization of Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) in Western Kenya. *Journal of Agri-Food and Applied Sciences,* 2(10), 308-316.

Melgarejo, T. A., Lehtonen, M. T., Fribourg, C. E., Rannali, M., & Valkonen, J. P. T. (2007). Strains of BCMV and BCMNV characterized from lima bean plants affected by deforming mosaic disease in Peru. Brief Report. *Archives of Virology*, 152, 1941–1949.

Petrović, D., Ignjatov, M., Nikolić, Z., Vujaković, M., Vasić, M., Milošević, M., & Ajduković, K. T. (2010). Occurrence and distribution of viruses infecting the bean in Serbia. *Archives of Biological Science,* 62(3), 595-601.

Rowhani, A., Maningas, M. A., Lile, L. S., Daubert, S. D., & Golino, D. A., 1995. Development of a detection system for viruses of woody plants based on PCR analysis of immobilized virions. *Phytopathology*, 85, 347–352.

Spence, N., & Walkey, D. (1995). Variation for pathogenicity among isolates of *Bean common mosaic virus* in Africa and a reinterpretation of the genetic relationship between cultivars of *Phaseolus vulgaris* and pathotypes of BCMV. *Plant Pathology,* 44, 527-546.

Wortmann, C. S., Kirkby, R. A., Elude, C. A. & Allen, D. J. (1998). Atlas of common bean (Phaseolus vulgaris L.) production in Africa. CIAT publication, No. 297.