Occurrence of *Alfalfa Mosaic Virus* (AMV) Infecting Bean Crop in Burdur Province, Turkey

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ABSTRACT— During surveys conducted in 2012- 2013 growing season, field-grown bean plants with symptoms of mosaic, chlorotic mottling, vein banding, blistering and leaf malformation were observed. A total of 118 leaf samples were collected from various locations in this region. *Alfalfa mosaic virus* (AMV), was detected in bean, using enzyme-linked immunosorbent assay (ELISA), and immunocapture-reverse-transcription-polymerase chain reaction (IC-RT-PCR) methods. ELISA tests showed that among 118 field samples tested, 7 were infected with AMV. Seven leaf samples that had tested positive in DAS-ELISA were used for IC-RT-PCR. IC-RT-PCR was carried out by using specific primer which amplified a 351 bp fragment of coat protein of AMV in samples. The presence of AMV in the leaf samples was further confirmed IC-RT-PCR using specific primers.

Keywords— Virus, detection, ELISA, IC-RT-PCR

1. INTRODUCTION

Bean (*Phaseolus vulgaris* L.) belongs to the family Leguminosae and is one of the most important legume crop cultivated over nearly 28 million hectares producing approximately 20 million tonnes worldwide (Sing *et al.*, 1991; Loebenstein and Thottappilly, 2004; El-Aal *et al.*, 2011). *Phaseolus vulgaris* L. is a common bean which is an important dietary vegetable and main protein source in the diet of people of many countries. It ranks third among legume crops after chickpea and lentils with a cultivated area acreage of 93,174 ha and production of 200,000 tonnes in Turkey (Çalışkan, 2014). The climatic, irrigation and soil conditions in Burdur province of the Turkey are suitable for vegetable production. Bean is an important crop in this region: It is affected by many biotic and abiotic factors, among these, virus diseases are most prominent ones leading to massive economic losses (Petrovic *et al.*, 2010).

AMV is one of the most important and wide spread plant viruses and is found to infect 599 species belonging to 245 genera of 68 families, most of which are of the Fabaceae family (Jaspars and Bos, 1980). Alfalfa mosaic virus is the type species of the genus *Alfamovirus* and belongs to the family Bromoviridae RNA_s 1,2,3 and subgenomic RNA 4 are separately encapsidated into bacilliform particles which are 18 nm wide and have lengths characteristic of the RNA encapsided (Zitikaite and Samuitiene, 2008).

AMV can cause various mosaic, scattered bright yellow dots on leaves and deformations on bean. AMV can be transferred from infected to healthy bean plants by seeds and aphids in non-persistent manner (Kaiser and Hannan 1983; McLaughlin, 1991).

This is the first report of detection of AMV on bean plants from Burdur Province, Turkey. In this study, DAS-ELISA and IC-RT-PCR methods were used to evaluate AMV incidence in bean plants grown in Burdur province, Turkey.

2. MATERIAL AND METHODS

2.1. Field survey

Survey was conducted in growing season 2012-2013 in bean growing areas of Burdur Province, Turkey. A total of 118 bean leaf exhibiting virus-like symptoms were collected during surveys in this region. Leaf samples from each plant were placed into plastic bags and labelled . The samples were brought to the laboratory by placing on ice and stored at − 20ºC until tested. For the control of virus diseases, primarily their diagnosis is required. Various methods are used for

this purpose. ELISA is widely used for the detection of plant viruses (Clark and Adams, 1977). PCR technique is much more sensitive than other methods (Clark and Adams, 1977; Hassan *et al.,* 2006).. In this study, ELISA and IC-RT-PCR methods are used for AMV diagnosis.

2.2. DAS-ELISA Tests

All leaf samples were tested for the presence of AMV by ELISA using specific ELISA detection kits for AMV (Bioreba AG, Switzerland). Absorbance values of alkaline phosphatase were measured at 405 nm with microplate reader (EL X 800 universal Microplate Reader Bio-Tek Instruments, Inc.B-2610, Wilrijk, Belgium). Samples with absorbance values greater than twice the mean absorbance reading of healthy controls were considered positive for virus [Choi et al., 2002].

2.3. Immunocapture Method

IC-RT-PCR assay was conducted for AMV according to the procedure described by Rowhani *et al.,* (1995) Microtubes were incubated over night at 4°C with 100 µl of antibody (1 µg/ml) and washed with PBS−Tween buffer and incubated with ELISA-positive plant tissue extract. After thorough three times washing with PBS−Tween and a final rinse with sterile water, the treated PCR tubes were ready for RT-PCR.

Reverse transcription was performed in a 50 µl reaction mixture containing 21 µl H_2O; 25 µl 2 x 1 PrimeScript OneStep RT-PCR buffer (containing 400 µM dNTP mixture and One Step Enhancer solution); 2 µl PrimeScript 1step enzyme mix (Primescript RTase, Taq DNA Polymerase , RNase Inhibitor) and 1 µl of each primers (20 µM) (Takara Bio Inc.).

RT-PCR of AMV coat protein gene portion of approximately 351 bp were amplified (Xu and Nie, 2006).
F-5'-CATCATGAGTTCTTCACAAAAG-3'
R-5'-TCGTCACGTCATCAGTGAGAC-3'

Thermocycling procedure performed for AMV was as follows: 50 ºC for 30 min, 94 ºC for 2 min, followed by 30 cycles of 94 ºC for 30 s, 55 ºC for 30 s and 72 ºC for 1 min and 72 ºC for 3 min. IC-RT-PCR products were separated by 1% agarose gel electrophoresis, stained with ethidium bromide (0.5 µg/ml) and visualized under UV transilluminator with a digital camera (UVP-Doc-It Imaging System, UK).

3. RESULTS AND DISCUSSION

In the surveys, several different virus or virus-like symptoms, including mosaic (Fig 1, 2), local and systemic chlorosis, leaf spots, vein clearing, leaf deformation, discoloration, and various degrees of dwarfing were observed on bean plants. Similar observations were reported in other studies (Güzel and Arlı-Sökmen, 2003; Ghorbani *et al.,* 2010).

Figure 1: Mosaic Symptoms Observed on AMV-Infected Bean Leaves.

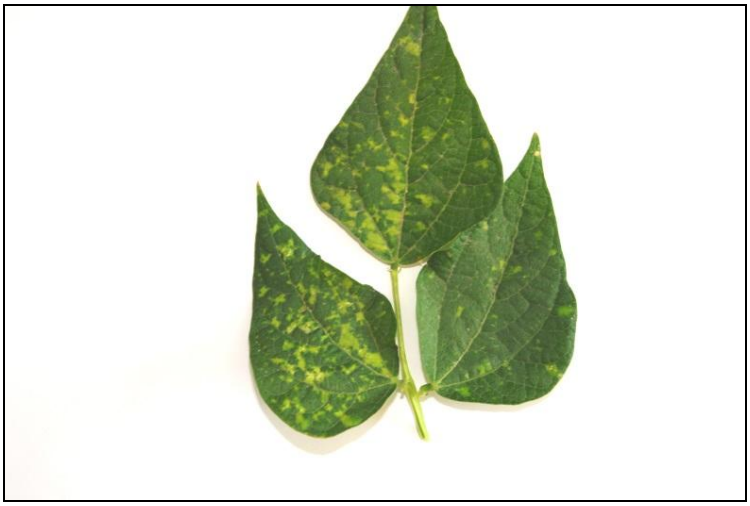

Figure 2: Yellow Spotting Symptoms Observed in Bean Leaves.

The results showed that 7 out 118 samples (5.9 %) were infected with AMV. ELISA test showed the presence of AMV infection in Burdur province in Turkey. Several researchers have used ELISA tests to reveal the plant viruses. ELISA is a routine and reliable test to diagnose plant viruses (Ghorbani *et al.*, 2010). In this study, 7 samples which were positive in DAS-ELISA were tested by IC-RT-PCR to confirm of AMV infection. The results confirmed the specificity of the primers used in this study. IC-RT-PCR test showed the presence of AMV with PCR products of the expected size (351 bp for AMV). No band was observed in healthy plants (negative controls). As a result of IC-RT-PCR, infection of all 7 samples, with serologically detected viruses were verified by this technique (Fig. 3).

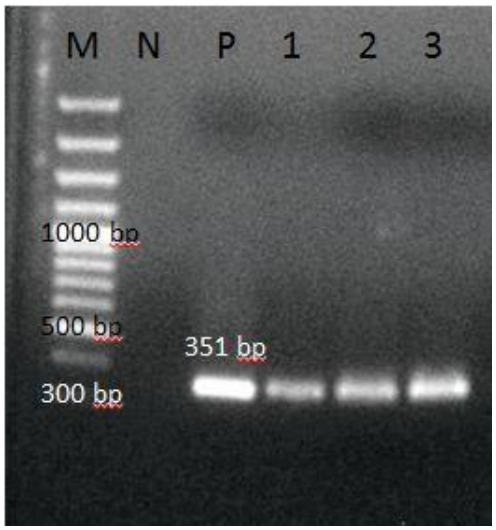

Figure 3: Agarose gel electrophoretic stained by ethidium bromide analysis of RT-PCR amplification of AMV cp gene from infected bean. M: Marker 100 bp DNA ladder (TAKARA). P: Positive control, N: Negative control, Lane1-3 infected field samples.

RT-PCR method is used for detecting plant viruses within many genera such as potyviruses and cucumoviruses (Sanchez-Navarro *et al.*, 2006; Choi *et al.*, 1999; Dietzgen, 2001). RNA extraction is troublesome and RNA molecules degrade readily because of the ubiquitous presence of RNase. In contrast, the IC-RT-PCR method avoid the extraction of viral or plant total RNA, and is easily performed in a single tube (Webster *et al.*, 2004). The results obtained in this study show the successful use of IC-RT-PCR as a rapid assay for direct detection of AMV in infected tissues of bean samples.

4. CONCLUSION

In this study, serological, and molecular assays revealed that *Alfalfa mosaic virus* was present in bean plants produced in this region. Biological, serological and molecular assays have generally been used for identification of vegetable viruses. Although ELISA is the preferred assay for routine virus detection, RT-PCR has increasingly been used for detection and identification of viruses due to higher level of sensitivity (Herranz *et al.*, 2005; Sanchez *et al.*, 1998; Saade *et al.*, 2000). Although the detection of AMV on beans in other regions of Turkey has previously been reported (Güzel and Arlı-Sökmen, 2003; Açıkgöz, 1984; Kutluk-Yılmaz *et al.*, 2002) AMV of infecting bean growing areas in Burdur province, Turkey were first identified and reported in this study.

Furthermore, *Alfalfa mosaic virus* has a wide host range among weed and crop plants (Kaiser and Hannan, 1983; McLaughlin, 1991). AMV is transmitted via seed or aphids. Spread from alfalfa to the surrounding crops via aphids is common (Loebenstein and Thottappilly, 2004). Later on, AMV completes its life cycle on bean plants at the end of growing seasons. Further characterization and the epidemiology of AMV needs to be investigated further. Besides, the farmers should be informed about how viruses spread from plant to plant and precautions for controlling virus transmission.

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