



Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 6(9) pp. 310-313, September, 2017 Issue.  
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*Full Length Research Paper*

# A new Rhabdo virus Infection in Maize Plantations in Turkey

Filiz Ertunc

Ankara University, Faculty of Agriculture, Department of Plant Protection, 06110 Ankara, Turkey  
Email:ertunc@agri.ankara.edu.tr

Accepted 05 October, 2017

**Maize mosaic rhabdo virus (MMV)** is devastating virus infection, causes serious crop losses, on the family *Poaceae* is especially on maize and transmitted by leaf hopper, *Peregrinus maidis*. Samples were collected from leaves and stems of maize plants, from Bartın, Düzce, Sakarya and Zonguldak provinces of Turkey, in 2012-2013. They were tested against MMV antiserum by DAS-ELISA and also RT-PCR assay with a primers specific to glycoprotein gene of the virus, designed in our laboratory. 15 of the collected maize samples were found to be infected with MMV. Positive samples were mechanically inoculated to maize plantlets for propagation and maintain. MMV inoculated maize plants, show light mosaic symptoms were tested by ELISA and RT-PCR again, obtained bands were 470bp long. In electron microscopic observations, rhabdovirus-like particules were detected. So far to our knowledge, it is the first report of Maize mosaic rhabdovirus presence in Turkey.

**Keywords:** Maize, MMV, Rhabdovirus, RT-PCR, electromicrocopy

## INTRODUCTION

Maize (*Zea mays* L.) is a culture plant, belonging to *Poaceae* family, and is grown as food and feed in Turkey. Turkey is one of the main producer of maize and it is cultivated in 6.800.000 da area with annual production is 6.400.000 tons (TUIK, 2016). It is widely grown in northern and southern parts of Anatolia peninsula (TUIK, 2016). It is mainly produced as grain and as silage for animal feeding in Turkey.

Many fungal, bacterial and viral diseases are detected in corn plantations according to the ecological conditions. They all cause some disorders in leaves, cobs and stem of corn plants and lower the quantity and quality of the production. Symptoms occurring by the virus infections are quite similar to each other: they all cause severe systemic streak mosaic on leaves, sheath and husk stripes are parallel to the main veins, severe stunting and deformation

on the cobs of the infected maize plants. More than forty virus infections are detected in corn plantations in the Mediterranean basin and in the world (Ivanovic et al. 1995; Lapierre and Signoret, 2004). *Maize dwarf mosaic potyvirus* (MDMV), *Sugarcane mosaic potyvirus* (SCMV), *Barley yellow dwarf luteovirus* (BYDV), *Maize stripe virus* (MSpV), *Maize mosaic rhabdo virus* (MMV), *Maize streak geminivirus* (MSV), *Maize white line mosaic virus* (MWLMV), *Johnsongrass mosaic potyvirus* (JGMV), *Wheat spindle streak mosaic virus* (WSSMV) and *Cucumber mosaic virus* (CMV) are the most widespread virus infections of corn plantations in the World (Lapierre and Signoret, 2004; Brunt et al., 1996). The widespread virus infection of corn plantations in Turkey is MDMV, and it is present in corn plantations since 1991 (Baloğlu et al. 1991). The viruses in corn plantations located in Thrace

region has been investigated by Ilbağı et al. (2006) and predominant infection has been detected as MDMV, JGMV, SCMV and BYDV-PAV strain. Widespread virus infections in maize fields located in the northern part of Turkey were detected as MDMV, MMV and BYDV-PAV strain (Erkan and Kutluk Yılmaz, 2009). Later the outbreaks of the MDMV infection was detected from the maize fields located in Bursa province (Ilbağı and Geyik, 2014). MDMV is the most important virus infection in United States and cause devastating epidemics in corn plantations (Josephson et al. 1969)

Virus infections belonging to *Rhabdoviridae* family are pathogens on humans, vertebrate and invertebrate animals and also plants. Some of the rhabdo viruses, infect plants are transmitted by insects and other arthropods. The genome structures of *Rhabdoviridae* family are negative-sense single stranded RNA, consisting of five core genes, with a length in the range of 11 000-13 000 nucleotides. (Tsai et al., 2005; Reed et al., 2005). Plant infecting rhabdoviruses are divided in two genera, *Cytorhabdovirus* and the *Nucleorhabdovirus* according to their multiplication sites in the infected cells.

Genus *Cytorhabdovirus*, which type species is *Lettuce necrotic yellows virus*, have relatively narrow particles about 60 nm and replicate in the perinuclear space or in the endoplasmic reticulum in plant cells. Members of genus *Nucleorhabdovirus*, which type species is *Potato yellow dwarf virus* and have relatively bigger particles about 90 nm, replicate in the nucleus and mature by budding through the inner nuclear membrane (Martelli and Russo, 1977). Plant rhabdoviruses are in bacilliform or bullet-shaped particles and can be easily distinguished from the constituents present in infected tissues by electron microscopic observations of infected plant saps or thin sectioning of the infected tissues.

Maize mosaic virus (MMV) is member of *Rhabdoviridae* family, *Nucleorhabdovirus* genus, that is transmitted by *Peregrinus maidis* and leafhoppers in a circulative, propagative manner. MMV, wide spread in the family *Poaceae*, is virus disease that causes serious crop losses, especially on maize (Lapierre and Signoret, 2004). MMV consists of four structural proteins, which are N (54 kDa nucleocapsid protein), M1 and M2 (Matrix protein), G (70 kDa Glycosylate) and L (241 kDa RNA polymerase) proteins. The surrounding membrane contains two proteins: the matrix, M protein (or proteins), which is in a hexagonal array, and the G protein (glycoprotein), which forms the surface projections. Size and details of negatively stained particle morphology was observed by electron microscopic investigations. Particles of these viruses readily deform and fragmented *in vitro* unless pH and other conditions are closely controlled (Martelli and Russo, 1977). Iranian isolates of MMV have been reported as distinct from the other isolates according to the nucleotide sequences (Massah et al. 2008).

This research has been conducted in order to verify the presence of rhabdovirus infections in Turkey.

## MATERIALS AND METHODS

### Collection of virus isolates

Symptomatic and asymptomatic leaf, and corn cob samples were collected from maize fields located in Bartın (Merkezve Ulus), Düzce (Merkez, Çilimli, Gölyaka, Kaynaşlı), Sakarya (Merkez, Akyazı, Söğüt, Kaynarca), and Zonguldak (Merkez, Çaycuma, Devrek, Ereğli, Gökçe) provinces (Western Black Sea Region), which are the provinces commonly maize produced in Turkey. Samples were collected in summer, in July and August, in 2012. Each plant sample was taken per 10.000 kilometer square area. Sampling has been done according to the enlargement of production areas of each provinces. They were kept in deep-freezer (-25 °C) until they were processed.

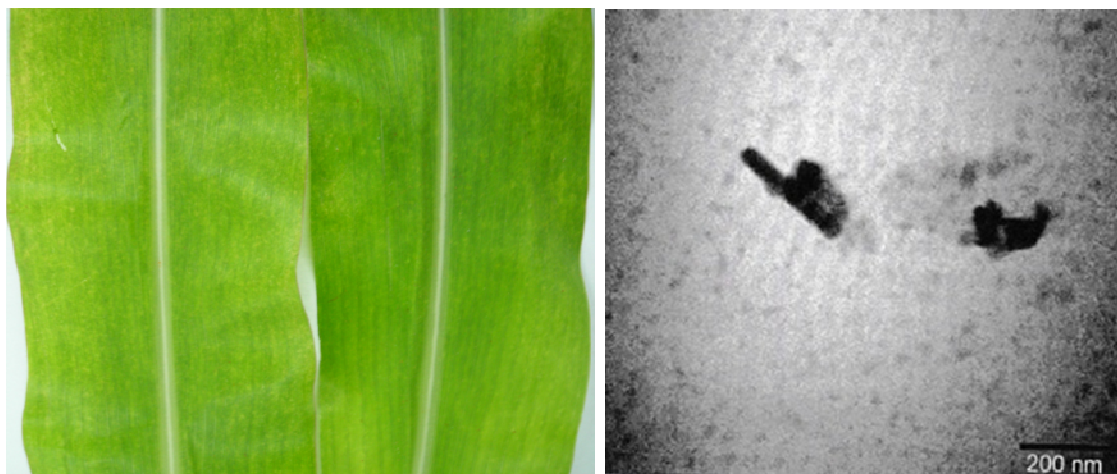
**DAS-ELISA Test:** All of the collected samples were subjected to DAS-ELISA test (Clark and Adams, 1977) against MDMV, SCMV, BYDV-MAV, BYDV-PAV, MSsV, MMV, MWLMV, JGMV, WSSMV, BSMV, MCMV, CMV polyclonal antisera kits of Agdia (USA) according to the manufacturer's instructions.

### Electron Microscopy

Electron microscopic observations were performed for to observe the bullet-shaped particles of MMV partially purified according to the method of Rana et al. (1988). MMV infected maize leaves were cut small pieces and infiltrated in 0,05 M glycine solution containing 0,02 M Mg Cl<sub>2</sub> (pH 8,5) under vacuum. Samples were homogenated and squeezed from cheese cloth. Solution was centrifuged at low speed (2000 rpm) for 5 minutes, and 1 mM DIECA and 3% active charcoal was added to supernatant, then it was centrifuged at 25.000 rpm for 1,5 hours. Pellet was suspended in extraction buffer (pH: 7,5) containing 5 % sucrose and density gradient centrifugation was performed in 30-60 % sucrose solution at 22.500 rpm for 1 hour. Maize mosaic virus particle band was detected (in 40-50% sucrose solutions) were collected. Partially purified MMV preparations were used for electron microscopic observations. Grids were stained 1% uranyl acetate solutions and observed with Jeol JEM 1400 electron microscope at magnification of 100.000 X.

### Total RNA extraction

Total RNA was extracted from MMV infected leaves by Foissac et al. (2001). 100 mg plant tissue was macerated in a sterile mortar and pestle in extraction buffer, 500 µl of



**Figure 1.** Symptoms of MMV on maize leaves and electron microscopical observations of particles (Bar represents 200 nm)

the extract has been transferred to a sterile tube and 10% sodium lauryl sulphate solution was added to the tube. Tubes were incubated at 70 °C for 10 minutes and on ice for 5 minutes then centrifuged at 14,000 rpm for 10 minutes. 300 µl of supernatant was transferred to a new tube and 150 µl ethanol, 25 µl silica suspension and 300 µl 6 M sodium iodine was added. The mixture was shaken for 10 minutes and centrifuged at 6000 rpm 1 minute. Pellet was washed with washing solution then again centrifuged at same speed. Pellet was solved in 150 µl RNase free water and incubated at 70 °C for 4 minutes. The mixture was centrifuged at 14,000 rpm for 3 min and supernatant were collected in a new tube and kept at – 20 °C until they were processed.

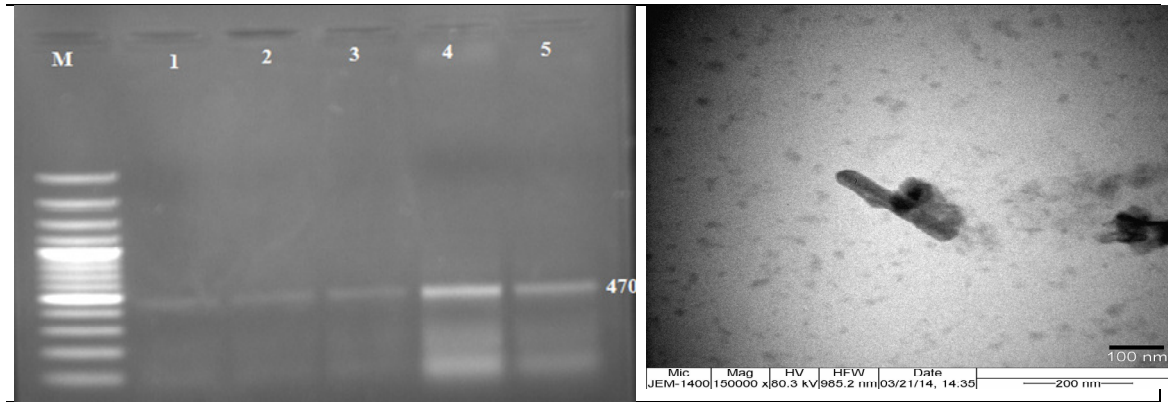
#### Primer Design and RT-PCR Assay

The primers used were designed by us with Primer three programme and were specific to the MMV glycoprotein gene. As primer, MMV-TF5'-CGGGATAGGGAGAGGAAAGT-3' and MMV-TR5'TAATGACGGAAGCCAAGGAC -3' were designed by us. The PCR mixture also contained 2.5 µl of 10x reaction buffer (Fermentas), 1 mM dNTP mixture, 3 mM MgCl<sub>2</sub>, 2.5 U Avian myeloblastosis virus (AMV) reverse transcriptase (Fermentas), 2.5 U Taq DNA polymerase (Fermentas), 2 pmol of each primers. Amplification reaction was as follows: one cycle of RT reaction at 50°C for 30 min, initial denaturation for 3 min at 94°C, followed by 35 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 3 min and the final extension was at 72°C for 10 min. RT-PCR amplifications was done in Eppendorf Mastercycler gradient thermocycler. Amplified

DNA fragments were applied to electrophoresis on a 1.5% agarose gel in TAE buffer and stained with ethidium bromide and visualized by Sygene Gene Genius image analyser (Cambridge-UK).

#### RESULTS AND DISCUSSION

During the surveys done in 2012, totally, 111 leaves and 111 corncob samples were collected from the maize fields and were all subjected to DAS-ELISA test. 15 of tested samples were found to be infected with MMV. The particle structure of MMVinfected particles were partially purified according to Rana et al. (1988) and were observed by electron microscopy (JEM-1400), and particle sizes have been evaluated as 283,6 nm in the electron microscopical observations (Figure 1). Infection was also confirmed by RT-PCR assay and amplified products were 470bp long, obtained by PCR amplification (Figure 2). Infected samples were mechanically inoculated maize plants, in 2-3 leaves stage in greenhouse conditions, using 0.02 M phosphate buffer. In green house condition, maize plants showed light mosaic symptoms, which are specific to MMV (Figure1). The inoculated maize plants were again tested for MMV presence by DAS-ELISA and RT-PCR tests. Thus, infection of MMV were again confirmed from mechanically infected maize plants. This results indicates that MMV is transmitted mechanically in maize plants. Previously, MMV infection has been reported from Samsun province by and Erkanand Kutluk Yilmaz (2009) and detection has been performed by DAS\_ELISA.



**Figure 2.** RT-PCR amplification of MMV isolates (470 bp) 1-3 :Duzce isolates, 4-5:Sakarya isolates

## CONCLUSIONS

In the present research we have confirmed the presence of MMV by mechanical inoculation, DAS-ELISA, particule morphology and also RT-PCR assay by the primer designed by us. So far to our knowledge, this is the first report on the presence of rhabdovirus infection, detected in maize plantations in Turkey.

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