International Scholars Journals

African Journal of Infectious Diseases Research ISSN 2756-3340 Vol. 10 (1), pp. 001-004, January, 2023. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

A new Rhabdo virus Infection in Maize Plantations in Turkey

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Accepted 05 October, 2022

Maize mosaicrhabdo virus **(MMV) is devastating virus infection, causes serious crop losses, on the family *Poaceae* is especially on maize and transmitedby leaf hopper, *Peregrinusmaidis.* 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 plant lets 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 producter 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 occuring by the virus infections are quite similar to each other: they all cause severe systemic streak mosaic on leaves, sheath and huskstripes 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 mozaicrhabdo 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 Turkeywere 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 (Ilbagı 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 arepathogens onhumans, vertebrate and invertebrate animals and also plants. Some of the rhabdo viruses, infect plants are transmitted by insectsand 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 particules about 90nm, replicate in the nucleus and mature by budding through the inner nuclear membrane(Martelli and Russo, 1977). Plant rhabdoviruses arein 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, *Nucleorhabdovirusgenus*, that is transmitted by *Peregrinusmaidis* 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 corncob samples were collected from maize fields located in Bartın (Merkezve Ulus), Düzce (Merkez, Çilimli, Gölyaka , Kaynaşlı), Sakarya (Merkez, Akyazı, Söğütlü, Kaynarca), and Zonguldak (Merkez, Çaycuma, Devrek, Ereğli, Gökçebey) provinces (Western Black Sea Region), which are the provinces commonly maize produced in Turkey. Samples were collected in summer, in July and August, in2012.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 ^{O}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,MSpV, MMV,MWLMV, JGMV, WSSMV, BSMV, MCMV, CMV polyclonal antisera kits ofAgdia (USA) according to the manufacturer's instructions.

Electron Microscopy

Electron microscopic observations were performed for to observe the bullet-shaped particules of MMV partially purified according to the method of Rana et al. (1988). MMV infected maize leaves were cut small pieces and infilitrated in 0,05 M glycine solution containing 0,02M Mg Cl_2 (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 particule band was detected (in 40-50% sucrose solutions) were collected. Partially purified MMV preparations were used for electron microscobic 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

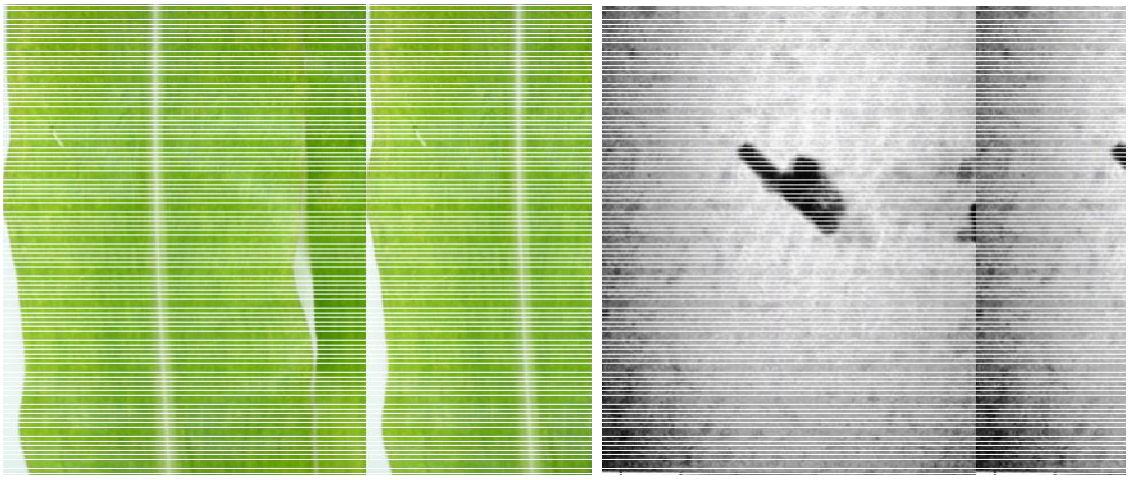

Figure 1. Syptoms of MMV on maize leavves and electron microscobic observations of particules (Bar repreesents 200 nm)

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 ^{o}C for 10 minuttes and on ice for 5 minutes then centrifuged at 14.0000 rpm for 10 minutes. 300 µl of supernatant was transferrred to a new tube and 150 µl ethanol, 25µl silica suspension and 300 µl 6 M sodium iodine was added. The mixturre was shaked for 10 minutes and centrifuged at 6000 r pm 1 minute. Pellet was washed with washing solutionn then again centrifuged at same speed. Pellet was solvved in 150 µl RNase free water and incubated at 70 ^{o}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 ^{o}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 MM V glicoprotein gene. As primer, MMV-TF5'-CGGGATAGGGAGAGGAAAGT-3' and MMV-TR5'TAATGACGGAAGCCAAGGAC -3' were designed by us. The PCR mixture also contaained 2.5 µl of 10x reaction buffer (Fermentas), 1 mM dNTP mixture, 3 mM $MgCl_2$, 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 denaturatioon for 3 min at 94°C, followed by 35 cycles of 94°C for 1 m in, 57°C for 1 min, and 72°C for 3 min and the final exteension was at 72°C for 10 min. RT-PCR amplifications was done in Eppendorf Mastercycler gradient thermocyccler. Amplified DNA fragments were applied to electrophoresis on a 1.5% agarose gel in TAE buffer annd stained with ethidium bromide and visualized by Syg ene Gene Genious image analyser (Cambridge-UK).

RESULTS AND DISCUSSION

During the surveys done in 20112, totally, 111 leaves and 111 corbcob samples were colleected from the maize fields and were all subjected to DAS-ELISA test. 15 of tested samples were found to be innfected with MMV. The particule structure of MMVinfectedparticules were partially purified according to Rana et al. (1988) and were observed by electron microscopy (JEM-1400), andparticule sizes have been evaluated as 283,6 nm in the electron microscobic observationns (Figure 1). Infection was also confirmed by RT-PCR assay andamplified products were 470bp long, obtained byPCCR amplification (Figure 2). Infected samples were mechaanically inoculated maize plants, in 2-3 leaves stage in greeenhouse conditions, using 0.02 M phosphate buffer. In green house condition, maize plants showed light mosaic symttoms, 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 plaants. This results indicates that MMV is transmitted mechanically in maize plants. Previously, MMV infection has b een reported from Samsun province by and Erkanand Kuutluk Yılmaz (2009) and detection has been performed byy DAS_ELISA.

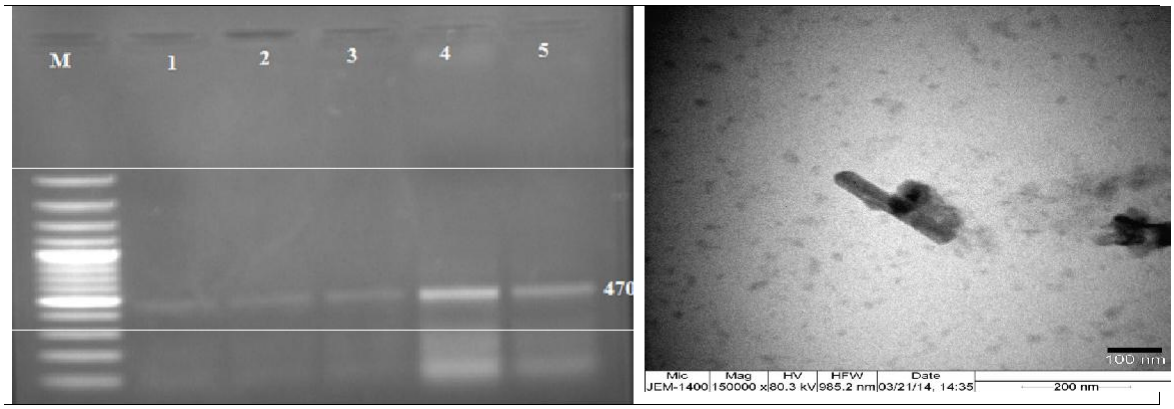

Figure 2. RT-PCR amplification of MMV isolates (470 bp) 1-3 :Duzce isolates, 4-5:Sakarya isolates

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 plantationsin Turkey.

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