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DETERMINATION OF *Tobacco mosaic virus* IN TOBACCO FIELDS IN DENIZLI PROVINCE, TURKEY

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Abstract

This study was carried out to detect of Tobacco mosaic virus (TMV) in tobacco growing areas in Denizli province, Turkey. Total of 94 plant samples including mosaic, curling of leaves, chlorotic lesion and stunding symptoms were collected from the fields in Denizli province during 2015-2016 years. Presence of Tobacco mosaic virus was investigated using Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) method. As a result of DAS-ELISA, it was found 30.85% of leaf samples with TMV.

Key words*: tobacco, TMV, DAS-ELISA.*

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is an annual plant from the family *Solanaceae. Nicotiana tabacum* is one of more than 60 species in the genus *Nicotiana*. By selecting locally adapted plants many variations have been stabilised as cultivars and different tobacco types have developed namely Virginia, flue-cured, air-cured, fire-cured, burley, oriental also called Turkish tobaccos are world wide variations of the same species. Disease resistances have been transferred by various breeding techniques from the related species of this genus, many of which have only a very small leaf on a small shrub. The types of tobacco vary in their morphology, quality, taste, aroma and final use in the blending of mixtures for use as base material for cigars, cigarettes, chewing tobacco or snuff (Ergün and Uğurlu, 2006).

Tobacco can be used in pipe, cigarette, paint, soap industry, cologne and perfume making (Şahin and Taşlıgil, 2013).

The homeland of tobacco is stated to be Central America. China is the leader in worldwide tobacco production as of 2014 with 3,000,000 tons. It is followed by Brasilia, India and USA. Whereas Turkey is ranked tenth with 90,000 tons. Denizli province is important for tobacco production due to their soil and climatic properties and rich irrigation facilities. Especially, high quality tobacco is produced in Kale and Tavas districts of Denizli (Tüik, 2015).

Significant efficiency losses occur in tobacco cultivation due to wrong or insufficient agricultural applications as well as biotic and abiotic disease factors. There are many fungi, bacteria and virus based diseases in the tobacco cultivation areas which limit cultivation.

Virus diseases are significant among these factors due to their chemical and physical structures, sizes, types of infection, symptom formation, transport and the lack of an effective struggle against them (Agrios, 1997).

The viruses that are observed in tobacco which cause significant losses in efficiency are as follows: *Tobacco mosaic virus (*TMV*), Tomato mosaic virus (*ToMV*)*, *Cucumber mosaic virus (*CMV*)*, *Potato Y virus (*PVY*)*, *Tobacco etch virus* (TEV) (Brunt et al., 1996; Zhu et al., 2002).

TMV belongs to the *Tobamovirus* type. Virus includes linear single helical RNA genome. It has a rod shaped particle structure. The virus has a length of about 300 nm and a width of about 18 nm. Virus particles consist of % 5 nucleic acid; % 95 protein. The genome is single pieced (Goelet et al., 1982).

Tobacco mosaic virus is an economically important disease infecting tobacco and other *Solanaceous* crops worldwide. TMV infects

199 different species from 30 families (Bagley, 2001).

TMV can be spread from plant to plant is on workers' hands, clothing or on tools.The disease is mechanically transmitted, resulting in quick and effective infection. TMV can be transmitted to seedlings from the contaminated seeds. First symptoms in infected plants is vein clearing. Soon after vein clearing, mosaic symptoms occur in the newer leaves. It was seen the malformation of leaves and stunting in tobacco plants (Baker and Adkins, 2000; Dawson, 1999).

TMV in different crops have previously been detected in Turkey (Yılmaz et al., 1983; Yılmaz and Davis, 1985; Bostan and Dursun, 2002; Arlı-Sökmen et al., 2005; Şevik and Köse-Tohumcu, 2011; Paylan et al., 2011; Sertkaya, 2012; Pazarlar et al., 2013).

The existence of virus diseases, their harms and their symptoms vary in accordance with the type of the tobacco plant as well as the environmental conditions. That is why, first the detection of the viruses in the culture plant that is cultivated should be made in order to minimize the damages caused by virus disease and to develop control methods. It is not correct to carry out diagnosis based only on observation for virus diagnosis.

The aim of this study is to determine TMV in the tobacco cultivation areas of Denizli province by DAS-ELISA method.

MATERIALS AND METHODS

The main material of this study consists of 94 leaf samples which show virus disease symptoms and which are thought to be infected with virus. The samples were collected from the tobacco cultivation areas (6 locations) during June-September in 2015-2016. The samplings were made from plants with leaf deformation, leaf curling, necrosis formation on the leaves, mosaic symptoms and stunting symptoms (Figure 1). The samples were labeled inside polyethylene bags and brought to the laboratory in ice boxes to be preserved in deep freezer (-20^{o}C) until the required tests were carried out.

Figure 1. Tobacco growing areas in Tavas District of Denizli province

ToMV-DAS-ELISA (Agdia, Inc, Elkhart, IN) commercial kit was used in the study. The application was carried out in accordance with the procedure of the commercial company. Accordingly, 100 µl of IgG was added to each of the hole of ELISA plate, which was diluted in the coating buffer at a ratio of 1:1000 which was then kept at +4º C for overnight.

Afterwards the ELISA plates were washed with the washing buffer. The washing was repeated 3 times. The plant extracts prepared by diluting at a ratio of 1/10 using extraction buffer was added to each hole as 100 µl and was kept overnight at +4º C. Washing was repeated the next day. After washing process, conjugated antibody was diluted at a ratio of 1:1000 in the conjugate buffer, 100 µl was added to each hole and was kept at 37ºC for 2 hours.

After the washing process, each substrate prepared as 1 mg/ml in the substrate solution was added to each hole as 100 µl and was left to wait at room temperature.

Values that give at least twice or higher readings in comparison with the negative control value according to the absorbance values read at 405 nm have been accepted as positive (Özaslan et al., 2006; Şevik and Köse-Tohumcu, 2011).

RESULTS AND DISCUSSION

Symptoms such as leaf deformation, leaf curling, decoloring of the leaves, necrotic local lesion on the leaves, mosaic symptoms, vein yellowing and stunting symptoms have been observed and these plants have been photographed during the survey (Figure 2; Figure 3). The symptoms were similar to those obtained in previous studies and support the suggestion that the virus may be TMV (Bagley, 2001; Khateri et al., 2008; Wei et al., 2014). DAS-ELISA tests were carried out on the leaf samples collected from 94 plants with virus infection symptoms in order to determine the existence of TMV. The results showed that 29 out 94 samples were infected with TMV. Whereas TMV infection rate for 94 of the collected samples was determined as 30.85% (Table 1).

Figure 2. Mosaic and leaf deformation symptoms on tobacco leaves

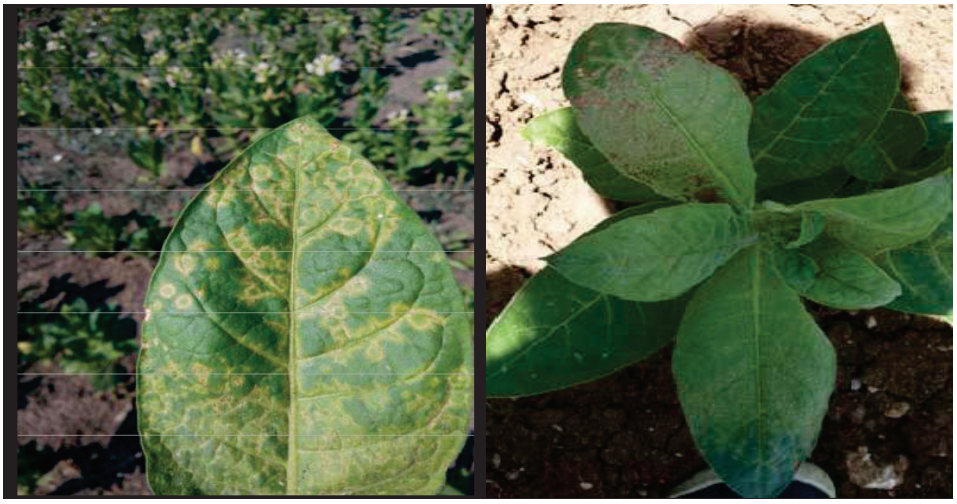

Figure 3. Ringspot and necrotic local lesion on tobacco leaves

Table 1. Samples tested, place where samples taken, number of infected samples determined by DAS-ELISA method, percentage of infection

Locations	Total Sample	TMV-infected samples	% TMV
Karaköy	35	6	17.14%
Altınova	22	9	40.91%
Sofular	6	2	33.33%
Gülözü	23	9	39.13%
Çalıköy	5	3	60.00%
Keçeliler	3	0	0.00%
TOTAL	94	29	30.85%

Nas et al. (1975) have used the sap inoculation method for identification of TMV of tobacco plants in Adana and Hatay provinces in Turkey. It has been found in this study as a result that the average disease ratio is 1.55% in Adana province and 2.04% in Hatay province.

Six tobacco samples collected from Karaköy location, nine tobacco samples collected from Altınova and Gülözü locations, two tobacco samples collected from Sofular location and three tobacco samples collected from Çalıköy location which tested positive for TMV by DAS-ELISA.

Samples collected from Keçeciler location were not found be infected with TMV (Table 1).

Virus based diseases are quite significant due to the facts that their control is mostly hard and indeed impossible, that there is no effective chemicals and that the viruses spread far and wide via vector insects.

It is essential to first diagnose the viruses found in the cultivated culture plant in order to minimize the damages due to viruses and to develop control strategies.

The symptomatologic studies that will be carried out based on observation for defining the viruses should be supported with serologic or molecular tests that will be carried out. It is possible to take precautions for the control of this virus disease only after carrying out the diagnosis of this factor (Agrios, 1997).

The ELISA tests used in the diagnosis work for many plant viruses have widely been used by researchers (Yılmaz and Davis, 1985; Khateri et al., 2008; Şevik and Köse-Tohumcu, 2011). The fact that it is fast, sensitive, economical and reliable makes this method a preference (Vinayarani et al., 2011).

In a survey conducted in the Azerbaijan and Guilan provinces of Iran for viruses infecting tobacco *Tobacco streak virus* (TSV), *Tomato spotted wilt virus* (TSWV), *Tobacco etch virus* (TEV), *Tobacco ringspot virus* (TRSV), *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) were detected among tobacco leaf samples, by ELISA.

It has been found that the most severe mosaic type symptoms including the deformation and blistering on leaves were mainly seen in the infections by CMV and TMV (Khateri et al., 2008).

Şevik and Köse-Tohumcu, (2011) reported that TMV was identified by DAS-ELISA in seed and seedling. It has been found in this study as a result of DAS-ELISA tests on the leaf samples that 93 of the 400 samples were infected with TMV.

In this study, the existence of TMV in tobacco cultivation areas at Denizli province have been found out using serological method. No study has been carried out previously in the region to detect TMV.

CONCLUSIONS

TMV can survive in seed and in soil. TMV contaminated soil should be discarded. During the growing season, infected plants should be removed from field.

Crop rotation is also very important. However, resistant plants or rotational crop should be employed to reduce the amount of inoculum in the field. Rotational crop should

not belong to the *Solanaceous* family. Farmers should be informed about how viruses spread from plant to plant and the precautions for controlling virus transmission. The results of this study showed that DAS-ELISA can be used for TMV diagnosis and certification. However, ELISA is easier, less expensive and less time- consuming.

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