

STUDY OF THE SPREAD OF TEV IN VIRGINIA, BURLEY AND BASMI TOBACCO

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Abstract

The subject of study in the present work is the spread of TEV from the potyvirus group in Virginia, Burley and Basmi tobacco varieties. The aim is to monitor the occurrence and development of TEV in these varieties grown in the central parts of southern Bulgaria and to analyze their resistance to this virus. During the period 2010 - 2019 immunological studies were carried out to prove the new viral tobacco disease in Bulgaria. A serological identification kit of the French company SEDIAG S.A.S. was used to demonstrate the virus. To establish TEV, a TAS-ELISA was performed at 1: 100 dilution of IgG. The virus was found to be sporadic. From the eight years of the study, TEV has been proven in four. The 2010-2011 period is characterized by a significant prevalence of TEV in Burley Group tobacco (14.3% and 12.7%, respectively). The virus is most strongly developed in 2019. It is evidenced in 60% of the tested samples from the Virginia variety. During the eight-year study period, only in 2010 TEV was found in isolates from the three tobacco varieties - Virginia, Burley and Basmi. As a result of the single-factor analysis of variance and Duncan's test, it was proven that tobacco from the Basmi variety was the most susceptible to infection with the TEV virus (0.165), followed by Virginia (0.136). Burley tobacco was found most resistant to the disease of TEV (0.126).

Key words: ELISA - test, TEV, Burley, Virginia, Basmi, analysis of variance.

INTRODUCTION

Plant viruses are one of the most important pathogens causing diseases of major economic importance in many basic agricultural crops, including tobacco, reducing yield and quality (Maiss, 2004). Tobacco is a natural host for more than 20 viruses, economically the most important of which and causing significant damage to tobacco production are TMV (Tobacco mosaic virus) genus Tobamovirus; TSWV (Tomato spotted wilt virus) genus Tospovirus; CMV (Cucumber mosaic virus) genus Cucumovirus; AMV (Alfalfa mosaic virus) genus Alfamovirus; TRSV (Tobacco ringspot virus) genus Nepovirus; PVY (Potato virus Y); TEV (Tobacco etch virus) and TVMV (Tobacco vein mottling virus) genus Potyvirus, (Dukić et al., 2006; Dimitrov & Bozhukov, 2004). Viruses of the type Potyvirus are the most common tobacco viruses and cause serious economic losses in this crop. The major vector responsible for the spread of viruses in this group are the aphids of the species *Myzus persicae* Sulz (Lukas, 1975; Gooding, 1985;

Kovachevski et al., 1999). The three potyviruses PVY (Potato virus Y), TEV (Tobacco etch virus) and TVMV (Tobacco vein mottling virus) are often considered by some authors as a viral complex (Greenwell, 2011; Dietrich & Maiss, 2003; Yonchev, 2014). They have been described as viruses of major economic importance for tobacco production in the United States, especially in regions such as Tennessee, Kentucky, Virginia, and North Carolina (Pirone, 1989).

PVY has been established in a number of countries, but it is proving to be the most serious problem for tobacco production in Chile, Hungary, Spain, South Africa. Last but not least, it is a problem on the Balkan Peninsula (Gooding, 1985; Kovachevski et al., 1999) Dukić et al., 2006; Duduk & Krstic, 2006; Dekic et al., 2007).

TVMV was not described until 1970, when Gooding & Sun (1972) and Pirone et al. (1973) found a new virus attacking Burley tobacco in regions of the United States such as Tennessee, Kentucky, Virginia and North Carolina. The virus is spread in North America, Canada,

Mexico, USA, Alaska, Italy, Portugal, Zambia, Colombia and China (Kenedy, 2011; Horowitz & Ishaaya, 2004).

Tobacco Etch Virus was first reported in 1928 in the United States - Kentucky by Valleau and Johnson (1928). It was then found in Canada, Venezuela, Nicaragua, India and Japan. Mickowski (1984) believes that Tobacco Etch Virus does not occur on tobacco grown in the countries of the Balkan Peninsula. In Bulgaria, Kovachevski and others (1999) report that the disease is found in tomato and thorn apple. Dimitrov and Bozukov (2004), on the basis of symptomatic diagnosis, reported the presence of TEV in the tobacco in Bulgaria.

In 2010, Tobacco Etch Virus - TEV was diagnosed serologically by ELISA in Virginia tobacco in the Plovdiv area (Yonchev et al, 2010). The virus is commonly found in tobacco mainly in mixed infections with other viruses of the genus Potyvirus, but is most commonly found in mixed infection with PVY. Potyviruses found in tobacco cannot be distinguished solely on the basis of symptoms. Accurate diagnosis is possible by applying ELISA or PCR.

The purpose of this study is to trace the occurrence and development of TEV in the three varieties of tobacco grown in the central part of southern Bulgaria and to make a comparative assessment of their resistance to TEV.

MATERIALS AND METHODS

During the period 2010-2019, immunological studies were carried out to prove the new viral disease in Bulgaria, caused by TEV (Tobacco etch virus).

For this purpose, fresh material is collected annually in the years 2010-2014 and 2017 and 2019 from the tobacco grown on the experimental fields of the Institute of Tobacco and Tobacco Products - Markovo - leaves with symptoms characteristic of the TEV virus. Samples (isolates) were obtained from 208 tobacco plants - 92 from Virginia variety, 66 from Burley and 50 from Oriental tobacco. Samples collected were subjected to an ELISA test to determine the presence of TEV.

A serological identification kit of the French company SEDIAG S.A.S. was used to prove

the potyvirus. A TAS-ELISA was performed to determine TEV with a 1: 100 dilution of IgG. The study was performed according to the methodology of the manufacturer of the ELISA kit.

Optical density (OD) reading in the ELISA test was performed using a Biotek Elx 808 spectrophotometer at a wavelength of 405 nm, 60 minutes after substrate deposition. Signals with OD values at least twice as high as those of the negative control are considered positive.

The serological evidence of TEV was obtained from the laboratory for immunity to viral diseases of the Maritza Institute of Vegetable Crops.

C. frutescens cv tobasco and *D. stramonium* are used for biotest screening for TEV, which respond with specific symptoms of TEV contamination, with pepper reacting with necrosis of the plants, wilting and death of plants, and thorn apple responds to the infection simultaneously with severe deformation and wrinkling of the leaves. The latter can take on a filamentous form.

In the present study, a comparative assessment of the impact of TEV on the different varieties was made by ANOVA and Duncan test to evaluate the differences. The mathematical processing of the data was performed using the IBM Statistics SPSS 25 statistical software (Cronk, 2016; Field, 2013; McCormick et al., 2017; Meyers et al., 2013).

RESULTS AND DISCUSSIONS

According to bibliographic data, three potyviruses TVMV, TEV, and PVY are common in tobacco, which cause similar symptoms (Shew & Lucas, 1991; Kennedy, 2011; Uzest, 2007). On the basis of these data, samples were collected from plants with differing symptoms of Potato Virus Y, which were subjected to an ELISA test for the presence of the new in Bulgaria potyvirus TEV (Photos 1 and 2).

The results of the conducted analyzes strongly prove that tobacco complexes or self-contained viruses cannot be distinguished solely on the basis of symptoms (Yonchev, 2015). A number of other authors draw similar conclusions (Gooding and Lapp, 1980; Kennedy, 2011; Chi et al., 2011; Gooding & Sun, 1972). Accurate

diagnosis is possible through the use of ELISA or PCR (Kennedy, 2011).



Photo 1. Symptoms of a disease caused by TEV with tobacco isolate from the Basmi variety



Photo 2. Symptoms of a disease caused by TEV with tobacco isolate from the Virginia variety

Figures 1-5 show the results of the ELISA test performed on all 208 samples.

From Figure 5 it can be seen that the TEV potyvirus is sporadic throughout the study period. From the eight years of the study, TEV has been demonstrated in four of the reporting years. The virus was proven by ELISA - test in the test samples for two consecutive years - 2010 and 2011, after which in 2012, 2013, 2014 and 2017 the virus was not detected, but in 2018 and 2019, TEV recurred. In 2010, TEV was proven on 12 (24.5%) of the samples of the three varieties tested during the reporting year. In 2011, the virus was detected in 10 samples (15.9%), in 2018, 7 of the tested samples (20%) responded positively, and in 2019 the positive isolates were 6 (60%) (Figures 1, 2, 3 and 4).

The period 2010-2011 is characterized by a serious spread of TEV in the large-leaf tobacco of the Burley variety, 14.3% and 12.7%

respectively, while in 2018 the virus is detected in 20% of the tested samples of the Basmi variety. The virus develops most strongly in 2019. It is evidenced in 60% of the tested samples from the Virginia variety.

During the eight-year study period only in 2010, TEV was demonstrated in isolates from the three tobacco varieties - Virginia, Burley and Basmi (Figure 5).

The results from the study show that TEV does not infect tobacco annually in the study area, such as PVY. It can be concluded that when TEV develops, it can be found in all three varieties of tobacco.

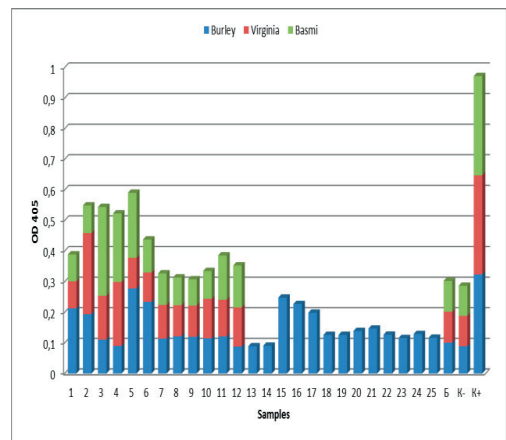


Figure 1. Identification of TEV in tobacco genotype plants of Burley, Virginia and Basmi varieties in 2010

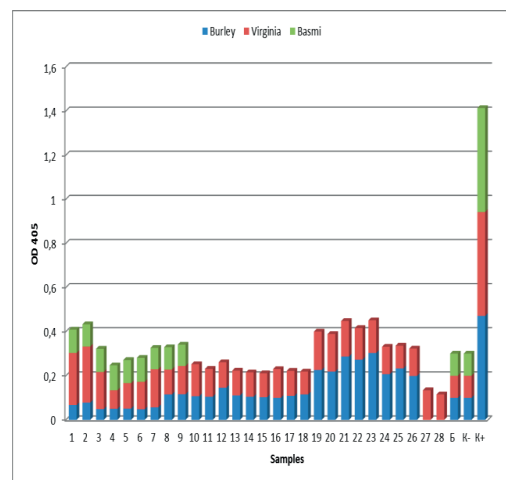


Figure 2. Identification of TEV in tobacco genotype plants of Burley, Virginia and Basmi varieties in 2011

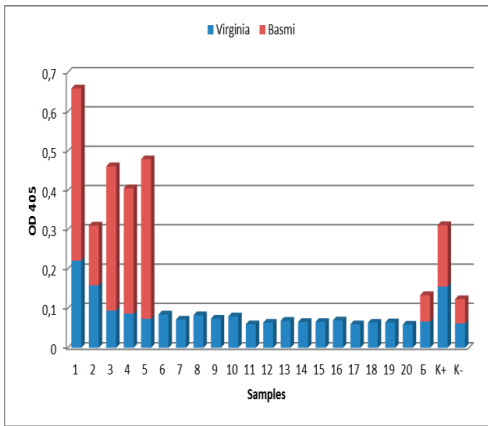


Figure 3. Identification of TEV in tobacco genotype plants of Burley, Virginia and Basmi varieties in 2018

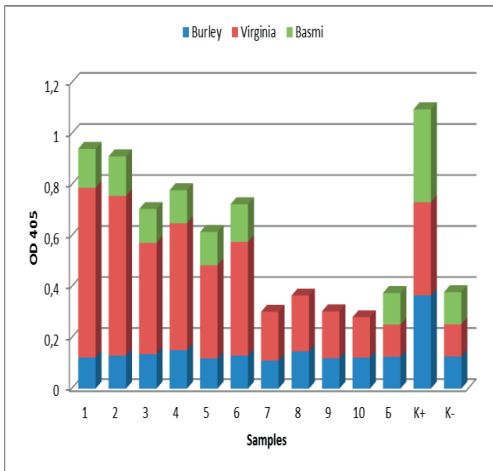


Figure 4. Identification of TEV in tobacco genotype plants of Burley, Virginia and Basmi varieties in 2019

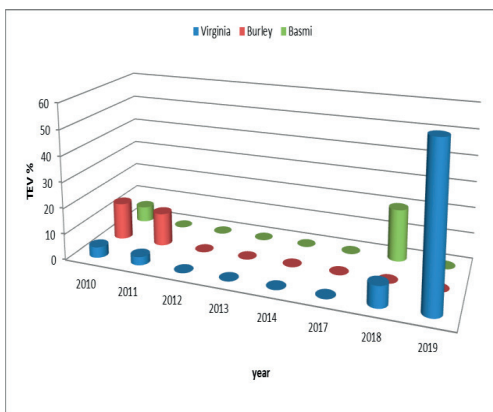


Figure 5. Percentage distribution of TEV by tobacco varieties in different years of the study

The spread of the TEV virus in different tobacco genotypes over the whole study period is at different scales, as evidenced by the comparative evaluation of Virginia, Burley and Basmi varieties. A prerequisite for its implementation is the homogeneity of the experimental data base. This was verified by the Levene's Homogeneity Test (Test of Homogeneity of Variances, Levene Statistic). It was found that the varieties studied could be compared according to the severity of TEV.

As a result of the One-way Analysis of Variance and Duncan's test, it was proved that tobacco from the Basmi variety was the most susceptible to infection with the TEV virus (0.165), followed by Virginia (0.136) (Table 1). The most resistant to TEV disease is Barley (0.126).

Taking into account the standard deviation values (0.042), it should be considered that Barley is not only the most resistant varietal group to TEV infection, but this stability is maintained over time. This fact proves that environmental factors do not have a significant effect on Barley morbidity. In Basmi it was proven that its high level of susceptibility to the virus is unstable over time, i.e. environmental changes have an impact on its sustainability. This fact warrants future research to determine the factors that have a positive effect on the prevention of tobacco from the Basmi variety from TEV infection.

Table 1. Comparative evaluation of tobacco varieties according to the disease rate of the TEV virus by one-way ANOVA and Duncan test at significance level $\alpha = 0.05$

Name	Duncan	Std. Deviation	Std. Error
Barley	0.126 ^b	0.066	0.008
Virginia	0.136 ^{ab}	0.042	0.007
Basmi	0.165 ^a	0.108	0.021

CONCLUSIONS

The TEV virus appears sporadically. From the eight years of the study, TEV has been demonstrated in four of the reporting years - 2010, 2011, 2018 and 2019.

The period 2010-2011 is characterized by a high prevalence of TEV in the large-leaf tobacco of the Burley variety, 14.3% and 12.7% respectively.

The highest spread of the virus is in 2019. It is evidenced in 60% of the tested samples from the Virginia varietal group. During the eight-year study period, only in 2010, TEV was proven in isolates from the three tobacco varieties - Virginia, Burley and Basmi. Basmi variety tobacco is the most susceptible to infection with the TEV virus, followed by Virginia, and the most resistant is Barley. In addition, environmental factors do not have a significant effect on Barley morbidity. TEV can be found in all three varieties of tobacco when it has the right conditions to manifest itself.

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