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SPREAD OF STOLBUR IN SOME TOMATO VARIETIES AND INDICATORS OF THEIR PRODUCTIVITY

Irina ZAMORZAEVA, Aighiuni BAHSIEV, Nadejda MIHNEA

Institute of Genetics, Physiology and Plant Protection, 20 Padurii Street, MD-2002, Chisinau, Republic of Moldova

Corresponding author email: izamorz@gmail.com

Abstract

The aim of this study was to identify the distribution of 'Ca. P. solani' in the four Moldavian tomato varieties during growing season and to evaluate their productivity and fruit quality. Molecular diagnosis of phytoplasma was used. The most significant difference in the percentage of infected tomato plants between the studied varieties was recorded at the stage of mass ripening of fruits. It has been found that the varieties Elvira and Desteptarea were more susceptible to 'Ca. P. solani' compared with Cerasus and Mary Gratefully. At the end of the period of vegetation all analyzed varieties were significantly loaded with phytoplasma infection. Differences in the indicators of productivity and quality of fruits in the studied tomato varieties were determined. In general, varieties that were more resistant to phytoplasma infection, respectively, had a higher yield and better fruit quality. Thus, we can conclude that, in addition to other plant diseases, climatic conditions of the year, some features of the genotype, etc., stolbur negatively affected productivity and, especially, reduced the quality of tomato fruits.

Key words*: phytoplasma, molecular diagnosis, tomato varieties, productivity, fruit quality.*

INTRODUCTION

'*Candidatus* Phytoplasma solani' is a pathogen that affects a wide range of crop plants including tomatoes (EFSA, 2014). This phytopathogen colonizes the phloem of plants and is transmitted by insects vector of the order Hemiptera, families *Psyllidae*, *Cicadellidae* and *Cixiidae* (Bertaccini & Duduc, 2009; Weintraub & Beanland, 2006).

Phytoplasmas belong to the Mollicutes class. The genus Candidatus Phytoplasma comprises more than 43 candidate species that fall into ribosomal groups and subgroups. Molecular tools have been developed that allow more precise differentiation of phytoplasma. In particular, non-conservative single-copy non-ribosomal genes, such as ribosomal protein (rp), secY, secA, rpoB, tuff, groEL (chaperonin) have been widely used to differentiate most phytoplasmas (Bertaccini, 2019).

Phytoplasmosis causes considerable losses in the quality and productivity of agricultural production (Bertaccini & Duduk, 2009). This disease (stolbur) is a widespread in tomato in Moldova. Its distribution in tomato fields is controlled by the climatic conditions of the year, being more or less abundant in different growing seasons. However, stolbur is presented in Moldavian tomato fields every year and has a negative effect on the fruits production. Disease control is possible. Modern agricultural techniques, biological control, use of resistant varieties, chemical treatments are considered as the basic components of disease management Chemical control is usually effective, but it has some negative consequences such as the reduction of amount of many beneficial organisms, as well as a toxic impact on the human health and the environment (Gavrilescu & Chisti, 2005). The use of tomato varieties resistant to pathogens for the control of phytoplasma infection has a number of economic and ecological advantages. The degree of damage, as well as the percentage of infected plants, vary from one variety to another.

Analyzing the sensitivity of some varieties of tomato to phytoplasma infection, it is necessary to take into account the combination of the environmental factors of the current year. From the other hand, these factors directly affect the productivity and quality of tomatoes.

The aim of this study was to detect the spread of '*Ca*. P. solani' in the four Moldavian tomato varieties and to evaluate their productivity and fruit quality during the growing season of 2018.

MATERIALS AND METHODS

The phytoplasma infection spread was studied on the Moldavian tomato varieties Elvira, Cerasus, Desteptarea and Mary Gratefully created in the Institute of Genetics, Physiology and Plant Protection (Chisinau, Moldova). The phytoplasma presence was identified by molecular analysis in individual tomato plants (20 plants in each variety) collected at the period of mass fruit ripening (August) and the end of the season of vegetation (September) of 2018.

DNA for the molecular identification was isolated from the fruit peduncle of each plant by express alkaline boiling method (Guo et al., 2003). This method consists of few fast consequent steps: thin sections of fruit peduncles are boiled in 10 µl 0.3 N NaOH for 5 minutes; this mix is neutralized by adding 10 µl 0.3 N HCl; obtained mix is centrifuged for 3 minutes at 10000 rpm. A 1 µl aliquot of obtained solution is used as a template DNA in the first round of nested-PCR. Two pairs of specific primers designed on the base of nucleotide sequence of '*Ca*. P. solani' chaperonin gene were used in both rounds of nested-PCR: cpn421F / cpn421R (round I) and cpn200 F / cpn200R (round II) (Zamorzaeva, 2015). The following programs for amplification were used: I - 94°C 5 minutes; II - 94°C 30 seconds, 60°C 30 seconds, 72°C 30 seconds × 30 cycles (round I) or × 35 cycles (round II); III - 72°C 10 minutes; IV - 4°C ∞. The products of round II of PCR were registered in UV light after the electrophoresis on 1.5% agarose gel in TBE×1 stained by ethidium bromide.

The productivity (t/ha) of the four tomato varieties were evaluated in the growing season of 2018. The yield of marketable fruits was additionally registered in each studied variety. The percentage of marketable fruits was calculated as an important indicator of fruits quality of analyzed tomato varieties.

A statistical processing the data obtained by molecular methods and quantitative measurements were carried out. Fisher's criterion was applied to qualitative traits in limited random sampling.

RESULTS AND DISCUSSIONS

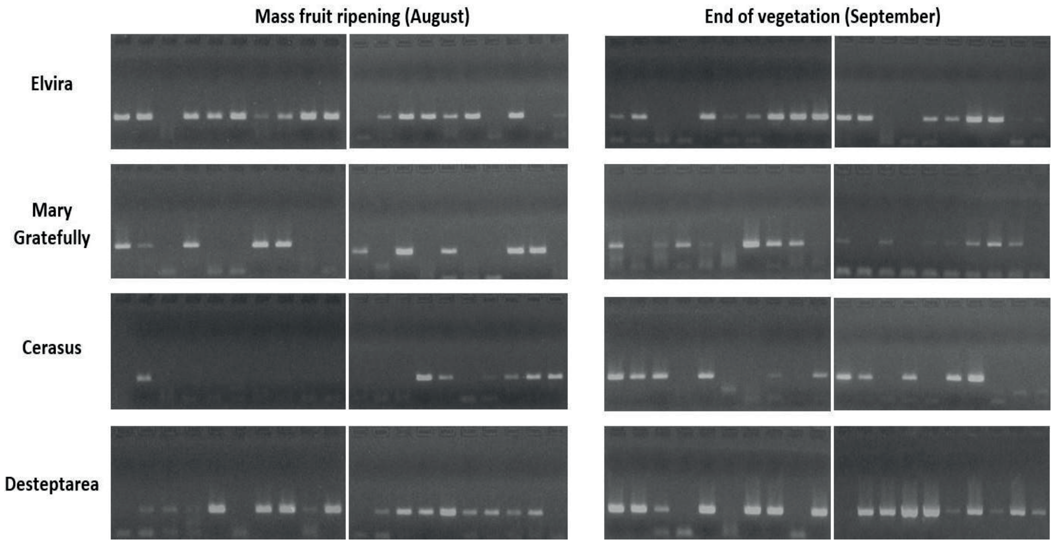

Figure 1. Results of the molecular diagnosis of '*Ca*. P. solani' presence in plants of the four tomato varieties at the period of mass fruit ripening and at the end of the season of vegetation: corresponding fragment 200 b.p. obtained by the electrophoresis of products of nested-PCR (round II) was registered

The evaluation and comparative analysis of the four tomato varieties demonstrated the difference in the phytoplasma infection spread between these varieties. Results of the molecular diagnosis of ‘*Ca*. P. solani’ presence in tomato plants are summarized in Table 1 and presented in Figure 1.

Table 1. Number of infected plants in the four analyzed tomato varieties (20 plants were analyzed in each variety)

Variety	Number of infected plants	
	Mass fruit ripening (August)	End of vegetation (September)
Elvira	17	17
Mary Gratefully	10	14
Cerasus	7	11
Desteptarea	16	16

It was found that the most significant difference in the number of infected tomato plants between studied varieties was recorded at the stage of mass fruit ripening. The spread of phytoplasma infection significantly increased in the tomato field to the end of the season of vegetation and the difference in the percentage of infected plants in studied varieties was less pronounced at this period (Figure 2).

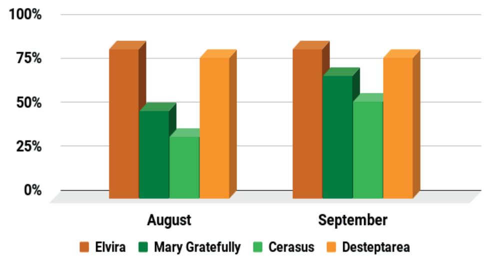

Figure 2. Distribution of ‘*Ca*. P. solani’ in the four tomato varieties

Analyzing the spread of phytoplasma infection in the tomato field at the stage of mass fruit ripening it was found that the varieties Elvira and Desteptarea were more susceptible to ‘*Ca*. P. solani’ (85% and 80% of infected plants, respectively) compared with Cerasus (35% of infected plants) and Mary Gratefully (50% of infected plants). Comparing pairs of the contrast in the susceptibility to ‘*Ca*. P. solani’ varieties one can see that the difference in the percentage of infected plants remained significant for all analyzed pairs with $P \leq 0.01$ (pairs Cerasus-Elvira, Cerasus-Desteptarea) or $P \leq 0.05$ (pairs Mary Gtatefully-Elvira, Mary Gtatefully-Desteptarea).

The comparison of ‘*Ca*. P. solani’ distribution in August and September allowed to see that the level of the infection in varieties Elvira and Desteptarea was no changed, it consisted of 80-85% of infected plants in September. The percentage of infected with phytoplasma plants significantly increased in variety Mary Gratefully having reached of 70%. Variety Cerasus manifested the highest resistance to ‘*Ca*. P. solani’ infection till the end of the season of vegetation: only about a half of plants (55%) were infected in September. Thus, the difference in the level of phytoplasma infection in studied varieties was not considerably pronounced in September: significant difference ($P \leq 0.05$) was recorded only in comparison of the best (Cerasus) and worst (Elvira) varieties.

Some variability of the indicators of productivity and fruit quality was established in studied tomato varieties in climatic conditions of 2018 in the Republic of Moldova. This vegetative season was in whole favorable for the tomato growing and ripening (warm and wet June, hot and wet July, hot and drought August). At the same time, these climatic conditions were advantageous for the phytoplasma infection spread in the tomato field. It is known that hot temperature increases the activity of insect vectors as well as the reproduction of the phytoplasmas in their bodies (Murral et al., 1996). In our study, 20% from 35 insects of families *Cixiidae* and *Psyllidae* collected in July-August of 2018 near the tomato field, were infected with ‘*Ca*. P. solani’ (unpublished data). These insects may be potential vectors of phytoplasma to tomato plants, increasing the level of infection in the field in August-September. It has to be emphasized that the incubation period of phytoplasmosis can be relatively long, consisting of one month or more (Blancard, 2012). Increasing the infected insects’ activity in July-August impacted on the phytoplasmosis spread in August-September.

So, the productivity and the yield of marketable fruits (important characteristic of fruits quality) were evaluated in the four tomato varieties (Table 2).

Table 2. Indicators of productivity and fruit quality of tomatoes in 2018

Variety	Productivity (t/ha)	
	total	marketable fruits
Elvira	58.0	40.1
Mary Gratefully	73.0	59.5
Cerasus	74.4	60.9
Desteptarea	67.8	50.9

In general, varieties that were more resistant to phytoplasma infection, respectively, had a higher yield. It has to be emphasized that the productivity is, firstly, a characteristic of the genotype. Secondly, it is under the influence of the climatic conditions of the year (changes in temperature, humidity, number of sunny or cloudy days during the growing season, *etc.*). Diseases of tomato plants other than stolbur can also affect the productivity and fruit quality. On the other hand, it is known that '*Ca*. P. solani' infection negatively influences on productivity and, especially, reduces the quality of tomato fruits (Bertaccini & Duduk, 2009).

Our results demonstrated that the highest productivity was registered in varieties Cerasus and Mary Gratefully (see Table 2, Figure 3). These varieties were less damaged by phytoplasmosis at the period of mass fruit ripening (August), when tomato fruits were mainly harvested. Worst productivity (58 t/ha) was recorded in the most susceptible to '*Ca*. P. solani' variety Elvira.

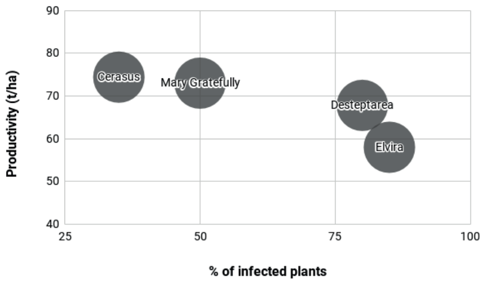

Figure 3. Correlation between the phytoplasma spread in the four tomato varieties in August and their productivity in 2018

The correlation of the level of phytoplasma spread in the four studied tomato varieties and yield of marketable fruits was additionally analyzed. It was established that the percentage of the marketable fruits varied in different varieties (Figure 4).

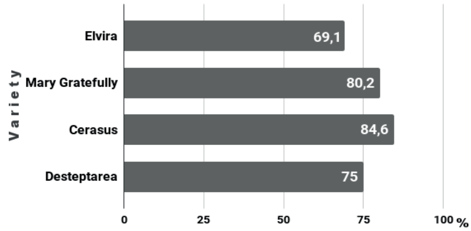

Figure 4. Percentage of the marketable fruits collected from the four tomato varieties in 2018

The correlation between the degree of phytoplasma infection in a variety and the yield of marketable fruits was perceptible in August (see Figures 4, 5). Namely, the percentage of marketable fruits in the varieties Cerasus and Mary Gratefully, which were more resistant to phytoplasma, was above 80%. The best indicator was registered in Cerasus (84.6% of marketable fruits from all harvested fruits). This indicator in the more susceptible to phytoplasma variety Desteptarea was 75%; the most susceptible to phytoplasma infection variety Elvira showed the worst yield of marketable fruits, it was less than 70%.

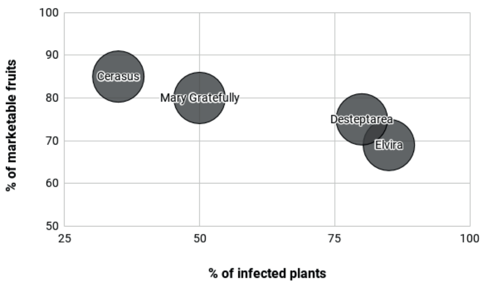

Figure 5. Correlation between the phytoplasma spread in the four tomato varieties in August and the yield of marketable fruits

In fact, analyzing the correlations between the phytoplasma spread in the tomato field in August of 2018 and the total productivity or the yield of marketable fruits, the four studied tomato varieties may be divided into two groups: (1) more resistant to '*Ca*. P. solani' Cerasus and Mary Gratefully which had better indicators of fruits quantity and quality; (2) more susceptible to '*Ca*. P. solani' Elvira and Desteptarea which had worse indicators of these traits (compare Figures 3 and 5).

The correlation between the degree of the phytoplasma presence in tomato field in September, at the end of the growing season, and the yield of marketable fruits in the variety had other pattern of distribution (Figure 6). It has to be mentioned that the infection distribution at the later stages of plants development, after a period of mass fruit ripening, does not affect the quantity and quality of fruits. Significant spread of '*Ca*. P. solani' in a tomato field in September is not important in terms of productivity of the variety and the yield of marketable fruits. This indicator may be useful for the assessing the degree of resistance of tomato varieties to '*Ca*. P. solani'.

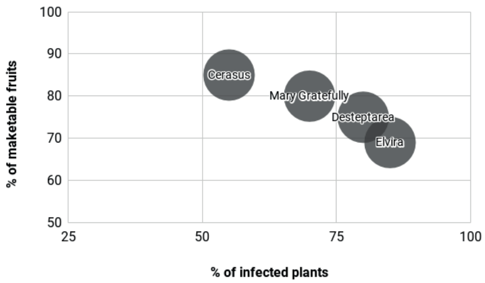

Figure 6. Correlation between the phytoplasma spread in the four tomato varieties in September and the yield of marketable fruits

Nevertheless, analyzing this correlation one can see that the best variety was Cerasus and the worst was Elvira. Varieties Mary Gratefully and Desteptarea were intermediate in terms of fruit quality and plants loading by phytoplasma infection in September.

CONCLUSIONS

The difference in the '*Ca*. P. solani' infection spread between the four tomato varieties was established in the growing season of 2018. This difference was more pronounced at the stage of mass fruit ripening, in August. At the end of the period of vegetation, in September, all analyzed tomato varieties were significantly loaded with phytoplasma. Nevertheless, the difference between the best (Cerasus) and worst (Elvira) varieties remained significant ($P \leq 0.05$) in September.

The difference in productivity and yield of marketable fruits was found comparing the four tomato varieties. In general, varieties that were more resistant to phytoplasma infection, respectively, had a higher yield.

Having in mind that stolbur was only one of the factors (along with other plants diseases, genotype features, adverse climatic conditions of the year, *etc.*), which negatively affected the economically important indicators of productivity and quality of tomato fruits, the correlations between these indicators and the level of phytoplasma spread in the tomato field provided an additional information for characterizing the four tomato varieties.

Analysis of correlations between the phytoplasma spread in the tomato field in August and total productivity or yield of marketable fruits allowed to divide the four studied tomato varieties into two groups: first group consisted of more resistant to '*Ca*. P. solani' Cerasus and Mary Gratefully with better indicators of fruits quantity and quality; second group consisted of more susceptible to '*Ca*. P. solani' Elvira and Desteptarea with worse indicators of productivity and yield of marketable fruits. These results have to be considered in the tomato breeding process. Additionally, summing up comprehensive study of the four tomato varieties in 2018 we may conclude that Cerasus was the best variety and Elvira was the worst.

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