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MONITORING THE SPREAD OF '*CANDIDATUS* PHYTOPLASMA SOLANI' IN MOLDAVIAN TOMATO VARIETIES

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INTRODUCTION

'*Candidatus* Phytoplasma solani' is a pathogen from the 16SrXII group of phytoplasmas that colonizes the phloem of many cultural and wild plants. Among the crops affected by '*Ca*. P. solani' are tomato, potato, eggplants, pepper and other species of the *Solanaceae* family [12]. This pathogen affects the plant systemically, leading to a profound disturbance in the growth and development of the infected plant. '*Ca*. P. solani' is considered as an endemic pathogen at the territory of Europe [7].

Phytoplasma epidemiology is a complex process, the transmission of the pathogen occurs mainly through polyphagous insect vectors from the families *Cixiidae*, *Cicadellidae,* and *Psyllidae* [15].In the same way, weeds play an important role in the spread of phytoplasma in crops: insect vectors overwinter on the roots of a number of wild plants[5].Typical symptoms of phytoplasmos is include dwarfism, yellowing, overgrowth of lateral shoots ("witch's broom"), fusion of sepals, formation of small fruits, or infertility of flowers. All this subsequently leads to a significant decrease in the quality and yield of many agricultural crops [2]. Thus, it is important to monitor the emergence and spread of phytoplasma in order to minimize economic losses. Determination of the disease by morphological symptoms is possible only at the later stages of development, when the plant is already systemically infected. Also, the confirmation of phytoplasma presence by visual indicators can be difficult in the case of mixed diseases [3]. *In vitro* cultivation also has some disadvantages, being quite laborious and requiring the use of commercial media [1]. Moreover, phytoplasmas do not form species specific colonies. In fact, only molecular methods allow the precise and reliable diagnostics of the pathogen at any stages of plant development [10].

Phytoplasma control is possible, it includes the use of insecticides, herbicides, antibacterial products, etc. All of them are based on minimizing the level and spread of infection, but effect negativelyto the environment [6]. A safer for the environment approach in the fight against phytoplasma infection is the identification, breeding and cultivation of the genotypes resistant to the pathogen [14, 8].

Thus, the aim of the study was to determine and compare the spread of '*Ca*. P. solani' infection in two local tomato varieties.

MATERIAL AND METHOD

Molecular diagnostics of '*Ca*. P. solani' was carried out on two local Moldaviantomato varieties grown in the field conditions: Desteptarea and Mary Gratefully. A comparative analysis of the distribution of phytoplasmain these two varieties was made in the two years: 2020 and 2022. For research, tomato fruits with peduncles from 12 plants of each varietywere collected at the stage of mass fruit ripening (August). These sampling volume and period are the best for the comparative analysis of the level of infection as it was established in our previous study [17].

DNA was isolated using the express method byboiling thin sections of each peduncle in 10 µl0.3 N NaOH (5 minutes) followed neutralizing this mix with 10 µl 0.3 N HCl and centrifugation of the obtained mix for 3 minutes at 10,000 rpm[9]. A 1 µl aliquot of obtained solution was used as template DNA in PCR.

The molecular diagnosis was carried out by the nested-PCR method with specific to '*Ca*. P. solani' primerscpn421F/Randcpn200F/R designed in Molecular Genetics Lab of the IGPPP (Chisinau, Moldova) on the base of the unique chaperonin (*groEL*) gene sequence[18]. The following amplification programs were used: I - 94°C 5'; II - 94°C 30", 58°C 30", 72°C 30"× n (n=30 in the first PCR;n=35 in the second PCR); III - 72°C 10'; IV - 4°C ∞.The results were registered in UV light after an electrophoresis of the stained with ethidium bromide amplified products in 1.5% agarose gel (1×TBE buffer). The size of the amplicon was measured using the marker of DNA fragment lengths "O'Gene 100 bp DNA Ladder Ruler Plus".

RESULTS AND DISCUTIONS

The molecular diagnosis of phytoplasma in tomato fruits collected at the stage of mass ripening in 2020 and 2022 showed a low level of infection of the both analyzed genotypes. The level of infection for each genotype was determined by counting the

number of amplicons of a specific length of 200 bp on the electropherograms, which indicated a positive signal of infection (Figure 1).

The results of the accessing phytoplasma infection in the both tomato varieties during two years of study are summarized in Table 1. Namely, in the growing season of 2020 the percentage of phytoplasma infection in the Mary Gratefully variety was 33%. The Desteptarea variety in the same year demonstrated complete immunity to '*Ca*. P. solani'. Compared to 2020, at the end of the growing season of 2022, a decrease in the level of infection of the Mary Gratefully variety to 8% and, at the same time, an increase of the infection of the Desteptarea plants to 25% was revealed. In fact, the susceptibility of plants of the studied varieties to '*Ca*. P. solani' varied under the different growing season conditions. The conclusion about the resistance of these genotypes to phytoplasma infection can not be made analyzing these data.

Following the obtained results, one can see a low level of the presence of phytoplasma infection in the tomato field in both years consisting of the same 17% of infected plants. It was interesting to compare levels of the '*Ca*. P. solani' infection in the varieties Desteptarea and Mary Gratefully in 2020 and 2022 with our previous study of the same genotypes in 2018 and 2019 [16, 18].

The comparison of the results of the present (2020, 2022) and previous (2018, 2019) studies demonstrates a sharp decrease in the level of phytoplasma infection in the both analyzed tomato varieties (Figure 2). Namely, the percentage of infected plants in the field fellfrom 80%-58% to 0%-25% in the Desteptarea and from 50%-92% to 33%-8% in the Mary Gratefully, respectively. Thus, in the both Desteptarea and Mary Gratefully varieties, the level of '*Ca*. P. solani' infection varies depending on the conditions of the growing season rather than on the genotype features.

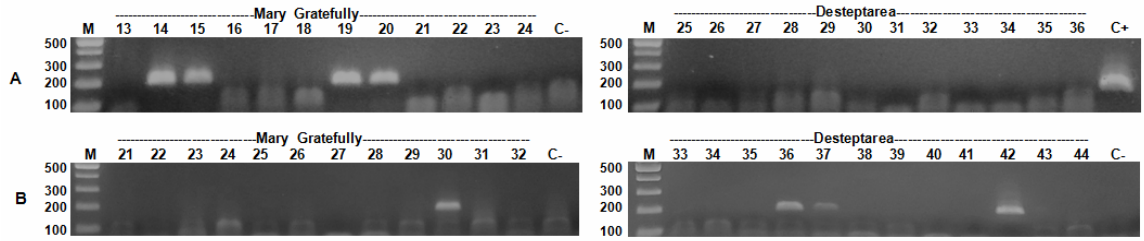

Figure 1. Results of nested-PCR analysis with chaperonin primers based on the DNA isolated from tomato fruits collected at the mass ripening stage. A – in 2020; B– in 2022. M – marker of DNA fragment lengths; C- – negative control; C+ – positive control

Table 1. Phytoplasma distribution in the tomato varieties Desteptarea and Mary Gratefully in the two years of study

Year / Variety	Analyzed plants	2020		2022	
		Infected plants	%	Infected plants	%
Desteptarea	12	0	0	3	25
MaryGratefully	12	4	33	1	8
TOTAL	24	4	17	4	17

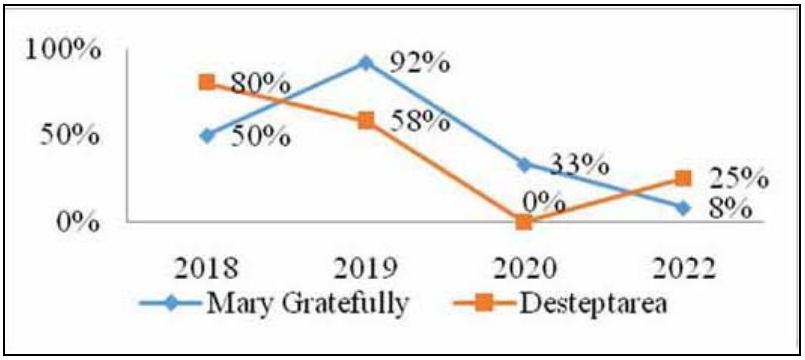

Figure 2. Phytoplasma distribution in the tomato varieties Desteptarea and Mary Gratefully for four years of the study

Significant reduction of '*Ca*. P. solani' infection rate of tomato plants that observed in recent years of the study can be due to several factors as the reduction of activity and density of vector insects that transmit the infection, the increase of non-specific resistance of tomato plants, the correct use of agricultural techniques as well as unfavorable climatic conditions for phytoplasma multiplication. Likewise, the presence of phytoplasma antagonists, the activity of certain substances (H_2O_2) or secondary plant metabolites such as ROS and the induction of acquired systemic resistance decrease phytoplasmosis of plants [11,13].

Expression of a number of genes which suppressed pathogenesis, heat shock and some metabolicgenes also include in the reduction of phytoplasmsa spread [4]. Finally, the appearance in recent years of a new strain of phytoplasma with a lower transfectability and pathogenicity can be a possible reason for the decrease of the infection level in tomato.

The fact of an abrupt decrease in the spread of phytoplasma in the field in the last two years of research compared to the research data of 2018 and 2019 serves as an indirect confirmation of this assumption. The study of the reasons for the decrease in tomato infection requires us to carry out further additional research, such as sequencing and comparison of phytoplasma isolates obtained in different years, comparative quantitative determination of fruit loading with the infection, etc.

CONCLUSIONS

The molecular diagnosis of '*Ca*. P. solani' in the tomato plants of the varieties Desteptarea and Mary Gratefully grown in the field during the 2020 and 2022 years revealed a relatively low spread of the infection. The comparative analysis with the data obtained in the growing seasons of 2018 and 2019 demonstrated a significant difference in the percentage of infected tomato plants as an abrupt decrease in the level of phytoplasmaspread in the field in the last two years of research (2020, 2022).

The obtained results did not allow to establish the genotype which is more orless resistant to '*Ca*. P. solani' because the variety with the highest / lowest level of infection alternated between the growing seasons. In fact, the character of the distribution of '*Ca*. P. solani' in those studied in 2018-2022 of two tomato varieties varied according to the conditions of the growing season, not being a characteristic of the genotype.

So, both studied varieties, Desteptarea and Mary Gratefully, are not resistant to '*Ca*. P. solani' and require strict monitoring of the appearance and spread of the infection during the growing season and the application, if necessary, of certain agricultural techniques against phytoplasma.

ABSTRACT

The molecular diagnosis of the spread of the pathogen '*Candidatus* Phytoplasma solani' was carried out in two local Moldavian varieties of tomato, Desteptarea and Mary Gratefully, at the stage of mass fruit ripening during two growing seasons (2020 and 2022). A relatively low level of infection of the tomato field was revealed for both studied varieties in these years, especially compared with the data of the phytoplasma distribution for the previous years of the study, 2018 and 2019. Possible reasons for the sharp decrease of tomato infection in recent years, requiring further additional study, are discussed.

The main result of the research is that both studied varieties, Desteptarea and Mary Gratefully, are not resistant to '*Ca*. P. solani' as the variety with the highest / lowest level of the infection alternated between the growing seasons. Thus, the level of infection with phytoplasma in two varieties of tomato varied according to the conditions of the year, without being a characteristic of the genotype.

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