PHYTOPLASMA TESTING IN SWEET PEPPER IN MOLDOVA

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Abstract

Phytoplasma infects a wide variety of crops, causing considerable economic losses. About half of the vegetable crops damaged by phytoplasma belong to the *Solanaceae* family including tomato, eggplant and pepper which play an important role in the agriculture economics of Moldova. Our previous research confirmed the presence of ‘*Candidatus Phytoplasma solani*’ (16SrXII-A subgroup) in tomatoes and also identified insect vectors. In this communication, we present for the first time in Moldova the results of molecular diagnosis of association of ‘*Ca. P. solani*’ in 4% of the analyzed sweet pepper samples. ‘*Ca. P. asteris*’ group was absent in the pepper field.

Key words: ‘*Candidatus Phytoplasma solani*’ group, ‘*Candidatus Phytoplasma asteris*’ group, sweet pepper, nested PCR.

Introduction

Phytoplasmas are wall-less bacteria colonizing the phloem of plants and causing considerable economic losses [2]. Diseases associated with phytoplasmas affect many vegetable crops in the world. The distribution of causative agents of phytoplasmosis in vegetables is dependent on the geographical region with 16SrI group being the predominant one worldwide [9]. About half of the vegetable crops reported to be affected by phytoplasmas belong to the *Solanaceae* family. Tomato, eggplant and pepper are the important agriculture crops in Moldova. The presence of ‘*Ca. P. solani*’ group in Moldova was confirmed recently from tomato [11]. In this communication, we attempted the molecular diagnosis of phytoplasma in sweet pepper plants (*Capsicum annuum*). Two phytoplasma associations were included in study: ‘*Ca. P. solani*’ (16SrXII-A subgroup) and ‘*Ca. P. asteris*’ (16SrI-B subgroup). ‘*Ca. P. solani*’ group was identified in pepper in a number of countries / regions in Europe such as Romania, southern Russia [4], Serbia [6], Macedonia [7]. ‘*Ca. P. asteris*’ was detected in pepper in India [8], Mexico [10], China [12] and other countries.

Materials and Methods

Phytoplasma detection was carried out in sweet pepper plants of Moldavian varieties grown in the experimental field of the Institute of Genetics, Physiology and Plant Protection (IGPPP, Chisinau, Moldova). Plants of four varieties for molecular diagnosis of phytoplasma were randomly selected from four non-contiguous rows in the field (12 plants in each row), and the fruits from these plants were collected during the period of mass fruit ripening of more than 50% (early September). A total number of 48 plants were tested for phytoplasmas presence. DNA was extracted by DNAzol kit (Thermo Fisher Scientific) from a pool of 12 plants (fruits) collected from the row. As a result, DNA samples from plants of four different varieties (rows I, II, III, IV) were obtained for future molecular diagnosis. Additionally, for assessing the percentage of pepper plants infected with phytoplasmas in the field, molecular diagnosis of individual plants from infected variety was carried out. To achieve this DNA was extracted from the fruit peduncle of each plant by rapid alkaline boiling [5]. Phytoplasma detection was carried out by nested PCR in MicroAmp 96-Well Tray in an Applied Biosystems Thermo Cycler. Specific primers for ‘*Ca. P. solani*’ and ‘*Ca. P. asteris*’ groups were used which were developed in Molecular Genetics Lab of IGPPP based on the unique chaperonin (*groEl*) gene sequences [11, 1]. The following programs for amplification were used: 94°C for 5 minutes; 94°C for 30 seconds, 58°C for 30 seconds, elongation at 72°C for 30 seconds for 30 cycles and for 35 cycles (for the nested PCR reaction); 72°C for 10 minutes. The products of amplification were separated by electrophoresis and analyzed in UV light using CSL UV-Transilluminator (Cleaver, England). A Gene Ruler 100 bp Ladder fragment length marker (Thermo Scientific) was used in electrophoresis to measure obtained fragment sizes.

Results and Discussions

The results of the nested PCR demonstrated the presence of association of '*Ca. P. solani*' in one of the four analyzed samples of pepper DNA but there was no specified amplification achieved for the '*Ca. P. asteris*' association (Figure 1). This result came as no surprise given that '*Ca. P. solani*' group was identified in our studies on tomato [11] and insects [1], while 16SrI-B subgroup was not found. Moreover, the presence of 'bois noir' ('*Ca. P. solani*', 16SrXII-A) was confirmed in grapevine in the vineyards of Moldova [3]. Stolbur ('*Ca. P. solani*', 16SrXII-A) was only detected in *Solanaceae* crops in Romania and southern Russia, in the territories located in close proximity to Moldova [4].

Figure 1. Results of nested PCR assay of '*Ca. P. solani*' group (A) and '*Ca. P. asteris*' group (B) in sweet pepper. M – marker of the fragment length; I-IV – the four samples each containing mixed DNA from 12 plants; C- – negative control of amplification

The data obtained showed that pepper plants were not significantly infected with phytoplasma: only in one out of four DNA samples 16SrXII-A subgroup was confirmed. Assessment of the proportion of infected pepper plants using molecular diagnosis of 12 individual plants from row I confirmed the low level of phytoplasma spread in the field. Namely, only two infected plants were found (Figure 2).

Figure 2. Results of nested PCR assay of '*Ca. P. solani*' group presence in individual sweet pepper plants. M – marker of the fragment length; 1-12 – individual plants from the row I; C- – negative control of amplification

Thus, the phytoplasma spread in the pepper field was really insignificant: the presence of the 16SrXII-A subgroup was diagnosed only in 4% of the 48 analyzed plants. The similar data were obtained for '*Ca. P. solani*' distribution in different pepper growing areas in Serbia being mainly of 3%-5% [6]. Also, the stolbur disease was registered as sporadic in Eastern Macedonia, and the damage of pepper crop was not substantial [7].

The final step of the present study was the collection and estimation of seeds from infected and uninfected pepper fruits. Firstly, significant visual difference was observed between seeds (Figure 3). Secondly, the germination of seeds in Lab conditions (in thermostat at +25°C) was assessed. It was revealed that the seeds of infected fruits were generally not viable, including the infected seeds that looked like the uninfected ones. The germination rate of seeds extracted from healthy fruits was about 87%.

Figure 3. Seeds extracted from phytoplasma-infected (A) and uninfected (B) pepper fruits

So, the lack of viability or low viability of pepper seeds damaged by phytoplasma, as well as their visually distinguishable disturbances, are favorable facts from the point of view of the collection and long-term conservation of healthy seeds.

Conclusions

The main result of the present study demonstrated the presence of the ‘*Ca. P. solani*’ (16SrXII-A subgroup) in sweet pepper plants in Moldova. ‘*Ca. P. asteris*’ belonging to the 16SrI-B subgroup was not found in the pepper field of IGPPP. The molecular diagnosis of phytoplasmas in *Capsicum annuum* was carried out in our country for the first time.

Additionally, the phytoplasma spread in the pepper field was revealed as minor: only 4% of the analyzed plants were damaged by stolbur. Seeds harvested from infected by ‘*Ca. P. solani*’ plants were not viable and visually differed from the seeds of healthy pepper, having abnormalities in color, shape, and size. This prevents phytoplasma-infected seeds from entering the IGPPP collection intended for long-term storage.

Research was carried out within the Project of the State Program 20.80009.5107.11 „Long-term *ex situ* conservation of plant genetic resources in the Gene Bank using the methods of molecular biology for plant germplasm health testing”, financed by the National Agency for Research and Development of the Republic of Moldova. The authors are grateful to the scientific researcher of IGPPP Focșa Nina for obtaining plant material in the field.

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