UDC: 632.3:635.21(478) https://doi.org/10.53040/gppb8.2024.03

# STOLBUR SPREAD IN SOME POTATO GENOTYPES GROWN IN THE REPUBLIC OF MOLDOVA

Bahsiev Aighiuni*, *https://orcid.org/0000-0002-1915-7652*
Zamorzaeva Irina, *https://orcid.org/0000-0002-6302-2069*
Melian Lolita, *https://orcid.org/0000-0002-9192-0560*
*Institute of Genetics, Physiology and Plant Protection, MSU, Chisinau, Republic of Moldova*
**e-mail*: *aighiuni.bahsiev@igfpp.md*

**Summary.** Testing of 11 potato genotypes for the stolbur disease was carried out in plants grown on the experimental plots of IGPPP, MSU during the 2023 season of vegetation. A low level of '*Candidatus* Phytoplasma solani' infection was determined by nested-PCR analysis in all analyzed genotypes. Overall, the percentage of infected plants in the potato field was only 14.6%. In addition, the presence of phytoplasma infection was detected in the tubers of infected potato plants, while it was not detected in the sprouts that appeared on these tubers.

**Key-words:** '*Candidatus* Phytoplasma solani', stolbur disease, molecular diagnostics, potato, infection spread.

## Introduction

Phytoplasmas are a group of wall-less microorganisms that colonize the phloem of more than 1000 plant species, including solanaceous plants [1]. An important role in phytoplasma transmission is played by vector insects like leafhoppers, planthoppers and psyllids. Numerous species among them are polyphagous, which facilitates the infection of new plant species. Thus, phytoplasmas can adapt to different agroecosystems due to their ecological plasticity [2]. Monitoring new agricultural crops that can be affected by phytoplasma is a tool for controlling the spread of infection in the region.

The geographical distribution of phytoplasmas is wide. They were found in North Africa, Asia Minor, and the Near East, and especially in the Euro-Mediterranean area. In the European Union '*Candidatus* Phytoplasma solani', the causal agent of stolbur and bois noir, is widespread and considered as an endemic pathogen [3]. In the Republic of Moldova this pathogen predominantly affects tomato and grapewine, and to a lesser extent pepper [4, 5]. Stolbur infestation causes a significant reduction in the quality and quantity of crops reaching up to 90% [6].

One of the important agricultural crops in Moldova is potato. Typical symptoms of stolbur in potato include upward rolling of the top leaves with reddish or purplish discoloration, proliferation of buds, shortened internodes, and swollen nodes [7]. Rapid diagnosis and precise identification of phytoplasmas responsible for emerging plant diseases are crucial for preventing further spread of infection and reducing economic losses [8, 9]. The methods for stolbur diagnosing can be based on symptomatic study, electron microscopy, *in vitro* cultivation, and molecular technology. Each of these methods has certain limitations. Morphological study is quite difficult because infection may occur simultaneously with other diseases that can mask the symptoms [10]. Also, disease identification is only possible with the appearance of symptoms approximately one month after infection. Detection using electron microscopy is limited by the fact that phytoplasma cells are morphologically polymorphic [11]. The *in vitro* cultivation is not efficient due to laborious to obtain specific colonies and requirments to use commercial media [8].

Reliable and timely diagnosis is possible using molecular methods. The advantages of molecular diagnosis include the possibility to determine the infection before the appearance of symptoms, high specificity and accuracy, in phytoplasma identification. Molecular methods allow

to evaluate quickly the specificity of disease that affects the crop and monitor the infection spread among new crops [12, 13].

The aim of the research was the molecular diagnosis of '*Ca*. P. solani' in plants of some potato genotypes from IGPPP collection grown on the experimental lots of the institute during the season of vegetation of 2023.

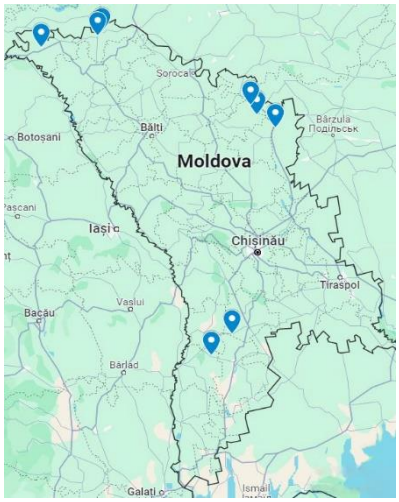
## Materials and methods

The molecular diagnosis of '*Ca*. P. solani' was carried out in 11 potato genotypes of the different origin (Table 1). The leaves were collected for analysis from potato plants growing on the experimental plots of IGPPP (MSU) at the end of June and in mid-August of 2023. The tubers of potato were colected at the end of August.

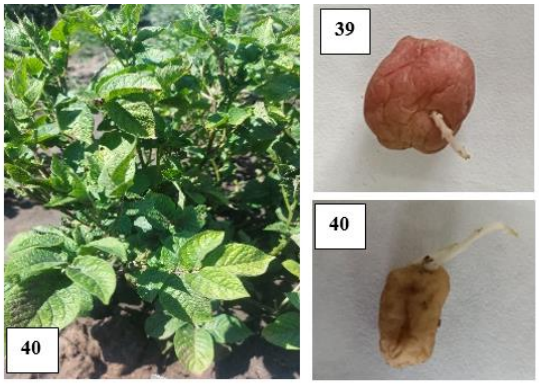
**Table 1**

**Potato genotypes and their origin**

| Nr. | Genotype | Origin of genotype |
|---|---|---|
| **1** | Summer potato | d.Rezina, v.Solonceni |
| **3** | Potato 40 days | d. Ungheni, v.Cioropcani |
| **8** | Cheyenne variety | Franta |
| **6** | Potato 40 days, pink | d. Soldanesti, v. Climautii de jos |
| **12** | Potato | d. Soldanesti, v. Poiana |
| **23** | White potato 40 days | d.Cantemir, v.Visniovca |
| **38** | Violet potato, 2019 | d. Soldanesti, v. Poiana |
| **39** | 74/MDI05707 red potato + americanca | d.Ochnita, v.Ochnita |
| **40** | 62/MDI05733 potato tsutsuki | d.Briceni, v. Berlinti. |
| **50** | Potato MDI05705 | d.Ochnita, v.Ochnita |
| **56** | Home white potato | d.Cimislia, v. Cenac |




DNA extraction from general sample of potato leaves, tubers and sprouts on tubers (Fig. 1) was done using DNAzol to analyze the presence of '*Ca*. P. solani' in plants of different genotypes. The general sample consists of 5-10 plants of each genotype. The high quality of purified DNA was confirmed using PCR with common plant ribosomal primers.




**A** **B**

**Figure 1. Potato plant material for DNA isolation: A – leaves; B – tubers**

DNA isolation from leaves of individual potato plants was performed by the boiling thin slices of leaves in an alkaline solution (express method).

Phytoplasma detection was carried out using the nested-PCR analysis with specific primer pairs cpn 421 F/R and cpn 200 F/R based on the unique sequence of the chaperonin gene (*groEL*) of '*Ca*. P. solani'. The amplification reaction proceeded according to the following program: I - 94°C for 5'; II - 94°C for 30", 58°C for 30", 72°C for 30" × 30 cycles (I round) or ×35 cycles (II round); III - 72°C for 10'; IV - 4°C ∞. The results were recorded by electrophoresis of the amplified products stained with ethidium bromide (1× TBE buffer, 1.5% agarose gel) with subsequent visualization under UV light. The size of the amplified fragment was determined using „GeneRuler 1kb DNA Ladder".

## Rezults and discusions

The data of the molecular diagnosis of the presence of '*Ca*. P. solani' in plants of potato genotypes at the end of June and in mid-August are presented in Figure 2. The results obtained demonstrate the absence of phytoplasma infection in all analyzed genotypes at the end of June (Fig. 2A). The presence of the pathogen was detected in 5 out of 6 potato genotypes in mid-August (Fig. 2B).

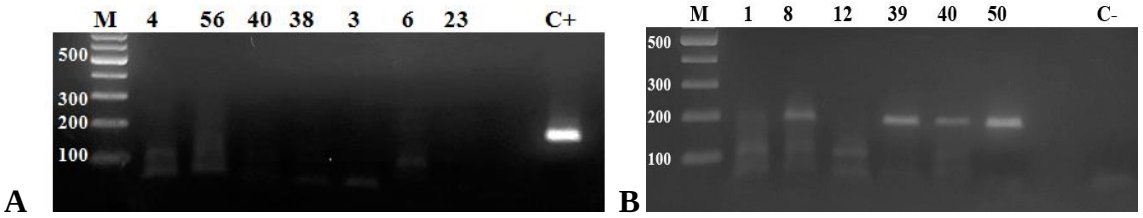



**Figure 2: Results of the nested-PCR analysis based on DNA isolated from general samples of leaves collected from potato plants at the end of June (A) and in mid-August (B):**
M – DNA fragment length marker; C+ – positive control of amplification;
C- – negative control of amplification.

Molecular analysis of individual potato plants was done in 5 genotypes infected with phytoplasma in mid-August. This genotypes were chosen in accordance with results of molecular testing of general samples (see Figure 2). Between 5-10 plants were analyzed in each genotype. The results of the nested-PCR are shown in Figure 3.

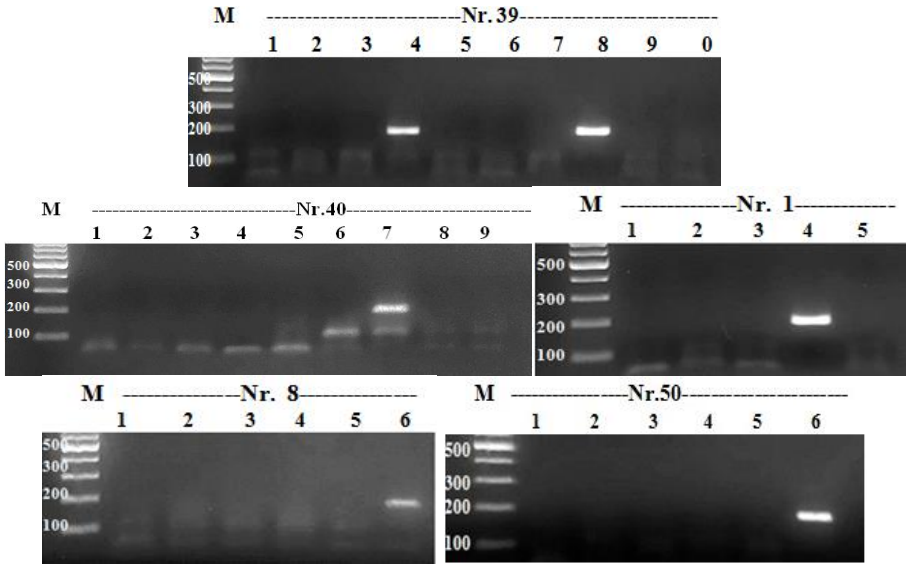


**Figure 3: Results of the nested-PCR analysis based on DNA isolated from individual potato plants:** M – DNA fragment length marker.

This step of the study allows to register the level of infection spread in the potato field in whole as well as in each analyzing genotype. According to the obtained results one can see a low level of phytoplasma spread in all studied potato genotypes (Fig. 4). The percentage of infected plants in the potato field consists of only 14.6%, At the same time, at the end of the season of vegetation 2023, the spread of this disease at the tomato field of IGPPP was significantly higher. In accordance to our study, tomatoes were infected with '*Ca*. P. solani' in the mass [14]. The assumption is reasonable that stolbur does not pose such a danger to potatoes grown in the field in Moldova as it does to tomato.

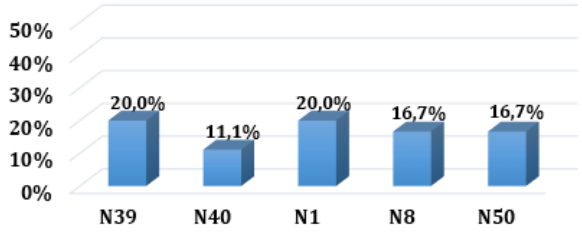



**Figure 4: Spread of stolbur at five potato genotypes in mid-august 2023**

In addition, the interesting results were obtained in the molecular analysis of the sprouted tubers of two potato genotypes. The presence of phytoplasma infection was determined in the tubers of both analyzed genotypes, while in the sprouts it was not identified (Fig. 5).

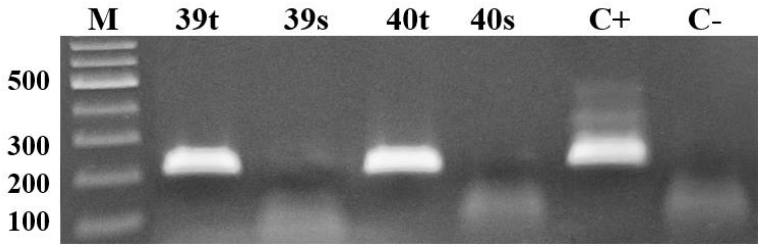



**Figure 5: Results of the nested-PCR analysis based on DNA isolated from the sprouted tubers of two potato genotypes:** M – DNA fragment length marker; 39t, 40t – tubers; 39s, 40s – sprouts; C+ – positive control of amplification; C- – negative control of amplification.

These results give hope for obtaining uninfected potato plants just in the case when infected tubers were planted. However, this assumption requires further extensive research.

## Conclusions

The presence of '*Ca*. P. *solani*' infection was determined in the potato plants in mid-August. Phytoplasma infection was not identified at the end of June. The level of infection in analyzed potato genotypes was low under the conditions of the season of vegetation 2023. The presence of stolbur infection in potato tubers was established, while the pathogen was not detected in the sprouts that appeared on the affected tubers. Thus, stolbur does not currently pose a major danger to potato cultivation in Moldova. However, further stolbur monitoring is needed to prevent outbreaks of this disease in this crop.

**Acknowledgements.** This study was supported by the subprogram 011101 „*Genetic and biotechnological approaches of management of agroecosystems under climate change conditions*", funded by the Ministry of Education and Research.

**References**

1. Wei, W, Trivellone, V., Dietrich, C.H. et al. Identification of phytoplasmas representing multiple new genetic lineages from phloem-feeding leafhoppers highlights the diversity of phytoplasmas and their potential vectors. *Pathogens*, 2021, vol.10, nr. 352, P. 1-17.
2. Weintraub, P.G., Trivellone, V., Krüger, K. Transmission and management of phytoplasma - associated diseases. *Phytoplasmas, Plant Pathogenic Bacteria – II*. Springer, 2019, P. 27-52.
3. EFSA PLH Panel (EFSA Panel on Plant Health), 2014. Scientific Opinion on the pest categorization of *Candidatus* Phytoplasma solani. *EFSA Journal*. 2014, vol. 12, nr. 12:3924, P. 1-27.
4. Заморзаева, И., Бахшиев, А. Молекулярная диагностика распространения столбура в некоторых важных для сельского хозяйства Молдовы культурах. *Агрофизический институт: 90 лет на службе земледелия и растениеводства*, Санкт-Петербург: ФГБНУ АФИ, 2022, С. 509-513.
5. Хаустов, Е. Распространение почернения древесины винограда в естественных условиях. *Theses of the: 74th scientific conference of students*, Ed. 74, 2021, Chisinau, P. 51.
6. Hogenhout, S.A. Plant Pathogens, Minor (Phytoplasmas). *Encyclopedia of Microbiology* (Third Edition), Editor(s): Moselio Schaechter, Academic Press, 2009, P. 678-688.
7. Ember, Z., Acs, I.E., Munyaneza, J.M. et al. Survey and molecular detection of phytoplasmas associated with potato in Romania and southern Russia. *European Journal of Plant Pathology*, 2011, vol. 130, P.367-377.
8. Bertaccini, A., Duduk, B. Phytoplasma and phytoplasma diseases: a review of recent research. *Phytopathologia Mediterranea*, 2010, vol. 48, nr. 3, P. 355-378.
9. Ministry for Primary Industries Amendments to the Import Health Standard 155.02.06: Importation of Nursery Stock. Consultation: Proposed measures to manage phytoplasmas on ornamental plants for planting Importation of Nursery Stock (155.02.06) https://www.mpi.govt.nz/dmsdocument/60817/direct
10. COST ACTION FA0807. Phytoplasmas and phytoplasma disease management: how to reduce their economic impact. Advances in knowledge about phytoplasma diseases in Argentina, P. 82-89. Assunta Bertaccini (ed.). Bologna, Italy, P. 82-89.
11. Namba, S., Molecular and biological properties of phytoplasmas. *Proceedings of the Japan Academy*, Series B, 2019, vol. 95, nr. 7, P. 401-418.
12. Delić, D. Polymerase Chain Reaction for phytoplasmas detection. Polymerase Chain Reaction. *InTech*. 2012, P. 91-120.
13. Marcone, C., Rao, G.P. Control of phytoplasma diseases through resistant plants. In: Phytoplasmas: *Plant Pathogenic Bacteria - II: Transmission and Management of Phytoplasma - Associated Diseases.* Springer Singapore, 2019, P. 165-84.
14. Bahsiev, A., Zamorzaeva-Orleanscaia, I. Estimation of '*Candidatus* Phytoplasma solani' prevalence in local tomato varieties. *Biology and sustainable development*, Ed. 21, 23 November 2023, Bacau, 2023, Ed. 21, P. 69.