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MOLECULAR DIAGNOSIS OF STOLBUR IN LOCAL TOMATO VARIETY MARY GRATEFULLY

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'Candidatus Phytoplasma solani' (16SrXII-A group), the causal agent of stolbur disease, is a phloem-limited, cell wall-less bacterium that infects numerous cultivated plant species including tomato, potato, grapevine, maize, and pepper. In tomato, the pathogen induces severe symptoms such as stunting, shortened internodes, virescence, phyllody, floral sterility, reduced fruit size, and phloem hypertrophy, resulting in significant yield and fruit quality losses. The pathogen is primarily transmitted by insect vectors feeding on plant sap and damaging the vascular system including phloem. Perennial plants impact in stolbur transmission playing the role of reservoirs for overwintering of insects. Additionally, the parasitic plant *Cuscuta* spp. can also contribute to phytoplasma epidemiology. Because phytoplasma infections are systemic and often remain latent or are masked by mixed infections, reliable molecular diagnosis is essential for disease surveillance and management.

The aim of this study was to determine the incidence of '*Ca*. P. solani' infection in the local Moldovan tomato variety *Mary Gratefully* during two growing seasons using nested-PCR analysis.

Tomato fruits with peduncles were collected from ten field-grown plants at the stage of mass fruit ripening (third decade of August) in the 2024 and 2025. Total DNA was extracted from thin peduncle sections using a rapid alkaline boiling protocol. Molecular diagnosis was performed by nested-PCR with the '*Ca*. P. solani-specific primer pairs cpn421F/R and cpn200F/R targeting the *groEL* (chaperonin) gene. These primers were designed in the Molecular Genetics Laboratory of the IGPPP. Amplification products were separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide and visualized under UV illumination.

Comparative molecular analysis revealed differences in phytoplasma incidence between the two growing seasons. In 2024, 80% of the analyzed plants tested positive for '*Ca*. P. solani' indicating a high level of the field infection. In 2025, the infection rate decreased to 50%. However, the significant difference in infection in-

cidences over the two-year study was demonstrated in different climatic conditions of these years. Obtained in this study results confirm the persistent occurrence of stolbur phytoplasma in the tomato variety Mary Gratefully under field conditions. Also, the essential dependence of the stolbur spread between plants of this variety growing in the field on the climatic conditions of the year was confirmed too. The persistence of earlier obtained data with the results of this study allows to conclude that the local tomato variety Mary Gratefully is a high sensitive to stolbur and that the phytoplasma spread is depending on the season conditions. This variety cannot be recommended to breeding programs directed to the creation of stolbur - resistant tomato genotypes. Also, taking into account that '*Ca*. P. solani' represents a significant danger to this tomato variety it should be strongly recommended monitoring the emergence and outbreak of infection in the field.

Keywords: *'Candidatus Phytoplasma solani',* stolbur, tomato, nested-PCR, molecular diagnosis, Mary Gratefully variety**.**

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