UDC: 634.11:632.4 DOI: https://doi.org/10.53040/ppap2026.92

MOLECULAR SURVEILLANCE OF MAJOR FUNGAL PATHOGENS IN APPLE USING SPECIES-SPECIFIC PCR

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Background: Fungal pathogens are among the major factors limiting apple production and postharvest fruit quality, causing substantial economic losses worldwide. Rapid and accurate molecular detection of phytopathogenic fungi is essential for effective disease monitoring and management. Most of them are belonging to the group of mycotoxin-producing fungi, that can contaminate food crops if storage conditions are unfulfilled. Therefore, the early detection of these pathogens is critical for minimizing crop losses and implementing timely control strategies.

The present study aimed to evaluate the molecular spectrum of fungal pathogens belonging to the genera *Alternaria*, *Penicillium*, *Aspergillus*, *Monilinia*, *Myrothecium*, and *Mucor* associated with apple tissues using species-specific polymerase chain reaction (PCR).

Methods: Plant material was collected from the apple cultivars 'Golden Spur' and 'Granny Smith' grown in lysimeter experiments and from 'Golden Delicious', 'Reinette Simirenko' trees cultivated in an orchard. Leaf samples were tested throughout the growing season, fruits were studied at the stage of biological maturity and after storage. Genomic DNA was extracted from plant tissues and analyzed using species-specific primers targeting *Alternaria alternata*, *Alternaria solani*, *Penicillium expansum*, *Penicillium brevicompactum*, *Penicillium citrinum*, *Monilinia yunnanensis*, *Myrothecium roridum*, *Mucor piriformis*, and *Aspergillus flavus*. Nested PCR was carried out under optimized amplification conditions. Amplified fragments were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide, visualized under UV illumination, and documented.

Results: Molecular analysis revealed that *Alternaria alternata* was the predominant fungal pathogen detected in apple tissues throughout the entire growing season, indicating its widespread occurrence in the studied genotypes. In contrast, *Alternaria*

solani was not detected in any of the analyzed samples. *Myrothecium roridum* was identified only once, in spring leaves of the cultivar 'Golden Delicious'. Pathogens belonging to the genus *Penicillium* were detected at a low frequency. *Penicillium expansum* and *Penicillium citrinum* were identified exclusively in leaf samples collected during the growing season from the apple cultivars 'Reinette Simirenko' and 'Golden Delicious'. In contrast, *Penicillium citrinum* was detected only in stored fruits of the 'Golden Delicious' cultivar. No PCR amplification products specific to *Aspergillus flavus*, *Mucor piriformis*, or *Monilinia yunnanensis* were obtained from any of the analyzed samples.

Conclusions: The results demonstrate the high prevalence of *Alternaria alternata* in apple tissues compared with the sporadic occurrence of *Penicillium* and *Myrothecium* species. The absence of *Aspergillus flavus*, *Mucor piriformis*, and *Monilinia yunnanensis* suggests that these pathogens were not involved in infections under the investigated environmental conditions. These findings confirm the usefulness of species-specific PCR as a rapid and reliable tool for phytopathogen monitoring in apple production systems. Continuous molecular surveillance and strict control of storage conditions are recommended to minimize the development of *Alternaria* and *Penicillium* infections during postharvest storage and to preserve fruit quality.

Keywords: Pathogens, fungi, apple, PCR.

Acknowledgments. This research was supported by the project/subprogram 011101 "Genetic and biotechnological approaches of management of agroecosystems under climate change conditions", funded by the Ministry of Education and Research.