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MOLECULAR DIAGNOSTICS OF SOME FUNGAL PATHOGENS IN GREENHOUSE GROWN TOMATOES

Angela DEAGHILEVA[1], ORCID: 0000-0003-3659-2450
angela.deaghileva@sti.usm.md
Galina BELOUSOVA[1], ORCID: 0000-0002-9977-1248
galina.belousova@sti.usm.md
Irina CUZNETOVA[1], ORCID: 0000-0001-5810-6244
irina.cuznetova@sti.usm.md
Zoia IGNATOVA[1], ORCID: 0000-0003-1171-5928
zoia.ignatova@sti.usm.md
Karel MULLER[2], ORCID: 0000-0002-0817-8810
e-mail: muller@ueb.cas.cz

[1]Institute of Genetics, Physiology and Plant Protection, Moldova State University, Chisinau, Republic of Moldova
[2]Institute of Experimental Botany of the Czech Academy of Sciences

Abstract. *Tomato production in protected environments such as greenhouses has become a popular trend in agriculture. The main advantage of greenhouse crop production over open field systems is the ability to control the environment, limiting the impact of abiotic and biotic factors. However, under greenhouse conditions tomatoes are still susceptible to certain soil-borne fungal diseases. Early detection of fungal pathogens is paramount for controlling the diseases and their spread. The aim of the study was to monitor fungal pathogens affecting tomato plants grown in protected conditions. Molecular diagnostics of pathogens Alternaria spp., Fusarium spp., Penicillium spp. and Aspergillus spp. was carried out during the vegetative season in various organs of tomato plants planting in greenhouse using nested-PCR assay. A. alternata was the predominant pathogen among all tested tomato genotypes. Fusarium and Aspergillus species were not detected in greenhouse tomato plantings during vegetative season.*

Keywords: fungi greenhouse, pathogens PCR Tomato.

INTRODUCTION

Global temperature rise is a trigger for climate change and, as a result, a multitude of risks to organisms all over the world. Climate change has a major impact on the agricultural sector, decreasing crop productivity. High temperatures and extreme fluctuations in precipitation quantities distort plant growth cycle, facilitate the emergence of new pests and diseases, their propagation and widening the host range [1, 2]. Threats of epiphytoties caused by various pathogens are

currently increasing in agroecosystems in temperate regions [3]. The increasing impact of pathogens has led to the excessive use of chemicals, which have negative consequences for the environment, human and animal health [4, 5]. Plant breeding programs and long-term use of pesticides have provoked the emergence of strains with increased virulence [6]. Numerous studies on the impact of climate change on biological processes demonstrate the need for effective measures to reduce the severity of diseases, using more environmentally friendly methods of plant protection. Mitigation methods include phytosanitary control of seed and planting material, the use of resistant varieties and microclimate modification. In the last decade, tomato production in protected environments such as greenhouses has become a new trend in agriculture. The main advantage of greenhouse crop production over open field systems is the ability to control the environment, limiting the impact of abiotic and biotic factors [7]. Greenhouse tomatoes have better quality and are uniform in shape, size and color compared to those grown in the open field. However, under greenhouse conditions tomatoes are still susceptible to certain microbial diseases, as the closed environment can promote the development of infections. Soil-borne fungi are the main limiting factor for tomato production and cause significant economic losses [8]. Early detection of fungal pathogens is paramount for controlling the diseases and their spread.

The aim of the study was to monitor fungal pathogens affecting tomato plants grown in protected conditions. Molecular diagnostics of pathogens *Alternaria* spp., *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. was carried out during the vegetative season in various organs of tomato plants grown in greenhouse using nested-PCR assay.

MATERIALS AND METHODS

Monitoring of phytopathogenic fungi was carried out during the 2024 growing season. The following tomato genotypes were studied: the wild tomato species Solanum pimpinellifolium, Elvira and Rufina varieties (selection of IGFPP). Asymptomatic tomato plants at different phases of development were tested: seeds before sowing (2023 harvest), leaves (phase of 4-5 leaves, flowering phase), fruits, “new” seeds. Total DNA from plant material were isolated by a modified CTAB method [9]. At all stages of the analysis, DNA extraction from tomato plants was performed in triplicate to ensure reproducibility of the results.

Molecular analysis by nested-PCR was performed using primers for the detection of *Alternaria* spp., *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. Primer design was done based on species-specific sequences of each pathogen from NCBI nucleotide collection presented in GenBank database using Primer-BLAST designing tool: glyceraldehyde 3-phosphate dehydrogenase gene (GPD) of *A. alternata* and *A. solani*; translation elongation factor 1-alpha (*tef1*) gene of *F.*

verticillioides, F. oxysporum, F. solani, F. avenaceum; beta-tubulin (*tub2*) gene sequences of *F. equiseti, F. sporotrichioides, Penicillium citrinum;* oxygenase (*fum6*) gene sequences of *F. proliferatum;* o-methyltransferase A (*aflP*) gene of *Aspergillus flavus*.

The PCR mixture, in a volume of 25 µl, contained: 66 mM tris-HCl (pH-8,4), 16 mM $(NH_4)_2SO_4$, 2,5 mM $MgCl_2$, 0,1% Tween 20, 7 % glicerol, 100 µg ml^{-1} *Bovin Serum Albumin*, 0,2 mM dNTPs of each, 1,25 U *Taq DNA* polymerase, 5 pM of each primer, and 5-10 ng DNA.

The first round of the *nested*-PCR included 1 cycle: 95 ^{0}C – 4 min, 60 ^{0}C – 1 min, 72 ^{0}C – 1 min; followed by 29 cycles: 95 ^{0}C –1 min, 60 ^{0}C – 1 min, 72 ^{0}C – 1 min. The second-round conditions of the *nested*-PCR were: 35 cycles: 95 ^{0}C – 1 min, 60 ^{0}C – 1 min, 72 ^{0}C – 1 min.

The amplification products were separated in 1,5 % agarose gel by electrophoresis (5-8 V/cm) and compared with molecular marker (100 bp DNA ladder, Thermo Fisher Scientific) in a migration buffer of Tris/borate/EDTA (pH 8.0) with ethidium bromide, viewed in the UV (302 nm) and photographed.

RESULTS AND DISCUSSION

Prior to molecular diagnostics of pathogens, total DNA extracted from plant material was tested for the presence of PCR inhibitors. DNA quality analysis was performed using primers designed based on plant 18S ribosomal RNA gene sequences [10]. The results of amplification selectively demonstrated in Figure 1.

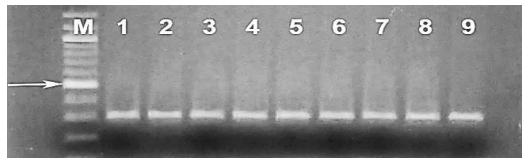


Figure 1. Electropherogram of PCR products obtained on total DNA extracted from tomato seeds using primers to the 18SrDNA gene.
1-3. Solanum pimpinellifolium, 4-6. Elvira, 7-9. Rufina. M - 100 bp DNA ladder. The arrow points at a 500 bp marker fragment.

Specific fragment of expected size (315 bp) was revealed for all tomato DNA samples. The analysis indicated that DNA was free of PCR inhibitors and was suitable for downstream pathogen testing.

Molecular analysis of pre-planting seeds using species-specific primers to *Alternaria* spp. revealed the presence of *Alternaria alternata,* but absence of *Alternaria solani* in all genotypes of tomato. *Penicillium citrinum* was detected only in the seeds of Solanum pimpinellifolium (Figure 2).

The positive signals in the second round of *nested*-PCR for *A. alternata*

were marked with the presence of a 288 bp amplicon on electrophoresis (Figure 2A.) and 250 bp – for *P. citrinum* (Figure 2B.). It is known that infected seeds can serve as a source of primary infection, increasing the pathogenic load in plants during the growing season.

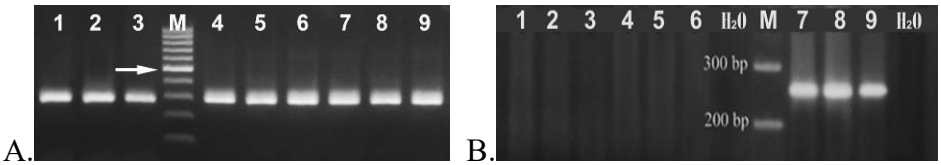


Figure 2. Electropherogram of nested-PCR products obtained using primers for detection of *A. alternata* (A.) and *P. citrinum* (B.) and total DNA extracted from the seeds.

1-3. Elvira, 4-6. Rufina, 7-9. Solanum pimpinellifolium. M - 100 bp DNA ladder. The arrow points at a 500 bp marker fragment.

Thus, in order to obtain healthy planting material, a phytosanitary assessment of their quality is necessary before storing and compliance with storage conditions. *A. alternata* was revealed in the tomato plants at the phase of 4-5 leaves in all studied tomato genotypes (Figure 3).

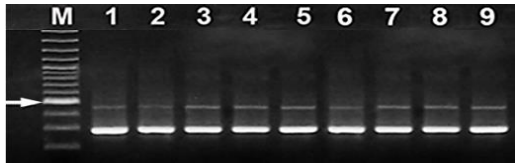


Figure 3. Electropherogram of nested-PCR products obtained using primers for detection of *A. alternata* in total DNA extracted from the tomato plants at the of 4-5 leaves.

1-3. Elvira, 4-6. Rufina, 7-9. Solanum pimpinellifolium. M - 100 bp DNA ladder. The arrow points at a 500 bp marker fragment.

Alternaria spp. was not found in leaves collected at the flowering phase. The single incidence of *A. alternata* infection was revealed in 'Rufina' fruits, but absent in "new" seeds of all tomato genotypes. We have previously shown that fungi of the genus Alternaria infect tomato plants in open field during all phases of development [11]. Therefore, in greenhouse conditions it is possible to contain the development of this pathogen.

During the vegetative season, *Fusarium*, *Aspergillus* and *Penicillium* species (*F. verticillioides, F. oxysporum, F. solani, F. avenaceum, F. equiseti, F. sporotrichioides, F. proliferatum, A. flavus, P. citrinum*) were not detected in greenhouse grown tomatoes. In previous studies we detected these fungal species

at different stages of tomato development in open field conditions [12]. It is known, that *Alternaria* can inhibit the growth and development of *Fusarium* [13]. However, the effectiveness of suppression can be influenced by many factors, including environmental conditions and the genotype of the host plant [14]. Thus, greenhouse growing conditions and the presence of *Alternaria* spp. probably limited the development of the named species of *Fusarium, Aspergillus* and *Penicillium*.

As shown, *A. alternata* was the predominant pathogen in tomato plants during some phases of development. So, it can be used as a representative pathogen for presymptomatic assessment of phytosanitary status of tomato plantations to optimize fungal disease control under greenhouse conditions.

The probability of tomato infection by soil-borne fungal pathogens exists at all stages of cultivation. To overcome this risk, it is necessary to use healthy seeds, genotypes with low susceptibility to pathogens and to optimize the planting conditions. Success in combating pathogens largely depends on the correct application of plant protection measures to obtain high-quality and healthy products. Of great importance is the detection of potential foci of infection before the appearance of external signs of disease in affected plants. Seasonal monitoring of pathogens using PCR-based diagnostic methods is necessary. Presymptomatic detection of tomato pathogens allows optimizing the use of chemical plant protection products and obtaining a more environmentally friendly and healthy product and seed material.

CONCLUSIONS

As a result of monitoring some fungal infections of tomatoes in greenhouse conditions, a decrease in the pathogenic load in plants was found, compared to open field systems. *A. alternata* was the predominant pathogen among all tested tomato genotypes. *Fusarium* and *Aspergillus* species were not detected in greenhouse tomato plantings during vegetative season. None of the pathogen species declared in the study were detected in the fresh seeds, which is favorable for their subsequent reproduction.

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