Colletotricum and *Fusarium* Spp. "Hitch -Hiking" on Imported Mango and Banana Fruits to Hungary

Colletotrichum *és* Fusarium *fajok jelentősége hazánkba importált mangó és banán gyümölcsökön*

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Abstract: Post-harvest losses caused by plant pathogens, both in general and during transport, are a major economic problem. Symptoms of fruit rot, caused by pathogens, are often observed in supermarkets. Importing fruits could be a pathway for pests and pathogens to enter a new region. We aimed to identify the pathogens introduced into Hungary through the fruit trade of bananas and mangoes. We confirmed the presence of *Colletotrichum* and *Fusarium* species in the fruits.

Keywords*: Colletotrichum; Fusarium; ITS*

Összefoglalás: A gyümölcsök nemzetközi kereskedelme megkönnyíti a növényi kórokozók és kártevők globális terjedését is. A boltok polcain gyakran találkozhatunk növénypatogének okozta tüneteket mutató mangó és banán gyümölcsökkel. Kutatásunk során a trópusokról importált mangó és banán gyümölcsökkel érkező *Colletotrichum* és *Fusarium* fajokat és azok jelentőségét vizsgáltuk.

Kulcsszavak*: Colletotrichum; Fusarium; ITS*

1. Introduction

Mangoes (*Mangifera indica* L.) and bananas (*Musa* × *paradisiaca*) are among the most popular fruits and are generally cultivated in tropical, subtropical and other regions and are distributed worldwide. Millions of tons of tropical fruits, including bananas and mangoes, are imported to the EU from more than 130 countries every year, which could become a potential pathway for plant pathogens (Suffert et al., 2018; Freshfel, 2021). Post-harvest losses caused by plant pathogens, both in general and during transport, are a huge economic problem. Fruits showing symptoms of fungal diseases are often observed in grocery stores. The aim of this study was to identify *Colletotrichum* and *Fusarium* species introduced into Hungary with the fruit trade.

2. Materials and Methods

For this study, tropical fruits (bananas and mangoes) showing symptoms of fungal diseases were selected from supermarkets and fruit merchants in Hungary. Isolates were obtained from infected fruit tissues placed into PDA medium. Macroscopic (mycelial colour, shape, edges and pattern of colonies) and microscopic (colour, shape and size of conidia) characteristics of the fungal isolates were recorded. Size and shape of 50 conidia per sample were characterized. Koch's postulates were fulfilled for all isolates. Genomic DNA was extracted from the growing margins of the colonies, using the cetyl-trimethyl-ammonium-bromide (CTAB) method. ITS1 and ITS4 primers were used to amplify the internal transcribed spacer (ITS) region. The partial beta-tubulin (TUB) gene was amplified using Bt1a and Bt1b primers. CAL1 and CAL2 primers were used to amplify a part of the calmodulin (CAL) gene. A part of the beta-tubulin (TUB) gene, the calmodulin (CAL) gene and the ITS region were amplified and sequenced Primers used in this study are listed in Table 1. Sequences were checked and edited according to the chromatogram and compared with the sequences stored in the NCBI database.

Table 1. The list of primers for amplification of internal transcribed spacer, the partial calmodulin gene and the partial beta-tubulin gene

Gene or DNA region	Primer	Primer sequence (5'-3')	References
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC	
calmodulin	CA_CAL1	TGAGTACAAGGAGGCCTT CTCCC	Glass és Donaldson 1995
	CA_CAL2	TTTGCATGAGTTGGACGAACTC	
beta-tubulin	Bt1a	TTCCCCCGTCTCCACTTCTTCATG	Glass és Donaldson 1995
	Bt1b	GACGAGATCGTTCATGTTGAACTC	

3. Results

3.1. Fungal pathogens isolated from banana fruits

Fusarium desaboruense

Symptoms on fruit: Spots on fruits were brown to back, sometimes sunken, 0.3-1 cm in size. Fruit tissues under the spots were mildly rotten.

Morphology and culture characteristics: Colony surface was cottony, white to pale violet. Colony reverse was white to pale violet and becoming violet pigmented over time. Microconidia were 6.7 × 2.6 µm in size, hyalin, ovoid, smooth and thin-walled with 1-2 septate. Macroconidia were 36.9 × 3.5 µm in size, hyalin, slightly curved with 3-4 septate. No chlamydospores were observed.

Molecular identification: BLAST analysis revealed that ITS and TUB gene had 99-100 % identity with the existing sequences of *Fusarium desaboruense.*

Fusarium oxysporum

Symptoms on fruit: Spots on fruits were brown to back, sometimes sunken, 0.3-1 cm in size. Fruit tissues under the spots were mildly rotten.

Morphology and culture characteristics: Colony surface was cottony, white to grey. Colony reverse was orange pigmented. Growth pattern of mycelia was in concentric rings. Microconidia were 2.95 × 7.21 µm in size, hyalin, ovoid, smooth and thin-walled with 0-1 septate. Macroconidia were 40.01 × 2.98 µm in size, hyalin, slightly curved with 2-3 septate. No chlamydospores were observed.

Molecular identification: Based on a BLAST analysis, ITS and TUB gene had 99.79-100% identity with the existing sequences of *Fusarium oxysporum.*

Colletotrichum musae

Symptoms on fruit: Spots on fruits were brown to back, sunken, 0.5-4 cm in size with salmon coloured spore masses and acervuli in the lesion.

Morphology and culture characteristics: Colony was cottony, white on the surface, pale orange to pale pink on the reverse side. The aerial mycelia were white then turned pale grey covered bright pinkish-orange conidial masses in the middle. Conidia were one-celled, 16.2 × 5.9 µm in size, hyaline, cylindrical in shape, with rounded ends.

Molecular identification: BLAST analysis revealed that ITS, TUB and CAL gene had 100% identity with the existing sequences of *Colletotrichum musae.*

3.2. Fungal pathogens isolated from mango fruits

Fusarium oxysporum

Symptoms on fruit: Spots on fruits were brown to back, sometimes sunken, 0.3-1 cm in size. Fruit tissues under the spots were mildly rotten.

Morphology and culture characteristics: Colony surface was cottony, white to grey. Colony reverse was orange pigmented. Growth pattern of mycelia was in concentric rings. Microconidia were 2.60 × 9.01 µm in size, hyalin, ovoid, smooth and thin-walled with 0-1 septate. Macroconidia were 38.7-3.02 µm in size, hyalin, slightly curved with 2-3 septate. No chlamydospores were observed.

Molecular identification: Based on a BLAST analysis, ITS and TUB gene had 99.79-100% identity with the existing sequences of *Fusarium oxysporum.*

Colletotrichum asianum

Symptoms on fruit: Brown to black rot appears at the stem end with orange coloured spore masses and acervuli in the lesion. Spots on fruits were brown to back, sunken, 0.3-1 cm in size sometimes also with spore masses and acervuli in the lesion.

Morphology and culture characteristics: Colony was cottony, white on the surface, pale orange with grey concentric rings and dark grey in the middle on the reverse side. The aerial mycelia were white with bright orange conidial mass in the middle. Morphological characteristics– Conidia were one-celled, 14.8 × 4.3µm in size, hyaline, cylindrical in shape, with rounded ends or with one acute end.

Molecular identification: Based on a BLAST analysis, ITS, TUB and CAL gene had 99.86-100% identity with the existing sequences of *Colletotrichum asianum.*

Colletotrichum fructicola

Symptoms on fruit: The symptoms were identical to those observed in *C. asianum*.

Morphology and culture characteristics: Colony was cottony, white on the surface, pale orange with grey concentric rings and dark grey in the middle on the reverse side. The aerial mycelia was white with bright orange conidial mass in the middle. Conida were one-celled, 14.3 × 4.1 µm in size, hyaline, cylindrical in shape, with rounded ends.

Molecular identification: BLAST analysis revealed that ITS, TUB and CAL gene had 99.79-100% identity with the existing sequences of *Colletotrichum frucicola.*

4. Discussion

The global trade of fruits facilitates the international movement of plant pathogens, with the introduction of new pathogens into a region potentially having economic consequences for local crop production.

In this study we reported the presence of *Colletotrichum asianum, C. fructicola,* on imported mango fruits and *Fusarium desaboruense* (*syn. F. sacchari*) on banana fruits for the first time in Hungary. *C. fructicola* is a polyphagous pathogen, member of the *C. gloeosporioides* complex causing anthracnose, bitter rot and leaf spot diseases of many cultivated plant species, including *Malus domestica, M. pumila, Prunus persica*, which are common fruit crops in Hungary (EFSA, 2021). *Fusarium desaboruense* was described in 2019 as a novel pathogen in the *Fusarium fujikuroi* species complex causing Panama disease in Indonesia (Maryani et al., 2019). Since, a study has shown that *F. desaboruense* should be considered synonymous with *F. sacchari* (Yilmaz et al. 2021). Beside sugarcane, *F. sacchari* infects *Solanaceae* crops, chilli peppers and strawberries (Saseetharan and Zakaria, 2014).

Infected fruit parts can end up in compost bins, providing an opportunity for pathogen establishment. With global warming, climatic factors will become more favourable for many pathogens, therefore more attention should be paid to newly introduced pathogens.

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References

EFSA PLH Panel (EFSA Panel on Plant Health), Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M.-A., Jaques Miret, J. A., Justesen, A. F., MacLeod, A., Magnusson, C. S. , Milonas, P., Navas-Cortes, J. A., Parnell, S., Potting, R., Thulke, H.-H., Van der Werf, W., Civera, A. V., Yuen, J., Zappalà, L., Migheli, Q., Vloutoglou, I., Campese, C., Maiorano, A., Streissl, F. and Reignault, P. L. 2021. Scientific Opinion on the pest categorisation of *Colletotrichum fructicola*. *EFSA Journal*. **19** (8) 6803 41. https://doi.org/10.2903/j.efsa.2021.6803

Freshfel 2021. Freshfel European Fresh Produce Association - activity report 2021. https://freshfel.org/wp-content/uploads/2021/06/Freshfel-Europe-Activity-Report-2021.pdf [accessed on 15 November 2023]

Glass, N. L. and Donaldson, G. C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and environmental microbiology.* **61** (4) 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Maryani N., Sandoval-Denis M., Lombard L., Crous P. W. and Kema G. H. J. 2019. New endemic *Fusarium* species hitch-hiking with pathogenic *Fusarium* strains causing Panama disease in small-holder banana plots in Indonesia. *Persoonia*. **43** 48–69. https://doi.org/10.3767/persoonia.2019.43.02.

Saseetharan, N. H. M. and Zakaria, L. 2014. Occurrence of *Fusarium* spp. on vegetable crops and assessment of their pathogenicity. *Pertanika J. Trop. Agri. Sci.* **37** (4) 445–455.

Suffert, M., Wilstermann, A., Petter, F., Schrader, G. and Grousset, F. 2018. Identification of new pests likely to be introduced into Europe with the fruit trade. *EPPO Bulletin*. **48** 144–154. https://doi.org/10.1111/epp.12462

White, T. J., Burns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M. A., Gelfand, D. H., Sninsky, J. J., White, T. J. (eds.), PCR Protocols: A guide to methods and applications. 315–322. San Diego: Academic Press. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Yilmaz N., Sandoval-Denis M., Lombard L., Visagie C. M., Wingfield B. D. and Crous P. W. 2021. Redefining species limits in the *Fusarium fujikuroi* species complex. *Persoonia*. **46** 129–162. https://doi.org/10.3767/persoonia.2021.46.05