Disease Note

PRESENCE OF BACILLUS PUMILUS CAUSING FRUIT ROT OF FICUS LACOR IN PAKISTAN

Hakim, S. Naz, F. Liaquat, S. Gul, H.J. Chaudhary and M.F.H. Munis

Department of Plant Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan

Ficus lacor (pakhar), a member of the family Moraceae, is known for its medicinal use. Whereas decoction of buds is used for ulcer and leucorrhoea, seeds are used to cure gastric problems, ulcer, typhoid fever, dysentery and boils (Rai et al., 2004). In 2014-15, some 40-50 fruits of F. lacor were collected from different areas of Islamabad and Rawalpindi, which showed dark and light brown spots, that progressed further rotting the whole fruit. For the isolation of the putative causal agent of the disease, the infected parts of the fruits were surface-sterilized with 2% sodium hypochlorite and placed directly on potato dextrose agar (PDA) and Luria-Bertani (LB) media. Bacterial growth was observed on LB plates only. The pathogenicity of bacterial isolates was assessed by injecting a cell suspension (calibrated at 10⁸ CFU/ml) in only. The pathogenicity of bacterial isolates was assessed (LB) media. Bacterial growth was observed on LB plates directly on potato dextrose agar (PDA) and Luria-Bertani (LB) media. Bacterial growth was observed on LB plates only. The pathogenicity of bacterial isolates was assessed by injecting a cell suspension (calibrated at 10⁸ CFU/ml) in healthy F. lacor fruits. Symptoms similar to those shown by naturally infected fruits developed 4 to 6 days post inoculation. Control fruits injected with sterilized distilled water remained healthy. A 16S RNA gene fragment of 1013 bp was amplified from bacterial isolates and sequenced, showing 99% similarity with the Bacillus pumilus strain TE07 16S ribosomal RNA gene. The sequence was deposited in GenBank under the accession No. KJ833786.1. B. pumilus is a spore-forming rod-shaped Gram-positive and aerobic bacterium which can infect several hosts including leaves of cabbage and peach, and fruits of apricot, apple, cucumber, olive and squash, and flower head of cauliflower and cloves (Galal et al., 2006). To our knowledge, this is the first report of B. pumilus causing fruit rot of F. lacor in Pakistan.


Disease Note

FIRST REPORT OF CURVULARIA SPICIFERA CAUSING BROWN ROT OF CITRUS IN SOUTHERN ITALY

F. Garganese, S.M. Sanzani, A. Mincuzzi and A. Ippolito

Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola165/A, 70126 Bari, Italy

In 2014, rot symptoms were observed on mandarin fruits (Citrus reticulata) in a grove of Metaponto (southern Italy). Five to 7 mm lesions, colonized by a brown mycelium characterized diseased tissues. Fruit was collected, and surface-sterilized in 2% sodium hypochlorite. Tissue portions, removed in between symptomatic and healthy areas, were placed on potato dextrose agar (PDA) selective for yeasts and fungi and incubated at 24°C. The obtained fungal isolate was deposited in the collection of DISSPA, University of Bari, Italy, in its monoconidial form. On PDA, colonies were fast growing, initially light brown, turning dark brown after 7 days, raised and lobate-undulate. The aerial mycelium was cottony. Conidia were 18.5 ± 3 μm long × 7 ± 3.4 μm wide, ellipsoidal or oblong, straight, with rounded ends, light brown, 3-transverse septated, with an evident hilum and walls fairly thick, thinner towards the ends. Conidiophore light to medium brown, of variable length, scattered or clustered, often branching were observed. Based on these morphological characters, the fungus was identified as Curvularia spicifera (Bainier) Boedijn. Curvularia is a hyphomycete associated with over 60 host genera (Manamgoda et al., 2011). Most Curvularia spp. are found in tropical regions, though a few have been reported in temperate zones. A molecular identification was also performed. Internal transcribed spacer regions ITS1 and ITS2, including the 5.8S gene, were amplified using the universal primers ITS5/ITS4, as reported by White et al. (1990), and sequenced (GenBank Accession No. KR229979). Blast analysis revealed 100% nucleotide similarity with other ITS sequences of C. spicifera available in GenBank. To fulfill Koch’s postulate, a pathogenicity test was conducted on mandarins. Fruit was surface sterilized with 2% sodium hypochlorite solution, rinsed with sterile water, dried, and wounded once at the equator with a sterile nail, by making a 3 × 2 mm injury that was then inoculated by the spore suspension (10⁵ conidia/ml). Fruit was placed at 24°C and 95% RH. Non-inoculated fruit was used as a control. Seven days after inoculation, fruit showed symptoms similar to those previously observed in the sampled fruit, while control fruit showed no symptoms. The colonies isolated from infected tissues showed the same morphological and molecular features of the identified strain. To our knowledge, this is the first report of citrus fruit infection by C. spicifera in Southern Italy. Further studies will be conducted to determine the incidence of the disease, as well as possible control means, since rot symptoms would make the fruit unmarketable.


Corresponding author: M.F.H. Munis
E-mail: munis@qau.edu.pk
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Corresponding author: S.M. Sanzani
E-mail: simonamarianna.sanzani@uniba.it
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