

DISEASE NOTE

FIRST REPORT OF GRAPEVINE LEAFROLL-ASSOCIATED VIRUS 2 IN MOROCCAN VINEYARDS

M. Afechtal¹, I. Bibi², A. Aarabe², M. Sbaghi³,
M. Ouantar² and Z. Faddoul²

¹National Institute for Agricultural Research (INRA), Regional
Center of Kénitra, Laboratory of Virology, Route de Sidi
Yahya du Gharb, Km 9, B.P. 257, Kénitra, Morocco

²Laboratory of Botany, Biotechnology and Plant pathology,
Faculty of Science, University of Ibn Tofail, Kénitra, Morocco

³National Institute for Agricultural Research (INRA),
Scientific Division, Rabat, Morocco

With an area of cultivation of about 44,600 ha, grapevines rank fourth among fruit crops of Morocco and represent one of the most valuable crops for the country's economy. Grapevine leafroll disease, caused by the grapevine leafroll-associated viruses (family *Closteroviridae*) is one of the most important viral diseases of grapevines (*Vitis vinifera*) worldwide (Fuchs *et al.*, 2009). During 2014, a number of vineyards of different sizes were visited in Gharb region in order to assess their sanitary status. Grapevines with typical leaf roll symptoms were reported on cultivar 'Red Globe' from one vineyard. The symptoms included red leaves and downwardly rolled leaf margins. A total of six samples were collected from vines showing virus symptoms in addition to ten samples from apparently symptomless plants. These samples were subjected to ELISA test, using commercially available kits (Bioreba, Germany), for the presence of *Grapevine leafroll associated virus 1*, 2 and 3 (GLRaV-1, -2 and -3). Symptomatic vines tested positive for GLRaV-2 and one symptomless plant tested positive to GLRaV-3, previously detected in Morocco (Digiario *et al.*, 1999); all other ELISA tests were negative. Total nucleic acid was extracted from the samples using RNeasy Plant Mini Kit (Qiagen, Germany) and tested by RT-PCR employing specific primers to amplify a 334 bp fragment of the HSP70h gene of GLRaV-2 (L2 F: 5'-ATAATTCGGCGTACATCCCCACTT-3' and U2 R: 5'-GCCCTCCGCGCAACTAATGACAG-3') (Bertazzon and Angelini, 2004). The RT-PCR assays were also positive for the six symptomatic vines and the ten asymptomatic plants were negative for GLRaV-2, thus confirming the results obtained by ELISA. To the best of our knowledge, this study represents the first report of the occurrence of GLRaV-2 in Morocco.

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Corresponding author: M. Afechtal
E-mail: mohamedafechtal.inra@gmail.com

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DISEASE NOTE

FIRST REPORT OF LEAF SPOT CAUSED BY *CHAETOPYRENA PENICILLATA* ON *MEDICAGO SATIVA* IN IRAN

N. Parhizi, D. Zafari, M.J. Soleimani and S. Bagherabadi

Department of Plant Protection, College of Agriculture,
Bu-Ali Sina University, Hamadan, Iran

Alfalfa (*Medicago sativa*) is a perennial flowering plant in the pea family Fabaceae, cultivated as an important forage crop in many countries around the world, including Iran. During a survey in spring and summer of 2016, symptoms of leaf spots on *M. sativa* were observed in an area of more than 20 ha in Hamadan province, Iran. It was estimated that 50-60% of plants were damaged by this disease. Leaf spots were small, dark brown, 0.3- 0.5 cm in diameter. Small pieces of infected tissues were taken from the margins of spots and after surface-disinfection, they were transferred to Potato Dextrose Agar (PDA) at 25°C. The isolates were purified and were transferred onto Oatmeal Agar (OA). On this medium aerial mycelium consisting of subhyaline, branched, septate, smooth, 2-3 µm wide hyphae, produced globose to subglobose, dark brown to black pycnidia. Conidia were hyaline, smooth to punctate, 1-celled, and cylindrical to subcylindrical, straight with rounded apex and truncate base, and measured 12-20 × 3-5 µm. Morphological characteristics of our isolates were in agreement with the description for *Chaetopyrena penicillata* (Arzanlou and Khodaei, 2012). Pathogenicity tests were performed three times. *Medicago* leaves were sprayed with conidial suspension (1 × 10⁶ spores/ml), while control plants were sprayed with sterile distilled water. Symptoms were reproduced 10 days after inoculation and *C. penicillata* was consistently reisolated from artificially infected plants only. To confirm the morphological identification, the internal transcribed spacer regions (ITS1, ITS2, and 5.8S gene) of rDNA were amplified with the primers ITS1/ITS4 and sequenced (White *et al.*, 1990). The sequence was deposited in GenBank (Accession No. KY681683) and BLAST search showed 99% similarity with sequence belonging to *C. penicillata* (JQ663990.1). To our knowledge, this is the first report of leaf spot caused by *C. penicillata* on *M. sativa* in Iran.

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Corresponding author: D. Zafari
E-mail: Zafari_d@yahoo.com

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