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NEW OR UNUSUAL DISEASE REPORTS

Macrophomina phaseolina associated with grapevine decline in Iran

Farnaz ABED-ASHTIANI, Abolfazl NARMANI and Mahdi ARZANLOU

Department of Plant Protection, Faculty of Agriculture, University of Tabriz, 5166614766 Tabriz, Iran

Summary. Grapevines exhibiting general decline symptoms were observed in July 2012 in Arbatan, a region in Marand county, north-western Iran. Leaf necrosis symptoms were also observed in affected grapevines. Dark brown necrotic lesions and wood discoloration were observed in the vascular tissues of affected cordons. Fungal isolates, all with similar cultural and morphological features, were obtained from symptomatic tissues. Morphological characteristics indicated that the isolates were *Macrophomina phaseolina*. Sequence analysis of the elongation factor (EF-1α) gene from isolates confirmed this identification, with 100% similarity to the reference *M. phaseolina* sequences obtained from GenBank. Pathogenicity assays of two *M. phaseolina* isolates on 2-year-old potted vines (cv. 'Keshmeshi') demonstrated that *M. phaseolina* was pathogenic on grapevine. This study confirmed the association of *M. phaseolina* with grapevine decline symptoms, and represents the first report of this fungus associated with grapevine trunk diseases in Iran.

Key words: grapevine trunk diseases, *Vitis vinifera.*

Introduction

Grapevine (*Vitis vinifera* L.) is a globally and economically important fruit crop, with grapes utilized as fresh fruit, and in other forms such as raisin, juice concentrate, fermented products, grape molasses, and grape seed oil, as well as industrial products such as ethanol and anthocyanin. In July 2012, general decline symptoms were observed on grapevines in vineyards of the Arbatan region, Marand county, East Azerbaijan province in north-western Iran. Incidence of the disease reached up to 25% in the investigated vineyards. Initial symptoms appeared as marginal leaf necrosis followed by complete foliar necrosis. Some of the infected cordons also dried out as the disease developed. In cross-sections of stems of diseased cordons, symptoms appeared as wood discolouration and unilateral brown necrotic lesions in the pith and vascular tissues. These eventually developed and surrounded whole cordons. Severely declining plants showed dark brown necrotic zones in the woody vascular rings as well as brown and black blotches on the cordon margins (Figure 1). The decline symptoms in affected vineyards led to complete vine wilting. The study reported here was initiated to establish the cause of these symptoms.

Materials and methods

Twenty two samples of diseased wood tissues from grapevines (cv. Keshmeshi) were collected from declining vines in eight different vineyards of the study area. Small sections of diseased stem samples were surface disinfected in 70% ethanol for 15–20 s and rinsed three times in sterilized distilled water. After drying on sterile filter paper, the segments were cultured onto potato dextrose agar (PDA, Fluka) plates amended with 100 mg L^{-1} streptomycin sulphate and 100 mg L^{-1} ampicillin (Arzanlou and Narmani, 2015). Pure cultures were obtained from hyphal tip subculturing. The cultures were deposited in the Culture Collection of Tabriz University (CCTU), Iran. The isolates were plated on PDA and incubated at 24°C under 12 h light/12 h dark fluorescent light

Corresponding author: M. Arzanlou
E-mail: arzanlou@hotmail.com

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Figure 1. A. Decline symptoms on grapevines from which only *Macrophomina phaseolina* was isolated. B–D. Various symptoms of wood discolouration and necrotic lesions in trunk cross-sections.

photoperiod, and their cultural and morphological characteristics were examined during seven days after inoculation of the culture plates.

DNA extraction from pure cultures was carried out following the protocol of Moller *et al*. (1992). The ITS region (including ITS1, 5.8S rDNA and ITS2) of the ribosomal DNA using universal primers ITS5 and ITS4 (White *et al*., 1990), and the elongation factor 1-alpha (EF-1α) gene using universal primers EF1-728F (Carbone and Kohn, 1999) and EF2 (O'Donnell *et al*., 1998), were chosen to characterize the isolates. PCR amplification of the ITS and EF-1α regions of two representative isolates was followed by sequencing of the PCR products with the same forward and reverse primers as in the PCR amplification, by commercial sequencing service provider (Pishgam Biotech Co.). The resulting sequences of each isolate were refined using SeqMan^{TM}II (DNASTAR), and a consensus sequence was generated for each of the sequences. A BLAST search against the GenBank nucleotide database was carried out to confirm the identity of the isolates. The alignment of sequences of the isolates used in this study and sequences of reference *Machrophomina phaseolina* isolates obtained from the GenBank (Sarr *et al*., 2014) was conducted using the ClutalW algorithm implemented in MEGA7 (Kumar *et al*., 2016). The alignments were checked visually, and if necessary they were improved manually. The DNA sequences of the ITS and EF-1α regions of the two representative *M. phaseolina* isolates were deposited in the GenBank (ITS acc. nos. KY680344, KY680345, EF-1α acc. nos. MG600247, MG600248).

A pathogenicity test was carried out by inoculating a 5 mm diam. mycelial plug of 7-d-old cultures of

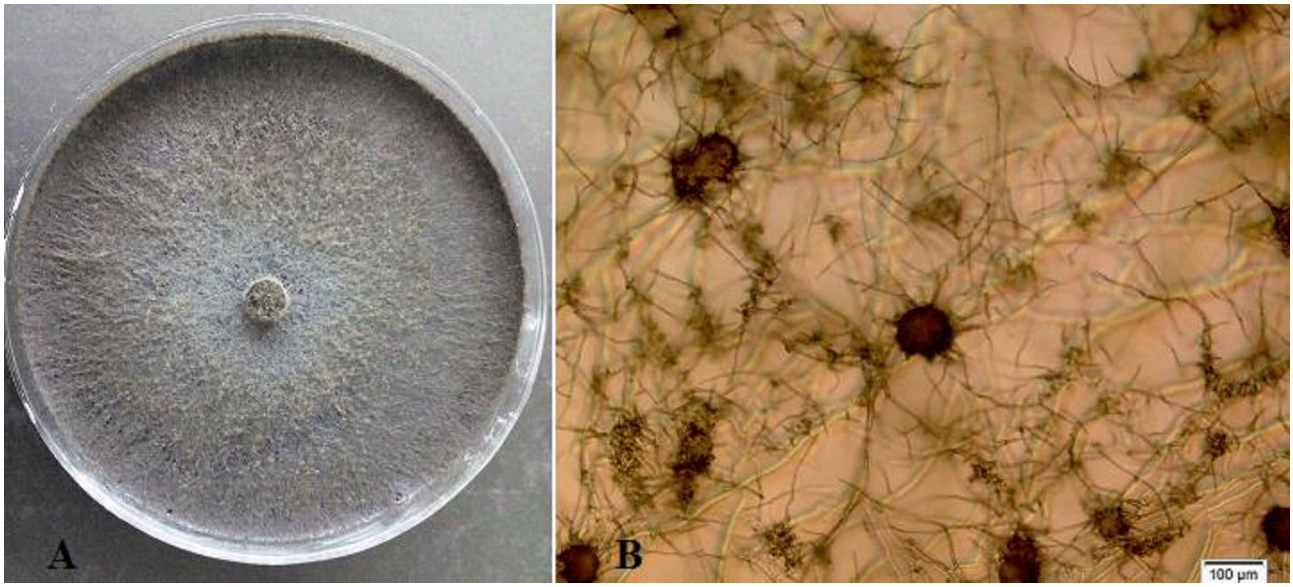

Figure 2. A. Colony morphology of *Macrophomina phaseolina* on PDA. B. Sclerotia on PDA.

two representative *M. phaseolina* isolates (CCTU1689 and CCTU1656) onto wounded shoots of 2-year-old potted grapevine (cv. Keshmeshi) plants. Parafilm was used to protect the inoculated sites. The shoots of healthy plants were inoculated with uncolonized sterile agar plugs as controls. Each vine was inoculated with one fungal isolate in three replications. The experiment was repeated twice. Disease development and symptoms were assessed 45 d after inoculation.

Results and discussion

Twenty fungal isolates were isolated from 22 collected grapevine samples, out of which five isolates with similar growth patterns were consistently isolated from the infected tissues. The samples from which *M. phaseolina* isolates were recovered, had been collected from four different vineyards. Colonies on PDA attained diameter of 70 mm after 3 d of incubation. These colonies were flat to slightly raised, with entire margins. Colony colour on both sides (upper surface and reverse) were white to gray, turning black with age. Hyphae were septate, 2–4 µm wide and sub-hyaline to dark brown. Black spherical to oblong or irregular microsclerotia ranging 90–180 µm (av. 128 µm) × 50–100 µm (av. 81 µm) (based on 30 randomly selected microsclerotia), developed in culture after 4–5 d incubation (Figure 2). Conidiomata were not observed in culture. Morphological features of the isolates were typical of those of *Macrophomina phaseolina* (Tassi) Goidanich (Holliday and Punithalingam, 1970).

PCR amplification of the ITS and EF-1α regions of two representative isolates (CCTU1689 and CCTU1656) was followed by sequencing of the fragments. The lengths of the ITS sequences was 518 and 555 bp, and the length of the EF-1α sequence was 264 bp. The ITS sequence data showed substantial homology with sequence data for both *M. phaseolina* and *M. pseudophaseolina, which is* a new species of *Macrophomina* described by Sarr *et al*. (2014). While, based on the EF-1α gene sequence data, the identity of our isolates was confirmed as *M. phaseolina*. The EF-1α gene sequences of our isolates (CCTU1689 and CCTU1656) showed 100% similarity to the published *M. phaseolina* sequences (GenBank acc. no. KF952069), while deviated from available sequence data for *M. pseudophaseolina* in Genbank (GenBank acc. no. KF952153, 91% similarity). The DNA sequences of the ITS and EF-1α regions of two representative *M. phaseolina* isolates were deposited in the GenBank (ITS acc. nos. KY680344, KY680345, EF-1α acc. nos. MG600247, MG600248, for isolates CCTU1689 and CCTU1656, respectively).

The results of the pathogenicity test confirmed *M. phaseolina* to be pathogenic on grapevines. Forty five

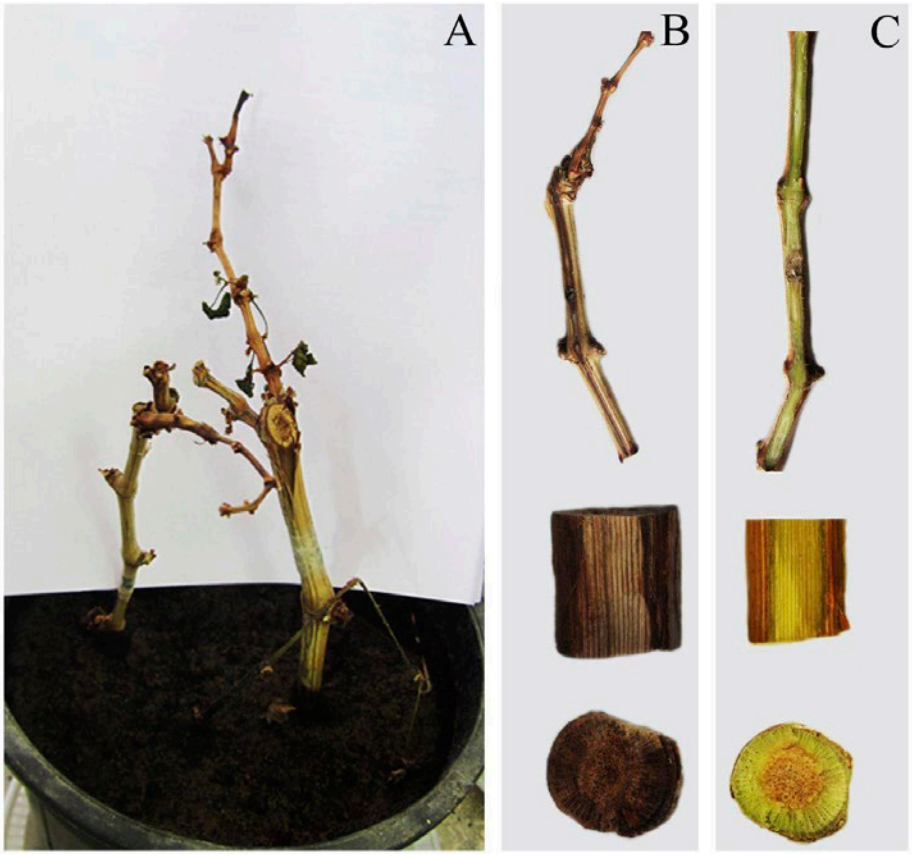

Figure 3. A. Pathogenicity assay of *Macrophomona phaseolina* on 2-year-old shoots of 'Keshmeshi' grapevines (inoculated with *M. phaseolina*). B. Disease symptoms and wood discolouration from an inoculated plant. C. Control (uninoculated) stem with no disease symptoms.

d after inoculation, disease symptoms on inoculated plants were similar to those observed on naturally infected plants. General decline symptoms and wilt were observed in the inoculated plants. Leaves dried out while they were green, and plants each had an overall wilted appearance. Wood necrosis was observed in longitudinal and cross-sections of inoculated shoots, extending from the inoculation site. Brown discolouration was observed under the bark of the shoots and the lesions (25–30 cm in length) developed all along the inoculated shoots. Control plants did not show any disease symptom (Figure 3). *Macrophomina phaseolina* was successfully re-isolated from inoculated grapevines and not from control plants. Thus, Koch's postulates were fulfilled and the pathogenic nature of *M. phaseolina* on grapevine was confirmed.

Macrophomina phaseolina has previously been reported on *Vitis vinifera* from Australia, Hawaii, Malawi, South Africa (Farr and Rossman, 2017), and Spain (Farr and Rossman, 2017; Gonzalez and Tello, 2011). To our knowledge, this is the first report of *M. phaseolina* associated with grapevine trunk disease in Iran.

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Compliance with ethical standards

This research did not involve any human and/or animal experimental subjects. The authors declare that they have no conflicts of interests.

Literature cited

Arzanlou M. and A. Narmani, 2015. ITS sequence data and morphology differentiate *Cytospora chrysosperma* associated with trunk disease of grapevine in northern Iran. *Journal of Plant Protection Research* 55, 117–125.

Carbone I. and L.M. Kohn, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91, 553–556.

Farr D.F. and A.Y. Rossman, 2017. *Fungal Databases*. U.S. National Fungus Collections, ARS, USDA. Retrieved December 5, 2017, from https://nt.ars-grin.gov/fungaldatabases/.

Gonzalez V. and M.L. Tello, 2011. The endophytic mycota associated with *Vitis vinifera* in central Spain. *Fungal Diversity* 47, 29–42.

Holliday P. and E. Punithalingam, 1970. *CMI Descriptions of Pathogenic Fungi and Bacteria*. No. 275. CAB International, Kew, Surrey, UK.

Kumar S., G. Stecher and K. Tamura, 2016. MEGA7: Molecular Evolutionary Genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33, 1870–1874.

Moller E.M., G. Bahnweg and H.H. Geiger, 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 20, 6115–6116.

O'Donnell K., H.C. Kistler, E. Cigelnik and R.C. Ploetz, 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Science of the United States of America* 95, 2044–2049.

Sarr M.P., M. Ndiaye, J.Z. Groenewald and P.W. Crous, 2014. Genetic diversity in *Macrophomina phaseolina*, the causal agent of charcoal rot. *Phytopathologia Mediterranea* 53, 250–268.

White T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols. A Guide to Methods and Applications* (M.A. Innis, G.H. Gelfand, J.J. Sninsky, T.J. White ed.), Academic press, New York, NY, USA, 315–322.

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