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NEW OR UNUSUAL DISEASE REPORTS

Identification of *Ulocladium atrum* causing potato leaf blight in Iran

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Summary. Leaf blight was observed on potato (*Solanum tuberosum* L.) affecting 19% of plants growing in fields in Iran. Symptomatic leaf samples were collected from potato plants in the main potato-growing regions of Iran, and isolations were made onto PDA medium. Single spore isolates were identified as *Ulocladium atrum* on the basis of morphological characteristics, and the identification was confirmed by sequence analyses of the ITS, *gpd* and Alt a1 gene regions. Pathogenicity tests demonstrated that *U. atrum* caused leaf blight on potato. This is the first report of leaf blight caused by *U. atrum* on potato in Iran.

Key words: morphological characteristics, pathogenicity, *Solanum tuberosum* L.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important world food crops (Ghebreslassie *et al*., 2016). During July–August 2013, extensive leaf blight was observed on potato plants that cultivated in main potato-growing regions of Iran, including Hamadan, Fars and Isfahan provinces. The disease was recorded on more than 19% of potato plants grown in these provinces. Symptoms of the disease were mostly at the edges of the leaves, first as small, dark brown to black lesions, and then extending into irregular patches that, in some cases, covered whole infected leaves or entire plants. To date, no similar disease on potato plants has been reported in Iran. Therefore, the objective of this study was to identify the causal agent of the leaf blight on potato plants in the main potato-growing regions of Iran.

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Materials and methods

Isolation and characterization of the pathogen

During July–August 2013, affected leaves with typical symptoms of leaf blight were collected from tenpotato fields in each of Hamadan, Fars and Isfahan provinces. Infected leaf tissue samples were cut into 3–5 mm pieces and surface-sterilized in 1% NaOCl for 2 min, rinsed in sterile water twice and dried on sterilized filter paper. A total of 30 leaf pieces from each field sample were placed on potato dextrose agar (PDA) and incubated at 25°C with a 12 h photoperiod for 10 d. The frequency of colonies recovered from these leaf pieces was 100%. A total of 30 morphologically similar isolates were purified by single spore culturing and sub-culturing on PDA at 25°C with a 12 h photoperiod for 10 d, in order to characterize fungal growth and morphology, including conidia produced. The dimensionswere measured for ten conidia from each of the the isolates.

Pathogenicity tests

Pathogenicity tests were conducted for the 30 isolates, in a greenhouse with average temperature of 27±2°C and relative humidity of 75–85%. The ex-

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periment was arranged in a completely randomized design in ten replications of inoculated and control plants. Each replication consisted of one potato plant cv. Agria. One-month-old plants were inoculated by spraying conidial suspensions of *U. atrum* (10^3 conidia mL^{-1} of water). Disease rating was assessed 2 weeks after inoculation, as leaf blight percentage of symptomatic plants, using the a standard disease severity score system (NIAB, 1985). The pathogen was re-isolated from affected plants, and compared with the original isolates. The experiments were repeated twice for confirmation of the results during October and November 2013.

Molecular identification

To confirm the results from morphological studies, the representative isolate E1 was grown on PDA, and genomic DNA was extracted using the CTAB method (Talbot, 2001). A NanoDrop ND-1,000 spectrophotometer (LMS Co., Ltd) was used to check the quality and concentration of Genomic DNA. Nuclear rDNA internal transcriber spacer (ITS) region (White *et al.*, 1990) and two protein coding genes, glyceraldehyde 3-phosphate dehydrogenase (*gpd*) and allergen alt a1 (Alt a1) (Runa *et al.*, 2009), were amplified. BLAST searches of the ITS-5.8S rDNA sequences were performed against the NCBI nucleotide database. The PCR products were purified using Gene JET TM commercial PCR Purification Kit (SinaClon BioScience Co.), and sequenced using a commercial sequencing service provider (RNABIOTEC. Isfahan, Iran). Sequences of the ITS-5.8S rDNA were searched in the NCBI database (http://www.ncbi.nlm.nih.gov).

Results and discussion

Isolation and characterization of the pathogen

The colonies of the isolated 30 pure fungal strains on PDA were black with white margins on PDA. The conidia were pale olivaceous to dark brown, spherical to subspherical, developing in two main septation patterns, which lead to septae forming above and below the median septum in each conidium and becoming three trans-septate, described as 4-celled. These conidia which may produce further septae to develop 8-celled conidia, of dimensions 17.3–23.8 × 14.3–16.8 μm (Figure 1a). The hypae were septate, pale olivaceous brown in colour, with a smooth in areas, and were 5–5.5 μm in diameter (Figure 1b). Conidiophores were simple or branched, erect, arising laterally, 5.4–8.2 μm wide and up to 122 μm long. Based on the conidial morphology, including variations in conidial shape, size, length/width ratios, colour, septation, and ornamentation, all 30 isolates were identified as *U. atrum* (Matsushima, 1975; O'Day, 1996; Simmons, 1998).

Pathogenicity test

Disease symptoms similar to those observed in the affected fields were observed in all of the inoculated potato plants. Symptoms consisted of small, dark coloured lesions that turned black with time, and extended into irregular patches, occasionally covering the whole surfaces of affected leaves. *Ulocladium atrum* was re-isolated, and was identical to the inoculated original isolates in cultural growth and morphological chracteristics. The nil-inoculated plants did not develop disease symptoms. These results confirm that the *U. atrum* were pathogenic to potato, and that this fungus was the causal agent of leaf blight.

Molecular identification

PCR amplification of the ITS, *gpd* and Alt a1 regions for the representative isolate E1 generated predicted sizes of 529, 580 and 473 bp, respectively for these genetic regions. Sequences of the regions shared 100% identity with the *U. atrum* ex-epitype strain ATCC 18040 (Accession Nos. AF229486, AY278818 and AY563318). These results confirmed the identity of the fungus as *U. atrum*.

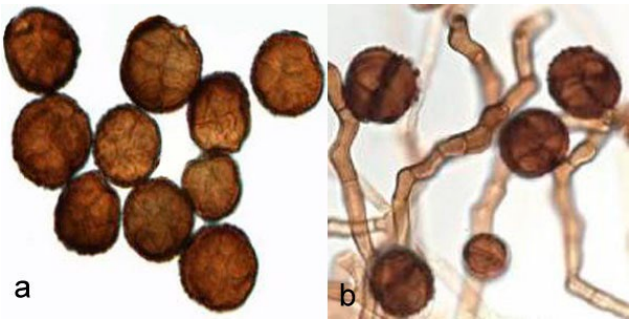

Figure 1. a) Multicellular conidia of *Ulocladium atrum*, isolated from potato leaves. b) Hyphae and conidiaof *U. atrum*.

Leaf blight caused by *U. atrum* is an important disease on potato, causing considerable damage worldwide (De Hoog *et al*., 2000; Hooker, 1981; Lapwood and Hide, 1971; O'Brien and Pice, 1976). *Ulocladium atrum* has also been isolated from *Cucumis sativus* (cucumber), *Helianthus annuus* (sunflower), *Lens culinaris* subsp. *culinaris* (lentil), *Prunus dulcis* (almond) and *Viola wittrockiana* (wild pansy) (O'Day, 1996; Li, 2002; Runa, 2009). All the isolates obtained from the affected potato plants in Iran were identified as *U. atrum*, based on morphological and molecular characteristics, as it was already defined by Woudenberg *et al*. (2013) previuosly. To our knowledge, this is the first report of leaf blight, caused by *U. atrum*, as a disease of potato in Iran.

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