

First Report of Leaf Spot of Strawberry Caused by *Alternaria tenuissima* in Iran

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Abstract

During a survey in autumn of 2013, leaf spots symptoms on strawberry (*Fragaria ananassa*) leaves were observed in Kurdistan province, Iran. From symptomatic leaves cultured on PDA medium totally 24 isolates of *Alternaria* were obtained. According to morphological and molecular studies obtained isolates were identified as *A. tenuissima*. To our knowledge, this is the first report of *A. tenuissima* on strawberry in Iran.

Keywords: *Fragaria ananassa*; *Alternaria*; Kurdistan province

Short Communication

The latest statistics by Iran ministry of agriculture shows about 2113 hectare of lands in the Kurdistan province is allocated for production of strawberry and annual production is estimated about 21130 ton. During a survey in autumn of 2013, leaf spots symptoms on strawberry leaves were observed in Kurdistan province, Iran (Figure 1A) with all major growing areas of the province affected. Disease incidence exceeded 50% in some regions in severely infected fields. Symptoms were further monitored on 35 selected plants. Initially, symptoms appeared on leaves as small, yellowish brown spots, becoming brown and finally necrotic with age. Small symptomatic leaf fragments were cut from healthy sections and were placed on Potato Dextrose Agar (PDA). Petri dishes were kept in an incubator at 25°C. Five to seven days after incubation, totally 24 *Alternaria* isolates were obtained from the strawberry symptomatic leaves in the growing areas. Obtained isolates after purification using single spore method were transferred to petri dishes containing Potato Carrot Agar (PCA) to study the morphological characteristics in an attempt to identify the cultures to species levels. These petri dishes were kept at 23-25°C under fluorescent light with light cycle of 8 hours light and 16 hours dark and were studied after five to seven days. Isolates on PCA medium, were brownish green and formed concentric rings, loosely cottony. The light exposed rings of colonies are dominated at 4 d by unbranched chains of 6-10 conidia. Longer chains and uncommon branching were found in colonies aging past 7 d. Mature conidia have only transepta reach size range 30-40 × 10-13 μm, ovoid, these median constricted conidia may be ovoid with a short apical secondary conidiophore. Muriformly septate conidia 32-40 × 14-16 μm. Conidia are median brown in color and rarely punctulate in ornamentation with 2-7 transverse and 1-2 longitudinal septa (Figure 1B-1E). Microscopic identification was achieved using Lica DMLB microscope. Based on morphological characteristics, the pathogen was identified as *Alternaria tenuissima* [1]. To confirm the morphological identification, molecular identification was conducted based on

DNA sequences of the glyceraldehyde-3-phosphate dehydrogenase gene (*gpd*) and its sequences was submitted to GenBank (Accession number KP276228). The BLAST similarity search using the sequences of the isolate revealed a high similarity to *Alternaria tenuissima* with GenBank entries JN634820. In order to confirm Koch's postulates, pathogenicity tests were conducted on strawberry leaves. Strawberry leaves, were sprayed with conidial suspension (10⁶ spores/ml), while control plants were sprayed with sterilized distilled water. After inoculation, leaves were kept in a growth chamber at 25°C. Leaf spot symptoms were observed on the inoculated leaves after 7 days and *A. tenuissima* was successfully reisolated from artificially infected leaves, while did not appear any spots on the control leaves. *A. tenuissima* was previously reported on *Fragaria ananassa* in Florida [2,3] and Korea [4]. To our knowledge, this is the first report of *A. tenuissima* on *Fragaria ananassa* (strawberry) in Iran. The culture has been deposited in the Public Collections of the CBS, The Netherlands, with Accession No. CBS (139417).

References

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Figure 1: A) Symptoms of leaf spot on Strawberry leaves. B) Surface of colony after 7 days on PCA. C) Simple conidiophore and long conidial chain. D) Branched conidial chain. E) Conidia with a distinct medium septum. F) Distinct and thick medium septum.

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