First report of leaf blight on Parthenium hysterophorus caused by Nigrospora sphaerica in Malaysia

ABSTRACT

Weeds may act as inoculum reservoirs for fungal pathogens that could affect other economically important crops (Karimi et al. 2019). In February 2019, leaves of the ubiquitous invasive weed, Parthenium hysterophorus L. (parthenium weed) exhibiting symptom of blight were observed at Ladang Infoternak Sg. Siput (U), a state-owned livestock center in Perak, Malaysia. Symptoms appeared as irregularly shaped, brown-to-black necrotic lesions across the entire leaf visible from both surfaces, and frequently on the older leaves. The disease incidence was approximately 30% of 1,000 plants. Twenty symptomatic parthenium weed leaves were collected from several infested livestock feeding plots for pathogen isolation. The infected tissues were sectioned and surface-sterilized with 70% ethyl alcohol for 1 min, rinsed three times with sterile distilled water, transferred onto potato dextrose agar, and incubated at 25°C under continuous dark for 7 days. Microscopic observation revealed fungal colonies with similar characteristics. Mycelium was initially white and gradually changed to pale orange on the back of the plate but later turned black as sporulation began. Conidia were spherical or sub-spherical, single-celled, smooth-walled, 12 to 21 µm diameter (mean = 15.56 ± 0.42 µm, n= 30) and were borne on a hyaline vesicle. Based on morphological features, the fungus was preliminarily identified as Nigrospora sphaerica (Sacc) E. W. Mason (Wang et al. 2017). To confirm identity, molecular identification was conducted using isolate 1SS which was selected as a representative isolate from the 20 isolates obtained. Genomic DNA was extracted from mycelia using a SDS-based extraction method (Xia et al. 2019). Amplification of the rDNA internal transcribed spacer (ITS) region was conducted with universal primer ITS1/ITS4 (White et al. 1990; Úrbez-Torres et al. 2008). The amplicon served as a template for Sanger sequencing conducted at a commercial service provider (Apical Scientific, Malaysia). The generated sequence trace data was analyzed with BioEdit v7.2. From BLASTn analysis, the ITS sequence (GenBank accession number. MN339998) had at least 99% nucleotide identity to that of N. sphaerica (GenBank accession number. MK108917). Pathogenicity was confirmed by spraying the leaf surfaces of 12 healthy parthenium weed plants (2-months-old) with a conidial suspension (106 conidia per ml) collected from a 7 day-old culture. Another 12 plants served as a control treatment and received only sterile distilled water. Inoculation was done 2 h before sunset and the inoculated plants were covered with plastic bags for 24 h to promote conidial germination. All plants were maintained in a glasshouse (24 to 35°C) for the development of the disease. After 7 days, typical leaf blight symptoms developed on the inoculated plants consistent with the symptoms observed in the field. The pathogen was re-isolated from the diseased leaves and morphological identification revealed the same characteristics as the original isolate with 100% re-isolation frequency, thus, fulfilling Koch's postulates. All leaves of the control plants remained symptomless and the experiment was repeated twice. In Malaysia, the incidence of N. sphaerica as a plant pathogen has been recorded on several important crops such as watermelon and dragon fruit (Kee et al. 2019; Ismail and Abd Razak 2021). To our knowledge, this is the first report of

leaf blight on P. hysterophorus caused by N. sphaerica from this country. This report justifies the significant potential of P. hysterophorus as an alternative weed host for the distribution of N. sphaerica.

Keyword: Causal agent; Crop type; Field crops; Fungi; Pathogen detection