First report of *Alternaria alternata* causing leaf spot on *Rumex vesicarius* in India

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Abstract A severe outbreak of leaf spot was observed on *Rumex vesicarius* for the first time in Andhra Pradesh state of India during 2009 to 2011. Based on pathogenicity, morphology and rDNA spacer sequences, the pathogen was identified as *Alternaria alternata* (Fr.) Keissler. This report is the first of *Alternaria* leaf spot of *Rumex vesicarius*.

Keywords *Rumex vesicarius* · *Alternaria alternata* · Pathogenicity · rDNA spacer sequence

Rumex vesicarius L is an annual, glabrous herb of the Polygonaceae, native to northern Africa and Asia (Pakistan and India), and is distributed widely in the tropics as an ornamental. It is also known as bladder dock, rosy dock, blister sorrel or country sorrel and is mostly cultivated as a leafy vegetable. This crop is widely used in the cuisine of different regions for its thick, semisucculent leaves, mild flavour, and mucilaginous texture. In south India, *R. vesicarius* is extensively grown for local markets and regional export. For the last 2 years (2009 to 2011), during rainy seasons (June to September), severe foliar symptoms were observed on *Rumex vesicarius* in Ranga Reddy, Chittoor

and Kadapa districts of Andhra Pradesh, India. Approximately 60 to 75% of the fields were affected with disease incidence ranging from 70 to 90%. Symptoms initially appeared as small light brown, circular spots with becoming irregular, dark brown lesions, while a few remained circular with concentric rings or zones (Fig. 1). On severely affected leaves several spots coalesced to form large necrotic areas (2 to 23 mm in diameter), ultimately leading to drying of the leaves. The disease first appeared in June, was widespread from the end of July through August when temperatures ranged from 25 to 30°C, and ended in September. Diseased leaves were cut into small fragments, surface sterilized in 1% sodium hypochlorite for 2 min, rinsed three times in sterile distilled water, and plated on potato dextrose agar (PDA), and then incubated at 25°C for 7 days under 12 h light and dark conditions. Hyphal tips from the margin of each developing colony were subcultured on PDA. Fungal colonies were initially white, becoming olivaceous, and turning brown with age. Conidiophores were short, simple or branched, golden brown in colour, measuring 15 µm long and 2–6 µm thick. Conidia were obclavate, obpyriform or ellipsoidal with a short conical beak, and borne in long chains, branched or unbranched, pale brown to golden brown and 22 to 32 µm long and 13 to 18 µm wide at the broadest point. Conidia had 3 to 8 transverse septa and 1 to 2 longitudinal septa (Fig. 2). On the basis of conidial morphological characteristics, the pathogen was identified as *Alternaria alternata* (Fr.) Keissler (Simmons 2007). Identification was confirmed by amplification of the internal transcribed spacer (ITS) region. Genomic DNA was extracted from pure cultures of an isolate and the internal transcribed spacer (ITS) region was amplified using the primers ITS4 and ITS5 (White et al. 1990). PCR amplicons

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Fig. 1 Leaf spot symptoms on *R. vesicarius*

of approximately 488 bp were obtained from isolate, and sequence comparisons with GenBank showed 100% similarity with *A. alternata* (GenBank Accession No. JF266575.1). Sequence from this study was submitted to GenBank nucleotide database (GenBank Accession No. AB675593). Moreover, *Alternaria alternata* has been deposited in the fungal identification service, (MACS-ARI), Agharkar Research Institute, Pune, India (Accession No. NFCCI-2637).

To determine pathogenicity, inoculations were performed on detached, surface sterilized, healthy leaves

Fig. 2 Conidia of *A. alternata* (scale bar=20 μ m)

following the method of Belisario et al. (1999). A 5- μ l drop of conidial suspension containing 1×10^5 CFU/ml was placed on each leaf and 12 leaves per isolate were used. Leaves were incubated in a growth chamber (90% relative humidity and a 12-h photoperiod). After 7 days, leaf spots similar to the original symptoms developed on all inoculated leaves and *A. alternata* was consistently re-isolated from symptomatic tissue, fulfilling Koch's postulates. Control leaves inoculated with sterile distilled water remained asymptomatic. *R. vesicarius* has been reported to be affected by leaf rust caused by *Uromyces longipedicellaris* (Ramachar and Rao, 1984) in India. *A. alternata* is a widespread pathogen affecting a wide range of hosts, and has previously been reported on apple (Johnson et al. 2000), citrus (Peever et al. 2002), tomato (Akhtar et al. 2004), *Stevia rebaudiana* (Maiti et al. 2006), *Rumex dentatus* (Siddiqui et al. 2009) and pomegranate (Ezra et al. 2010), but a new record as a plant pathogen on *R. vesicarius* is very significant. Thus, leaf spot on *R. vesicarius* caused by *A. alternata* is the first report from India and worldwide.

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