Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Phakopsora euvitis* Causing Leaf Rust Disease on Grapevine (*Vitis labrusca*) in India

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Plant Dis. 107:2868, 2023; published online as https://doi.org/10.1094/ PDIS-04-23-0651-PDN. Accepted for publication 5 May 2023.

Grapevine (Vitis labrusca L.), a member of the family Vitaceae and native of North America, is grown as a table grape. During the survey for grapevine diseases in May 2022, we noticed numerous yellow pustules of rust on the lower side of leaves of "Bangalore Blue" in Nandi Village (13°22'59.7"N, 77°42'33.4"E), Chikkaballapur District, Karnataka, India. The crop was at the maturity stage, and the rust disease severity was determined using the scale developed by Angelotti et al. (2008), which was up to 10%. The disease symptoms were numerous small, raised yellow pustules on the abaxial surface corresponding to adaxial surface chlorotic spots. In severe conditions, spots cover the entire leaf, and defoliation occurs. Similar disease symptoms were reported by Ono (2000), Primiano et al. (2017), and Weinert et al. (2003). The pathogenicity test was performed on cuttings of Bangalore Blue grapevine in a glasshouse at $25 \pm 1^{\circ}$ C. Urediniospores were collected from diseased leaves using a brush and suspended in distilled water, and the urediniospore suspension $(3 \times 10^4 \text{ spores/ml})$ was used for inoculation on the abaxial surface of leaves. Control plants were sprayed with distilled water. The leaves developed symptoms 15 to 17 days after inoculation, and the pathogen was confirmed by symptomatology and microscopic observation of urediniospores. Urediniospores were short-pedicellate, sessile, obovoid to obovoid-ellipsoid, and uniformly echinulate with 42.98 to

 32.54×31.37 to $25.15 \ \mu\text{m}$ in size. The aecial stage of *Phakopsora* sp. has been reported on an alternate host, Meliosma simplicifolia (Hosagoudar 1988). As the internal transcribed spacer (ITS) region offers some utility in the molecular detection of the Phakopsora genus (Rush et al. 2019), the pathogen was confirmed by studying different regions in the ITS such as ITS1, 5.8S rRNA, and ITS2. Total DNA was extracted from urediniospore mass using the Macherey-Nagel kit (Duren, Germany) by following the manufacturer's protocol. The quantity of isolated DNA was checked using an Qubit 3.0 fluorometer (Invitrogen) before being subjected to polymerase chain reaction (PCR) amplification in a thermocycler (Eppendorfvapo.protect) using ITS1 and ITS4 primers (IDT, Singapore) targeting ITS1, 5.8S rRNA, and ITS2 regions, and the obtained amplicon (~700 bp) was purified using the Macherey-Nagel NucleoSpin Gel and PCR Clean-up kit (Duren, Germany) as per the manufacturer's protocol and sequenced by Sanger's dideoxy chain-termination method (ABI 3730 [48 capillaries] electrophoresis). The sequence was edited in BioEdit (https://bioedit. software.informer.com/7.2/) and aligned in MUSCLE, and the phylogenetic tree was constructed in MEGA 11 using the neighbor-joining method by following the maximum likelihood criterion (Kumar et al. 2018). The sequence data were deposited in NCBI (accession no. OP221661). The BLAST search sequence of the isolate Nandi-KA in GenBank revealed 97.91% homology with the sequence of Phakopsora sp. (accession no. KC815548.1) and 96.87% with that of Phakopsora euvitis (accession no. AB354790.1). Based on disease symptoms, fungal morphology, the pathogenicity test, and the ITS sequence, the fungus was identified as P. euvitis, the pathogen causing grapevine leaf rust disease. Though similar disease symptoms are observed on grapevine in India (EPPO 2016), the pathogen was not confirmed. To our knowledge, this is the first report of P. euvitis causing leaf rust disease in grapevine (V. labrusca) in India.

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The author(s) declare no conflict of interest.

Keywords: India, ITS, pathogenicity, Phakopsora euvitis, Vitis labrusca

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