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First Report of Charcoal Rot Caused by *Macrophomina phaseolina* in *Basella alba* in India

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Malabar spinach (*Basella alba* L.) belongs to the family Basellaceae, which is native to Southeast Asia. It is widely cultivated as a perennial leafy vegetable in India. The leaves are rich sources of vitamin A, vitamin C, iron, calcium, soluble fiber, and protein per calorie. It possesses immense potential in androgenic and nephroprotective activity, antioxidant and antibacterial activity, anti-inflammatory activity, and central nervous system depressant activity (**Kumar et al. 2013**). During the period of August to October 2016, plants were observed with charcoal rot symptoms at the research farm of ICAR–Indian Institute of Vegetable Research and in six farmers' fields ranging in size from 200 to 1,000 m^2 in Varanasi district of Uttar Pradesh, India. The symptoms, observed on 30 to 40% of the plants, consisted of brownish to black discoloration at the collar region of the stem and branches that progressed into wilting and drying of the entire plant. Infected plant stems appeared shredded and contained black microsclerotia. Symptomatic plants were collected

for isolation of the causal agent. Samples were rinsed twice in running tap water and once in double-distilled water. Symptomatic excised stem fragments were further surface sterilized with 2% NaOCl and washed twice in autoclaved double-distilled water. The surface-sterilized stem fragments were plated on potato dextrose agar (PDA) media plates and were incubated at 28 ± 1°C for 48 h. Mycelial growth was black in color. Under a compound microscope, black round to oblong or irregular shaped black colored microsclerotia with mycelial attachment were observed. The average diameter of microsclerotia was 76.26 ± 8.06 µm (*n* = 50). Inoculum was multiplied in sand–maize medium. Five pieces of 15-day-old PDA culture were added to 250 g of sand–maize medium and incubated at 28 ± 1°C for 15 days. One part of inoculum was mixed with 10 parts of sterilized soil and used to fill 10-kg pots. Stem cuttings 15 cm long from healthy plants were obtained. Three cuttings were planted in each pot filled with inoculated and mock-inoculated soil, replicated three times, and maintained at 33 to 35°C and at 40 to 50% soil moisture content under glass house conditions. Cuttings were observed on a daily basis for the development of symptoms. Symptoms typical of charcoal rot on the collar region were first observed 12 days after planting, whereas plants in mock-inoculated soil remained healthy. The causal agent reisolated from the symptomatic plant tissue was found to be morphologically and culturally identical to the inoculated isolate. On the basis of morphological characteristics and a pathogenicity test the isolated charcoal rot causing pathogen was identified as *Macrophomina phaseolina* (**Tanaji et al. 2017**). For further confirmation, total genomic DNA was extracted using the HiPurA Fungal DNA Purification Kit (HiMedia, India). The elongation factor (EF1a) gene was amplified with primers TEF1-983F (5′GCYCCYGGHCAYCGTGAYTTYAT3′) and TEF1-2218R (5′ATGACACCRACRGCRACRGTYTG3′) (**Rehner and Buckley 2005**). Polymerase chain reaction products were sequenced and submitted to GenBank with accession number MG733372. In BLAST analysis, EF1a gene showed 100% sequence homology with *M. phaseolina* (DQ677929). *M. phaseolina* has been recorded worldwide infecting many agricultural and horticultural crops, but to our knowledge, this is the first report of *M. phaseolina* causing charcoal rot on *B. alba* in India.

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