Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Spot on Oat (*Avena sativa*) in India

Boda Praveen,¹ M. K. Prasanna Kumar,^{1,†} Pramesh Devanna,^{2,†} K. B. Palanna,¹ P. P. Buella,¹ Gutha Venkata Ramesh,¹ Namburi Karunakar Reddy,¹ and A. Nagaraja¹

¹University of Agricultural Sciences, GKVK, Bengaluru, 560 065, India

²Rice Pathology Laboratory, All India Coordinated Rice Improvement Programme, Gangavathi, University of Agricultural Sciences, Raichur, 584 104, India

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Oat (*Avena sativa* L.) is an important cereal crop grown worldwide primarily for food and animal feed. In November 2019, a leaf spot disease was observed on the oat plants at Mandya (12.5218° N, 76.8951° E), Karnataka, India. The disease incidence on plants was ranged between 43 and 57%. Initially, the symptoms appeared on leaves as small dark-brown spots surrounded by a yellow halo and later turned to irregular necrotic spots with a yellow halo. A total of 30 leaves showing typical symptoms were collected from 10 plants (three leaves per plant) and were cut into 4- to 5-mm pieces at the junction of infected and healthy tissues. Cut tissues were soaked in 75% ethanol for 45 s, followed by 1% sodium hypochlorite solution for 1 min, rinsed five times in sterile distilled water, air dried, placed on PDA, and incubated at 25 ± 1°C. After 7 days of incubation, greyish fungal colonies appeared on PDA. The single-spore isolation method was employed to recover pure cultures for five isolates. The colonies initially produced light-greyish aerial mycelia and then turned to dark-greyish upon maturity. Conidia were obclavate to pyriform and measured 17.34 to 46.97 µm long, 5.38 to 14.31 µm wide, with zero to three longitudinal and one to six transverse septa with a short beak (2.73 to 10.17 µm) ($n = 50$). Based on the morpho-cultural characteristics, the isolates were identified as *Alternaria* spp., and PCR assay using species-specific primers (AAF2/AAR3; Konstantinova et al. 2002) confirmed the taxonomic identity of all five isolates as *Alternaria alternata*. To further confirm the identity, the internal transcribed spacer (ITS), small subunit (SSU), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), RNA polymerase second largest subunit (*rpb2*), *Alternaria* major allergen (*Alt a1*), endopolygalacturonase (*endoPG*), an

anonymous gene region OPA10-2, KOG1058, and translation elongation factor 1-alpha (*tef1*) of two isolates (MAAS-1 and MAAS-2) were PCR amplified using the primers described previously (Praveen et al. 2021; Woudenberg et al. 2015), and the resultant PCR products were sequenced and deposited in NCBI GenBank (ITS: MW487388, MW741962; SSU: MW506220, MW752854; *gapdh*: MW506221, MW752855; *rpb2*: MW506222, MW752856; *Alt a1*: MW506223, MW752857; *endoPG*: MW506224, MW752858; OPA10-2: MW506225, MW752859; KOG1058: MW506226, MW752860; and *tef1*: MW506227, MW752861), which showed (99.62, 99.81%), (100, 100%), (100, 99.66%), (100, 100%), (99.58, 99.15%), (99.55, 99.32%), (99.53, 99.68%), (99.23, 99.56%), and (99.17, 99.58%) identity with ITS (AF347031), SSU (KC584507), *gapdh* (AY278808), *rpb2* (KC584375), *Alt a1* (AY563301), *endoPG* (JQ811978), OPA10-2 (KP124632), KOG1058 (KP125233), and *tef1* (KC584634) genes/genomic regions of type strain CBS916.96 of *A. alternata* respectively, confirming the identity of MAAS-1 and MAAS-2 as *A. alternata*. For the pathogenicity assay, a conidial suspension (2×10^6 conidia/ml) of MAAS-1 isolate was artificially sprayed until runoff on 10 healthy oat plants (cv. Kent, 35 days old), and 10 plants sprayed with sterile water served as a control. All plants were covered with polyethylene covers and kept under the greenhouse at 28 ± 1°C. The pathogenicity assay was repeated three times. After 6 days postinoculation, small dark-brown spots with a light-yellow halo appeared on leaves of MAAS-1 inoculated plants. In comparison, no symptoms were observed on control plants. The fungal pathogen was reisolated from the artificially infected plants and confirmed as *A. alternata* based on morpho-cultural characteristics and PCR assays. Leaf spot disease of oat caused by *A. alternata* has already been reported from China (Chen et al. 2020); to our knowledge, this is the first report of *A. alternata* causing leaf spot on oat in India. Leaf spot disease is an emerging threat to oat cultivation, and it reduces the grain yield and leaf quality; therefore, its management is essential for increasing productivity.

References:

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[†]Indicates the corresponding authors.

M. K. Prasanna Kumar; babu_prasanna@rediffmail.com, and P. Devanna; pramesh84@uasraichur.edu.in