Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Trichoderma afroharzianum* Causing Seed Rot on Maize in Italy

M. Sanna,[1] M. Pugliese,[1,2] M. L. Gullino,[1] and M. Mezzalama[1,2,†]

[1] AGROINNOVA – Centre of Competence for the Innovation in the Agro-environmental Sector, Università di Torino, 10095 Grugliasco (TO), Italy
[2] DISAFA - Dept Agricultural, Forestry and Food Sciences, Università di Torino, 10095 Grugliasco (TO), Italy

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Maize (*Zea mays* L.) is a cereal crop of great economic importance in Italy; its production is currently 60,602,320 t, covering 588,597 ha (ISTAT 2021). *Trichoderma* species are widespread filamentous fungi in soil and are well known and studied as biological control agents (Vinale et al. 2008). Seeds of a yellow grain hybrid (class FAO 700, 132 days) were collected in September 2020 from an experimental field in Carmagnola (TO, Italy; 44°53′11.0″ N 7°40′60.0″ E) and tested with the blotter test (Warham et al. 1996) to assess their phytosanitary condition. Among the 400 seeds tested, more than 50% showed rotting and the development of green mycelium typical of the genus *Trichoderma*. Because of the unexpectedly high percentage of decaying kernels, 10 colonies were identified by morphological and molecular methods. Single conidia colonies of one *Trichoderma* (T5.1) strain were cultured on potato dextrose agar (PDA) for pathogenicity tests and on PDA and synthetic nutrient-poor agar (SNA) for morphological and molecular identification. The colonies grown on PDA and SNA showed green, abundant, cottony, and radiating aerial mycelium and yellow pigmentation on the reverse. Colony radius after 72 h at 30°C was 60 to 65 mm on PDA and 50 to 55 mm on SNA. The isolates produced one-celled conidia 2.8 to 3.8 µm long and 2.1 to 3.6 µm wide (n = 50) on SNA. Conidiophores and phialides were lageniform to ampulliform and measured 4.5 to 9.7 µm long and 1.6 to 3.6 µm wide (n = 50); the base measured 1.5 to 2.9 µm wide, and the supporting cell measured 1.4 to 2.8 µm wide (n = 50). The identity of one single-conidia strain was confirmed by sequence comparison of the internal transcribed spacer (ITS), the translation elongation factor-1α (*tef-1α*), and RNA polymerase II subunit (*rpb2*) gene fragments (Oskiera et al. 2015). BLASTn searches of GenBank using ITS (OL691534), the partial *tef-1α* (OL743117), and *rpb2* (OL743116) sequences of the representative isolate T5.1 revealed a 100% identity for *rpb2* to *T. afroharzianum* TRS835 (KP009149) and 100% identity for *tef-1α* to *T. afroharzianum* Z19 (KR911897). Pathogenicity tests were carried out by suspending conidia from a 14-day-old culture on PDA in sterile H_2O to 1×10^6 CFU/ml. Twenty-five seeds were sown in pots filled with a steamed mix of white peat and perlite, 80:20 v/v, and maintained at 23°C under a seasonal day/night light cycle. Twenty primary ears were inoculated by injection into the silk channel with 1 ml of a conidial suspension of strain T5.1 7 days after silk channel emergence (BBCH 65) (Pfordt et al. 2020). Ears were removed 4 weeks postinoculation, and disease severity, reaching up to 75% of the kernels of the 20 cobs, was assessed visually according to the EPPO guidelines (EPPO 2015). Five control cobs inoculated with 1 ml of sterile distilled water were healthy. *T. afroharzianum* was reisolated from kernels showing a green mold developing on their surface and identified by resequencing of the *tef-1α* gene. *T. afroharzianum* has already been reported on maize in Germany and France as the causal agent of ear rot of maize (Pfordt et al. 2020). Although several species of *Trichoderma* are known to be beneficial microorganisms, our results support other findings of *Trichoderma* spp. causing ear rot on maize (Munkvold and White 2016). The potential production of mycotoxins and the losses that can be caused by the pathogen during postharvest need to be explored. To our knowledge, this is the first report of *T. afroharzianum* as a pathogen of maize in Italy.

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[†]Indicates the corresponding author.
M. Mezzalama; monica.mezzalama@unito.it