Morphological and Molecular Characterisation of European Species of the *Diaporthe/Phomopsis* Complex Associated with Soybean Seed Decay

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Abstract

The genus *Phomopsis* (teleomorph *Diaporthe*) comprises phytopathologically relevant fungi which cause diseases on a wide range of economically important crops including soybean. This group of pathogens has been reported to be involved in several soybean diseases, including *Phomopsis* seed decay (PSD) (*Phomopsis longicolla*), stem blight (*D. phaseolorum* var. *sojae*) and stem canker (*D. phaseolorum* var. *caulivora* and *D. phaseolorum* var. *meridionalis*), resulting in significant yield and quality losses. Accurate species identification of DPC is critical in understanding disease epidemiology and for developing effective control measures. Also, it has been documented that MAT primers are useful in mating-type diagnosis in a wide range of *Diaporthe* and *Phomopsis* species. In this study, we focused on morphological (colour and shape of colonies, existence of alpha, or beta conidia, or both, and their characteristics, production of perithecia, and size of conidia) and molecular analyses of species from DPC-damaged European soybean seeds obtained from several locations throughout Austria, France, and Germany. In addition, the European DPC isolates were classified according to their mating-type loci using Primers MAT1–1-1 FW/RV and MAT1–2-1 FW/RV. Surface sterilized soybean seeds were placed on APDA and incubated for 30 d at 24°C. Putative isolates of the DPC were purified using the single spore method. Genomic DNA was extracted from mycelium of each single-spore isolate. Thirty-two strains of *Diaporthe* and *Phomopsis* were isolated and phylogenetic relationships were determined using the translation elongation factor 1-alpha (TEF1) and nuclear ribosomal DNA internal transcribed spacers (ITS) sequences. By combining morphological and molecular data, four species including *Phomopsis longicolla*, *Phomopsis* sp., *Diaporthe caulivora* and *Diaporthe eres* could be distinguished on soybean seeds. Also, results from mating-type experiments revealed that MAT primers used in this study allowed mating-type diagnosis of the 28 isolates. Further studies for controlling these pathogens using biological control agents are currently in progress.

Keywords: *Diaporthe/Phomopsis* species complex, morphological and molecular identification, soybean seed decay

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