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Species of the *Diaporthe/Phomopsis* complex (DPC) in European soybean and establishment of quadruplex real-time PCR for diagnosis

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Abstract

Phomopsis seed decay is known as one of the most destructive soybean diseases, affecting seed quality and causing massive yield losses worldwide. The disease is caused primarily by *Diaporthe longicolla* along with other DPC species. Precise identification of the species of this complex is necessary for understanding the epidemiology of the disease and for optimal control. Based on the isolation of 32 DPC strains from DPC-damaged European soybean seeds we identified four species: *D. longicolla, D. caulivora, D. eres* and *D. novem*. These four species can be considered the principal DPC species on soybean in Central Europe. We now aim to develop a fast and accurate method to detect these pathogens via quadruplex Real-Time PCR. Based on sequences of translation elongation factor 1-alpha (*TEF1*), four specific TaqMan primer-probe sets were designed and tested for specificity and efficiency using DNA from pure cultures of these species and other important soybean pathogens from the genera *Sclerotinia, Colletotrichum, Fusarium, Uromyces*, and *Phakopsora*. Our primer-probe sets allow excellent discrimination of the different DPC species and can be used to detect and distinguish them in parallel using quadruplex real-time PCR. The quadruplex assay was tested on different plant material including healthy and infected soybean seeds or seed coats, soybean stems, and leaves. Moreover, the quadruplex real-time PCR was adapted to quantify these pathogens relative to the amount of plant material. Standard curves were created from serial dilutions of genomic DNA from each of the pathogens and from soybean plants. To quantify the amount of fungal DNA (ng) per plant DNA (ng) with the help of the standard curves, DNA samples from six soybean seed lots were tested in the quadruplex real-time PCR and SYBR Green-based Real-Time PCR assays. The results indicated that the amount of fungal biomass seems to be highly variable between individual seeds. We now want to deveolop the assay into a standard procedure for testing soybean seeds, plant material, and soil, and are planning comprehensive sampling to study the epidemiology of DPC species in Germany and testing different soybean cultivars for their resistance against the different DPC species.

Keywords: *Diaporthe* spp., European soybeans, quadruplex real-time (q)PCR

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