Molecular detection of *Stagonospora nodorum* and *Septoria tritici* – causal agents of septoria leaf spot diseases in wheat

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Abstract: The objective of this work was to use the polymerase chain reaction assays for detection of *Stagonospora nodorum* and *Septoria tritici* in artificially inoculated grain and leave samples of wheat. PCR analyses were done using primers derived from sequences of β-tubulin gene. In order to validate the results of PCR, using the ELISA provided a reliable detection of pathogen DNA in plants and seed tissues infected with these pathogens.

Key words: ELISA, PCR, plant pathogens, molecular detection, glume blotch.

Introduction

Septoria tritici Roberge in Desmaz. [teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn], the causal agent of *Septoria tritici* blotch, and *Stagonospora nodorum* (Berk.) E. Castellani and E. G. Germano syn. *Septoria nodorum* (Berk.) in Berk and Broome [teleomorph *Phaeosphaeria nodorum* (E. Muller) Hedjarounde, syn. *Leptosphaeria nodorum* E. Muller] (Cunfer, 1997), causing *Stagonospora nodorum* blotch, are widespread and economically important pathogens of wheat (Eyal et al., 1987; Meien-Vogeler et al., 1994; Lovell et al., 1997). Both pathogens can cause considerable reductions in yield and quality of grains (Halama, 1999) and both have been reported from all continents with the exception of some regions in South-west Asia (Eyal et al., 1987). The control of septoria diseases is primarily focused on foliar application of fungicides and growing of partially resistant cultivars. Effective chemical control is aimed to protect the upper three leaves, which provide most of grain-filling capacity (Royle et al., 1995). However, as soon as pycnidium formation is initiated in these leaves, none of the currently available fungicides is effective (Joshi & Sternberg, 1995).

Kendall et al. (1998) showed that accurate presymptomatic detection and quantification of *S. tritici* using the ELISA method could improve disease control. However, PCR seems to be a more sensitive method, since it allows detection of *S. tritici* earlier, in the latent phase of disease (Beck & Ligon, 1995; Fraaije et al., 1999). PCR-based assays are rapid with high specificity and sensitivity and have been used for detection and identification of several wheat pathogenic fungi, including *Blumeria graminis* f. sp. *tritici*, *Fusarium* spp., *Gaeumannomyces graminis*, *Microdochium nivale*, *Puccinia* spp., *Rhizoctonia cerealis*, *Tapesia* spp., and *Tilletia tritici* (McCartney et al., 2003). Furthermore, these assays have been applied to assess the genetic variation of pathogens (Park et al., 2000), fungicide resistance (Koenraadt & Jones, 1992), mating types (Foster et al., 1999), and host plant resistance (Willits & Sherwood, 1999).

The objective of this work was to apply the PCR assay for detection of *Stagonospora nodorum* and *Septoria tritici*-specific DNA sequences from solitary pathogens and from wheat leaves and seeds artificially infected by the pathogens.

Material and methods

The culture of *S. nodorum* was collected from primary leaves of wheat in Slovakia. The culture of *S. tritici* used in this study was provided by Prof. W. Bockus (Kansas State University, Manhattan, KS, USA). Both pathogens have been maintained on potato dextrose agar at 20 °C.

Seedlings (8 days old) of two wheat cultivars (Hana and Blava) were inoculated by spraying spores suspension (at concentration 1.5×10^6 per mL) of *S. nodorum* and *S. tritici*. Seedlings were placed in water-saturated trays, covered with polyethylene bags immediately after inoculation, and then grown in a controlled environment at 20 °C under continual illumination. Leaf samples (each weighing 1.5 g) were taken for testing 3, 6, and 11 days after inoculation. Seeds of wheat cultivar Brea were artificially inoculated by

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