Rapid detection and quantification of *Mycosphaerella graminicola* in wheat using PCR and RT-PCR assays

Abstract

Mycosphaerella graminicola is an economically important fungal disease in wheat. To develop a simple molecular diagnostic method which can qualitatively or quantitatively detect this pathogen in wheat, three PCR-based methods were tested. Three different methods were improved to simplify the process of DNA or RNA extraction. Altogether 13 *M. graminicola*-specific sequences were identified by searching in a public gene bank and by RAPD-PCR. The four highly specific and sensitive primer sets ST-rRNA F/R, ST-act F/R, R5870-1F/R5870-1R and R5870-1F/R5870-2R were developed based on these sequences. Their PCR conditions were optimized. Real-time PCR was successfully used to monitor the disease development in inoculated plants and naturally infected F, F1 and F2 leaves during the epidemic period using different primer sets. *M. graminicola* DNA could be quantitatively detected on the inoculation day, and a clear growth tendency were obtained during the disease development. The DNA amounts were significantly correlated with the disease index and the number of pycnidia. The efficacies of two fungicide treatments (Input and Fandango) on *M. graminicola* leaf blotch in different leaf positions were assessed using real-time PCR. Both treatments revealed remarkable efficacies of control against *M. graminicola* in most leaves. The Input treatment had a relatively better efficacy in F1 and F2 leaves than the Fandango treatment. RT-PCR assay was used to detect and identify *M. graminicola* in wheat with the three specific primer set E1/STSP2R, ST-rRNA F/R and ST-act F/R. The results by Northern hybridization and RT-PCR indicated that three target genes were basically stably expressed. Three primer sets had specificity and sensitivity high enough for the detection of *M. graminicola* in wheat plants. Two-step PCR was approximately 50 times more sensitive much more sensitive than one-step PCR. The disease development in inoculated wheat plants and in naturally infected F1 leaves during the epidemic period was monitored using RT-PCR with the primer sets E1/STSP2R.