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MULTIPLEX-TOUCHDOWN PCR FOR RAPID SIMULTANEOUS DETECTION OF *Rhizoctonia cerealis* AND *Rhizoctonia solani*

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[Abstract](#)

The aim of the study was to develop rapid and sensitive assay for the simultaneous detection of *R. cerealis* and *R. solani*. Pure cultures of fungi were grown on a potato dextrose agar for 5 days at 28 °C, and mycelium was harvested and used for DNA extraction. Total DNA was extracted using a commercial test-systems. Molecular identification of phytopathogenic fungi was performed using a multiplex-touchdown PCR with further electrophoretic separation of amplification products in agarose gel. The specific sequence characterized amplified region primers RtubR4/RtubF4 for *R. cerealis* and ITS1/GMRS–3 for *R. solani*

were tested for their specificity and useability in PCR multiplex capacity. The specificity of the multiplex-touchdown PCR was tested using DNA from wide range of fungal species and non-target DNA from healthy wheat. The used primer pairs provided only specific fragments for *R. cerealis*

and

R. solani

. No PCR products were obtained during amplification with the negative control or non-target DNA templates from other species. Coupled to this we have optimized the temperature regime for the multiplex PCR protocol. Taken together, our protocol convincingly demonstrated the simultaneous ability to detect

Rhizoctonia cerealis

and

Rhizoctonia solani

and can be used for the diagnosis of compound

Rhizoctonia
root rot.

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