Detection of *Tilletia controversa* and *Tilletia caries* in wheat by PCR method

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ABSTRACT

Tilletia controversa and *Tilletia caries* were specifically detected in wheat plants by PCR using primers TILf (5′-CAC AAG ACT ACG GAG GGG TG-3′) and TILr (5′-CTC CAA GCA ACC TTC TCT TTC-3′). DNAs from uninfected wheat, rye, barley and triticale were not amplified. Natural infection of control plants by other species of fungi as *Alternaria* spp., *Erysiphe graminis* and *Fusarium* spp. proved the specificity of the test because even in this case no unspecific products were formed. This method can be very useful both for seed producers and for state officers checking the seed quality.

Keywords: detection; primers; PCR; DNA; *Tilletia controversa*; *Tilletia caries*; wheat

Fungi from the genus *Tilletia* are basidiomycetous plant pathogens, belonging to the order *Ustilaginales.* In the Czech Republic there can occur three species of this genus, *Tilletia controversa* Kűhn, *Tilletia caries* (DC.) Tul. and *Tilletia foetida* (Wallr.) Liro.

The main symptom of infection of plants by these species is absence of grain underneath lemma. There is just mass of teliospores, karnal bunt. It imparts fishy odour and bad taste to flour.

Tilletia controversa is the most frequent species in the last years and also the most dangerous, especially in large cereal fields. Infestation is different in individual places in the field because of crop density. In open stand attack is lower than in the dense one, in which the growing point of plants is probably able to escape from the infecting hypha. That is why occurrence of the disease is higher in stand margins (Benada 1995). *Tilletia controversa* causes 30% loss of the yield, but in some cases this loss can reach 95% (Horák 2003). Plants of wheat and rye attacked by this species are smaller, lighter and have more shoots than healthy ones.

Tilletia caries was the most frequent in the past time, mainly in small fields. Farmers cultivated cereals without any precaution and control of cereal health (Benada 1997). This species attacks wheat, rye, barley and triticale. *Tilletia foetida* is rather rare in the Czech Republic.

Tilletia species are seed infectants mainly. Typical symptoms can be found on formed spike only. For seed producers, buyers and for foreign trade it can be important to identify *Tilletia* species on grain or juvenile plants. One smutty spike contains one hundred and fifty million spores. It is enough for contamination of three million seeds. Presence of *Tilletia* species can be a complication for certification of seeds. In the Czech Republic *Tilletia controversa* may not be found in reproducing material (Horák 2003).

Molecular methods based on DNA analysis have provided very useful information for species identification of these important plant pathogens (Martin and Kistler 1990, Correl et al. 1993). Polymerase chain reaction (PCR) is a powerful tool that has made a significant contribution to plant disease diagnosis (Ferreira et al. 1996). PCR methods can be used to detect and identify pathogens, because of their high sensitivity and accuracy. They are particularly useful for the detection and identification of regulated seed-borne pathogens (Goodwin and Annis 1991, McManus and Jones 1995, Shaad et al. 1995).

In this paper we describe using of PCR for specific detection of *Tilletia controversa* and *Tilletia caries* in plant material.

MATERIAL AND METHODS

Plant material was obtained from the Agricultural Research Institute, Kroměříž. Material included two samples of winter wheat (*Triticum aestivum*). The first one was from Kroměříž, field U malého větrolamu and was infected by *Tilletia caries*. The second one originated from trial field artificially

Supported by the Ministry of Agriculture of the Czech Republic, Grant QC 1264.

contaminated by soil from Vsetín, field Za ohrádkou, where *Tilletia controversa* occurs. As negative controls for our testing we used plants of wheat, rye, barley and triticale from the demonstration field of the Czech University of Agriculture in Prague-Suchdol. All these samples were naturally infected by *Alternaria* spp., *Erysiphe graminis* and *Fusarium* spp.

For DNA extraction a whole frozen plant was homogenized in a commercial blender with cetyltrimethylamonium bromide (CTAB) buffer and incubated in water bath in 60°C. Then homogenate was vortexed with chloroform-isoamylalcohol (24:1) and centrifuged. Upper phase was taken away, isopropanol was added and DNA was precipitated in liquid nitrogen. After this step it was centrifuged, isopropanol was decanted and pellet washed in 80% ethanol, 10mM LiCl, 1mM Tris. This solution was centrifuged, decanted again and pellet dried under vacuum. Then DNA pellet was resuspended in ddH_2O.

Primers TILf (5′-CAC AAG ACT ACG GAG GGG TG-3′) and TILr (5′-CTC CAA GCA ACC TTC TCT TTC-3′) were designed on the basis of known *Tilletia* ITS I DNA sequence from NCBI database (Zhang et al. 2001) using program Primer Design. Expected length of PCR product with these primers is 361 bp.

PCR solution contained 2.5 µl of buffer for Dynazyme II, 0.25 µl dNTP (0.4mM of each nucleotide), 0.4 µl primer mix (0.1mM), 0.5 µl Dynazyme II (1.5 U, Finnzymes), 1 µl of extracted DNA, 20,35 µl ddH_2O (Zouhar et al. 2002). Amplifications were performed in a MJ Research PTC-200 thermocycler. Samples were firstly heated at 95°C for five minutes, followed by fifty cycles of denaturation at 94°C for one minute, annealing at 58°C for one minute and extension at 72°C for one minute, and a final extension at 72°C for four minutes.

Amplified PCR products were examined by electrophoresis in agarose gel containing ethidium bromide and visualized using UV transilluminator.

Our experiment was repeated many times with the same results.

RESULTS AND DISCUSSION

Amplification of DNA extracted from plants infected by both *T. controversa* and *T. caries* using primers TILf and TILr gave product of expected length 361 bp, whereas no product was obtained from amplification of negative controls – wheat, rye, barley, and triticale (Figure 1). As these controls originated from field conditions, they were naturally infected by *Alternaria* spp., *Erysiphe graminis* and *Fusarium* spp.. In spite of the presence of these contaminating species there were no unspecific bands in our gels. It means that our primers are quite specific for *Tilletia* species and amplify neither DNA from healthy plants of different species nor DNA from other fungal species. So, they are more useful than primers ITS1 and ITS4 of Pimentel et al. (1996), which produce two fragments – 550 bp and 750 bp long. The former is specific for *Tilletia* species, but the latter is amplified wheat DNA. It can mean problems with

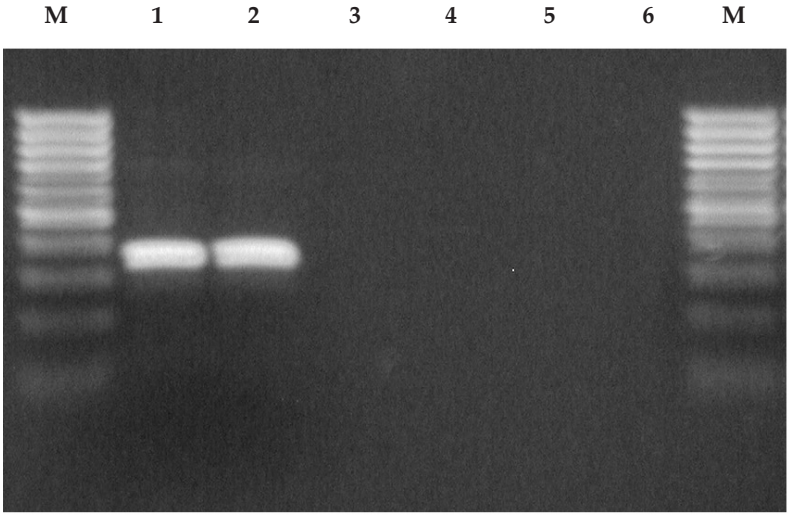

Figure 1. Electrophoresis of PCR products; amplified fragment is 361 bp long; number one is *Tilletia caries*, number two is *Tilletia controversa*; the other numbers are cereals not infected by *Tilletia* species – wheat, triticale, barley and rye; the absence of bands means specifity of primers to smut and not to plant

practical diagnosis. Unfortunately, we were not able to include *T. indica* and *T. foetida* into our tests, but from the practical point of view for the conditions of the Czech Republic it is not so important, because presence of *T. indica* is highly improbable and *T. foetida* occurs only exceptionally. We plan to test both these species in the future together with broader spectrum of various contaminating species of fungi to prove even better specificity of our method. Nevertheless, for the moment, it is already practically usable for the fast and easy detection of most frequent and dangerous *Tilletia* species in wheat plants from the field. It can be very useful both for seed producers and for state officers checking the seed quality.

Acknowledgement

We would like to thank Ing. Marie Váňová, CSc., from Agricultural Research Institute, Kroměříž, for plant material.

REFERENCES

Benada J. (1995): Odrůdová citlivost pšenice vůči sněti mazlavé a ječmene vůči pruhovitosti a prašné sněti. Rostl. Výr., *41*: 185–188.

Benada J. (1997): Příčiny rozšíření sněti zakrslé v České republice. Obiln. Listy, *6*: 111–112.

Correl J.C., Rhoads D.D., Guerber J.C. (1993): Examination of mitochondrial DNA restriction fragment length polymorphisms, DNA fingerprints and randomly amplified polymorphic DNA of *Coleotrichum orbiculare*. Phytopathology, *83*: 1199–1204.

Ferreira M.A.S.V., Tooley P.W., Hatziloukas E., Castro C., Schaad N.W. (1996): Isolation of a species-specific mitochondrial DNA sequence for identification of *Tilletia indica*, the karnal bunt of wheat fungus. App. Environ. Microbiol., *62*: 87–93.

Goodwin P.H., Annis S.L. (1991): Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by random amplified polymorphic DNA assay. Appl. Environ. Mycol., *57*: 2482–2486.

Horák A. (2003): Možnosti moření osiv obilovin a řepky. Úroda, (1): 14–15.

Martin F.N., Kistler H.C. (1990): Species-specific banding patterns of restriction endonuclease-digested mitochondrial DNA from the genus *Pythium*. Exp. Mycol., *14*: 32–46.

McManus P.C., Jones A.L. (1995): Detection of *Erwinia amylovora* by nested PCR and PCR-dot-blot and reverse-blot hybridisation. Phytopatology, *85*: 618–623.

Pimentel G., Carris L.M., Levy L., Meyer R.M. (1996): Genetic variability among isolates of *Tilletia barclayana*, *T. indica* and allied species. Mycologia, *90*: 1017–1027.

Shaad N.W., Cheong S.S., Tamaki S., Hatziloukas E., Panopoulos N.J. (1995): A combined biological and enzymatic amplification technique to detect *Pseudomonas syringae*. Phytopathology, *85*: 243–248.

Zhang G.M., Yao Y.J., Cheng Y.H. (2001): Phylogenetic analysis of nucleotide sequences from the ITS region of fourteen species of *Tilletia*. www.ncbi.nlm.nih.gov.

Zouhar M., Ryšánek P., Tesařová B., Marek M. (2002): Metodická příručka pro diagnostiku karanténních háďátek rodů *Globodera*, *Meloidogyne* a *Ditylenchus*. ČZU, Praha.

Received on September 25, 2003

ABSTRAKT

Detekce *Tilletia controversa* a *Tilletia caries* v pšenici pomocí PCR

Tilletia controversa and *Tilletia caries* byly specificky detekovány v celých rostlinách pšenice pomocí PCR s primery TILf (5′-CAC AAG ACT ACG GAG GGG TG-3′) a TILr (5′-CTC CAA GCA ACC TTC TCT TTC-3′). DNA z neinfikovaných rostlin pšenice, žita, ječmene a tritikale nebyla amplifikována. Přirozená infekce rostlin houbami *Alternaria* spp., *Erysiphe graminis* and *Fusarium* spp. prokázala specifitu testu pro houby z rodu *Tilletia*, neboť ani v tomto případě nevznikal žádný nespecifický produkt. Tato metoda může být velmi užitečná pro producenty osiva a pracovníky ÚKZÚZ pro kontrolu kvality osiva.

Klíčová slova: detekce; primery; PCR; DNA; *Tilletia controversa*; *Tilletia caries*; pšenice

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