Early Detection of Common Bunt in *Triticeae* by PCR Method

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Abstract: Polymerase Chain Reaction (PCR) using primers TILf (5′-CAC AAG ACT ACG GAG GGG TG-3′) and TILr (5′-CTC CAA GCA ACC TTC TCT TTC-3′) was used to detect infection of members of the *Triticeae* by common bunt (*Tilletia caries* and *T. foetida*). Infected plants yielded a product of expected length 361 bp with these primers. Uninfected control plants failed to produce such a product. The efficacy of the PCR test was confirmed by examining stained tissue with a light microscope. These results show that it is possible to use PLC for detection of mycelium also in other species of *Triticeae* than common wheat.

Keywords: PCR detection; *Tilletia*; common bunt; resistance; *Triticeae*

In the Czech Republic there are three species of *Tilletia*, *T. caries* (DC.) Tul., *T. foetida* (Wallr.) Liro and *T. controversa* Kühn. Resistance to the three species is controlled by the same resistance genes (Hoffmann & Metzger 1976; Metzger & Hoffmann 1978). Cultivars with tolerance against *T. caries* race have tolerance for *T. foetida* race that has the same virulence genes. Breeders looking for tolerance against *T. controversa* use *T. caries* or *T. foetida* for preliminary testing. It is easier to work with these two species because the techniques for inoculation and experimentation are simpler.

Because resistant genes can be repressed by virulent strains or new virulence combinations (Holton & Vogel 1952), it is necessary to keep looking for new sources of resistance that can be integrated into existing cultivars (Goates 1996). We can obtaine hybrids with a new gene virulence combination by crossing *Tilletia caries* and *T. foetida*. These hybrids can be useful for recognizing of resistance genes (Goates 1996).

MATERIAL AND METHODS

Plant material was obtained from Gene Bank of Research Institute of Crop Production Prague-Ruzyne and included 16 wild members of the *Triticeae* and spring wheats Aranka and Saxana. Plants were infected with common bunt, *Tillletia caries* and *T. foetida*, by shaking seeds in seed-coat with teliospores for one minute. The seeds were vernalised at 4°C for six weeks then kept outdoors for four weeks. As controls of primer specificity we used healthy plants of spring wheat Aranka and *Aegi-lops cylindrica* that were cultivated in the separate flowerpots and not infected with bunt. They were compared with mycelial culture..

For DNA extraction apical meristem of one shoot was homogenized in a commercial blender with cetyltrimethylamonium bromide (CTAB) buffer and incubated in a water bath at 60°C. The homogenate was then vortexed with chloroform-isoamylalcohol (24:1) and centrifuged. The upper phase was removed, isopropanol was added, and DNA was precipitated in liquid nitrogen. After this step it was centrifuged, isopropanol was decanted, and the 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 ddH2O.

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* ribosomal DNA sequence from NCBI database (Zhang *et al*. 2001). The expected length of the PCR product with these primers is 361 bp. The 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 (11 pmol), 0.5 µl Dynazyme II (1.5 U, Finnzymes), 1 µl of extracted DNA, 20.35 µl ddH_2O. Amplifications were performed in a MJ Research PTC-200 thermocycler. Samples were first heated at 95°C for five minutes, followed by thirty five 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.

The second shoot of the same plant was used for tissue staining. Plant tissue was cleared by boiling in 30% KOH for five minutes and stained in 1% trypan blue for 10 minutes then examined by light miscroscopy.

RESULTS AND DISCUSSION

Testing of specificity of primers TILf and TILr for amplification of common bunt DNA proceeded the tests of presence of mycelia in the plant. While the products of 361 bp appeared in the DNA samples isolated from mycelial cultures of *T. caries* and *T. foetida*, no product occurred in DNA samples of uninoculated wheat and *Ae. cylindrica*.

Testing of mycelium presence in plant by PCR was carried out on DNA extracted from apical meristem. PCR amplification with the primers TILf and TILr resulted in a 361 bp product in 5 samples: 2 accessions of *Triticum boeticum*, 1 accession of *T. araraticum*, 1 accession of *Heteranthelium piliferum* and one sample of spring wheat Aranka. The PCR approach did not prove the presence of common bunt in 12 of the tested *Triticeae* accessions and in spring wheat Saxana.

In one of the accessions of *T. boeticum*, in *T. araraticum* and in *Heteranthelium piliferum* staining as well as PCR analysis showed the presence of mycelium.

Moreover, staining detected presence of hyphae also in other accessions: *Aegilops speltoides*, *Ae. cylindrica*, *Ae. comosa* var. *heldreichii*, *Ae. umbellulata*, *Ae. neglecta*, *Ae. geniculata*, *T. boeoticum*, *T. monococcum* var. *clusianum*, *T. urartu*, *T. dicoccoides*. *Dasypyrum villosum*, *Eremopyrum boenaepartis*, Saxana. Neither of the methods revealed the mycelium in the sample of *T. triuncialis*.

Our experiments proved that PCR used for the detection of bunt mycelium in common wheat can be applied also in at least some genera/species of *Triticeae*. Further experiments are necessary to prove reliability of the used method before application for bunt resistance tests of wheat can be considered.

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