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MOLECULAR APPROACHES FOR THE IDENTIFICATION AND DIFFERENTIATION OF *TILLETIA* SPECIES CAUSING WHEAT BUNT

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Introduction

Wheat bunt is an important fungal disease affecting wheat production and grain quality, with several *Tilletia* species being associated with bunt symptoms. Morphological examination of infected grains and spikes can provide useful preliminary information, but it is not always sufficient for reliable species-level discrimination. Molecular methods, particularly PCR-based assays using species-specific or species-associated primers, provide an efficient approach for the detection and differentiation of *Tilletia* spp. The aim of this study was to investigate the presence of *Tilletia* species responsible for wheat bunt and to evaluate molecular markers for their identification and differentiation.

Materials and methods

Wheat spikes showing macroscopic symptoms of bunt were provided by the National Agricultural Research and Development Institute Fundulea (INCDA Fundulea). Teliospores were manually collected from infected grains, surface-disinfected with 5% sodium hypochlorite for 25 minutes and repeatedly rinsed with sterile distilled water. Genomic DNA was extracted from teliospores using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research) following the manufacturer's protocol. Three primer pairs described in the literature were evaluated: Tcar-F/Tcar-R for *T. caries* (expected product 276 bp) as literature indicates (Eibel et al., 2005); TIL-F/TIL-R for *T. caries*/*T. controversa* (361 bp) as described by Kochanová et al., (2004); L60-F/L60-R for *T. laevis* (660 bp) mentioned by Ren et al., (2021). Primer sequences were additionally checked in silico using Primer3Plus, OligoAnalyzer and sequence alignment tools. PCR amplification was performed using DreamTaq DNA Polymerase (Thermo Fisher Scientific). PCR products were separated by electrophoresis on 1.2% agarose gels, stained with ethidium bromide and visualized under UV light using a 1 kb DNA ladder for fragment-size estimation.

Results and conclusions

Results confirmed the expected amplification regions for all three primer systems. The Tcar primers generated a predicted fragment of approximately 276bp confirming *T. caries* presence in the analysed samples. The TIL-F/TIL-R primer set generated a 361 bp amplification product, unveiling a higher electrophoretic band intensity compared with the products generated by the Tcar-F/Tcar-R and L60-F/L60-R primers. However, this result does not exclude *T. controversa* presence from the samples since TIL primers can amplify *T. caries* and/or *T. controversa*. Therefore, the strong 361 bp band cannot by itself be used to say that *T. caries* is quantitatively dominant over *T. laevis* or that *T. controversa* is absent. Using the L60-F/L60-R primers a 660 bp amplification product was obtained confirming *T. laevis* presence in the bunt infected wheat.

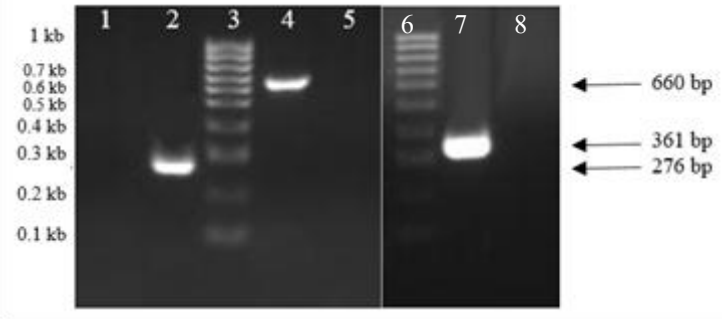


Figure 1 - Electrophoretic profile of the species-specific PCR amplification using the Tcar-F/Tcar-R, and L60-F/L60-R primer sets for *T. caries* (276 bp), *T. laevis* (660 bp) differentiation; TIL-F/TIL-R primer set (361 bp).

Legend: 1, 5, 8 - Negative control (no-DNA), 2 - *T. caries* specific amplification product, 3, 6 - Ladder 1 kb, 4 - *T. laevis* specific amplification product, 7 - *T. caries* and/or *T. controversa* specific amplification

The results indicate that studied wheat samples are infected by a mixt population of *T. caries* and *T. laevis* responsible for common bunt. The integration of bunt-specific molecular markers allowed the detection of the two mentioned *Tilletia* species. However, studies should be extended on finding also *T. controversa* specific markers, for better differentiation of stunt bunt from common bunt pathogens at teliospore stage.

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