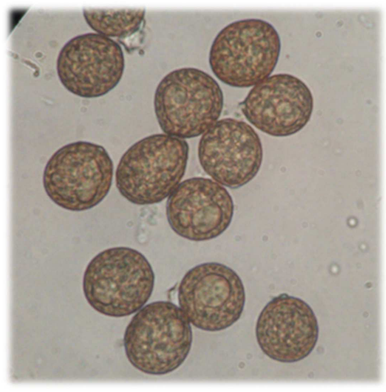
Early detection and quantification of *Tilletia* spp. infections in wheat



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INTRODUCTION

Wheat bunt is caused by several species of *Tilletia.* The pathogen is an obligate parasite organism, which restricts studies to *in vivo* experiments that must be conducted on plants. Moreover, the typical symptoms of infection become visible only at advanced stages of crop development, very close to the harvest time, which complicates the evaluation of treatment efficacy. This aspects prolong the time required to identify effective preventive measures. The development and validation of rapid molecular methods for the early detection of *Tilletia* spp. infections in young wheat plants would considerably reduce the time needed to assess the efficacy of protective measures.

AIM OF THE STUDY

The aim of the study was to develop and validate a laboratory testing system for the early identification and quantification of *Tilletia* spp. infections in young wheat seedlings.

EXPERIMENTAL DESIGN

The bunt-susceptible wheat cultivar Capo was used in this study. Surface disinfected kernels were experimentally inoculated with bunt spores and incubated under laboratory conditions in two cultivation systems. After seven weeks, fungal DNA was extracted from surface-sterilized young plants. The extracted material was quantified and subsequently employed in conventional PCR reactions using primers specific to the genus *Tilletia* and to the species *T. caries* and *T. laevis*. For early quantification of bunt infection in wheat, Real-Time PCR was additionally applied, employing species-specific primers for *T. caries*.

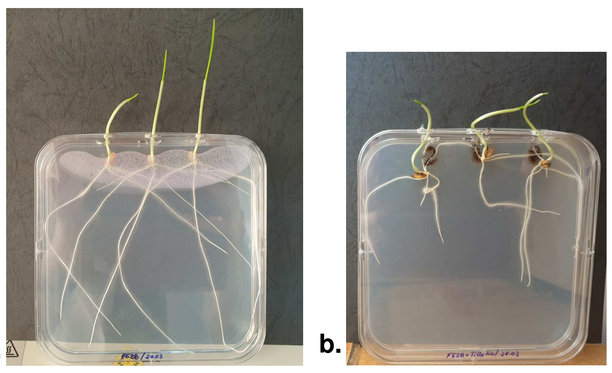

Figure 1. Trials in controlled conditions
a. Healthy plants, **b.** *Tilletia* artificial infection

Figure 2. Wheat plants for molecular analysis - healthy (right) and artificially infected (left)

RESULTS AND DISCUSSIONS

Among the two wheat cultivation methods, tested under laboratory conditions, only one enabled the establishment of bunt infection. This method involved maintaining the culture vessels for four weeks at 6 °C, followed by an additional three weeks under diurnal conditions, with 20 °C during the day and 15 °C at night, thereby allowing infection to develop under controlled laboratory conditions.

The presence of infection was validated through molecular biology analyses. Using genus-specific primers, amplicons of 361 bp characteristic of Tilletia spp. were obtained. When species-specific primers were applied for the detection of T. caries and T. laevis, amplification products of 276 bp and 660 bp, respectively, were generated.

Conventional PCR was thus validated as a method for the early identification of wheat bunt in plants at the early growth stage (BBCH 12). The protocol was further validated for T. caries through the application of Real-Time PCR for semi-quantitative determination, employing the SYBR Green method.

Perspectives

Early detection of *Tilletia* infections in various trials designed to select efficient integrated pest management methods.

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MATERIAL AND METHODS

Mixed local populations of *Tilletia.* Bunt teliospores collected from naturally infected wheat plants were used.

Bunt-susceptible wheat cultivar Capo was used in two cultivation systems

DNA extraction and purification of *Tilletia* teliospores were performed with the ZR Fungal/Bacterial MiniPrep™ (Zymo Research, SUA).

PCR-detection of *Tilletia caries, T. controversa* and *T. laevis.*

PRC mix		PCR conditions		Primers	Annealing	Amplicon	Target	Refernces
Dream Taq Buffer	1 X	94°C, 5 min.	1 cycle	TILf	58°C	361 bp	*T.caries& T.controversa* detection	Kochanová et al., 2004
dNTPs	0.2 mM	94°C, 30 sec.	35 cycles	TILr				
Each primer	0.5 µM	57÷60°C, 45 sec.		Tcar2A	60°C	276 bp	*T.caries* detection	Eibel et al., 2005
DNA polymerase	0.2 U	72°C, 45 sec.		Tcar2B				
DNA template	0.5 µL	72°C, 7 min	1 cycle	L60F	57°C	660 bp	*T.laevis* detection	Ren et al., 2021
Reaction volume	25 µL	8°C	∞	L60R				

Real-time PCR-detection of *Tilletia caries* using SYBR green method.

RESULTS AND DISCUSSIONS

PCR detection of wheat infectious *Tilletia* species

Results have shown that the bunt infection were generated by mixt populations of *Tilletia* species (figure 3 and 4).

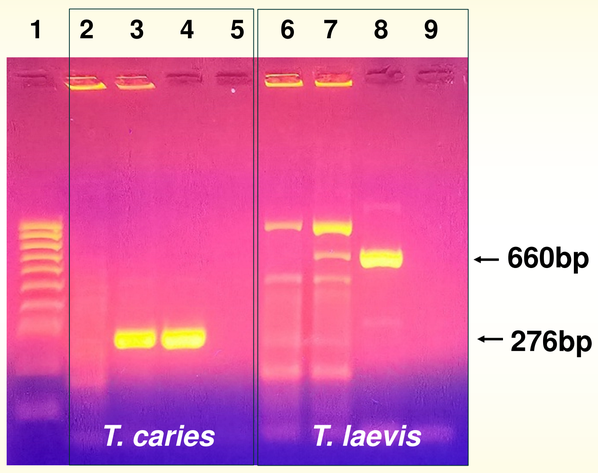
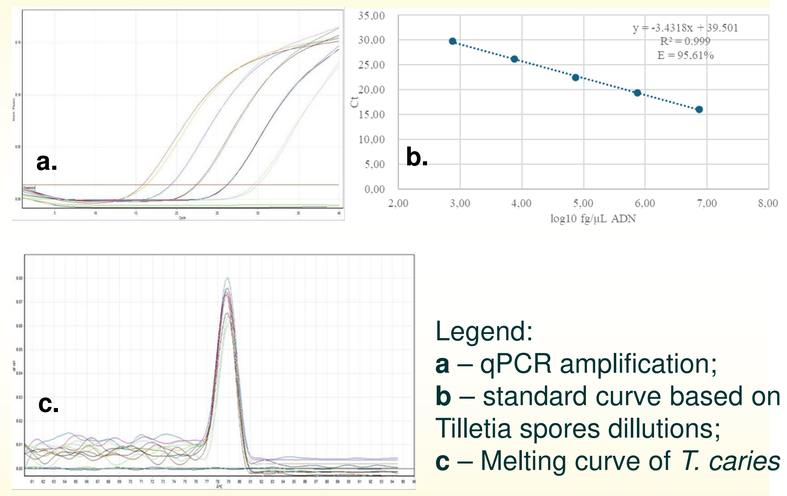


Figure 3. PCR-detection of bunt infected plants.
Legend: 1 – 100 bp DNA ladder; 2,6 – Healthy Capo wheat; 3,7 – Capo wheat, experimentally infected with bunt; 4,8 – natural population of *Tilletia* spp.; 5,9 – negative PCR control without DNA.



Legend:
a – qPCR amplification;
b – standard curve based on Tilletia spores dillutions;
c – Melting curve of *T. caries*

Figure 4. Real-Time semi-quantitative *PCR* for bunt detection.

CONCLUSION

The results indicate that **wheat infection by Tilletia spp. is dependent on low temperature.** Under laboratory conditions, experimental bunt infections were successfully simulated by maintaining cultures for **one month at 6 °C**, prior to cultivation under a diurnal regime of **20 °C during the day and 15 °C at night**, which enabled the establishment of infection.

The integration of **bunt-specific molecular markers** allowed the detection of infected plants through **conventional PCR** and **semi-quantitative PCR** techniques, even at early developmental stages. The proposed protocol significantly reduces the time required for validating plant protection strategies.

References:

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