RAPID MOLECULAR ASSAY FOR THE EVALUATION OF SEED TREATMENTS WITH CLOVE ESSENTIAL OIL AGAINST WHEAT COMMON BUNT

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Introduction and aim of the work

Common bunt, caused by the fungus *Tilletia laevis*, is an important disease of wheat, causing considerable yield loss and reduction in seed quality. The pathogen produces powdery dark teliospore in the kernels, usually called "bunt balls" (Figure 1). Infected and ripen kernels will release the teliospores in the soil, representing the source of inoculum for the following sowing season. Symptoms of infection become evident only at the final stages of plant growth, after the formation of the spike. The spread of organic and low-input farming led to the increase of the disease incidence and to a growing search for bio-compounds replacing chemical treatments for pest management. The aim of this work consists in the development of a quantitative, early, rapid and low-cost method based on the molecular detection of *T. laevis* in the wheat plant which allows to quantitatively evaluate the effectiveness of different seed treatments with clove oil (*Eugenia caryophyllata* Thunb).

Figure 1 Comparison of healthy (left) and bunted (right) wheat grain, and enlarged image of a "bunt ball" (below)

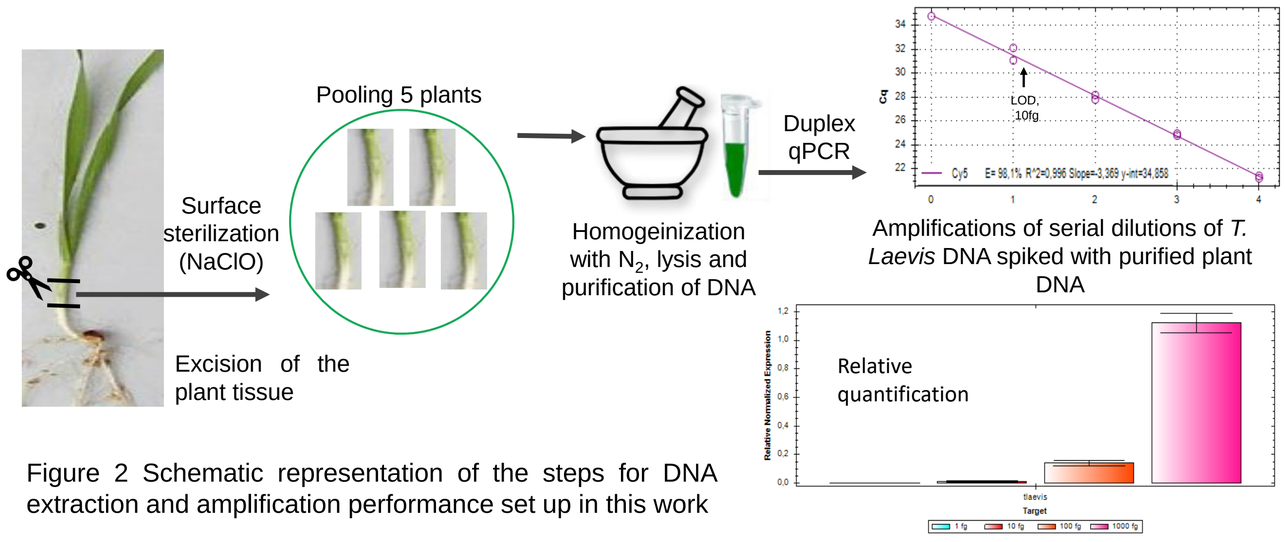

Figure 2 Schematic representation of the steps for DNA extraction and amplification performance set up in this work

Table 1 Seed treatment scheme. The products dilutions were performed in water.

	1	2	3	4	5	6	7	8
Product	*Bioxeda A (2.5% v/v)*	*Bioxeda B (2.5% v/v)*	*Bioxeda A (5% v/v)*	*Bioxeda B (5% v/v)*	*Clove oil (0.5% v/v)+pinolene (0.05% v/v)*	*Clove oil (1% v/v)+pinolene (0.05% v/v)*	*Water*	*Copper sulphate (Cutril)*
Treatment	*Seed immersion*	*Seed immersion*	*Spray application*	*Spray application*	*Seed immersion*	*Spray application*	*Seed immersion*	*Seed immersion*

Materials and methods

The trial was set up with 6 different treatments (table 1), performed either by seed immersion or spray application of clove oil and two commercial products, BIOXEDA A (microencapsulated) and BIOXEDA B (nanoencapsulated) from Xeda Italia S.r.l, containing 20% (w/w) of clove oil, plus two controls (infected/untreated and infected/copper-treated). For each treatment 90 seeds of durum wheat (cultivar Grifoni) naturally infected were sown in 3 repetitions, for a total of 30 seeds/ pot. The germination rate was evaluated after 14 days. The seedlings were sampled just before the tillering stage, the DNA was purified and analyzed by a duplex qPCR (Figure 2) targeting a reference gene (18S rDNA Universal) and the *T. laevis* specific ITS region, in order to normalize the quantity and quality of each DNA sample and to obtain a relative quantification of the fungal biomass in the sample. The results obtained from qPCRs were analysed by ANOVA and with the Tukey HSD multiple comparison test (p = 0.05).

Results and Discussion

The duplex qPCR showed an optimal efficiency, a good coefficient of determination (R^2>0.99) and a high sensitivity (10 fg). The correlation between Ct and infected plants frequency was verified analyzing plants bulks with a known number of infected plants (Figure 3). The results were statistically analysed (ANOVA) and showed that immersion and spray treatments with both the experimental formulations significantly reduced the percentage of plant infection compared to the untreated and water control (Figure 4). The immersion treatments with clove oil at a concentration of 0.5% reduced the infection but showed phytotoxicity. A comparable result was obtained with the use of the BIOXEDA A and BIOXEDA B formulation as seed immersion and BIOXEDA A as spray treatment. On the contrary, the treatment with clove oil at a concentration of 1% and the treatment with the BIOXEDA B formulation, both sprays, did not affect the presence of the infection, showing data comparable to those of the untreated control. Given the promising results further tests are ongoing to confirm the data obtained.

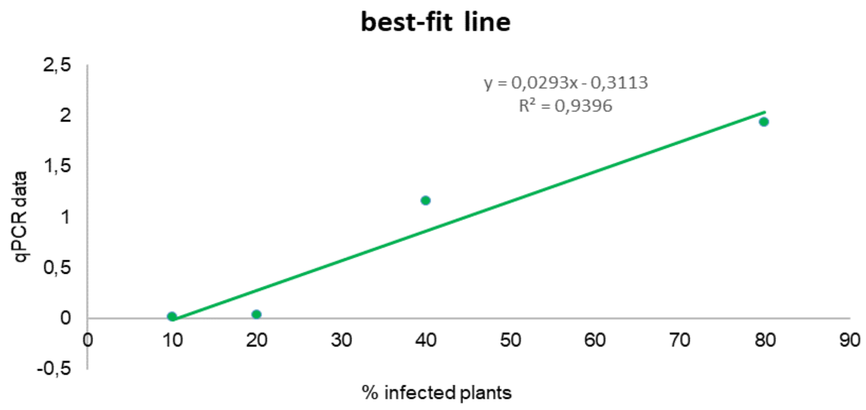

Figure 3 Positive linear correlation between qPCR detection of *T. laevis* and percentage of infected plants. The coefficient of determination (R^2) corresponds to the best fitted line

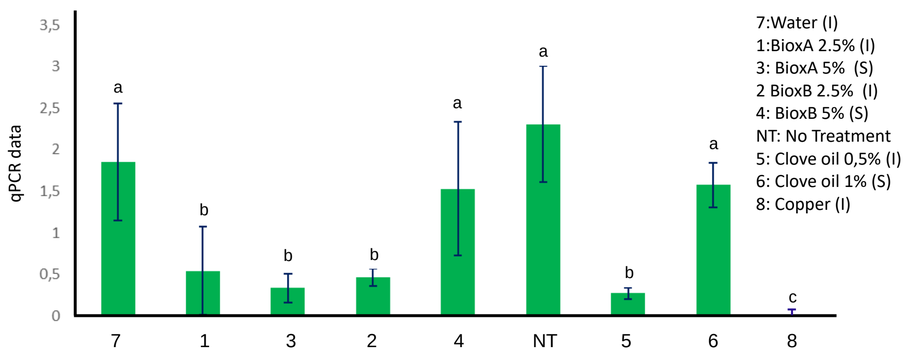

Figure 4 ANOVA among the different treatments. The mean values of the columns marked with different letters are significantly different; (I)=Immersion; (S)=Spray

Aknowledgements: the present study was carried out within the project CERESBIO (DIBIO) founded by MIPAAF