Thermoanalytical study of barley seeds infected with 

*Pyrenophora graminea*

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**ABSTRACT**

Leaf stripe caused by *Pyrenophora graminea* has been the major yield-reducing factor for barley production during the last decade. In this work, the changes of heat patterns in the infected barley seeds with *P. graminea* were studied by thermogravimetry (TG) and differential scanning calorimetry (DSC) techniques. The TG curves of the infected seeds show mass losses in three steps respectively which corresponded to the thermal events. DSC technique demonstrated the starch gelatinization process and determined the enthalpy in infected and non-infected seeds, which should be observed carefully. The TG and DSC curves were highly repeatable. Hence, the thermal approach presented here constitutes no prior assumption about the thermal decomposition changes in barley seeds infected with *P. graminea*.

**Keywords**: *Pyrenophora graminea*, barley, thermal analysis, DSC, TG.

**INTRODUCTION**

*Pyrenophora graminea* Ito & Kuribayashi [anamorph *Drechslera graminea* (Rabenh. ex. Schlech.]) is a seed-borne pathogen. It is the causal agent of leaf stripe in barley (*Hordeum vulgare* L.), which often leads to yield reductions (Porta-Puglia *et al.*, 1986; Arabi *et al.*, 2004). The fungus survives on kernels as a mycelium between the parenchymatival cells of the pericarp, in the hull, and the seed coat, but not in the embryo (Arru *et al.*, 2002). Fungal hyphae grow intercellularly from the coleorhiza up all sides to the roots and scutellar node where they start infecting the shoot (Haegi *et al.*, 1998). In susceptible plants, the disease usually results in severe stunting, premature and complete loss of grain (Tekauz and Chiko, 1980).

*P. garminae* could modify barley starchy endosperm cell walls after infection process (Noots *et al.*, 2001). However, starch molecules in the raw barley are large and reside in tightly structured units called starch granules. Brewing articles and texts often refer to the gelatinization of starch. This is the point at which a starch granule looses its ordered structure due to heat, making it accessible for enzymatic conversion. (Qiao *et al.*, 2005). The gelatinization process, which is a function of the starch: water ratio, can be studied by differential scanning calorimetry (DSC). Thermogravimetry (TG) can be also helpful to show the behavior of starch granules when heating leads to depolymerization (Bicudo *et al.*, 2009).

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The changes of heat patterns in the infected barley seeds with *P. graminea* have not been studied. This study was conducted on an universal susceptible genotype WI2291 infected with the virulent isolate PgSy3 using TG and DSC techniques.

**MATERIALS AND METHODS**

**Isolation of fungus**

Barley leaf tissue naturally infected with *P. graminea* was randomly sampled during 1998 and 2003. In preliminary studies (Arabi et al., 2005), PgSy3 isolate was selected based on morphological and physiological criteria (virulence) and consequently chosen for this study. Fungal isolate was cultured on potato dextrose agar (PDA, DIFCO, Detroit, MI USA) and incubated for 10 days, at 22 ±1°C in the dark to allow mycelial growth and sporulation.

**Plant material**

During the period 1996-2006 the Australian genotype WI 2291 was proved to be the most susceptible one to leaf stripe disease in the greenhouse, the laboratory and later in the fields (Arabi et al., 2001; 2006). This genotype was used for studying the thermal changes in this research. Inoculation tests of PgSy3 isolate was performed using the method described by Hammouda (1986). Inoculated and un-inoculated seeds were planted under field conditions. Healthy and diseased plants were recorded at heading stage. No plants showing leaf stripe symptoms were found among the un-inoculated controls. Before seed analysis, and to be certain whether harvested seeds have been originally infected with *P. graminea*, random seeds from the same infected plant used for TG and DSC analysis were surface sterilized as mentioned above, plated on PDA dishes and incubated for 72h at 23±1°C in the dark. The Petri dishes were then incubated for additional 4 days in a cycle of 12 h darkness and 12 h under light. After incubation, the seeds were examined under microscope for the presence of *P. graminea*; seed examination confirmed the presence of this pathogen on chosen samples.

**TG and DSC analysis**

TG curves were recorded using a simultaneous TG 60 system (Shimadzu) under a 100 mL min-1 air flow with a heating rate of 10 °C min-1. The initial sample mass was about 9 mg. Alumina crucibles were used for the TG experiments. DSC curves were recorded using a DSC 60 (Shimadzu) under an air flow of 100 mL min-1, heating rate of 5 °C min-1. Sealed aluminum crucibles were used in order to study the gelatinization process with a 5:1 (water:starch w/w) ratio prepared directly by weighting 2.0 mg of each starch and 10 mL of water was added; the aluminum crucible was sealed and after one hour a new DSC curve was realized.

**RESULTS AND DISCUSSION**

The simultaneous TG and DSC curves of the infected and non-infected barley seeds are shown in Figures 1 and 2. The TG curves (Fig 1a, b), show mass losses in the infected seeds in three steps respectively and thermal events corresponding to these losses. The first mass loss between 20 –135 °C which is attributed to the dehydration, that occurs in a single step. The second mass loss, between 200–485 °C corresponding to the change in thermal peak is ascribed to the oxidation of the organic matter. The last step, between 510 – 580 °C, the mass loss occurs through a slow process corresponding to the small and broad heat changing between 370 and 450 °C and a sharp change in peak at 510 °C is attributed to the total oxidation of the organic matter. These three steps were very obvious in the infected seeds as compared with the controls.

DSC curves of the indium, and an empty aluminum crucible was used as reference. The characteristics of the transitions, including onset temperature (Tₒ), peak temperature (Tₚ),
Table 1. Experimental values obtained of gelatinization enthalpy ($\Delta H_{gel}$), onset temperature ($T_o$), peak temperature ($T_p$) and initial mass ($m_i$) for studies barley seeds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Delta H_{gel}$</th>
<th>$T_p$ (°C)</th>
<th>$T_o$ (°C)</th>
<th>$m_i$ (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>28.0</td>
<td>55.0</td>
<td>46.5</td>
<td>38.32</td>
</tr>
<tr>
<td>II</td>
<td>26.1</td>
<td>43.7</td>
<td>41.0</td>
<td>36.91</td>
</tr>
</tbody>
</table>

I: infected, and II: non-infected seeds.

Fig. 1. TG curves of; (a) non-infected, and (b) infected seeds of barley genotype W1 2291 with *P. graminea*.

Fig. 2. DSC curves of; (a) non-infected, and (b) infected seeds of barley genotype W1 2291 with *P. graminea*. 
and gelatinization enthalpy ($DH_{gel}$) were calculated and shown in Table 1.

The results show that sharp changes in DSC curves were occurred in the infected seeds at 50-60 °C (Fig 2b). Moreover, the onset temperature ($T_o$), peak temperature ($T_p$) were higher in the infected seeds as compared with the non-infected ones (Table 1). In fact, fungi degrade plant cell wall polymers to obtain nutrients and to aid in penetrating cells and spreading through plant tissue (Nus and Shashikumar, 1993). Tsujiyama (2004) attributed these thermal changes to be due to the hemicelluloses degradation of cell wall by fungus directly after infection.

TG curves of infected seeds show mass losses in three steps respectively; helped to observe the dehydration and the thermal patterns changes as compared with the controls. By using DSC technique the starch gelatinization process was observed and the enthalpy was determined in infected (28.0) and non-infected (26.1) seeds, which should be observed carefully.

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REFERENCES


