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Susceptibility factors in wheat infected with the hemibiotrophic pathogen *Septoria tritici*

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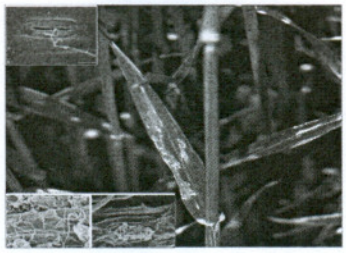
INTRODUCTION

- *Septoria tritici* (teleomorph *Mycosphaerella graminicola*), is one of the most important constraints of wheat production worldwide.
- *S. tritici* is considered to be a hemibiotrophic pathogen. It penetrates through stomata and grows intercellularly in the mesophyll. Leaves remain green until formation of pycnidia, from about 11 days after inoculation (dai) where the tissue turns necrotic. By 15 dai, mature pycnidia are seen.
- Production of Reactive Oxygen Species (ROS) is one of the earliest defence reactions in the plant. ROS can function as an antimicrobial agent, can be involved in structural defence mechanisms, *e.g.*, cell wall fortification, expression of defence-related genes and synthesis of phytoalexins.
- Symptom development and expression are in accordance with production of toxins by the pathogen (Shetty *et al.* 2003,2007). We have indications that host selective toxins may be involved in susceptibility to this fungus.



MATERIALS & METHODS

- Wheat cultivars Sevin (susceptible) and Stakado (resistant) to isolate IPO323 of *S. tritici*.
- IPO 323 was grown in Fries medium for 21 days, the culture filtrate was filtered through cheese cloth and passed through an ion exchange column. The collected fractions were infiltrated into susceptible and resistant cultivars.
- The necrosis inducing fractions were run on SDS-page and prominent bands were cut and analysed by MS-spectrometry
- Infiltration of the wheat mapping population Senat x Savannah with culture filtrate to locate sensitivity in the wheat genome.

RESULTS

H_2O_2 accumulation (Fig. 1, Table 1)

- Significantly higher in Stakado 1-11 dai. Penetration through stomata in Stakado always associated with accumulation of H_2O_2 and arrest of fungal growth. Significantly higher at 13-15 dai in Sevin, coinciding with tissue collapse and sporulation of *S. tritici*.

Host selective toxin (Figs. 2 and 3)

- The partially purified culture filtrate was seen giving necrosis only in cultivars susceptible to IPO 323.
- The band excised and analysed showed a sequence in the data base of *Mycosphaerella graminicola* gene sequence
- QTL analysis showed that the locus for sensitivity was over chromosome 5B.

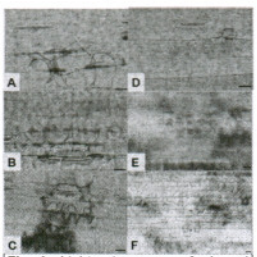


Fig 1. Light microscopy of cleared leaves after staining with DAB in Stakado (A-C) and Sevin (D-F). A). H_2O_2 in epidermal cells 1 dai. B). H_2O_2 in and around a progressing hypha in the mesophyll 5 dai. C). H_2O_2 in mesophyll and arrest of hyphae 7 dai. D). H_2O_2 in epidermal cells 7 dai. E-F). Massive accumulation of H_2O_2 15 dai. Bars=20 µm (Shetty *et al.* 2003).

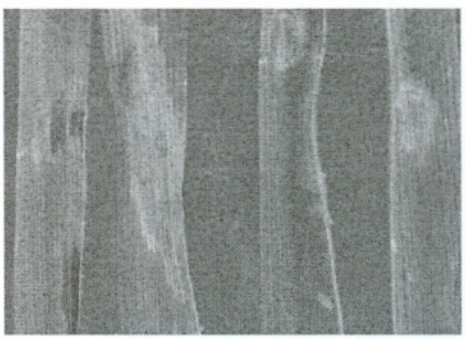


Fig. 2. Necrosis in cv. Sevin caused by infiltration of partially purified culture filtrate

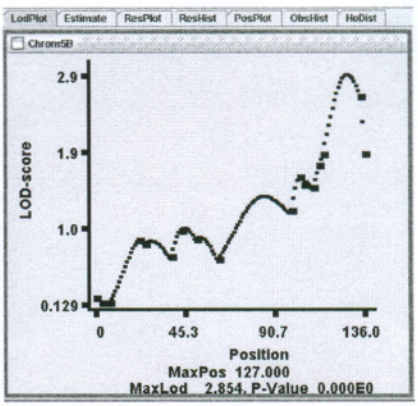


Fig 3. QTL analysis of the population Senat x Savannah after infiltration of culture filtrate. Figure shows the presence of a QTL on chromosome 5B

Table 1. Percentage leaf cells in Stakado and Sevin accumulating H_2O_2 (DAB-staining) after inoculation with *S. tritici* (Shetty *et al.* 2003).

Time (dai)	Stakado	Sevin	Odds ratio
1	0.7	0.1	11.35**
3	1.4	0.4	3.85**
5	3.5	1.1	3.56***
7	2.2	0.4	7.30***
9	4.4	0.1	34.28***
11	4.3	0.2	18.92***
13	4.0	79.5	0.01***
15	2.3	81.1	0.00***

Odds ratio for comparison of treatments in Sevin (Sevin used as a reference, odds ratio = 1.00).
The number of asterisks indicates the degree of significance, ***: Significant at $P \leq 0.001$;
**: significant at $P \leq 0.01$.

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