Characterization of the prehaustorial resistance against leaf rust (*Puccinia triticina* f. sp. *tritici*) in Einkorn (*Triticum monococcum*) by massive analysis of cDNA ends (MACE)

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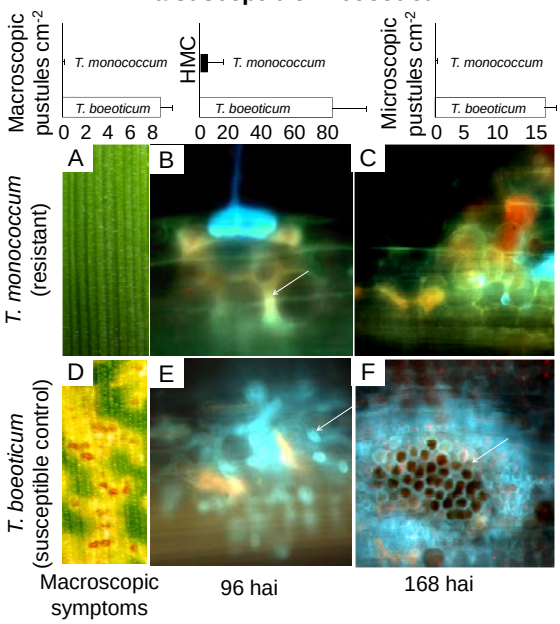
Introduction

Leaf rust caused by *Puccinia triticina* f. sp. *tritici* is the most common rust disease of wheat and causes high yield losses worldwide. *Triticum monococcum* accessions are valuable sources for improving leaf rust resistance in hexaploid wheat. In extensive screening programs *T. monococcum* has been identified showing prehaustorial resistance against all *Puccinia triticina* f.sp. *tritici* isolates with virulence against leaf rust resistance genes (*Lr*-genes) located on the A -genome. This race non-specific (horizontal) prehaustorial resistance (PHR) prevents the infection by *P. triticina* prior to the formation of haustorial mother cells (HMC). Hence the goals of our studies are (i) to analyze the biochemical background of this resistance by microscopy and measurement of the H_2O_2 accumulation in inoculated leaves and (ii) to determine the molecular background by genome wide expression studies using the massive analysis of cDNA ends (MACE).

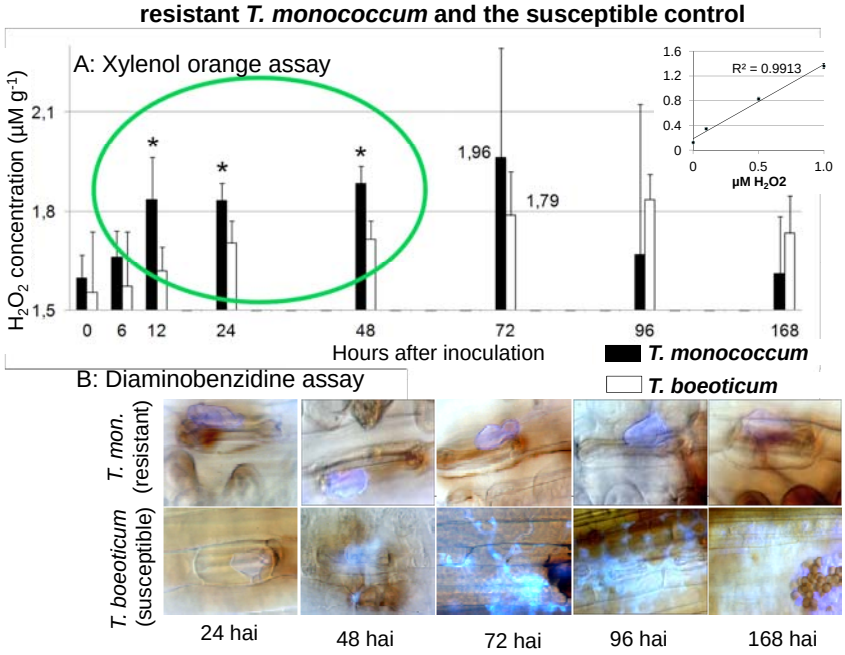
Comparison of symptoms and defense reactions of the resistant *T. monococcum* with a susceptible *T. boeoticum*



A: Green phenotype without any leaf rust symptoms
B: Autofluorescence around the infection site (arrow).
C: Inhibition of HMC generation and in consequence of the development of haustoria and uredospore pustules
D: Uredospore pustules
E: Numerous hmc (arrow)
F: Uredopsore pustules (arrow).

Lignification (C, arrow) and reduced flurorescence of the fungal cell wall suggest the involvement of H_2O_2, peroxidases and chitinases in effective defense reactions of *T. monococcum*.

Accumulation of hydrogen peroxide in inoculated leaves of resistant *T. monococcum* and the susceptible control



A: H_2O_2 concentration in leaf rust inoculated leaves. The green circle highlights the period with increased concentrations in *T. monococcum*. The embedded picture shows the calibration curve.
B: Diaminobenzidine stain shows the acccumulation of H_2O_2 (brown colored spots) and the development of fungal structures (blue colored) despite the accumulation of H_2O_2 in the susceptible control.

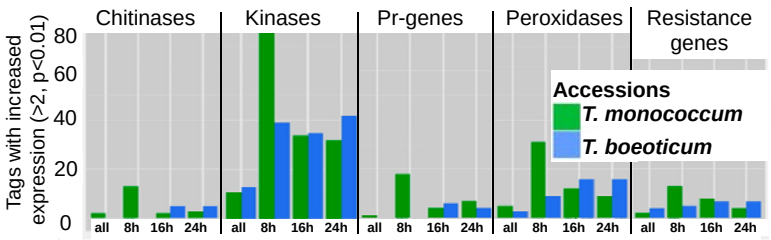
The early increase of the H_2O_2 concentration (green circle in A) in *T. monococcum* results in an effective defense reaction. which prevents growth of leaf rust in leaf tissue.

Serial analysis of gene expression- Massive Analysis of cDNA ends (MACE)

Number of differentially expressed tags after comparison of inoculated *T. monococcum* and *T. boeoticum*

	Time after inoculation			
	0 to 8 hai	8 to 16 hai	16-24 hai	entirty of all
quantitative	6810	6780	4832	1648
qualitative	4413	3592	3592	340

Comparison between the non inoculated and leaf rust inoculated genotype



Conclusion

Results obtained up to now elucidate that *Triticum monococcum* is a valuable source for horizontal resistance against *P. triticina* which may be efficiently employed in wheat breeding in the future. Microscopic analyses showed clearly an earlier and more effective defense reaction in the resistant *T. monococcum*. MACE showed in detail the increased expression of chitinases, kinases, peroxidases and pathogenesis related genes in the first 8 hai. The high number of differentially expressd tags which could be annotated to databases and the knowledge about SNPs facilitates in silico mapping and the development of polymorphic markers which after being mapped in a phenotyped segregating population may accelerate the transfer of this prehaustorial resistance to wheat.

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