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# Quantitative resistance of barley against scald – A candidate gene approach

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**SUMMARY** – Scald, caused by the fungus *Rhynchosporium secalis*, is a major disease of barley (*Hordeum vulgare* L.). Several resistance genes and QTLs have been described, but little is known about defence mechanisms and genes involved. By combining QTL analysis with the identification and mapping of candidate genes, the project aims at the development of molecular markers for scald resistance. Overall, two QTL on chromosome 2H and 7H were detected explaining together more than 80% of the phenotypic variance. Identification of candidate genes was carried out by global expression profiling using the Affymetrix GeneChip® Barley Genome Array in addition to qRT-PCR analysis. Later was performed to validate newly identified candidate genes as well as potential candidates from literature. For example, differential expression of barley PR5 genes was studied and the involvement of some TLP5-isoforms was proven. However, TLP5-genes did not map in detected QTL regions. Therefore, candidate gene analysis is still ongoing.

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## Introduction

Scald, caused by the fungal pathogen *Rhynchosporium secalis*, is an important global disease of barley (*Hordeum vulgare* L.). Several resistance genes and quantitative resistances have been described in barley, but until now little is known about the defence mechanisms and genes involved. Target of the current project is the detection of quantitative resistance loci (QTL) in a segregating DH population (138 lines) derived from the cross 'Igri' (*rrs1*) x 'Triton' (*Rrs1*) after inoculation with the virulent *R. secalis* isolate 271. Further on, detection of candidate genes for development of functional molecular selection markers is conducted by two different strategies. On one hand, identification of candidate genes was carried out by investigating differential gene expression of already known differentially expressed candidates from the literature. On the other hand, Affymetrix GeneChip® Barley1 Genome Array was used to identify differentially expressed candidate genes and beside to analyse global gene expression profiling for further discovery of physiological processes and pathways involved in resistance reaction.

## Material and methods

### Phenotyping, linkage map and QTL-analysis

A segregating DH-population (138 lines) derived from the cross 'Igri' (*rrs1*; susceptible) x 'Triton' (*Rrs1*, resistant) was chosen for analysis of quantitative resistance against scald. Phenotyping of disease severity was carried out under controlled growing conditions in the greenhouse (16°C) four times based on four single plants per genotype. Scald infection was performed by spray inoculation (250,000 spores/ml) of three weeks old plants (3-leaf-stage) using an aggressive isolate 271 (virulent against *Rrs1*<sub>Triton</sub>). According to Jackson and Webster (1976) the visual ranking ranged between 0 (no symptoms) to 4 (collapse of infected leaf).

As a prerequisite for QTL-mapping a genetic linkage map was generated comprising 237 SSR-, AFLP-, RAPD- and STS-marker using Kosambi function (LOD>3.0, max. 37.5 cM) of the software JoinMap 3.0. QTL-analysis was carried out by using PlabQTL-software (Utz & Melchinger, 1996) based on composite interval mapping (CIM) procedure. Threshold for QTL detection was set to LOD>3.0.

## Candidate gene analysis

Hybridisation of Affymetrix GeneChip® Barley1 Genome Array was done based on isolated RNA of 'Igri' and 'Triton' 96 h after inoculation with *R. secalis*-Isolate 271 in comparison to mock-infection. Normalisation of the Affymetrix hybridisation events were performed by using Affymetrix GCOS software. Detection of differential gene expression was determined by using the Affymetrix data mining tool, dChip-analysis software and own statistical analysis (Welch-test on normalized data and pairwise t-test after additional log-transformation) with a threshold of the significance level at  $p \leq 0.05$  and at least a 2-fold change of gene expression. Validation of identified promising candidate genes by using fluorescence labelled (SybrGreen) quantitative RealTime-PCR (qRT-PCR) on two separate biological experiments is still ongoing. On the other hand, known differentially expressed genes in the pathosystem barley-*R. secalis* like different PR proteins (Steiner-Lange *et al.*, 2003) as well as known resistance-associated genes of barley against the biotrophic powdery mildew fungus (Schultheiss *et al.*, 2003) are investigated by Reverse Transcriptase (RT)-PCR and qRT-PCR.

Those candidate genes differentially expressed between 'Igri' and 'Triton' after inoculation are furthermore sequenced to point out polymorphisms (SNPs or INDELS). Mapping of these promising candidates was carried out to determine co-localisation with those in parallel identified QTL.

## Results and discussion

After two of four inoculation experiments three QTL (LOD >3.0) on chromosome 2H and 7H were detected explaining 55% and 63.2% of the phenotypic and genotypic variance, respectively. Briefly, QTL-analysis comprising the third and fourth inoculation experiment will be finished. The QTL on chromosome 2H mapped in a similar region like the resistance gene *Rrs15*<sub>C18288</sub> (Schweizer *et al.*, 2004) whereas the two QTL of chromosome 7H are in comparable chromosomal regions like described for *Rrs2* (Grønnerød, 2000).

Global expression profiling using Affymetrix Barley1 GeneChip revealed 76 significantly differentially expressed genes of interest between 'Igri' (*rrs1*, susceptible) and 'Triton' (*Rrs1*, resistant) whereof seven are either knocked out or specifically expressed in only one genotype after *R. secalis* infection. Validation of these most promising genes by qRT-PCR of two independent experiments is still in process.

Literature based selection of differential expressed genes of the barley-*R. secalis* interaction like PR5-isoforms (Reiss and Horstmann, 2001) were investigated by RT- and qRT-PCR. First results suppose a genotypic differential expression 96 h after inoculation of the basic TLP5 and TLP6. Verification of the data by using at least two more biological replications will be done.

Mapping of barley TLP/PR5-genes reveals a single hit on chromosome 3H beside the already mapped resistance gene *Rrs1* and a cluster on chromosome 5H, that does not confirm a direct relation to already detected QTLs. Further candidate genes derived from the Affymetrix Barley1 GeneChip hybridization experiment will be included in the genetic map in the near future.

All in all, the study will finally aim at the identification of candidate genes directly associated with quantitative *R. secalis*-resistance, the identification of involved physiological processes in the *R. secalis*-barley interaction and the development of selection markers for breeding purposes.

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