

Towards isolating the wheat gene enhancing mycotoxin detoxification at the major Fusarium resistance QTL *Fhb1*

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Fhb1 - most prominent Fusarium resistance QTL

it confers not only strong resistance to spreading of the disease but also to the major mycotoxin deoxynivalenol (DON) by conjugation into the non-toxic DON-3-O-glucoside (D3G) (Lemmens et al. 2005). While three studies describe isolating the gene responsible for resistance to fungal spreading at the locus with contradictory results (Lagudah and Krattinger 2019), the gene encoding resistance against DON has not yet been characterized.

TILLING excluded eleven analyzed candidates

We established the *Fhb1* contig region from the donor line CM-82036 and fine-mapping the QTL to an 860 kb interval comprising 28 candidate genes (Schweiger et al. 2016). For eleven candidates mutant lines were detected - no loss of FHB/DON resistance phenotypes led to the rejection of all tested as the causal *Fhb1* gene.

Forward genetics identified three DON and

Fusarium susceptible mutants. F₂ co-segregation analysis confirmed for all three EMS-mutants the QTL region as responsible for the altered phenotype after Fusarium and DON treatment. Candidate gene sequencing resulted in 2-3 SNPs per line, but no common factor was found.

MutChromSeq results in two candidate scaffolds.

3B chromosomes of CM-82036 and the susceptible mutants were sequenced, a chromosome assembly of the wild-type established (130,108 scaffolds, 722.6 Mb) and sequencing reads of the mutants were mapped to it and to the 1 Mb QTL interval. We identified hundreds of scaffolds with SNPs in all three mutants; two were positioned in the *Fhb1* interval and are further analysed.

DON/FHB susceptible mutants convert less DON

into D3G. DON and D3G contents were determined to compare conversion rates of CM-82036 and the susceptible mutants, detecting about 15 times higher amounts of D3G compared to DON in the wild-type lines whereas in the susceptible mutants including F₂ progenies most of the applied DON was recovered (Fig. 2). DON and Fusarium infiltration gave similar DON/D3G ratios proposing that DON detoxification is regulated by mutations in the *Fhb1* interval controlling both traits.

Materials and Methods

CM-82036 mutant populations: 1,200 gamma-irradiated, 6,000 EMS-lines

Phenotyping for FHB and DON susceptibility: at anthesis, lines were either point-inoculated with *F. graminearum* or infiltrated with DON solution (Fig.3), symptomatic spikelets were evaluated.

Genotyping: SNP detection in candidate genes: 8-fold 2D pool screening, heteroduplex analysis on Fragment Analyzer System followed by Sanger-sequencing. Genotyping of FHB/DON-susceptible lines: gene-specific primers for *Fhb1* region, sequencing of fragmented amplicons on MiSeq System.

MutChromSeq: following the approach Sánchez-Martín et al. (2016). CM-82036 and susceptible mutants were flow-sorted for 3B chromosomes and sequenced.

Literature: Lemmens et al. (2005) *Molecular Plant-Microbe Interactions* 18:1318; Lagudah and Krattinger (2019) *Nature Genetics* <https://doi.org/10.1038/s41588-019-0454-3>; Sánchez-Martín et al. *Genome Biology* (2016) 17:221.; Schweiger et al. (2016) *TAG* 129:1607; **External cooperations:** Hélène Berges, CNRGV-INRA, France: BAC library screening; Bradley J. Till, now: Plant Genomics Unit at Centro de Genómica Nutricional Agroalucicola Chile: gamma-irradiation, NGS. **Funded by the Austrian Science Fund: SFBF3711**

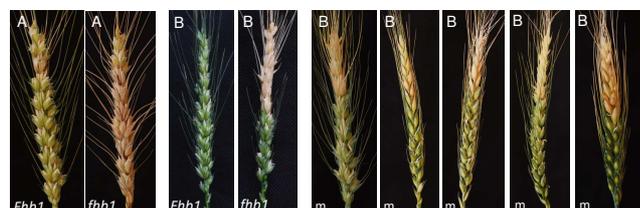
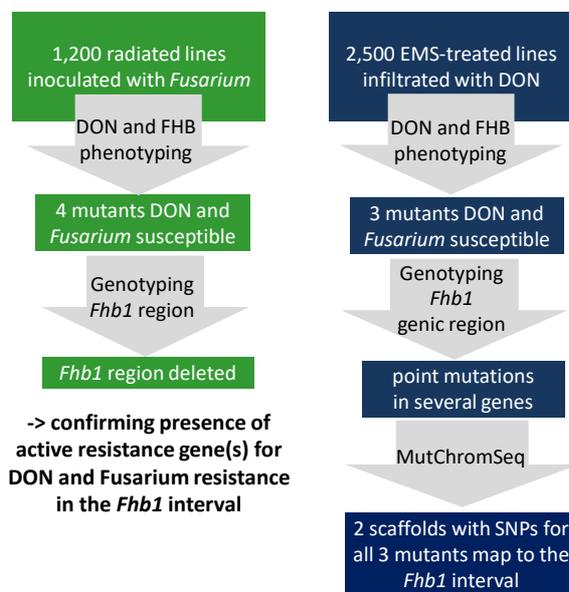


Fig. 1: Phenotypes of control lines differing in the possession of *Fhb1* (*Fhb1*, *fhb1*) and mutant lines (m) 26 days after *F. graminearum* point-inoculation (A) or DON infiltration (B).

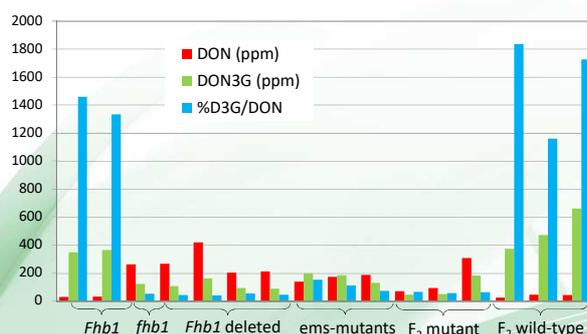


Fig. 2: DON, D3G and the ratio D3G/DON of control and susceptible mutant lines after infiltrating 1mg DON/head. Three greenhouse experiments with 8-15 heads/experiment.



Fig. 3: Fusarium conidia or DON solution were pipetted in wheat florets, treated heads covered with water-sprayed bags to promote infection/toxin absorption.