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Fine-mapping of the *Rpt5* net blotch resistance gene region in barley

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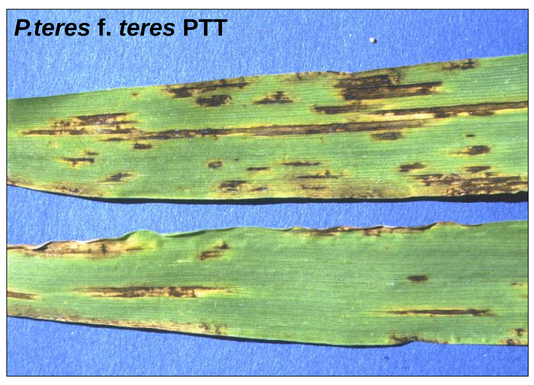
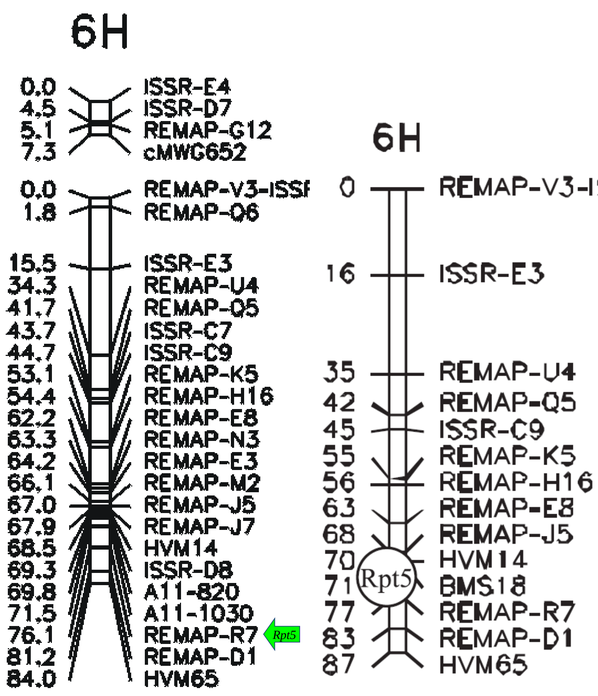
Abstract

The net blotch disease, caused by the *Pyrenophora teres* f. *teres*, is one of the most important fungal diseases of barley (*Hordeum vulgare*) in Finland. During testing of a collection of barley accessions, including landraces, for resistance to net blotch, the Ethiopian landrace CI9819 turned out to be 98% resistant, an optimal level for providing sustainable resistance. The resistance gene in CI9819 was designated *Rpt5.* We are using a variety of mapping strategies, including collinearity exploitation, to fine-map *Rpt5*. So far we have narrowed the *Rpt5* containing segment to 0.03cM. We have also carried out association genetics on a wide set of barley cultivars and made expression analyses of resistant and susceptible mapping parents. These approaches, combined with the genetically and physically mapped barley gene space ("gene-ome") and emerging barley genome sequence will greatly enhance the efforts to positionally clone *Rpt5.*



Introduction:

The net-type net blotch disease caused by *Pyrenophora teres f. teres* was first described by Drechsler (1923). Net blotch is common worldwide and causes great yield losses (Liu et al., 2011; Manninen et al., 2006). Net-type net-blotch is the most important barley disease in Finland. Testing of 47 different barley landraces against *P. teres f. teres* Finnish isolates resulted in identification of the Ethiopian landrace *H. vulgare* CI9819 having 98% resistance (Jalli, 2010). Preliminary genetic mapping of *H. vulgare* CI9819 revealed two genes, *Rpt5* and *Rpt6*, conferring resistance to the net-type and the spot-type, respectively (Manninen et al., 2006). *Rpt5* was separated from *Rpt6* by crossing CI9819 with cv. Rolfi, picking a recombinant line that contains only *Rpt5* and making a doubled haploid from it. The resulting line, C40, was crossed with cv. Rolfi to produce an F_2 mapping population of over 5,000 individuals for refinement of the genetic map of the *Rpt5* region. Currently, the map is being improved by exploiting the collinearity of the *Rpt5* syntenic region in *Brachypodium distachyon* chromosome 3.

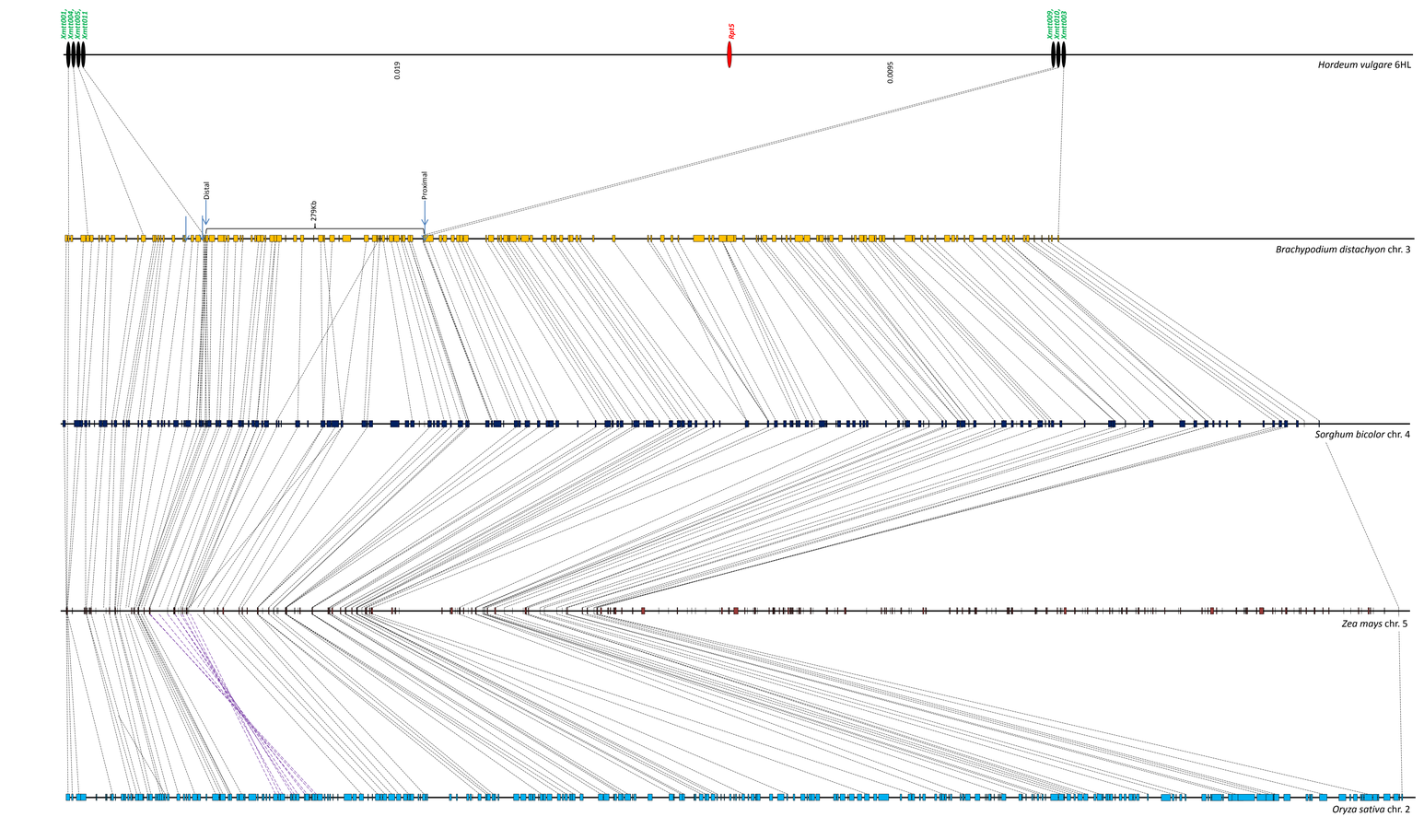


1. Genetic mapping of the *Rpt5* region

The genetic mapping of the *Rpt5* region proceeded by exploiting retrotransposon-based genetic markers (Manninen et al. 2006). Subsequently, Illumina iSelect SNP platform containing 7,864 markers (Comadran et al., 2012; Tondelli et al. 2013) was used to refine the *Rpt5* map. These genic SNP markers were exploited for anchoring of the *Rpt5* region to the collinear region in *B. distachyon*.

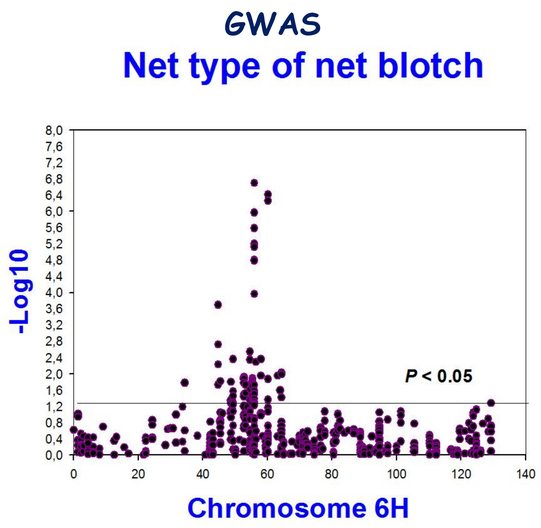
	Xmtt001	Xmtt004	Xmtt005	Xmtt011	Rpt5	Xmtt009	Xmtt010	Xmtt003	Xmtt002	Xmtt007	Xmtt008	Xmtt006
92.-15-1	R	R	R	R	S	S	S	S	S	S	S	S
80.-5-3	R	R	R	R	R	S	S	S	S	S	S	S
77.-9-9	S	S	S	S	S	S	S	S	S	S	R	R
21.-2-3	R	R	R	R	R	R	R	R	R	R	R	S

R Allele originating from the *Rpt5* containing accession *H. vulgare* C40
S Allele originating from *H. vulgare* cv. Rolfi not containing *Rpt5*



2. Refinement of the genetic mapping of the *Rpt5* region using *B. distachyon* collinearity

The goal of anchoring the *Rpt5* region to the collinear region in *B. distachyon* was to develop additional genetic markers based on *B. distachyon* genes for fine-mapping *Rpt5*. The segment on chromosome 3 of *B. distachyon* between the two SNP markers anchoring *Rpt5* was 2,160 Kb. Barley sequence data stemming from the IBSC genome sequencing project (IBSC, 2012) was used to develop markers corresponding to *B. distachyon* genes inside that segment. The genetic CAPS markers were developed as described by Raats et al. (2014). Currently, the size of the *B. distachyon* segment defined by the genes corresponding to the two nearest *Rpt5* flanking markers (*Xmtt011* and *Xmtt009*) is 279 Kb, narrowing the *B. distachyon* segment by almost eight fold. This *B. distachyon* region contains 39 annotated genes, offering the potential to further exploit collinearity for refinement of the *Rpt5* region. The collinear regions *Oryza sativa*, *Sorghum bicolor*, and *Zea mays* are being examined for this purpose.



3. Association genetics of the *Rpt5* region

Association genetics for the characterization of the *Rpt5* region was undertaken as a compliment to Mendelian mapping. A selection of 216 two-rowed barley cultivars representative of the European diversity of two-rowed barley was screened with the iSelect platform of 7,864 SNPs that is described above. A second population, comprising a collection of Nordic landraces and European old and new varieties, was examined using 15 BOPA1 SNPs (Close et al., 2009). The cultivars were tested for resistance or susceptibility to *P. teres f. teres* by inoculation with four net-type isolates from different parts of the world at the seedling stage. Scored for resistance was as described by Manninen et al. (2006) and Tekauz (1985). Association analyses were performed with the mixed linear model (Yu et al., 2006) approach by using TASSEL version 3.0 (Bradbury et al., 2007). Minor SNP markers with allele frequencies lower than 5% were removed. P values lower than 0.05 were considered significant. The estimated positions of the SNPs were based on the IBSC (2012) map. For both populations, the most important loci for net blotch resistance was located on chromosome 6H: at 44.77 cM, 55.94 cM, and 60.23 cM. The QTL at 55.94 cM seems to be exactly at the same locus as the *Rpt5* gene that we have mapped earlier in the Rolfi x CI9819 cross. The figure focuses on the chromosome 6HL region containing *Rpt5.* It shows the level of association of the net-type net blotch resistance of the barley cultivars with the different SNP-based markers per chromosomal location of the SNP-based markers as estimated according to the maps of the IBSC (2012). The highest level of association of the net-type net-blotch resistance with SNP allelic polymorphism corresponded to the *Rpt5* location.

4. Conclusions

Association genetics of the *Rpt5* region with a high-density Infinium iSelect SNP array has enabled anchoring of the *Rpt5* region to the syntenic region in *B. distachyon* chromosome 3. Genes in the collinear *B. distachyon* region served to refine the Mendelian map of the *Rpt5* region through development of CAPS makers based on gene and pseudo-gene sequences assigned to barley 6HL by the ongoing genome sequencing project (IBSC, 2012). Iterative application of this approach has narrowed the corresponding segment in *B. distachyon* to 279Kb. Additional potential exists for saturating the *Rpt5* with more molecular markers derived both from the 39 *B. distachyon* genes on this segment and from genes on corresponding segments in other sequenced grasses. Additional F_2 lines are currently being screened to increase the resolution. Ultimately, a CI9819 pooled BAC library (Chalhoub et al., 2004; Isidore et al., 2005; Simková et al. 2008; Vrána et al. 2000) will enable positional cloning of the *Rpt5* gene.

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