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Characterization and chromosomal location of powdery mildew resistance genes from wild barley PI282605

Charakterisierung und Chromosomenlokalisierung von Mehltau-Resistenzgenen der Wildgersten-Akzession PI282605

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Abstract

The objective of this work was to find the identity of three resistance genes against powdery mildew by mapping in an F_2 population derived from a cross between winter barley (*Hordeum vulgare* L.) variety 'Tiffany' and the wild barley (*H. vulgare* ssp. *spontaneum*) accession PI282605, an effective powdery mildew resistance source.

Key words: *Blumeria graminis* f.sp. *hordei*, genetic analysis, *Hordeum vulgare*, molecular mapping

Zusammenfassung

Das Ziel dieser Untersuchung bestand in der genetischen Kartierung und Charakterisierung von drei Mehltau-Resistenzgenen in einer F_2-Population der Kreuzung der Wintergerstensorte 'Tiffany' (*Hordeum vulgare* L.) und der Akzession PI282605 der Wildgerste (*H. vulgare* ssp. *spontaneum*), einer effektiven Mehltau-Resistenzquelle.

Stichwörter: *Blumeria graminis* f.sp. *hordei*, genetische Analyse, *Hordeum vulgare*, molekulare Kartierung

1 Introduction

Blumeria graminis (DC.) Golovin ex Speer f.sp. *hordei* Em. Marchal (= *Bgh*) is an obligate biotrophic fungus that causes powdery mildew in barley (*Hordeum vulgare* L.), a common disease in temperate climates and the most frequent disease of barley in the Czech Republic (Dreiseitl 2007a). Control of powdery mildew can be achieved through the use of resistant varieties; however, the high mutation rate of the pathogen can result in overcoming of race-specific resistance genes in new cultivars within a few years. Considering the limited number or complete lack of new resistance genes in cultivated barley, potential related sources such as wild barley have been screened for effective resistance genes against powdery mildew. A screen of a set of 1,383 wild barley accessions of *H. vulgare* ssp. *spontaneum* (Dreiseitl and Bockelman 2003) revealed 25 accessions to be an effective powdery mildew resistance sources.

The present study was undertaken to genetically characterize the accession PI282605 of wild barley resistant to powdery mildew (Dreiseitl and Bockelman 2003) for prospective exploitation in breeding. The objectives of this investigation were: (1) to find the number of genes/loci in wild barley PI282605 conferring powdery mildew resistance; (2) to find the identity of these resistance genes by means of their chromosomal locations; and (3) to identify linked polymorphic DNA markers.

2 Materials and methods

The tested population F_2 was obtained from a cross between the variety 'Tiffany' and the wild barley (*H. vulgare* ssp. *spontaneum*) accession PI282605. 'Tiffany' is a two-rowed winter barley carrying powdery mildew resistance genes *Mla7* and *MlaMu2* (Dreiseitl 2007a; 2007b), which have already been overcome. Twelve parental, nine F_1 and 229 F_2 plants were grown in the greenhouse. The resistance tests were done on leaf segments as described by Řepková et al. (2006): the virulent (*Va7*, *VaMu2*) pathotype 5715 of *Bgh* was used to find the number of genes conferring resistance; the avirulent (*Aa7*) pathotype 1002 was used to test the allelism for the *Mla* locus. The results of resistance tests of F_2 populations with *Va7* and *Aa7* pathotypes were compared and conclusion on allelism for the *Mla* locus was drawn.

The molecular mapping was carried out using 65 polymorphic simple sequence repeat (SSR) markers mainly after Ramsay et al. (2000). In addition, the primers for the *cMWG682* marker were generated by the Primer3 program using the *cMWG682-5* sequence (10 – 513 bp; http://wheat.pw.usda.gov/GG2). The primers for *RGH1aE2a* marker were designed from the sequence of the *RGH1a* gene (exon 2; 2199 – 2706 bp) at the *Mla* locus (http://www.ncbi.nlm.nih.gov; accession number AF427791). The primer sequences of *cMWG682* were as follows: 5'-gcacacgccaacacaaagt-3' and 5'-tctcagcatccaacaatcca-3', and of *RGH1aE2a*: 5'-caggaacaatttaggcagtcg-3' and 5'-agtcccttgattt-ccctggt-3'. For the *cMWG682* marker, the PCR cycle corresponded to cycle E by Ramsay et al. (2000); for the *RGH1aE2a* marker, the same cycle was used as designed Řepková et al. (2009). To identify polymorphic sites for *cMWG682*, the restriction enzymes *Alu*I, *Ava*II, *Bst*UI, *Btg*I, *Dde*I, *Hae*III, *Hpa*II, *Nla*III, and *Taq*I were used to digest the resulting amplicons of both parents. For *RGH1aE2a*, the polymorphism was tested with *Alu*I, *Bsa*JI, *Dpn*I, *Hin*fI, *Mbo*I, and *Rsa*I.

The linkage between DNA markers and resistance genes was detected using a modified bulk segregant analysis (BSA) in which each resistant (RT0) and susceptible (RTs 3–4 and 4) bulk consisted of 19 and 16 F_2 plants and their DNAs without DNA pooling, respectively. For the markers found to be linked with the resistance genes by BSA, the significance of the linkage was statistically evaluated by the MapQTL 5 software (Van Ooijen 2004) using random sample of 115 F_2 plants of all genotypes. The LOD score was calculated by means of marker regression.

3 Results and discussion

In analyzed cross, 213 resistant (RT0 to RT3) and 16 susceptible (RT3–4 and 4) plants were scored after inoculation with the virulent pathotype. The segregation ratio obtained corre-

sponded to a theoretical ratio of 15 : 1 ($\chi^2 = 0.21$), which was consistent with two independent resistance genes with dominant inheritance. The distribution of reaction types found in the F_2 generation and the RTs of the parents and the F_1 generation are shown in Figure 1. Variation attributed to the powdery mildew resistance trait resembled nearly a continuous scale typical for quantitative trait loci (QTL). The allelism test confirmed one resistance gene at the *Mla* locus because no susceptible F_2 plants were detected after avirulent pathotype inoculation.

The new developed CAPS (cleaved amplified polymorphic sequence) marker *cMWG682* amplified a 470-bp fragment in both 'Tiffany' and PI282605. The marker was polymorphic after digestion with the restriction enzyme *Nla*III: a parental non-digested 470-bp DNA fragment was found in 'Tiffany', and 280-bp and 190-bp DNA fragments were found in PI282605. The new developed CAPS marker *RGH1aE2a* amplified a 508-bp fragment in both 'Tiffany' and PI282605. The marker was polymorphic after digestion with the restriction enzyme *Alu*I: DNA fragments of 220-bp, 130-bp, 120-bp and 38-bp were found in 'Tiffany', while DNA fragments of 258-bp, 130-bp and 120-bp were found in PI282605. By interval mapping on the short arm of chromosome 1H, one resistance gene was found to be linked with *GBM1007* and *Bmac0213* (LOD 3.32). A second gene was mapped between *EBmag0794* and *Bmag0206* (LOD 3.26) on the short arm of chromosome 7H at a distance of 2 cM from *Bmag0206*. A third gene was assigned to a linkage with *cMWG682* (LOD 22.43) on the short arm of chromosome 2H (Fig. 2).

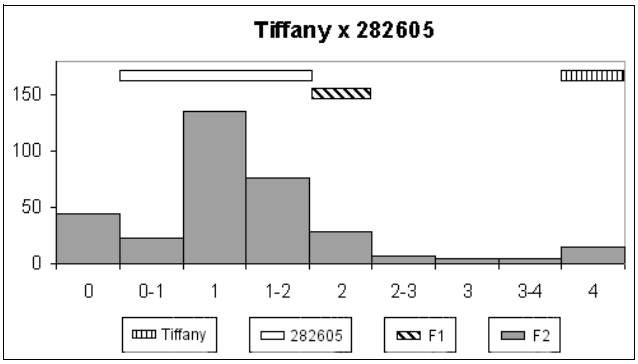

Fig. 1: Distribution of reaction types of the F_2 plants of the cross variety 'Tiffany' x *Hordeum vulgare* ssp. *spontaneum* following inoculation with virulent *Va7* pathotype of *Blumeria graminis* f. sp. *hordei* and comparison with the parental and the F_1 generation. x – reaction types (RTs), y – number of plants of the F_2 generation for individual RTs.

The resistance gene on chromosome 2HS was mapped to place where QTLs for powdery mildew resistance had been reported before in different populations by Backes et al. (2003) and Von Korff et al. (2005). In addition, the gene *Mlhb* transferred from *H. bulbosum* was mapped close to the marker cMWG682 (Pickering et al. 1995). A comparison of qualitative gene positions and mapped QTLs for powdery mildew resistance revealed strong correspondence also for the *Mla* locus (Backes et al. 2003; Emebiri et al. 2005; Von Korff et al. 2005). In the upper part of chromosome 7HS, however, no QTL has been detected so far. This sub-telomeric position corresponds to the known gene *mlt* (Schönfeld et al. 1996). Its recessive mode of inheritance excludes an identity corresponding to the dominant resistance gene from PI282605. No other dominant major powdery mildew gene is known in this chromosomal region.

Knowledge on genetic and molecular bases of resistance might simplify the breeding. The most effective gene participating in powdery mildew resistance was that on chromosome 2H (67% of phenotypic variation), two other genes participated with only 7.7% and 7.9%. In addition to PI282605, a powdery mildew resistance gene corresponding to a locus on the 2HS chromosome was detected in four resistant accessions of *H. vulgare* ssp. *spontaneum* (PI466197, PI284752, PI391126 and PI296935) out of 23 accessions analyzed so far in our laboratories. This locus was found to be significant in powdery mildew resistance and prospective for breeding.

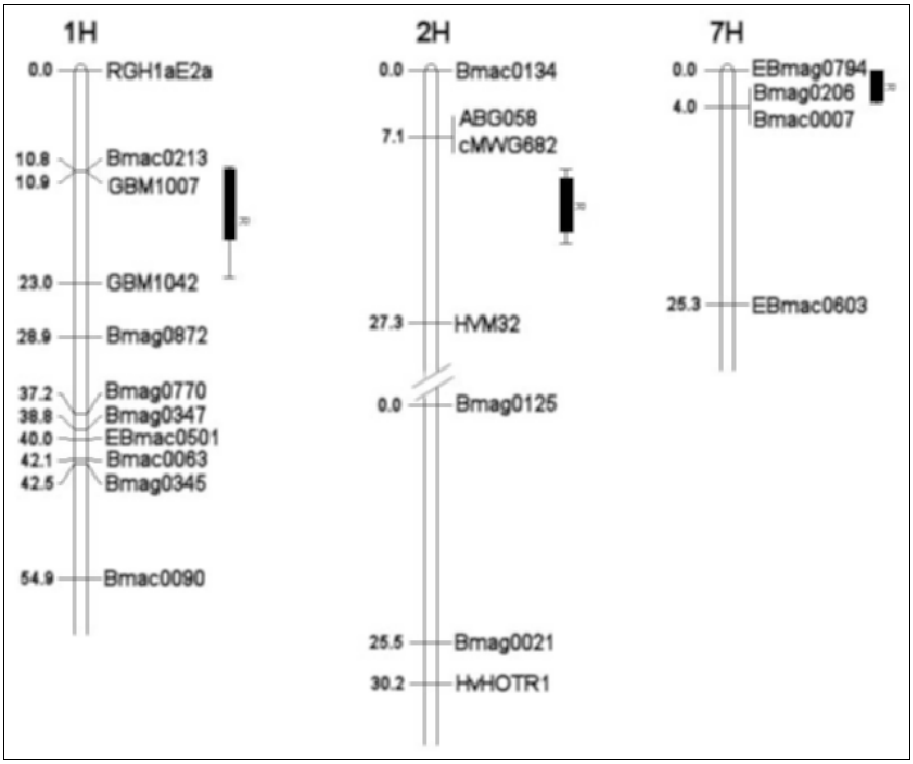

Fig. 2: A partial genetic map of the barley chromosomes 1HS, 2HS and 7HS based on the analysis of F_2 plants from the cross 'Tiffany' × PI282605 showing the positions of the genes (*R*) conferring resistance to powdery mildew. Map intervals are given in centiMorgans (cM) to the left of chromosomes, DNA marker loci are assigned to the right of chromosomes, the bars to the right of chromosomes indicate the *R* gene positions (with confidence intervals).

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