IDENTIFICATION OF CANDIDATES OF THE BARLEY LEAF STRIPE RESISTANCE GENE Rdg2a BY MEANS OF MAP–BASED CLONING

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The barley gene Rdg2a confers resistance against several isolates of the seed borne fungal pathogen Pyrenophora graminea (the causal agent of barley leaf stripe) and immunity against isolate Dg2, the most virulent isolate of a collection of monoconidial isolates. Firstly, this gene was finely mapped to the distal region of the short arm of barley chromosome 1 (7H), by using a segregating population of 2800 F1 gametes. In addition, a rice-barley syntenic relationship, and a physical contig based on cv. Morex barley BACs clones that overlaps the Rdg2a interval map were established.

In order to characterize the genetic bases of leaf stripe resistance, subsequent steps of a map-based cloning approach for the Rdg2a gene were carried out. For this purpose the genomic region of Rdg2a was saturated with molecular markers developed by using information derived from shotgun sequencing of the Morex BACs covering the region. Because the cv. Morex does not carry a functional allele of the resistance gene, a 5X cosmid library of barley cv. Thibaut (bearing a functional allele of the gene) was constructed. The screening of the cosmid library with markers co-segregating and tightly associated to Rdg2a allowed the identification of a cosmid contig encompassing the genomic region of the gene. Sequencing of cosmids belonging to this contig led to the identification of two sequences coding for NBS-LRR (Nucleotide Binding Site-Leucine Rich Repeats) proteins, the most common class of plant disease resistance proteins. The comparison of the sequences present in the candidate genomic region of cv. Thibaut and of two distinct susceptible cvs. (Morex and Mirco) revealed genomic rearrangements both in coding and non-coding sequences. These rearrangements suggest that the two NBS-LRR sequences identified could represent good candidates for Rdg2a.