CANDIDATES OF THE BARLEY LEAF STRIPE RESISTANCE GENE
Rdg2a ARE INCLUDED IN A CLUSTER OF NBS-LRR ENCODING GENES

BISCELLI C.*, BULGARELLI D.**, CONSONNI G.***, STANCA A.M.*, VALE’ G.*

*) CRA - GPG-Genomic and Post-Genomic Research Centre, Via S. Protaso 302, 29017
Fiorenzuola d’Arda (PC), Italy
**) Max Planck Institute for Plant Breeding Research, Carl-von-Linne-Weg 10, 50829 Cologne, Germany
***) DiPROVE, University of Milan, Via Celoria 2, 20133 Milano, Italy

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Rdg2a is a mono-mendelian barley resistance gene that 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.

In order to characterize the genetic basis of Rdg2a-mediated leaf stripe resistance, a map-based cloning approach was undertaken for this gene. For this purpose the Rdg2a genomic region was saturated with molecular markers developed from shot-gun sequencing of 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 Rdg2a) was constructed. Screening of the cosmid library with markers co-segregating and tightly associated to Rdg2a yielded the identification of a 72Kbp cosmid contig encompassing the genomic region of the gene. Low-pass shotgun sequencing of this contig led to the identification of three sequences coding for NBS-LRR (Nucleotide Binding Site-Leucine Rich Repeats) proteins. Transcription analyses revealed that the three predicted genes are expressed only in the Rdg2a-near isogenic line (NIL) resistant genotypes but not in the corresponding susceptible NIL. The cloning of the full length cDNAs of the candidates confirmed the computational prediction for two of them, while in the third one a predicted intron is retained in the mRNA, causing a frameshift in the transcript that, most likely, lead to the production of non functional protein. Southern-blot analyses conducted on Rdg2a-resistant and three different susceptible genotypes as well as sequencing of the two NBS-LRR susceptible alleles highlighted genomic rearrangements in the locus suggesting that two NBS-LRR genes out of the three identified could represent good candidates for Rdg2a. Interestingly, single-cell transient assay revealed that the two predicted candidate proteins exist in the cell either inside and outside of the nuclei. The TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick end labelling) analysis of pathogen-challenged embryos of a resistant NIL revealed the absence of DNA fragmentation indicating that Rdg2a-mediated leaf stripe resistance may not involve programmed cell death at the host pathogen interface.