TOWARDS THE POSITIONAL CLONING OF THE LEAF RUST RESISTANT \textit{LR14} LOCUS IN DURUM WHEAT


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Leaf rust is a main disease that affects durum wheat production. Resistance to this fungal pathogen is therefore a main objective for durum wheat breeding. The leaf rust resistant allele \textit{Lr14-Cresco} from the durum wheat cv. Creso and its derivative Colosseo is one of the most important leaf rust resistance sources present in the modern durum germplasm and it has been located in the distal portion of chr. 7BL (Maccaferri et al. 2008, TAG 91:731-738), with the identification of linked SSR markers (gwm146, gwm344) suitable for marker-assisted selection (MAS). The Colosseo x Lloyd RIL population (176 lines) has been used to enrich the QTL region with new SNP-based molecular markers. Phenotypic data were collected in the field and also at the seedling stage. A high heritability of the disease response was observed in both cases ($h^2 > 0.8$). Our target is to fine map and eventually clone \textit{Lr14-Cresco}. Twelve conserved orthologous sequence (COS-SNP) derived markers (UBW) were developed by exploiting the conserved collinearity between the most distal portions of rice chr. 6, \textit{Brachypodium} chr. 1 and wheat chr. arm 7BL. Using the coding sequence of the rice and \textit{Brachypodium} collinear genes, the corresponding wheat orthologs were retrieved and specific PCR assays (ca. 1 kb) targeting the intron/exon boundaries of the genes were designed. Then, genomic DNA of the parents Colosseo and Lloyd were amplified, cloned and sequenced allowing the identification of the SNPs differentiating the two homeologous copies of each gene (genome-specific SNPs) as well as the varietal-SNPs between Colosseo and Lloyd. These SNPs were then used to develop HRM (High Resolution Melting) assays and ASO (Allele Specific Oligonucleotide) markers that were 7B-specific and polymorphic between the two parents. Also, using DArT sequences available in Triticarte (http://www.triticarte.com.au), five new DArT markers mapped in the QTL region in other bread wheat mapping populations and one DArT marker already mapped near to the phenotype in the Colosseo x Lloyd RILs were converted in HRM assays. All the developed SNP-based markers were mapped within the interval that includes the QTL peak; in particular two DArT markers (wPt-4038 and wPt-4140) were mapped through HRM genotyping 0.1 and 1 cM away from the \textit{Lr14-Cresco} locus, respectively. The results are supported by an independent association mapping study carried out using a panel of 183 elite accessions (Maccaferri et al. 2010, Molecular Breeding 26:189-228). This allowed us to validate the presence of \textit{Lr14} and to further improve mapping resolution.