

Fine-mapping of the durable leaf rust resistance gene *Lr34* using sequence information from *Brachypodium* and *Aegilops tauschii*

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THE DURABLE LEAF RUST RESISTANCE GENE *LR34*

One of the most relevant genes in wheat disease resistance breeding is *Lr34*. This locus has contributed towards durable resistance to leaf rust, stripe rust, stem rust and powdery mildew, making *Lr34* a unique resource for breeding and a model for understanding the molecular basis of partial horizontal resistance. *Lr34* has been used in breeding programs since many decades and has not been overcome. A detailed QTL study mapped this gene to the short arm of chromosome 7D (Schnurbusch et al. 2004). The gene explained up to 42% of the phenotypic variance observed in the field. *Lr34* is associated with the morphological marker 'Leaf Tip Necrosis' (Ltn1). Ltn1 can be used as a morphological marker to assist breeding for *Lr34* (Figure1), but is limited by variable expression under different environmental conditions and genetic background.



Fig.1: A wheat plant carrying the durable resistance gene *Lr34* and exhibiting the morphological marker 'Leaf Tip Necrosis'.

INCREASING MARKER DENSITY AROUND *LR34*

Previous work identified the two SSR markers gwm1220 (distal) and SWM10 (proximal) as the closest flanking markers for *Lr34* (Lagudah et al. 2006, Bossolini et al. 2006). To increase marker density around *Lr34*, we used molecular information from the orthologous region in *Brachypodium sylvaticum* and rice. *Brachypodium* was recently proposed as a new model organism for temperate grasses and it showed closer homology to wheat than rice (Bossolini et al. 2007). In *Brachypodium* there are four coding sequences spanning the

homologous *Lr34* target interval between the two flanking markers, namely a serine kinase, a pectate lyase, a hypothetical protein and a GTP binding protein. Wheat expressed sequence tags (wESTs) were identified by homology with these *Brachypodium* genes. These wESTs were then used to screen a fingerprinted bacterial artificial chromosome (BAC) library from *Aegilops tauschii* (Jan Dvorak, UC Davis), which is the donor of the wheat D-genome. Three BAC contigs of *Ae. tauschii* consisting of 124 BAC clones and spanning about 2.1 Mb of the *Lr34* target interval were identified by hybridization screening. They contained all the orthologous genes of *Brachypodium* as well as the two flanking markers gwm1220 and SWM10. We therefore concluded that a significant portion of the *Lr34* target interval was covered by these contigs. Thirteen BAC clones were selected for low-pass sequencing, resulting in a total of 790 kb of sequence information. These sequences were mined for simple sequence repeats (SSR), since these elements are known to be very powerful tools for genetic mapping and marker assisted selection even in genomes with a low degree of polymorphism. In addition locus-specific sequence-tagged sites (STS) were developed and their sequences were analysed for single nucleotide polymorphism (SNPs) and insertion / deletion (InDel) markers on the parents of the mapping populations. A third strategy was to develop RFLP markers based on low-copy probes. Using these strategies we were able to develop ten new molecular markers in the region of interest. All of them mapped to the expected position according to the homology with *Brachypodium*. Hence, *Brachypodium sylvaticum* proved to be a good model organism to derive locus-specific molecular markers in wheat. While the targeted gene region from *Brachypodium* was also identified in rice, the latter species carried a large inverted segment relative to the orthologous regions in *Ae. tauschii*, the D genome of hexaploid wheat and *Brachypodium* (Bossolini et al 2007, Spielmeier et al 2008). Using sequence information from the diploid D-genome progenitor *Ae. tauschii* turned out to be a successful strategy, since most PCR-based probes were highly transferable to hexaploid wheat cultivars. Of more significance were the additional markers that were obtained from *Ae. tauschii* but absent from the corresponding region in *Brachypodium*; these additional markers mapped closer to, as well as at the *Lr34* locus.

HIGH RESOLUTION GENETIC MAP

Three high-resolution back-cross populations were used to determine the mapping positions of the newly derived molecular markers. Phenotypic analysis was done independently on all the populations over several years. *Lr34* showed close linkage to markers csLVMS, csLVE17, SWM10 and SWSNP3. The two microsatellite markers csLVMS and SWM10 showed the same allele in the three independent sources of *Lr34* ‘Frontanta’, ‘Chinese Spring’ and ‘Forno’ as well as in many additional cultivars containing *Lr34* (Lagudah et al. 2006, Bossolini et al. 2006). Hence, these are highly useful markers to assist selection for *Lr34* in breeding programs worldwide. Genetic mapping defined a 0.15 cM interval that is flanked by the two new markers csLVE17 and SWSNP3. Five markers showed co-segregation with *Lr34*. Figure 2 summarizes the mapping data based on a consensus map from three fine-mapping populations.

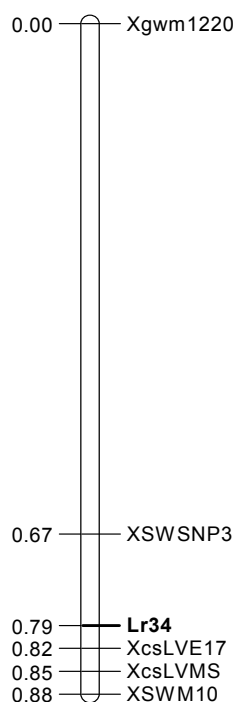


Fig.2: High resolution genetic consensus map of the *Lr34* region based on three fine-mapping populations. The map displays the position of the two newly developed flanking markers csLVE17 and SWSNP3 as well as the earlier described markers SWM10, csLVMS and gwm1220.

FROM GENETIC TO PHYSICAL INFORMATION

The two newly derived markers csLVE17 and SWSNP3 define a 0.15 cM target interval for *Lr34*. This interval was spanned physically by a BAC contig from *Ae. tauschii*. To get corresponding sequence information from hexaploid wheat, BAC libraries from the wheat cultivars ‘Chinese Spring’ (INRA, Toulouse) and

‘Glenlea’ (Sylvie Cloutier, Agriculture and Agri-Food, Canada) were screened using locus-specific PCR and RFLP probes derived from the *Ae. tauschii* contig. Four ‘Chinese Spring’ BAC clones spanning the whole target interval were identified and fully sequenced. These sequencing data revealed the presence of a gene-rich region surrounded by repeat-rich sequences. This finding corroborates the hypothesis that the wheat genome is organized in gene-rich islands surrounded by large stretches of repetitive sequences. The gene-rich region consists of ten candidate genes. None of these coding sequences has a homolog in *Brachypodium sylvaticum* or *Brachypodium distachyon*. Probably several independent insertion events led to the complexity of this gene-rich island. We propose that one of these coding sequences is responsible for the durable resistance conferred by *Lr34*.

REFERENCES

- Bossolini E, Krattinger SG and Keller B (2006) Development of simple sequence repeat markers specific for the *Lr34* resistance region of wheat. *Theor. Appl. Genet.* 113: 1049-1062.
- Bossolini E, Wicker T, Knobel PA and Keller B (2007) Comparison of orthologous loci from small grass genomes *Brachypodium* and rice: implications for wheat genomics and grass genome annotation. *Plant Journal* 49: 704-717.
- Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS and Spielmeier W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theor. Appl. Genet.* 114: 21-30.
- Schnurbusch T, Bossolini E, Messmer M and Keller B (2004) Tagging and validation of a major QTL for leaf rust resistance and leaf tip necrosis in the winter wheat cultivar ‘Forno’. *Phytopathology* 94: 1036-1041.
- Spielmeier W, Singh RP, McFadden H, Wellings CR, Huerta-Espino J, Kong X, Appels R, Lagudah ES (2008) Fine scale genetic and physical mapping using interstitial deletion mutants of *Lr34/Yr18*: A disease resistance locus effective against multiple pathogens in wheat. *Theor. Appl. Genet.* 116: 481-490.