TOWARDS CLONING $Yd_2$, A BARLEY RESISTANCE GENE TO BARLEY YELLOW DWARF VIRUS

Thesis submitted for the degree of
Doctor of Philosophy

by

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March, 2001
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Literature Cited
Summary

The $Yd_2$ gene in barley provides protection against barley yellow dwarf luteovirus (BYDV), the most economically devastating virus of cereals worldwide. The aim of this thesis was to investigate strategies to enable the $Yd_2$ gene to be cloned. These strategies included a map-based approach to cloning $Yd_2$ in barley, a syntenous map-based approach to cloning a $Yd_2$ orthologue in rice and the use of PCR primers designed to amplify conserved regions of resistance genes in an attempt to amplify a candidate $Yd_2$ gene.

Essential for cloning $Yd_2$ was the development of a resistance assay system to reliably characterise $Yd_2$ genotypes. Aphids reared on 3-week old oat plants grown at 22°C days and 18°C nights were highly viruliferous resulting in 100% infection rates for barley cultivars tested. Infected $Yd_2$ and non-$Yd_2$ barley cultivars were able to be reliably distinguished as early as 4 weeks post infection based on symptom expression. F2 progeny homozygous-positive for $Yd_2$ were resistant and distinguishable from other F2 progeny. Cultivars heterozygous and homozygous-minus for $Yd_2$ were susceptible and unable to be distinguished from each other. Resistance assays were required to be performed on the F3 progeny of these F2 individuals to distinguish between homozygous and heterozygous susceptible F2 individuals.

A quantitative dot-blot assay relating to viral titre levels in infected plants was developed to quickly distinguish between resistant and susceptible genotypes of different $Yd_2$ sources. Significant differences between viral titre in both shoot and root material were evident between non-$Yd_2$ and $Yd_2$ genotypes, with the differences being even more pronounced in the root material. The dot-blot assay was able to effectively distinguish between resistant and susceptible individuals 5 days post infection from a range of barley cultivars differing in $Yd_2$ status. This assay was unable to distinguish between homozygous and heterozygous susceptible individuals. Therefore, dot-blot analysis was performed on the F3 progeny of F2 individuals to identify homozygous susceptible and heterozygous susceptible F2 individuals. In combination with the qualitative resistance screening assay this quantitative assay was used to help genotype individuals for $Yd_2$ in our mapping populations.
To undertake map-based cloning in barley, genetic linkage maps of the \( Yd_2 \) region were constructed using 106 F\(_2\) individuals of the Proctor (\( Yd_2-\)minus) × Shannon (\( Yd_2-\)plus) cross and 572 F\(_2\) individuals of the Atlas (\( Yd_2-\)minus) × Atlas68 (\( Yd_2-\)plus) cross. These maps contain 29 molecular markers, the \( Yd_2 \) gene and the centromere. Twelve of these markers are within 1cM of \( Yd_2 \). From the Atlas × Atlas68 cross, 18 F\(_2\) individuals were carrying a recombination event within a genetic interval of 1.6cM between two \( Yd_2 \) flanking loci \( YLM \) and \( Xmgw952 \). F\(_3\) progeny derived from these F\(_2\) individuals which were homozygous for the recombinant chromosome were identified and characterised for use in bulked segregant analysis and fine scale mapping of the \( Yd_2 \) region.

Pulsed field gel electrophoresis (PFGE) was used to establish the relationship between genetic and physical distance in the region of \( Yd_2 \). Physical mapping of the \( Yd_2 \) region revealed a physical linkage between the two loci \( Xbcd134 \) and \( Ylp \). Because these loci are separated by 0.5cM on the Proctor × Shannon genetic map, a relationship of physical to genetic distance around this immediate area could be calculated as 1cM being less than to 360kb. This relationship suggested that there was a good chance of identifying a single genomic clone with our co-segregating marker Ylp which may also contain the \( Yd_2 \) gene.

A rice yeast artificial chromosome (YAC) library established by the Japanese Rice Genome Project was utilised as a tool for cloning the \( Yd_2 \) gene. Two YACs from an area of rice Chromosome 1 syntenous with the \( Yd_2 \) region of barley Chromosome 3 were identified. A physical linkage of 9kb between two loci \( XrYlp \) and \( Xrc122 \), syntenous with the loci \( Ylp \) and \( Xc122 \) which co-segregate with the \( Yd_2 \) gene in barley was established in rice. The YAC contig of this area of the rice genome however, was not continuous. Other molecular markers in close genetic proximity to \( Yd_2 \) with the exception of \( Xwg889 \) from both our barley maps could not be placed on the rice YAC clones identified as syntenous with \( Yd_2 \) region of barley. With the possibilities of mircosynteny breakdown, a favourable relationship between genetic and physical distance in the region of barley \( Yd_2 \) and the development of a publicly available large genomic insert barley library, a syntenous map-based approach in rice was not continued.
Barley bacterial artificial chromosome (BAC) filters were obtained, screened and a number of barley BACs from the $Yd_2$ region were identified. Two BACs were identified as encompassing the $Ylp$ locus which co-segregates with $Yd_2$, 5 BACs were identified as encompassing the $Xbcd134$ loci, a loci 0.5cM proximal to $Yd_2$ and 4 BACs identified as encompassing the $YLM$ loci, 0.7cM distal of $Yd_2$. A partial contig over the $Yd_2$ gene was established with BACs positive for the $Ylp$ locus showing a small overlap with 4 of the 5 BACs positive for the locus $Xbcd134$. No physical linkage was identified between the BACs positive for $YLM$ and $Ylp$, and it was concluded that $YLM$ may be to distal to $Yd_2$ to begin a chromosome walk from this side. All other markers identified within 2cM of $Yd_2$ on the genetic maps were absent from these clones. Based on this physical information it was concluded that a number of chromosome walks using end-clones from the BACs positive for $Ylp$ and distal to $Xbcd134$ may complete a contig spanning the $Yd_2$ region.

Concurrent with the map-based approaches in barley and rice, a strategy aimed at cloning $Yd_2$ using PCR primers shown to amplify conserved areas of resistance genes was undertaken. In order to amplify possible $Yd_2$ candidates, primers were targeted to the template of a rice YAC identified from the syntenous region of rice Chromosome 1, at barley Near Isogenic Lines (NILs) and also to resistant and susceptible bulks derived from critical recombinants identified from the Atlas x Atlas68 mapping population. While an number of the products amplified were identified as closely linked genetically or physically to $Yd_2$, sequence information of these products showed no identity to resistant gene analogues. However, a number of the products cloned from rice showed low identity matches to genes or transcripts which could be considered potential candidates for $Yd_2$. Northern analysis using these clones as probes however, detected no mRNA in infected or noninfected barley cultivars.