THE GENETICS OF BARLEY YELLOW DWARF VIRUS RESISTANCE IN BARLEY AND RICE

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by

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SUMMARY

Barley yellow dwarf virus (BYDV), an aphid transmitted luteovirus, is the most widespread and economically damaging virus of cereal crops. The work in this thesis aims to characterise the basis of the naturally occurring resistance to BYDV in cereals in three ways: Firstly, by facilitating the isolation of the *Yd2* gene for BYDV resistance from barley by a map-based approach. Secondly, by determining if a BYDV resistance gene in rice is orthologous to *Yd2*. Thirdly, by establishing if other BYDV resistance genes in non-Ethiopian barleys are allelic to *Yd2*. It is hoped that the information generated in this study will ultimately assist in the production of BYDV resistant cereal cultivars.

A detailed genetic map of the *Yd2* region of barley chromosome 3 was constructed, containing 19 RFLP loci, the centromere and the *Yd2* gene. *Yd2* mapped on the long arm, 0.5 cM from the centromere, and in the mapping population of 106 F_2 individuals, perfectly cosegregated with the RFLP loci *XYlp*, and *Xwg889*. This map represents the first stage in a project to isolate the *Yd2* gene by a map-based approach. The isolation of *Yd2* could help to elucidate the molecular mechanism of the *Yd2*-mediated BYDV resistance, and may allow the production of BYDV resistant cereals by genetic transformation. The RFLP markers mapped closest to *Yd2* could also be useful in barley breeding, by enabling selection for both the presence of *Yd2* and the absence of agronomically undesirable traits known to be closely linked to *Yd2*.

Genetically Directed Representational Difference Analysis (GDRDA) is a technique based on subtractive hybridisation, which can be used to identify RFLP markers closely linked to a gene of interest. Two GDRDA experiments were performed with the intention of generating additional RFLP markers close to *Yd2*. However, the first experiment yielded RFLP probes that were not derived from the barley genome, while the second experiment

yielded probes that detected repetitive sequences. It was concluded that GDRDA is of limited use in generating further markers close to *Yd2*.

To isolate the *Yd2* gene by a map-based approach, a much larger mapping population will need to be analysed to genetically resolve markers tightly linked to *Yd2*. If the two morphological markers *uzu dwarf* and *white stripe,,j* flank *Yd2*, then they could assist in this task by enabling the visual identification of F_2 seedlings resulting from recombination close to *Yd2*. However, in this study, both morphological markers were found to be located distal to *Yd2*.Therefore, these two morphological markers can not be used together to facilitate high resolution genetic mapping of the *Yd2* locus.

It may be possible to use large-insert genomic DNA clones from the relatively small genome of rice to generate further RFLP markers close to the *Yd2* gene in barley, provided that the order of orthologous sequences in barley and rice is conserved close to the *Yd2* locus. To assess the feasibility of this approach, RFLP probes used to identify loci close to *Yd2* were mapped in rice using a segregating rice F_2 population. Five of the RFLP loci mapped together and in the same order as RFLP loci mapped close to *Yd2* in barley using the same probes. By comparing the location of RFLPs mapped by other researchers in rice using probes mapped close to *Yd2*, the region of conserved linkage between rice and the *Yd2* region was tentatively identified as the central portion of rice chromosome 1. The collinearity shown by orthologous sequences in barley and rice indicated that it may indeed be possible to use rice to assist in generating RFLP markers close to *Yd2*.

Of all the cereals, rice is the most amenable to map-based gene isolation, due to its small genome, well developed physical and genetic maps, and its ability to be genetically transformed with high efficiency. If a BYDV resistance gene that is orthologous to *Yd2* could be identified in rice, this gene could be isolated with relative ease, and then used to identify barley cDNA clones corresponding to *Yd2* gene by virtue of the sequence homology expected between these genes. To test if a BYDV resistance gene from an Italian rice line is orthologous to *Yd2*, recombinant-inbred rice lines previously characterised for this gene were

analysed using probes mapped close to *Yd2* in barley. No genetic linkage was detected between the RFLP loci and the BYDV resistance gene, indicating that the gene is unlikely to be orthologous to *Yd2*.

BYDV resistance alleles at the *Yd2* locus which are of a non-Ethiopian origin may show interesting differences to Ethiopian *Yd2* resistance alleles. To identify barleys which may contain resistance alleles of *Yd2*, ten BYDV resistant barleys not known to contain *Yd2* were assessed for their resistance to the PAV_{adel} isolate of BYDV in the glasshouse. CI 1179, Rojo, Perry, Hannchen, Post and CI 4228 were found to be the most resistant under these conditions, and were analysed further. If the resistance from these barleys is controlled by alleles of *Yd2*, RFLP markers close to *Yd2* will be expected to cosegregate with the resistance in F_2 families derived from crosses between these resistant barleys and the BYDV susceptible barleys Atlas and Proctor. RFLPs suitable for use in these allelism tests were identified using probes mapped close to *Yd2*. However, time did not permit the analysis of these F_2 populations.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I consent to this thesis being made available for photocopying and loan.

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