Theor Appl Genet (1996) 92: 858–864

N. C. Collins · N. G. Paltridge · C. M. Ford
R. H. Symons

The *Yd2* gene for barley yellow dwarf virus resistance maps close to the centromere on the long arm of barley chromosome 3

Received: 25 May 1995 / Accepted: 13 October 1995

Abstract Barley yellow dwarf luteovirus (BYDV) causes serious yield losses in all cereals worldwide. The *Yd2* gene from a number of Ethiopian barleys (*Hordeum vulgare* L.) has been the most effective means of providing resistance against BYDV in cultivated barley. Isolation of the *Yd2* gene will enable characterisation of the molecular basis of the *Yd2*-BYDV interaction. This paper describes the first stage in a project to isolate the gene: the construction of a detailed linkage map of the *Yd2* region. The map encompasses 27.6 centiMorgans (cM) of chromosome 3 and contains 19 RFLPs, 2 morphological marker loci, the centromere and *Yd2*. In the mapping population of 106 F_2 individuals, *Yd2* perfectly cosegregated with the RFLP loci *Xwg889* and *XYlp*, which were located on the long arm, 0.5 cM from the centromere. The two morphological marker loci, *uzu dwarf* and *white stripe„j*, both mapped distal to *Yd2*. The protein product of the gene at the *XYlp* locus will provide a convenient assay for the selection of *Yd2* during the breeding of BYDV-resistant barley varieties.

Key words Restriction fragment length polymorphism · Barley yellow dwarf · Resistance · *Yd2* · Morphological marker

Introduction

Barley yellow dwarf luteovirus (BYDV) infects all cereal types and causes significant yield losses worldwide. Annual yield reductions due to BYDV average 1–3%, although losses are tenfold greater in some seasons (Burnett 1984). Symptoms include stunting and leaf yellowing (Oswald and Houston 1953). BYDV encompasses a number of virus types within the Luteovirus group, which have been characterised on the basis of aphid vector specificity, serology, ultrastructural symptomology and molecular biology (Rochow 1979; Gill and Chong 1979; Miller et al. 1995).

The most effective means of minimising BYDV-induced yield loss in barley (*Hordeum vulgare* L.) has been the *Yd2* gene, which has been incorporated into at least seven barley cultivars (Schaller 1984). The *Yd2* gene was discovered as a result of a BYDV resistance screen of 6689 barley accessions from a worldwide collection, in which over 100 Ethiopian accessions showed the mildest BYDV symptoms (Schaller et al. 1963). In all of the Ethiopian lines analysed genetically, the resistance was shown to be due to a single locus (Schaller 1964; Rasmussen and Schaller 1959; Damsteegt and Bruehl 1964). Further genetic studies by Catherall et al. (1970) demonstrated the existence of a number of *Yd2* resistance alleles. In these studies, *Yd2* alleles from different Ethiopian accessions expressed different degrees of resistance and dominance when bred into common genetic backgrounds. The effectiveness of the *Yd2* gene also varies against different types and isolates of BYDV. For example, all BYDV-PAV isolates, but not all isolates of BYDV-RPV or BYDV-MAV, appear to be affected by *Yd2* (Banks et al. 1992; Skaria et al. 1985; Herrera and Plumb 1989). ELISA studies have shown that *Yd2* significantly reduces the rate of virus accumulation of affected BYDV types in plant tissue (Banks et al. 1992; Skaria et al. 1985). However, the mechanism by which *Yd2* limits BYDV replication remains to be elucidated.

In our laboratory, we aim to isolate the *Yd2* gene from barley in order to characterise the molecular basis of the BYDV-*Yd2* interaction. As there is no known way of identifying the *Yd2* protein product, we adopted a map-based approach to isolating *Yd2*. Schaller et al. (1964) had already located *Yd2* to the long arm of barley chromosome 3 using morphological markers. In this study, we have created a more detailed map of the *Yd2* region of barley chromosome 3, as a first step toward isolating the *Yd2* gene. The map described here contains 19 restriction fragment

Communicated by G. E. Hart

N. C. Collins · N. G. Paltridge · C. M. Ford · R. H. Symons (✉)
Department of Plant Science, Waite Institute,
University of Adelaide, Glen Osmond, S.A. 5064, Australia

length polymorphism (RFLP) loci mapped using clones obtained from a wide range of sources, two morphological markers, the centromere and *Yd2*.

Materials and methods

Sources of plant lines, virus and aphids

Seeds of the barley lines 'Shannon', 'Proctor' and 'CI 3208-1' were kindly provided by Wayne Vertigan of the Tasmanian Department of Agriculture. Rafiq Islam of the University of Adelaide generously supplied seed of 'Chinese Spring' wheat (*Triticum aestivum* L.), 'Betzes' barley and the 'Chinese Spring' wheat – 'Betzes' barley disomic and ditelosomic addition lines (Islam et al. 1981; Islam 1983). The mutant barley line OUM231 was obtained through the Barley Germplasm Centre at Okayama University, Japan. Andrew Barr, formerly of the South Australian Research and Development Institute, generously provided the New Zealand Cape oats (*Avena sativa* L.). The BYDV-PAV isolate and *Rhopalosiphum padi* (L.) aphids were originally obtained from Monique Henry, formerly of the University of Adelaide.

RFLP clones

Seventy-eight RFLP clones known to hybridise to loci on Triticeae linkage group 3 were obtained from a wide range of sources and used as probes to identify RFLP loci closely linked to *Yd2*. The Australian Triticeae Mapping Initiative RFLP clone collection at the University of Sydney supplied 71 clones representing a range of cDNAs and genomic DNAs derived from wheat, barley, *Triticum tauschii* (Coss.) and oat (Devos et al. 1992; Kleinhofs et al. 1993; Liu and Tsuniwaki 1991; Heun et al. 1991; Gill et al. 1991). Other clones obtained for screening were AWBma15, AWBma18 and AWBma20; AWJa8 and AWJa47; and AWPh7d and AWPhA12a, which were developed by P. Murphy, L. H. Ji and U. Baumann, respectively, in the laboratory of Peter Langridge at the University of Adelaide. The AWBma clones are cDNAs derived from barley roots, the AWJa clones are wheat anther cDNAs and AWPh clones are cDNAs from *Phalaris coerulescens* (Desf.) pollen.

A protein previously identified as a potential marker for the *Yd2* gene (Holloway and Heath 1992) was purified and partially sequenced to enable the design of a pair of DNA primers for the polymerase chain reaction (PCR) amplification of a portion of the corresponding gene (manuscript in preparation). Beginning with total cDNA derived from coleoptiles of the barley variety 'Shannon', we amplified and cloned a fragment of approximately 670 bp. The resulting '*Yd2*-linked protein (YLP)' cDNA clone was used as an RFLP probe to determine the position of the protein marker gene relative to *Yd2*.

RFLP clones developed by the laboratory of Liu and Tsunewaki (1991) and the RFLP loci they detected have the prefixes Tag and *Xglk*, respectively. The name of each other RFLP locus in this study is italicised and consists of an '*X*' prefix and, in lower-case, the name of the RFLP clone that was used to detect it.

Preparation of [^{32}P]-labelled RFLP probes

Cloned DNA inserts were PCR-amplified from plasmid minipreparations using M13 forward and reverse sequencing primers or primers complementary to the SP6 and T7 RNA polymerase promoters. Alternatively, inserts were excised from plasmids by restriction digestion. Inserts were purified from agarose gel slices using the Bresa-Clean kit (Bresatec), and approximately 20 ng of the DNA was used with 30 μCi of α-[^{32}P]-dCTP in the synthesis of probes by random priming, essentially as described by Feinberg and Vogelstein (1983). Probes were diluted to 150 μl in 10 m*M* TRIS-HCl, 1 m*M* EDTA, 0.1 *M* NaCl, pH 8.0, and unincorporated dNTPs removed using spin columns filled with Biogel P-10 (Pharmacia).

Plant genomic DNA preparation

The method for genomic plant DNA preparation was based on the protocol used by Guidet et al. (1991), as modified by Peter Langridge (unpublished). Leaf tissue was placed inside 2-ml snap-top Eppendorf tubes and frozen by dropping the tubes into liquid nitrogen. Frozen tissue was pulverised with a spatula to make 0.8 ml of powder, stirred with 0.75 ml of extraction buffer (1% sarkosyl, 0.1 *M* TRIS-HCl, 0.1 *M* NaCl, 10 m*M* EDTA, pH 8.0) and extracted with 0.75 ml of phenol:chloroform:isoamyl alcohol (25:24:1; the phenol was equilibrated to pH 8.0 with 0.1 *M* TRIS-HCl, pH 8.0 prior to use). The aqueous phase was re-extracted with 0.8 ml of chloroform, and the nucleic acid precipitated by the addition of 0.1 volume of 3.0 *M* sodium acetate, pH 5.2, followed by 1.0 volume of isopropanol. After centrifugation, nucleic acid pellets were washed in 1 ml 70% ethanol at room temperature for 15 min on an rotator, recovered by centrifugation, dried completely and resuspended in 45 μl of 5 m*M* TRIS-HCl, 0.05 m*M* EDTA, pH 8.0, containing 40 μg/ml pancreatic RNase A (Sigma).

DNA restriction, electrophoresis and Southern hybridisation analysis

Plant genomic DNA was digested with a range of restriction enzymes according to the suppliers of the enzymes (Bresatec; Promega; Boehringer). Approximately 3 μg of each restricted DNA sample (7 μl of the original DNA preparation) was electrophoresed in 0.9–1.3% agarose gels and transferred to Hybond N+ nylon membrane (Amersham) by Southern blotting using the protocols provided with the membrane.

The prehybridisation and hybridisation procedure used was based on the protocol supplied with the membrane. Following hybridisation, membranes were washed (1) three times for 5 min each in 2×SSC, 0.1% SDS at room temperature, and then (2) two times for 10 min each in 0.2×SSC, 0.1% SDS at 65°C. Two final washes of 10 min in 1×SSC, 0.1% SDS at 55°C were used instead of step (2) when probes derived from non-barley species were used. Autoradiography was performed with Konica X-ray film at –80°C with an intensifying screen for 1–5 days.

RFLP analysis

Each RFLP clone was used to probe membranes containing DNA from the barley varieties 'Shannon' and 'Proctor' that had been cut separately with a range of restriction enzymes. Clones that showed RFLPs between the two varieties were subsequently mapped using an F_2 family derived from a single 'Proctor' (non-*Yd2*)×'Shannon' (plus-*Yd2*) cross.

Southern banding patterns of the F_2 individuals were determined by probing membranes containing restricted DNA from the F_2 individuals or pooled DNA of their progeny. A population of 50 individuals was initially scored for all markers, and a further 56 plants were then characterised for the 10 markers found to be closest to *Yd2* (*Xglk223* to *Xpsr116* and *Xbcd263*). RFLP loci were mapped using the computer program MAPMAKER (Lander et al. 1987), and the Kosambi Mapping Function (Kosambi 1944) was chosen to convert recombination fraction values to centiMorgans (cM).

Wheat-barley disomic and ditelosomic addition lines (Islam et al. 1981; Islam 1983) were used to verify the chromosome 3 location of RFLP loci and to locate each locus to the long or the short arm of chromosome 3.

Morphological markers

The mutant barley line OUM231 was originally generated by inducing the *white stripe„j* mutation in 'Akashinriki', a Japanese *uzu dwarf* variety (Konishi et al. 1984). In temperatures of less than 20°C, individuals homozygous for the recessive *white stripe„j* mutation produce white leaves, while the recessive *uzu dwarf* mutation has many effects, including halving coleoptile length (Takahashi 1951). The

Ethiopian barley CI 3208-1 has normal length coleoptiles and green leaves (and was the source of *Yd2* used in the breeding of the BYDV-resistant cultivar 'Shannon').

F_2 seeds from a cross between OUM231 and CI 3208-1 were germinated on perlite at 4°C for 4 weeks with normal room lighting. After 4 weeks, seedlings were scored for leaf colour and coleoptile length, and a number of short-green and long-white recombinants were transferred to soil and grown in the glasshouse. The selected recombinants were analysed with RFLP probes in order to determine the positions of *uzu dwarf* and *white stripe„j* relative to the RFLP markers.

BYDV resistance assays

The BYDV-PAV virus was maintained in the oat cultivar 'New Zealand Cape' and transferred by means of *Rhopalosiphum padi* aphids using virus acquisition and inoculation periods of 2 days each. The *Yd2* genotypes of F_2 individuals from the 'Proctor' (non-*Yd2*)×'Shannon' (plus-*Yd2*) mapping population were determined by infecting 12–30 F_3 or F_4 progeny of each F_2 individual with BYDV-PAV, a type of BYDV known to be affected by *Yd2* (Scaria et al. 1985; Herrera and Plumb 1989). Seeds were sown in 6-inch pots (four seeds per pot), and 6 days later 30–50 viruliferous aphids were transferred to each seedling. Aphids were allowed to feed for 2 days and then killed by spraying with dimethoate (Rogor; Hortico, Australia). Infected plants were grown in a glasshouse at 20°–25°C or outside during May to September. By comparing the degree of stunting of individuals in the infected families with the stunting of infected control plants of 'Shannon' and 'Proctor', we were able to classify the families as resistant, susceptible or segregating for resistance, thereby defining the genotypes of the corresponding F_2 individuals as *Yd2/Yd2*, –/– or *Yd2*/–, respectively. In addition, RFLP analysis of infected plants was used to help in interpreting the resistance assays by showing whether the variation in stunting observed within each family was due to genetic segregation or experimental variation.

Results

RFLP analysis

The *Yd2*-containing, BYDV-resistant barley cultivar 'Shannon' was bred by crossing the Ethiopian barley CI 3208-1 to the BYDV susceptible cultivar 'Proctor', followed by 4 backcrosses to 'Proctor' (Symes 1979; Vertigan 1980). As such, 'Shannon' is expected to possess a segment of chromosome 3 from CI 3208-1 containing the *Yd2* gene in a genetic background derived principally from 'Proctor'. RFLP probes were screened in order to identify restriction fragments that were polymorphic for 'Shannon' and 'Proctor' and therefore likely to be closely linked to *Yd2*. Of the 79 RFLP probes screened, 22 identified polymorphisms suitable for mapping (Table 1).

Segregation analysis of the RFLPs in the 'Proctor' (non-*Yd2*)×'Shannon' (plus-*Yd2*) F_2 population placed 20 of the polymorphisms within a single, 27.6 cM-long linkage group. Only the loci detected by the probes AWBma20 and CDO419 yielded maximum LOD scores smaller than 3.0, and these were not included in this cluster. The main RFLP linkage group represents the *Yd2*-containing portion of chromosome 3 in 'Shannon' that originated from CI 3208-1 and formed a framework upon which *Yd2* and the morphological markers were later placed.

The wheat cDNA probe PSR116 and the barley cDNA probe BCD263 gave identical hybridisation patterns for a number of barley varieties and restriction enzymes and detected loci that perfectly cosegregated in the 'Proctor'×'Shannon' F_2 population. It is therefore likely that these clones originated from orthologous genes. Eight other RFLPs, indicated in the complete map (Fig. 2), perfectly cosegregated with 1 or more loci in the mapping population. These 8 RFLP loci are distinct, as the probes showed unique hybridisation patterns.

Most of the probes detected only 1 locus in barley as they hybridised to a single restriction fragment for 1 or more of the restriction enzymes used. Eight of the probes may detect multiple loci in barley as they hybridised to more than 1 restriction fragment for each of the restriction enzymes tested (Table 1).

Chromosome and chromosome arm designations

The chromosome and chromosome arm locations of 10 RFLP loci were determined using 'Chinese Spring' wheat – 'Betzes' barley disomic and ditelosomic addition lines (Islam et al. 1981; Islam 1983; Table 1). For the probes that detected multiple restriction fragments in barley, care was taken to base locus positions on restriction fragments corresponding to those mapped (i.e. fragments of similar size and hybridisation intensity) using the same restriction enzymes that were used for mapping. The relevant restriction fragments identified by each of the 10 probes in 'Betzes' barley were also present in the 3H disomic addition line, confirming that the main RFLP linkage group was on barley chromosome 3. The absence of the restriction fragments from either the 3HS or 3HL ditelosomic addition lines allowed localisation of 7 loci to the long arm, and 3 to the short arm. The RFLP locus *Xbcd127* and the *Xbcd134-XksuA3-Xawbm7d* locus group perfectly cosegregated in the mapping population and were found to be on the short and the long arm of chromosome 3, respectively. This enabled the centromere to be located between *Xbcd127* and *Xbcd134-XksuA3-Xawbm7d*.

Morphological marker loci

Of 390 OUM231 × CI 3208-1 F_2 seedlings scored for leaf colour and coleoptile length, the numbers of long-green, short-white, long-white and short-green seedlings obtained were 257, 99, 16 and 18, respectively. When the product ratio and tables of Stevens (1939) were used, these figures gave a recombination fraction of 8.6±1.5% between *uzu dwarf* and *white stripe„j*, which is consistent with the value of 10.0±1.1% observed by Konishi et al. (1984) for recombination between these two markers.

To determine the positions of the morphological markers, we analyzed a sample of 13 recombinants for the *uzu dwarf – white stripe„j* interval grown from 6 long-white and 7 short-green seedlings with 9 RFLP probes found to detect polymorphism between OUM231 and CI 3208-1

Table 1 RFLP clone data. The table includes the restriction enzymes used for mapping in the 'Proctor'×'Shannon' and OUM231×CI 3208-1 F_2 populations, the maximum number of loci detected by each probe and the chromosome 3 arm designations of a number of the corresponding loci

Clone name	Clone type	Source species	Source laboratory [a]	Informative restriction enzyme		Maximum loci number[b]	Arm[c]
				Proctor × Shannon	OUM231 × CI 3208-1		
AWBma15	cDNA	*Triticum aestivum*	g	*Hin*dIII	n. m.[d]	5	3HS
Tag223	Genomic DNA	*Triticum tauschii*	c	*Dra*I	n. m.	2	3HS
BCD127	cDNA	*Hordeum vulgare*	a	*Eco*RI	*Eco*RI	1	3HS
BCD134	cDNA	*Hordeum vulgare*	a	*Eco*RI	*Hae*III	1	3HL
KSUA3	Genomic DNA	*Triticum tauschii*	e	*Bam*HI	n. m.	6	3HL
AWPh7d	cDNA	*Phalaris coerulescens*	g	*Bam*H1	n. m.	1	3HL
YLP	cDNA	*Hordeum vulgare*	f	*Hae*III	n. m.	1	3HL
WG889	Genomic DNA	*Triticum aestivum*	a	*Dra*I	n. m.	3	3HL
BCD828	cDNA	*Hordeum vulgare*	a	*Bam*HI	*Bam*HI	2	3HL
ABG396	Genomic DNA	*Hordeum vulgare*	d	*Xba*I	n. m.	1	n. d.
PSR116	cDNA	*Triticum aestivum*	b	*Xba*I	n. m.	1	n. d.
BCD263	cDNA	*Hordeum vulgare*	a	*Xba*I	n. m.	1	n. d.
ABG703	Genomic DNA	*Hordeum vulgare*	d	*Hin*dIII	n. m.	2	3HL
Tag80	Genomic DNA	*Triticum aestivum*	c	*Xba*I	*Xba*I	1	n. d.
BCD809	cDNA	*Hordeum vulgare*	a	*Hin*dIII	n. m.	1	n. d.
PSR156	cDNA	*Triticum aestivum*	b	*Xba*I	*Xba*I	1	n. d.
WG178	Genomic DNA	*Triticum aestivum*	a	*Eco*RI	*Eco*RI	1	n. d.
PSR543	Genomic DNA	*Triticum aestivum*	b	*Xba*I	*Xba*I	1	n. d.
KSUG59	Genomic DNA	*Triticum tauschii*	e	*Dra*I	*Dra*I	1	n. d.
CDO718	cDNA	*Avena sativa*	a	*Xba*I	*Xba*I	2	n. d.
CDO419	cDNA	*Avena sativa*	a	*Hin*dIII	n. m.	6	n. d.
AWBma20	cDNA	*Triticum aestivum*	g	*Xba*I	n. m.	1	n. d.

[a] a, M. Sorrells, Cornell University; b, M. D. Gale, John Innes Centre, Norwich; c, K. Tsunewaki, Kyoto University; d, A. Kleinhofs, Washington State University; e, B. Gill, Kansas State University; f, C. Ford, University of Adelaide; g, P. Langridge, University of Adelaide

[b] Maximum number of loci detected in barley, as defined by the minimum number of restriction fragments detected for any one restriction enzyme

[c] 3HS, short arm of barley chromosome 3; 3HL, long arm of barley chromosome 3; n. d., arm location not determined

[d] n. m., not mapped in the OUM231×CI 3208-1 F_2 population

(Table 1). A comparison of the morphological marker genotypes of the recombinants with their RFLP genotypes showed that the morphological markers were located within the region spanned by the RFLP markers. Recombination events were detected on both sides of the morphological marker pair, as well as between the two morphological markers, thereby enabling *white stripe„j* to be located between *XksuG59* and *Xpsr543*, and *uzu dwarf* to be placed between *Xglk80* and *Xbcd828* (Fig. 1).

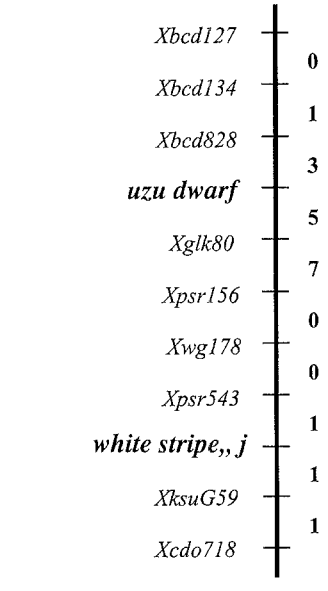

Fig. 1 Recombination events observed between the RFLP and morphological loci *uzu dwarf* and *white stripe„j* in 13 individuals from the OUM231× CI 3208-1 F_2 population. The numbers of recombination events observed in each interval are shown on the *right*

Resistance bioassays

Throughout all of the resistance assays, the numbers of aphids used to inoculate seedlings with BYDV were high enough to give an infection rate of 100%. Although the degree of stunting varied, almost all infected plants developed some yellowing. Those individuals lacking yellow colouration were analysed by dot blot hybridisation (not shown) and found to be infected.

Catherall et al. (1970) demonstrated that particular *Yd2* alleles could be codominant or recessive depending on the temperature at which infected plants were grown. In this study, susceptible and resistant individuals were observed in a 3:1 ratio in segregating families, showing that in the relatively cool growth conditions used, the *Yd2* allele from 'Shannon' was recessive. Upon infection, *–/–* and *–/Yd2* individuals typically developed half the fresh weight of *Yd2/Yd2* individuals 4 weeks after infection.

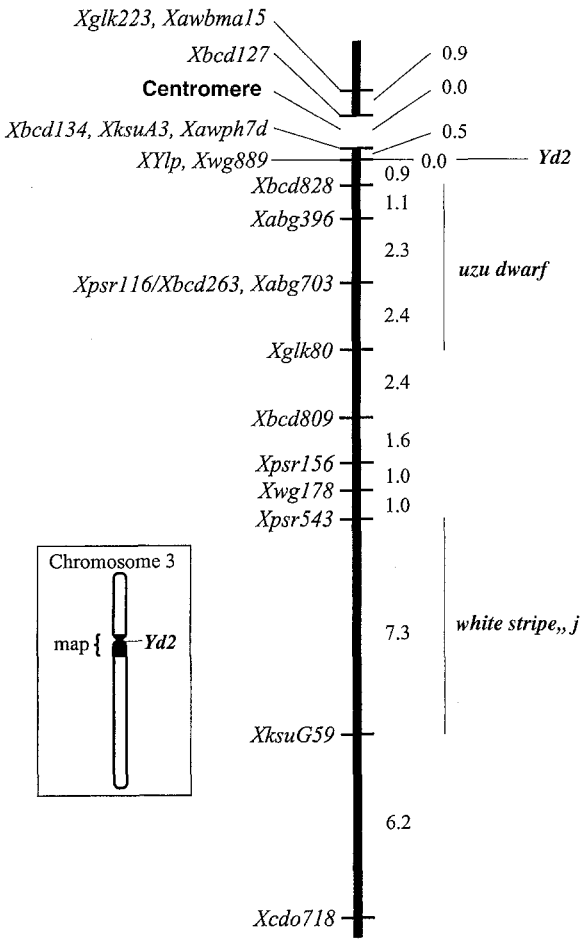

Fig. 2 Linkage map of the *Yd2* region of barley chromosome 3. RFLP loci separated by *commas* perfectly cosegregated in the mapping population. As *Xpsr116* and *Xbcd263* almost certainly represent orthologous loci (see text), they are presented as the one locus, *Xpsr116/Xbcd263*. Intervals to which the morphological markers *uzu dwarf* and *white stripe„j* were localised are shown by *vertical lines*. Genetic distances are shown in centiMorgans. The *gap* at the centromere separates cosegregating markers that were located to different chromosome arms. The *inset* shows the position and size of the map relative to the total genetic map of barley chromosome 3

The resistance assay was used to determine the *Yd2* genotypes of all of the individuals from the 'Proctor' (non-*Yd2*)×'Shannon' (plus-*Yd2*) mapping population that resulted from recombination in the region spanned by the RFLP loci. For each of these recombinants, an RFLP locus from the heterozygous region was used to confirm the *Yd2* status of the respective progeny families. In families that were homozygous for *Yd2* or the susceptibility allele, the small variation observed in the size of individuals had no correlation with individual RFLP genotype, and was attributed to experimental variation. In families that were segregating for *Yd2* (*Yd2/Yd2*), plants homozygous for the RFLP allele from the resistant parent 'Shannon' were much less stunted than the other (*Yd2/–* and *–/–*) plants in the family.

By comparing the *Yd2* genotypes of the F_2 recombinants with the F_2 RFLP data, we were abale to locate *Yd2* relative to the RFLP loci.

A genetic map of the *Yd2* region

The results obtained by mapping the RFLP loci, the two morphological markers, the centromere and *Yd2* are summarised in a single genetic map (Fig. 2). The *Yd2* gene could not be resolved with the RFLP loci *XYlp* and *Xwg889*, which were positioned 0.5 cM from the centromere on the long arm of chromosome 3. The morphological markers *uzu dwarf* and *white stripe„j* both mapped distal to *Yd2*. A comparison of the 27.6-cM-long map of the *Yd2* region with the complete 223-cM-long map of barley chromosome 3 constructed by Kleinhofs et al. (1993) shows that the region mapped in this exercise spans 12% of the total genetic length of the chromosome.

Discussion

In this paper we report the construction of a detailed linkage map of the region of barley chromosome 3 around the barley yellow dwarf virus resistance gene, *Yd2*. Within the mapping population of 106 individuals, no recombination was observed between the *Yd2* locus and RFLP loci *XYlp* and *Xwg889*, which were located 0.5 cM from the centromere. Although it is possible that these 3 loci are physically close, it is more likely that the failure to resolve them was due to their close proximity to the centromere, where the rate of recombination in cereal chromosomes is known to be relatively low (Moore et al. 1993, and references therein). In this study, the *Yd2* gene was found to be located between the centromere and the morphological marker *uzu dwarf* on barley chromosome 3. Using chromosome C-bands as genetic markers, Linde-Laursen (1982) obtained direct evidence for reduced recombination in this same interval by observing that the ratio of genetic to physical distance between *uzu dwarf* and the centromere was extremely small compared to the more distal part of the long arm of barley chromosome 3.

To order *Yd2*, *XYlp* and *Xwg889*, and to determine the genetic distances between them, we need to analyse many more recombinants for the region immediately surrounding *Yd2*. To make such an exercise feasible, it was originally thought that such recombinants could be visually identified at the early seedling stage using the morphological markers *uzu dwarf* and *white stripe„j*. *Yd2* had previously been placed between these two morphological marker loci (von Wettstein-Knowles 1990), although this placement was unreliable because it was obtained using data from two separate crosses (Konishi et al. 1984; Schaller et al. 1964). In this study, it was shown that both morphological loci were distal to *Yd2* and therefore of no use in increasing genetic resolution close to *Yd2*. An alternative strategy for identifying recombinants would be to

convert 2 RFLP markers closely flanking *Yd2* into PCR markers so that they could be scored more efficiently (Talbert et al. 1994).

A straightforward, map-based approach to isolating the *Yd2* gene is unlikely to succeed due to the large size of the barley genome. However, the identification and isolation of the gene in rice that is orthologous to *Yd2* may be possible due to the synteny between rice and other cereals (Kurata et al. 1994a; Ahn et al. 1993), the relatively small size of the rice genome (Bennett and Smith 1991), and the significant advances that have been made in the genetic and physical mapping of rice chromosomes (Kurata et al. 1994b; Causse et al. 1994; Umehara et al. 1993; Wang et al. 1995). We therefore intend to isolate the *Yd2* homologue from rice and then use it to identify a *Yd2* cDNA clone from barley. To begin this exercise, we are mapping RFLP clones from the rice map of Kurata et al. (1994b) relative to *Yd2* in barley to determine the precise location of the *Yd2* homologue in the rice genome.

The *XYlp* locus perfectly cosegregated with *Yd2* in this study and corresponds to a gene that encodes a protein which varies in isoelectric point between barley varieties with and without *Yd2* (Holloway and Heath 1992). A western blot assay for the detection of the protein isoforms within single barley plants, currently being developed in our laboratory, will therefore provide an ideal alternative to resistance assays for the selection of *Yd2* during the breeding of new BYDV resistant barley varieties. The protein assay will be much more convenient and reliable than resistance assays in the field which require progeny testing, the presence of virus and aphid vectors, cool sunny weather conditions and the absence of mineral deficiency symptoms which resemble, and are often confused with, BYDV symptoms (Burnett 1984).

Acknowledgments The authors are sincerely grateful to all people involved in supplying the biological materials and DNA clones necessary for this exercise. We especially thank Dr. P. Sharp and his staff at the Australian Triticeae Mapping Initiative (ATMI) RFLP clone collection for their continued and frequent support, and M. Sorrells, M. Gale, B. Gill, A. Kleinhofs, K. Tsunewaki, P. Murphy, U. Baumann and L. H. Ji for permission to use their clones. Technical assistance and advice provided by Kevin James, Angelo Karakousis and the late Peter Ellis are also greatly appreciated. We also thank Peter Langridge for his helpful advice during the study and for his assistance in the preparation of the manuscript. This study was funded by the Australian Research Council Special Research Centre for Basic and Applied Plant Molecular Biology.

References

Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

Banks PM, Waterhouse PM, Larkin PJ (1992) Pathogenicity of three RPV isolates of barley yellow dwarf virus on barley, wheat and wheat alien addition lines. Ann Appl Biol 121:305–314

Bennett MD, Smith JB (1991) Nuclear DNA amounts in angiosperms. Philos Trans R Soc London Ser B 334:309–345

Burnett PA (1984) vv. In: Barley yellow dwarf. A proceedings of a workshop. CIMMYT, Mexico, pp 6–13

Catherall PL, Jones AT, Hayes JD (1970) Inheritance and effectiveness of genes in barley that condition tolerance to barley yellow dwarf virus. Ann Appl Biol 65:153–161

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley D (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Damsteegt VD, Bruehl GW (1964) Inheritance of resistance in barley to barley yellow dwarf. Phytopathology 54:219–224

Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD (1992) RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gill CC, Chong J (1979) Cytopathological evidence for the division of barley yellow dwarf virus isolates into two subgroups. Virology 95:59–69

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Guidet F, Rogowsky P, Taylor C, Song W, Langridge P (1991) Cloning and characterisation of a new rye-specific repeated sequence. Genome 34:81–87

Herrera G, Plumb R (1989) The response of winter and spring barleys, with and without the *Yd2* gene, to 3 British isolates of BYDV. BYD Newsl 2:38–40

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Holloway PJ, Heath R (1992) Identification of polypeptide markers of barley yellow dwarf virus resistance and susceptibility genes in non-infected barley (*Hordeum vulgare*) plants. Theor Appl Genet 85:346–352

Islam AKMR (1983) Ditelosomic additions of barley chromosomes to wheat. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Maruzen, Kyoto, pp 233–238

Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161–174

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86: 705–712

Konishi T, Hayashi J, Moriya I, Takahashi R (1984) Inheritance and linkage studies in barley. VII. Location of six new mutant genes on chromosome 3. Ber Ohara Inst Landwirtsh Biol Okayama Univ 18:251–264

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kurata N, Moore G, Nagamura Y, Foote T, Yano M, Minobe Y, Gale M (1994a) Conservation of genome structure between rice and wheat. Bio/Technology 12:276–278

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang Z-X, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994b) A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for maps of experimental and natural populations. Genomics 1:174–181

Linde-Laursen I (1982) Linkage map of the long arm of barley chromosome 3 using C-bands and marker genes. Heredity 49: 27–35

Liu Y-G, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

Miller WA, Dinesh-Kumar SP, Paul CP (1995) Luteovirus gene expression. CRC Crit Rev Plant Sci 14:179–211

Moore G, Gale MD, Kurata N, Flavell RB (1993) Molecular analysis of small grain cereal genomes: current status and prospects. Bio/Technology 11:584–589

Oswald JW, Houston BR (1953) The yellow-dwarf virus of cereal crops. Phytopathology 43:128–136

Rasmusson DC, Schaller CW (1959) The inheritance of resistance in barley to the yellow-dwarf virus. Agron J 51:661–664

Rochow WF (1979) Field variants of barley yellow dwarf virus: detection and fluctuation during twenty years. Phytopathology 69:655–660

Schaller CW (1984) The genetics of resistance to barley yellow dwarf virus in barley. In: Barley yellow dwarf. A proceedings of a workshop. CIMMYT, Mexico, pp 93–99

Schaller CW, Rasmusson DC, Qualset CO (1963) Sources of resistance to the yellow dwarf virus in barley. Crop Sci 3:342–344

Schaller CW, Qualset CO, Rutger JN (1964) Inheritance and linkage of the *Yd2* gene conditioning resistance to the barley yellow dwarf virus disease in barley. Crop Sci 4:544–548

Skaria M, Lister RM, Foster JE, Shaner G (1985) Virus content as an index of symptomatic resistance to barley yellow dwarf virus in cereals. Phytopathology 75:212–216

Stevens WL (1939) Tables of the recombination fraction estimated from the product ratio. J Genet 39:171–180

Symes KJ (1979) Shannon. J Aust Inst Agric Sci 45:269

Takahashi R (1951) Studies on the classification and the geographical distribution of the Japanese barley varieties. II. Correlative inheritance of some quantitative characters with the ear types. Ber Ohara Inst Landwirtsch Forsch Okayama Univ 9:383–399

Talbert LE, Blake NK, Chee PW, Blake TK, Magyar GM (1994) Evaluation of "sequence-tagged-site" PCR products as molecular markers in wheat. Theor Appl Genet 87:789–794

Umehara Y, Tanoue H, Momma T, van Houten W, Yoshimura S, Wu J, Kurata N (1993) Selection of YAC clones for contig formation and gene isolation. Rice Genome 2/2:10

Vertigan WA (1980) Shannon: the first Tasmanian-bred barley. Tasmanian J Agric 51:33–35

von Wettstein-Knowles P (1990) Barley (*Hordeum vulgare*) 2N=14. In: Genetic maps, 5th edn. Cold Spring Harbour Laboratory Press, Cold Spring Harbor, N.Y.

Wang G-L, Holsten TE, Song W-Y, Wang H-P, Ronald PC (1995) Construction of a rice bacterial artificial chromosome library and identification of clones linked to the *Xa-21* desease resistance locus. Plant J 7:525–533