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Development of YLM, a codominant PCR marker closely linked to the *Yd2* gene for resistance to barley yellow dwarf disease

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Abstract The *Yd2* gene in barley provides protection against barley yellow dwarf luteovirus (BYDV), the most economically devastating virus of cereals worldwide. Because resistance assays to identify *Yd2*-containing individuals from breeding populations are often difficult, we have developed a closely linked, codominant PCR-based marker for *Yd2* using AFLP marker technology. The marker, designated YLM, can be amplified from barley genomic DNA prepared using a rapid and simple extraction procedure and, in a survey of more than 100 barley genotypes, was found to be polymorphic between most *Yd2* and non-*Yd2* lines. The YLM therefore shows excellent potential as a tool for selecting *Yd2*-carrying segregants in barley breeding programmes.

Key words AFLP · Barley yellow dwarf virus · *Hordeum vulgare* · PCR marker · *Yd2*

Introduction

Barley yellow dwarf (BYD) disease is caused by a suite of aphid-transmitted *Luteoviruses* which are collectively referred to as barley yellow dwarf luteovirus (BYDV). Recognised as the most economically damaging viral pathogen of cereals worldwide (Burnett et al. 1995), BYDV affects all major cereal crops and is found wherever cereals are grown (Lister and Ranieri 1995). Annual yield losses attributed to the disease average 1–3%, but are up to tenfold greater in exceptional years (Burnett 1984). Whilst cultural, chemical and biological methods can be used to limit such losses (Plumb and Johnstone 1995), the use of BYD-resistant germplasm is generally regarded as the cheapest and most effective means of controlling damage caused by the virus.

In barley (*Hordeum vulgare* L.), *Yd2* has been the only BYD-resistance gene used in breeding programmes. The gene was originally discovered in a number of Ethiopian genotypes (Schaller et al. 1963, 1964; Rasmusson and Schaller 1959; Damsteegt and Bruehl 1964) and confers tolerance to the PAV and MAV isolates of BYDV (Skaria et al. 1985; Ranieri et al. 1993), which are the most prevalent isolates in cereal growing regions (Lister and Ranieri 1995). To date, *Yd2* has been incorporated into at least 17 barley cultivars worldwide and has provided useful levels of protection for over 20 years (Burnett et al. 1995).

A major constraint to the breeding of BYD-resistant barley has always been the inconvenience and unreliability of the biological assay for resistance. Such assays require the appropriate isolate of the virus to be carried by aphid vectors of the appropriate species. In addition, symptoms of BYDV infection are easily confused with damage due to environmental stresses such as frost, waterlogging and nitrogen deficiency (Conti et al. 1990). Furthermore, distinguishing *Yd2*-containing individuals from non-*Yd2* individuals is often hampered by variation in the effectiveness of the gene in different genetic backgrounds and growth conditions, and by the fact that the *Yd2*-mediated resistance is often expressed in an incompletely dominant or recessive manner (Rasmusson and Schaller 1959; Jones and Catherall 1970; Catherall et al. 1970). During breeding programmes, a molecular marker tightly linked to *Yd2*

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could therefore provide a more convenient means of selecting for the gene than BYD-resistance assays.

Earlier work (Holloway and Heath 1992) reported the development of an immunologically based assay for a protein showing *Yd2*-associated variation in iso-electric point and, in addition, several *Yd2*-linked restriction fragment length polymorphism (RFLP) markers have been identified (Collins et al. 1996). However, both marker systems are time-consuming and expensive to assay and are impractical for routine use in breeding programmes. Molecular markers based on the polymerase chain reaction (PCR) (Saiki et al. 1985) offer a favourable alternative; genotype analysis is fast and economical and can be conducted at the single-leaf stage, as only small amounts of genomic DNA are required.

We describe here the process by which amplified fragment length polymorphism (AFLP) analysis (Vos et al. 1995) was used to develop a PCR marker for *Yd2* which is simple to use and codominant. The marker is designated YLM, and its potential for use in barley breeding is demonstrated. During the present work, another PCR marker for *Yd2* was developed in this laboratory (Ford et al. 1998) from the protein marker (Ylp) first identified by Holloway and Heath (1992). The relative advantages of the PCR markers developed at the *Ylp* and YLM loci are also discussed in this article.

Materials and methods

Plant lines

Barley seed was provided by Wayne Vertigan of the Tasmanian Department of Agriculture (cvs. 'Shannon' and 'Proctor') or was obtained from the Australian Winter Cereal Collection at Tamworth, NSW (cvs. 'Atlas 68', 'Prato', 'Sutter', 'Vixen' and 'UC 566') or the barley seed collection at the University of Adelaide (all other lines).

DNA pools and genetic mapping

BYD-susceptible and -resistant DNA pools, and other genetic material used for AFLP analysis (see Results), were derived from F_2 individuals from the 'Proctor' (non-*Yd2*) × 'Shannon' (plus-*Yd2*) mapping population as previously described (Collins et al. 1996). Initial segregation analyses were conducted using this population. In addition, the barley cultivars 'Atlas' (non-*Yd2*) and 'Atlas 68' (plus-*Yd2*) (Schaller and Chim 1969a) were used to construct a new F_2 mapping population for high-resolution mapping of the *Yd2* region (Paltridge et al., manuscript in preparation). Detailed information on the genetic linkage between the YLM and *Yd2* loci was obtained from this mapping population.

RFLP clones used were kindly provided by Prof. M. Gale, John Innes Centre (PSR116), Prof. K. Tsunewaki, Kyoto University (Tag223) and Prof. M. Sorrells, Cornell University (BCD134 and BCD828). The RFLP locus detected by the RFLP clone Tag223 is represented as *Xglk223* in this study. The name of each other RFLP locus is italicised and comprises an '*X*' prefix and, in lowercase, the name of the RFLP clone that was used to detect it.

AFLP analysis and cloning of AFLP bands

AFLP analysis was carried out as described by Thomas et al. (1995) and Brigneti et al. (1997), using the same *Pst* and *Mse* adaptors and complementary PCR primers as Thomas et al. (1995). The *Pst* and *Mse* primers contained two and three selective nucleotides, respectively, and are designated $P + NN$ and $M + NNN$. AFLP bands of interest were cloned and sequenced as described by Brigneti et al. (1997).

PCR analysis of barley DNA for YLM genotype

Template for PCR amplification comprised approximately 100 ng of DNA prepared using the method of Collins et al. (1996) or, alternatively, was prepared using the simple NaOH extraction method of Wang et al. (1993). In the latter procedure, a tissue homogenate was prepared by grinding 5 mg of leaf tissue in 50 µl of 0.5 *M* NaOH. One microlitre of the homogenate diluted tenfold in 0.1 *M* TRIS-HCl pH 8.0 was then used in PCR reactions.

Oligonucleotide primers were synthesised on an Applied Biosystems (ABI) Model 392 DNA/RNA synthesiser and conditions for PCR amplification were based on those described by Penner et al. (1996); 0.63 µ*M* each primer, 1.5 m*M* $MgCl_2$, 0.2 m*M* each dNTP, 1 × *Taq* DNA polymerase activity buffer [67 m*M* TRIS-HCl pH 8.8 at 25°C, 16.6 m*M* $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg/ml Gelatin] and 1 U of *Taq* DNA polymerase (Biotech International). Reactions were performed with oil overlay in a 20 µl volume, and thermal cycling comprised 40 cycles of 30 s at 94°C, 30 s at 58°C and 1 min at 72°C in an MJ Minicycler™ or a PTC-100™ thermal cycler (MJ Research, USA). Electrophoretic analyses of the PCR products were performed using 0.75% agarose/2.25% NuSieve GTG agarose (FMC Bioproducts) gels and 1 × TBE electrophoresis buffer.

Results

AFLP in barley genome analysis

Markers closely linked to *Yd2* were identified using a strategy developed by Giovannoni et al. (1991) in which DNA pools homozygous for opposing alleles of a targeted chromosomal interval are constructed from an existing mapping population. Using RFLP analysis and BYD-resistance bioassays, Collins et al. (1996) showed that in a mapping population of 106 F_2 individuals from the 'Proctor' × 'Shannon' cross, the RFLP loci *Xglk223* and *Xpsr116* flank *Yd2* by 5.7 cM. In the present work, F_2 individuals which were homozygous for 'Proctor' (non-*Yd2*) or 'Shannon' (plus-*Yd2*) DNA at both RFLP loci and at the *Yd2* locus were selected from this mapping population and used to construct susceptible and resistant DNA pools (Fig. 1a). Susceptible and resistant pools were constructed from 7 and 6 F_2 individuals, respectively. The use of 72 different combinations of $P + NN$ and $M + NNN$ primers (each amplifying, on average, 70 visible DNA bands) resulted in the amplification of approximately 5000 DNA bands from each pool. Thirty-seven such bands were polymorphic between the two pools.

To establish which of these AFLPs were likely to exhibit the tightest genetic linkage to *Yd2*, we then used primer combinations revealing polymorphisms to

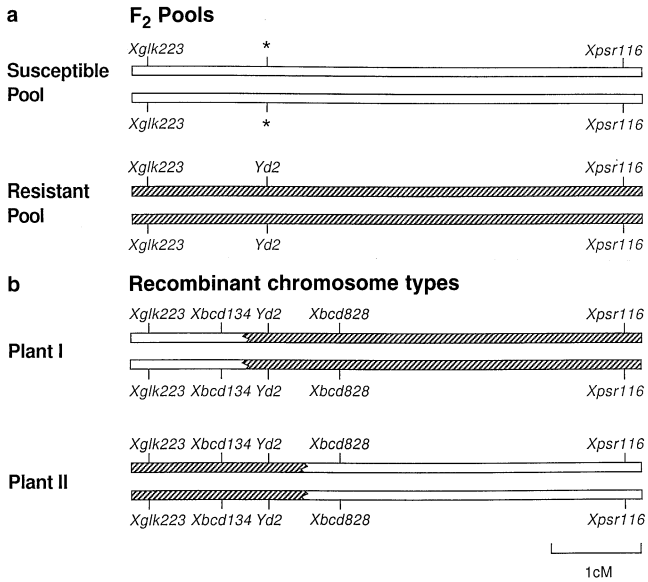

Fig. 1a, b Schematic representation of chromosome types used for AFLP analysis. Chromosomal regions derived from 'Proctor' (non-*Yd2*) and 'Shannon' (plus-*Yd2*) are shown as *open* and *crosshatched bars*, respectively, and the BYD-susceptible allele of the *Yd2* gene is represented by an *asterisk*. **a** BYD-susceptible and -resistant DNA pools, **b** recombinant chromosome types used to identify AFLP markers most closely linked to *Yd2*

screen DNA from the 2 plants represented schematically in Fig. 1b. Previous RFLP analysis of the 'Proctor' × 'Shannon' F_2 mapping population with the proximal RFLP marker *Xbcd134* and the distal RFLP marker *Xbcd828* had identified F_2 individuals resulting from the closest recombination events to either side of *Yd2* (Collins et al. 1996). RFLP analysis of the F_3 progeny of 2 such plants was used to identify individuals homozygous for these recombinant chromosomes (plants I and II). The only genetic material common to both plants in the *Yd2* region was the resistance-associated DNA within the interval spanned by these closest proximal and distal cross-over points. AFLP bands specific to the susceptible pool which were absent from plants I and II, or AFLP bands specific to the resistant pool which were amplifiable from plants I and II, were therefore expected to map closest to *Yd2*.

Three AFLP bands fulfilled either of these criteria. The first of these, identified by the primer pair *P* + *GC*/*M* + *GGA*, was amplifiable from the resistant but not susceptible pool and was present in both plants I and II. Though a potentially useful marker for *Yd2*, this AFLP band has not yet been taken on for further analysis. Interestingly, the other 2 AFLP bands were amplified by the *P* + *GC*/*M* + *AGG* primer pair, and exhibited only slight differences in mobility on polyacrylamide gels: 1 of the 2 bands, designated YLM-S, was amplified from the susceptible pool only and was absent from both plants I and II; the other, designated YLM-R, was amplified from the resistant pool only and was present in plants I and II. Cloning and sequencing of the fragments YLM-S and -R revealed them to be of identical sequence, except for 2 single base differences and an 11 base-pair insertion/deletion (Fig. 2). The 2 fragments therefore appeared to be allelic, and provided an appropriate starting point for the development of a codominant PCR marker for *Yd2*. Genbank Accession Numbers for the YLM-S and YLM-R DNA sequences are AF030407 and AF030408, respectively.

Development of a PCR marker for *Yd2*

A system was then developed for the PCR amplification of the 2 putatively allelic fragments from genomic DNA. A single pair of PCR primers was designed (YLMF and YLMR, Fig. 2) which span the polymorphic region of the YLM locus in 'Proctor' and 'Shannon' by 101 and 90 nt, respectively (Fig. 2); fragments were kept deliberately short so that the size difference between alleles would be proportionally greater. When used on 'Proctor' and 'Shannon' DNA template, single fragments of the expected sizes (101 and 90 nt) were amplified (Fig. 3a, lanes 1 and 2, respectively). The marker was also tested on 'Atlas' (non-*Yd2*) and 'Atlas 68' (plus-*Yd2*), the parents of a second F_2 mapping population developed in this laboratory (Paltridge et al., manuscript in preparation). The same polymorphic fragments were amplified from 'Atlas' and 'Atlas 68' (Fig. 3a, lanes 3 and 4, respectively) as from 'Proctor' and 'Shannon'. Neither fragment was amplified in the control reaction where genomic DNA was omitted from the mixture (Fig. 3a, lane C).

Initially, the DNA used as template for PCR amplification was prepared using the method of Collins et al. (1996). However, to simplify the protocol for large-scale application, we conducted test amplifications on DNA prepared using the simple NaOH extraction method of Wang et al. (1993). Optimal amplification of the bands YLM-S and -R was found to occur when 1 µl of a tenfold dilution of homogenised tissue was used as PCR template. Using this simplified protocol, we conducted test amplifications on 'Atlas' and 'Atlas 68' and on 12 F_2 segregants from the 'Atlas' × 'Atlas 68' mapping population (Fig. 3b). The desired sequences were amplified as efficiently as from template prepared by the method of Collins et al. (1996). As expected, the YLM genotype was closely associated with the *Yd2* genotype, determined using progeny resistance assays and represented in Fig. 3b as homozygous susceptible S (-/-), heterozygous H (*Yd2*/-), or homozygous resistant R (*Yd2*/*Yd2*). Significantly, both bands were amplified with equal intensity from *Yd2*-heterozygous template.

Segregation analysis

Initially, the YLM genotype was determined in the F_2 progeny from the 'Proctor' × 'Shannon' mapping

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YLM-S (Proctor)   5'-CTGCAGGCGC  GTTGAGGAGA  TGTTACCGTG  AATCGCATCA  TCGGGGCTCG  TCTATGGCGG
YLM-R (Shannon)   5'-CTGCAGGCGC  GTTGAGGAGA  TGTTACCGTG  AATCGCATCA  TCGAGGCTCG  TCTATGGCGG
                                                                      *
                                          YLMF
YLM-S                TCTATGCGCA  GCGGAGAACA  GGAGCTGGTG  AAATAGTGCC  TTTTACCTTT  TCGCATACAA
YLM-R                TCTATGCGCA  GCGGAGAACA  GGAGCTGGTG  AAATAGTGCC  TTTTACCTTT  TCGCATACAA
                                                                     YLMR
YLM-S                CCTATTGTAT  TTGTTTCCGG  AAATTGCTTT  TGGCTCCCGA  GCTTCACGGA  GCCCTTTAA-3'
YLM-R                CCTATTGTAT  TTGTTTCCCG  AAATTGCTT-  ----------  GCTTCACGGA  GCCCTTTAA-3'
                                          *             *  **********
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Fig. 2 DNA sequence of the fragments YLM-S, derived from Proctor (non-*Yd2*), and YLM-R, derived from Shannon (plus-*Yd2*). The two single-base polymorphisms and the 11 base-pair insertion/deletion polymorphism are denoted by *asterisks*. The PCR primers YLMF and YLMR are denoted by *arrows*

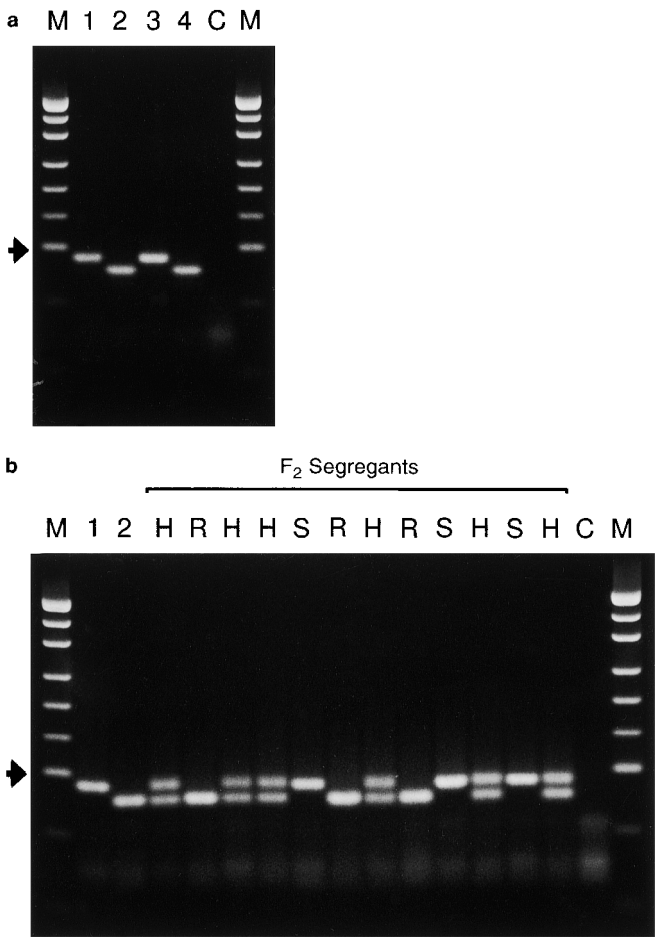

Fig. 3a, b Polymorphic DNA bands amplified from the YLM locus. The 110 base pair-band of the marker pUC19/*Hpa*II (Bresatec, Australia) is indicated by an *arrowhead* (*lanes M*). Negative control lanes are labelled *C*. **a** Amplification products derived from the non-*Yd2* barleys 'Proctor' and 'Atlas' (*lanes 1* and *3*, respectively), and the *Yd2*-carrying barleys 'Shannon' and 'Atlas 68' (*lanes 2* and *4*, respectively). Template for amplification was prepared using the method of Collins et al. (1996). **b** Test amplifications of the YLM from 'Atlas' and 'Atlas 68' (*lanes 1* and *2*, respectively) and from F_2 segregants of the 'Atlas' × 'Atlas 68' mapping population. Template for amplification was prepared using the simplified DNA extraction method of Wang et al. (1993). The *Yd2* genotype of F_2 segregants is represented as homozygous susceptible *S* (-/-), heterozygous *H* (*Yd2*/-) or homozygous resistant *R* (*Yd2*/*Yd2*)

population which were recombinant for the RFLP markers *Xglk223* and *Xpsr116*, shown previously to span a genetic distance of 5.7 cM and to flank *Yd2* (Collins et al. 1996). The YLM and *Yd2* loci cosegregated amongst these plants.

More detailed information on the genetic distance separating the YLM locus and *Yd2* was subsequently obtained as part of a programme in this laboratory to isolate *Yd2* using a map-based approach. A total of 572 F_2 individuals of the 'Atlas' × 'Atlas 68' mapping population were analysed, and the genetic distance between the YLM locus and *Yd2* was estimated to be 0.7 cM (Paltridge et al., manuscript in preparation).

Validation of the assay for use in breeding

To determine the suitability of the YLM locus for application in the marker-assisted selection of *Yd2*, we assessed the YLM genotype in 102 different barley lines. These included: (1) 93 BYD-susceptible barley cultivars grown in Australia and BYD-susceptible lines of importance in Australian breeding programmes (S. Jefferies, personal communication); and (2) 9 *Yd2*-containing barley genotypes. The results are presented in Table 1.

Each of the barley lines tested yielded an amplification product of length similar to 1 of the 2 DNA fragments characterised in 'Proctor' and 'Shannon' and illustrated in Fig. 3a. All 9 *Yd2*-carrying genotypes contained the same length variant of YLM as the *Yd2*-containing barley 'Shannon'. Of the 93 BYD-susceptible barleys, 85 yielded the larger fragment characteristic of 'Proctor' (non-*Yd2*), and only 8 the fragment characteristic of 'Shannon' (plus-*Yd2*).

Discussion

BYD-resistant barley cultivars have traditionally been bred using backcrossing programmes designed to introgress *Yd2* into the genetic backgrounds of agronomically adapted barley cultivars (Schaller and Chim 1969a,b; Schaller et al. 1973, 1977, 1979; Vertigan 1979). However, these programmes have long been hampered by the inconvenience and unreliability of the BYD-resistance assays required to select *Yd2*-carrying genotypes after each backcross generation (see

Table 1 YLM genotype of 102 different barley lines. **a** Ninety-three BYD-susceptible barley cultivars and lines grown in Australia or of importance to Australian breeding programmes, **b** nine *Yd2*-containing cultivars and lines

	YLM		YLM[a]
(a)			
BYD-susceptible lines (non-*Yd2*)			
Alexis	S	Malebo	S
Amaji Nijo	S	Maltine (Fr)	S
Andre	S	Moondyne	S
Arapiles	S	Morrell	R
Bandulla	S	Mundah	S
Barque	S	Namoi	S
Bearpaw	S	Natasha (Fr)	S
Blenheim	S	Norbet	S
Brindabella	S	Noyep	S
Camargue	S	O'Connor	S
Caminant	S	Onslow	S
Cantala	S	Osiris	S
Carina	S	Parwan	S
Chariot	S	Prisma	S
Chebec	S	Proctor	S
Cheri	S	Puffin	S
CI3576	R	Research	S
CIMMYT 42002	S	Resibee	S
Clipper	S	Richard	R
Corvette	S	Rubin	S
Dampier	S	Russia 39788	S
Dera	S	Sahara	S
Ellice	S	Sapporo Shin 5	S
Ethiopia 183	S	Schooner	S
Fergie	S	SH302	S
Forrest	R	Sissy	S
Galleon	S	Skiff	S
Gilbert	R	Steffi	S
Gimpel	S	Stirling	S
Golden Promise	S	Sultan	S
Grimmett	S	SumireMochi	R
Grit	S	Tallon	S
HS71284-48 (mountains)	S	Triumph	S
HS71285-27 (desert)	S	Turkey 741	S
HS77129-2 (Israel coast)	S	Ulandra	R
HS77137-11 (temperate)	S	Vic860458	S
HS77141-14 (desert)	S	Vic9104	S
Halycon	S	WA73S276	S
Harrington	S	WA82S522	S
Haruna Nijo	S	Waranga	S
HE 3631	S	Weeah	S
Igri	S	Windich	S
Kaputar	S	WI2776 (SB 85216)	R
Ketch	S	WI2875-22	S
KI Techenthal	S	Yagan	S
KMBR/52	S	Yerong	S
Lara	S		
(b)			
BYD-resistant lines (*Yd2* donor accession)[b]			
Shannon (CI3208-1)	R	Sutter (CI1237)	R
CM67 (CI2376)	R	UC566 (CI2376)	R
CM72 (CI2376)	R	Frankin (CI3208-1)	R
Atlas68 (CI3920-1)	R	Vixen (CI3906-1)	R
Prato (CI2376)	R		

[a] S represents the length variant YLM-S, characteristic of the non-*Yd2* cultivar 'Proctor'; R represents the length variant YLM-R, characteristic of the *Yd2*-carrying cultivar 'Shannon'

[b] Within brackets: the source of *Yd2* used in the breeding of the cultivars and lines (reproduced in part from Burnett et al. 1995)

Introduction). In this study, a codominant PCR marker to *Yd2* was developed using AFLP analysis. The marker allows for the indirect selection of *Yd2*-carrying genotypes and provides a simple and economical alternative to BYD-resistance assays in breeding programmes.

AFLP analysis was found in the present work to be a reliable and sensitive method for the generation of molecular markers close to the *Yd2* gene. The analysis of approximately 5000 amplification products from homozygous susceptible (-/-) and homozygous resistant (*Yd2*/*Yd2*) pools of F_2 segregant DNA revealed 37, or 0.7%, to be polymorphic. This level of polymorphism is considerably lower than that observed in published AFLP analyses in barley (Becker et al. 1995; Qi and Lindhout 1997) and arises from the fact that the template used in this study comprised pools of F_2 DNA designed to carry opposing alleles of only the targeted chromosome interval.

Polymorphisms identified in the initial screening of susceptible and resistant F_2 bulks were subsequently located relative to the two closest proximal and distal *Yd2*-flanking recombination events in the 'Proctor' × 'Shannon' mapping population. This strategy is similar to one used by Thomas et al. (1995), who sought AFLP markers tightly linked to the *Cf-9* gene in tomato for resistance to *Cladosporium fulvum*. Of the 37 polymorphisms originally identified 2 were localised to the interval between these closely *Yd2*-flanking crossover points. In the work described here, only 1 of the 2 tightly linked markers was taken on for further study; however, both markers will be valuable during future efforts in this laboratory to clone *Yd2* using a map-based approach.

One of the tightly linked AFLP markers was characterised as an 11-base-pair insertion/deletion polymorphism and was easily converted into a simple, codominant PCR marker to *Yd2*, designated YLM. It should be noted, however, that the conversion of AFLP markers to dominant or codominant PCR markers is rarely as straightforward as in the present work. Only 2 of the 37 polymorphisms identified in this study were evident as slight differences in band mobility; this is a similar frequency to that observed by Becker et al. (1995), who found 4 out of 118 polymorphisms identified between the barley lines 'Proctor' and 'Nudinka' to be of this type.

The conversion of the more commonly observed presence/absence-type AFLP markers into simple PCR assays is expected to be more difficult. Such polymorphisms are presumed to originate from sequence differences within restriction sites, or in flanking nucleotides which fail to match the selective nucleotides of AFLP primers and are therefore close to the ends of the AFLP fragments. Single primer-template mismatches only provide a suitable basis for allele-specific amplification when at the terminal 3′ base of PCR primers (Kwok et al. 1990). As a consequence, Inverse PCR (Ochman

et al. 1988; Triglia et al. 1988) must often be used to obtain additional sequence outside the original clone before allele-specific primers can be designed for a simple PCR assay (Brigneti et al. 1997). Alternatively, polymorphic restriction sites within the sequences of characterised AFLP markers must be sought for the development of systems of post-amplification restriction analysis (Konieczny and Ausubel 1993).

A detailed analysis of the genetic linkage between the YLM and *Yd2* revealed the two loci to be 0.7 cM apart. Typically, up to seven backcrosses have been used during the breeding of BYD-resistant barley cultivars (Schaller and Chim 1969a,b; Schaller et al. 1973, 1977, 1979; Vertigan 1979). To have a greater than 95% chance of retaining the *Yd2* gene during seven backcrosses, whilst avoiding BYD-resistance bioassays, any marker used to predict *Yd2* status should be within 0.7 cM of the gene[1]. The linkage between the YLM and *Yd2* is therefore sufficient for the marker to be used to assist in the selection of *Yd2*-carrying lines, even in breeding programmes involving seven backcrosses to a susceptible recurrent parent.

In order to develop the simplest possible method for assaying YLM genotype, and so facilitate the large-scale application of the marker, we explored the method of Wang et al. (1993) as a means of preparing DNA template for amplification. Once optimised for use in barley, this method yielded template which was amplified as satisfactorily as that prepared using more involved procedures. The method is fast and economical and, since it does not require the use of specialised or expensive equipment, will be suitable for widespread application in breeding programmes.

To assess the potential of the YLM for use in BYD-resistance breeding programmes involving barley lines other than 'Proctor' and 'Shannon', the marker was tested on a large collection of *Yd2* and non-*Yd2* barley genotypes (Table 1). Significantly, all 9 barley lines tested which are known to carry *Yd2* contained the same length variant of YLM as the *Yd2*-carrying 'Shannon'. These lines contain *Yd2* genes from the five accessions of Ethiopian barley so far used in BYD-resistance breeding programmes (Burnett et al. 1995). Amongst 93 BYD-susceptible barley lines, 85 were shown to carry the same length variant of the YLM as the non-*Yd2* line 'Proctor'. These data demonstrate that the marker is likely to be polymorphic for most crosses between *Yd2* and non-*Yd2* genotypes that breeders may wish to use and therefore has widespread potential for use in barley BYD-resistance breeding programmes.

It should be noted, however, that the 'Shannon' length variant of the YLM locus was amplified from 8 of the 93 lines in the BYD-susceptible barley collection. Though none of these barleys has been tested in this laboratory for BYDV reaction, they have not to our knowledge been reported to be BYD-resistant and are not expected to carry resistance to BYD at the *Yd2* locus. The association between YLM and *Yd2* is therefore unlikely to be complete and, as a result, the YLM is unlikely to be polymorphic for all pairs of *Yd2* and non-*Yd2* genotypes that breeders may wish to use.

During the present study, another PCR marker for *Yd2* was developed in this laboratory (Ford et al. 1998), at a locus designated *Ylp*. Two assays were developed at the locus: one was a codominant assay which used post-amplification restriction analysis to differentiate resistance- and susceptibility-associated alleles; the other, a dominant assay, employed allele-specific PCR to detect only the resistance-associated allele of the *Ylp* gene. The assay system presented here for YLM genotype analysis has significant practical advantages over both assays developed for analysis of the *Ylp* genotype.

Firstly, the codominant assay developed at the *Ylp* locus is based on the dual steps of PCR amplification and restriction analysis. In contrast, the assay presented here for the YLM involves only a single PCR amplification step. Heterozygotes can therefore be distinguished from either homozygote in a single reaction, providing the maximum amount of genetic information without the use of restriction enzyme analysis.

Secondly, the dominant assay developed for *Ylp* genotype analysis relies on the presence or absence of DNA bands for genotype designation and, as a consequence, provides a less robust assay than the codominant system developed for the YLM. In dominant marker systems, negative results arising from errors in reaction assembly can lead to false genotype assignment, whereas in codominant systems errors in reaction assembly are immediately recognised as the lack of any signal. An additional advantage of codominant marker systems is that they are less likely to result in false positive signals than dominant systems: low levels of DNA contamination are only ever likely to represent a small proportion of template available for primer binding and should not compete significantly with non-contaminant template during PCR amplification.

The PCR assays developed at the *Ylp* locus do have two significant advantages over the YLM described here, however. Firstly, the assays at the *Ylp* locus identified polymorphism between all *Yd2* and non-*Yd2* barley lines tested, whereas the YLM was only polymorphic between most lines tested; the *Ylp* assays therefore have the potential to be applied in more BYD-resistance breeding programmes than the YLM. Secondly, the *Ylp* locus has so far exhibited a perfect

[1] This figure was obtained by assuming that the probability of recombination occurring at each opportunity equals the genetic distance between loci expressed as a recombination fraction; i.e. 0.007. Because of the small genetic distances involved, recombination percentage can be regarded as equivalent to genetic distance in centiMorgans

genetic linkage to *Yd2* (Ford et al. 1998), whereas the YLM maps 0.7 cM from the gene (Paltridge et al., manuscript in preparation). Predictions of *Yd2* genotype made using the assays at the *Ylp* locus are therefore more likely to be correct than those made using the YLM.

It is envisaged that the PCR markers developed at the *Ylp* and YLM loci will complement each other in BYD-resistance breeding programmes. Because of its simplicity and codominance, the assay at the YLM locus is the more amenable of the two to large-scale application, and will be the method of choice in most breeding programmes; indeed, the marker is already in use in five such programmes in Australia and internationally. However, assays at the *Ylp* locus will still have application in breeding programmes where the YLM is not polymorphic between the *Yd2* and non-*Yd2* parent lines; additionally, assays for the *Ylp* genotype may be used, along with final BYD-resistance assays, to provide confirmation of predicted *Yd2* genotype in any BYD-resistance breeding programme.

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