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ORIGINAL PAPER

Wheat stripe rust resistance genes *Yr5* and *Yr7* are allelic

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Abstract Stripe rust is one of the most destructive diseases of wheat. Breeding for resistance is the most economical and environmentally acceptable means to control stripe rust. Genetic studies on resistance sources are very important. Previous inheritance studies on *Triticum aestivum* subsp. *spelta* cv. album and wheat cultivar Lee showed that each possessed a single dominant gene for stripe rust resistance, i.e., *Yr5* and *Yr7*, respectively. Both were located on the long arm of chromosome 2B, but due to the complexities caused by genetic background effects there was no clear evidence on the allelism or linkage status of these genes. Our study, involving an intercross of Avocet S near-isogenic lines possessing the genes, provided clear evidence for allelism or extremely close linkage of *Yr5* and *Yr7* based on phenotypic and molecular studies.

Introduction

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (*P. s. tritici*), is one of the most destructive diseases of wheat worldwide. In recent years, this disease has become increasingly important in some wheat-growing areas, where it had previously been absent or not regularly destructive, such as South Africa (Pretorius et al. 1997), the central states of the United States of America (Chen et al. 2002), Western Australia (WA) (Wellings et al. 2003), and some regions in China (Wan et al. 2004). These occurrences in new areas have resulted in both significant yield losses and large expenditures on fungicides aimed at reducing losses. The underlying causes of this appear to be further foreign incursions of the pathogen itself as was the case in WA in 2002, or of new pathotypes with wider virulence spectra combined with greater aggressiveness or adaptiveness compared to pre-existing pathotypes as would apply to the USA (Milus et al. 2009) and to eastern Australia following the WA incursion. In all cases mutation or adaptation of local pathotypes was almost certainly not involved.

Resistant cultivars are the most economical and environmentally acceptable approach to control stripe rust. Currently, there are 40 named stripe rust resistance loci in wheat (http://shigen.lab.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp), but there are several additional genes with temporary designations, pending more complete genetic analyses, and a considerable number of factors more appropriately designated as quantitative trait loci (QTL). Many within this last group are likely to be resolved as single genes following detailed genetic analysis. Many of the designated and temporarily designated genes have been overcome by at least some variants of the pathogen and are not considered useful in breeding for resistance. Instead, increased emphasis has been directed at resistance sources shown to be effective over wide areas, or with a reputation of having shown durable protection. Many of these latter sources are of the adult plant type. Nevertheless, there is also an ongoing interest in the genetics of resistance to pathogens regardless of the immediate application in resistance breeding.

The stripe rust resistance gene *Yr5* was originally identified in hexaploid *Triticum aestivum* ssp. *spelta* cv. album (TSA). Macer (1966) located it on chromosome 2B and

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Law (1976) further localised it to the long arm of that chromosome, 21 cM from the centromere. While *Yr5* confers resistance to almost all isolates of *P. s. tritici* in the world, except for Australia (Wellings and McIntosh 1990) and probably India (Nagarajan et al. 1986), there is no evidence that the gene has been utilised in a wheat cultivar and therefore no real test of its likely durability in an agricultural situation.

Yr7, named by Macer (1966) and originating from tetraploid *T. turgidum* ssp. *durum* cv. Iumillo, was transferred to hexaploid wheat cv. Thatcher and its derivatives, including Lee. Lee was likely derived from a cross of Timstein with Thatcher (rather than Hope; McIntosh et al. 1981). *Yr7* was located on chromosome 2B by Johnson et al. (1969) who reported it was either allelic or very closely linked with *Yr5* because no susceptible segregant was found in the cross of Thatcher × TSA. *Yr7* also showed very close linkage with *Sr9g* (McIntosh et al. 1981), placing it in a region approximately 20 cM from the 2B centromere.

Further expanded attempts to understand the relationship of *Yr5* and *Yr7* in intercrosses of source germplasms/accessions resulted in complexities in the identification of genotypes due to genetic background effects (Johnson and Dyck 1984; McDonald 1996). In the present work we intercrossed *Yr5* and *Yr7* near-isogenic lines (NILs) in the genetic background of Avocet S (AVS). This paper presents the results on phenotypic and molecular marker studies on the F_3 progenies. We found distinct segregation patterns for each gene and strong evidence for allelism between *Yr5* and *Yr7*.

Materials and methods

Plant materials

The wheat stripe rust *Yr5* (TSA/6*AVS) and *Yr7* (Lee/6*AVS) NILs were developed by backcrossing the *Yr5* and *Yr7* donors with the susceptible spring wheat Avocet S (AVS) (Wellings and McIntosh 1998). These NILs are maintained at the Plant Breeding Institute, University of Sydney, Australia. Intercrosses between the *Yr5* and *Yr7* NILs were made and F_2 and F_3 populations were developed.

Seedlings of TSA, Lee, the *Yr5* and *Yr7* NILs, and F_2 and F_3 progenies of the cross between the *Yr5* and *Yr7* NILs comprising at least 15–20 seedlings per F_3 line were tested for stripe rust response.

Plant materials used in molecular marker analyses included 20 *Yr5* and 17 *Yr7* homozygous resistant F_3 lines, TSA, Spaldings Prolific (SP), Lee, Thatcher, two cultivars carrying *Yr7* (Celebration and Cranbrook), 15 AVS NILs (with *Yr1*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, *Yr24*, *Yr26*, *Yr27*, *Yr32*, and *YrSp* (derived from SP)), Avocet R (*YrA*), and AVS.

P. striiformis pathotypes

The *P. striiformis* f. sp. *tritici* pathotypes (pt.) used in this study included 104 E137 A− (Culture 415) and 108 E141 A+ (Culture 420) (avirulent for both *Yr5* and *Yr7*), 360 E137 A− (Culture 430; virulent for *Yr5*, avirulent for *Yr7*), and 110 E143 A+ (Culture 444; avirulent for *Yr5*, virulent for *Yr7*).

Scoring for stripe rust response

Plant materials for assessing stripe rust responses were sown in 10-cm pots filled with a pine bark and sand potting mix. Prior to sowing, pots were fertilised with the complete fertiliser, Aquasol®. Approximately 7 days later, after seedling emergence, pots were fertilised with urea. Seedlings were grown in the glasshouse at 16–20°C and inoculated when they were at the 1.5 to 2 leaf stage. They were inoculated with a urediniospore suspension in light mineral oil and incubated in humidity chambers at 11 ± 2°C for 24 h. Seedlings were then moved to a greenhouse room with a temperature of 17–24°C. Infection types (ITs) were scored 12–16 days after inoculation according to a 0 to 4 scale, on which 0 indicates no visible symptoms and 4, complete susceptibility (McIntosh et al. 1995).

DNA isolation, PCR amplification, and restriction enzyme digestion for the cleaved amplified polymorphic sequence (CAPS) marker

Genomic DNA from approximately 100 mg of plant material collected from five plants was isolated using the DNeasy Plant Mini Kit (Qiagen Pty Ltd, Vic., Australia). DNA was quantified using a Nanodrop Spectrophotometer (Thermo Scientific, Wilmington, DE) and diluted to approximately 50 ng/µl.

The sequence tagged site (STS) primers, Yr5STS-9/10, developed by Chen et al. (2003), co-segregate with the *Yr5* locus. A 95% confidence interval for the distance between this STS marker and *Yr5* is +/− 0.7 cM (Chen et al. 2003). Each 20 µl of PCR solution contained 50 ng of DNA, 10 µl of HotStarTaq *Plus* Master Mix (Qiagen) (with a final concentration of 1 U of HotStarTaq *Plus* DNA polymerase, 1× PCR buffer, and 200 µM of each dNTP), and 0.5 µM of each primer. DNA amplification was performed in a Mastercycler (Eppendorf AG, Hamburg, Germany) programmed for 5 min at 95°C for initial activation and 35 cycles, each consisting of 30 s at 94°C, 30 s at 45°C, and 1 min at 72°C, followed by a final extension of 10 min at 72°C.

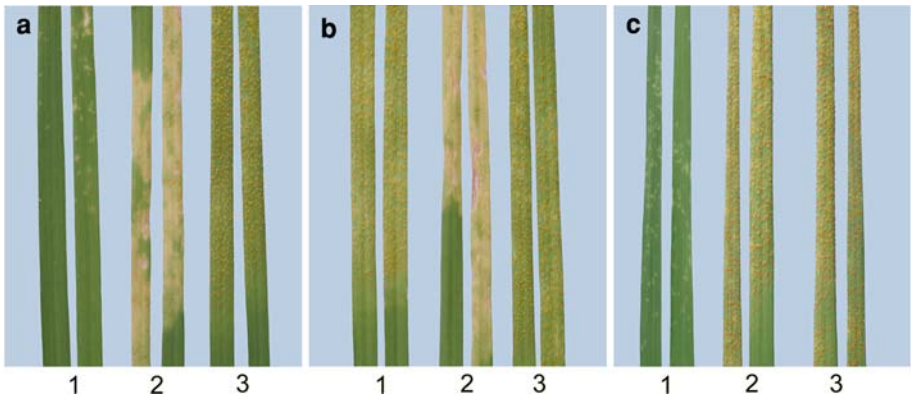

Fig. 1 Seedling leaves of AVS + Yr5 (*1*), AVS + Yr7 (*2*), and AVS (*3*) infected with pt. **a** 104 E137 A− (IT 0;, 1N, and 3+), **b** 360 E137 A− (IT, 3+, 1N, and 3+), and **c** 110 E143A+ (IT 0;, 3+, and 3+). The low IT conferred by *Yr5* is 0; (**a** and **c**) and that conferred by *Yr7* is 1N (**a** and **b**)

After amplification, 10 µl of PCR product was used to check the success of amplification. For the CAPS marker, 0.5 µl (5 U) of restriction enzyme *Dpn*II and 1.3 µl of 10× buffer for *Dpn*II (New England Biolabs, Beverly, MA) were added to the remaining 10 µl of PCR product. Samples were incubated at 37°C for 2 h and digestion products were separated in either 2% or 2.5% (w/v) agarose gels.

Twenty microlitres of PCR products amplified from AVS + Yr5, AVS + Yr7, and Lee were purified using Wizard SV gel and PCR clean-up system (Promega Corporation, NSW, Australia) and then sequenced in both directions.

Results

Phenotypic analyses

TSA and the lines carrying *Yr5* were resistant to pt. 104 E137 A−, 108 E141 A+, and 110 E143A+ with IT 0; (no or faintly visible uredinia), but susceptible to 360 E137 A−; Lee and the *Yr7*-carrying lines were resistant to pt. 104 E137 A−, 108 E141 A+, and 360 E137 A− with IT 1 N (necrotic areas with restricted sporulation), but susceptible to 110 E143A+. AVS was susceptible to all the above races with IT 3+ (heavy sporulation without necrosis or chlorosis) (Fig. 1).

Three F_2 families were analysed. As data were not significantly different across families, they were pooled. In total, 143 F_3 lines were scored for their responses to different rust strains. Thirty-seven lines were genotyped as *Yr5Yr5yr7yr7*, 36 were *yr5yr5Yr7Yr7*, and 70 were *Yr5yr5Yr7yr7*. When tested with pt. 108 E141 A+ no susceptible lines or individual plants within lines were found indicating that the genes must be allelic or closely linked in repulsion.

Molecular marker analyses

The plant materials amplified fragments of 439 or 433 bp for the resistant (Fig. 2a) or susceptible plants (data not shown), respectively. Polymorphism was better revealed after *Dpn*II digestion of the PCR products. The CAPS marker then provided distinct patterns for the 20 *Yr5* and 17 *Yr7* homozygous resistant lines (Fig. 2b), which are subsets of all the homozygous resistant lines. The sizes of the top bands were 289 bp for *Yr5* and approximately 200 bp for *Yr7*. We obtained a perfect match between the respective marker bands and the host response genotypes for 36 homozygous F_3 progenies tested, except line 2152, which was homozygous for *Yr7*, but appeared to amplify both the '*Yr5*' and '*Yr7*' bands. Sequencing results on PCR products from AVS + Yr5 and AVS + Yr7 revealed approximately 350 bp clear traces. Among them, AVS + Yr5 and AVS + Yr7 differed by only two nucleotides. One of the nucleotides is located at the *Dpn*II restriction site, thus explaining the band size differences after digestion.

The gel patterns of TSA and SP were the same as that of AVS + Yr5; Thatcher, Celebration, and Cranbrook had the same gel pattern as AVS + Yr7; Lee had two bands, one having the same size as the AVS + Yr5 band and the other having the same size as the AVS + Yr7 band (Fig. 3a).

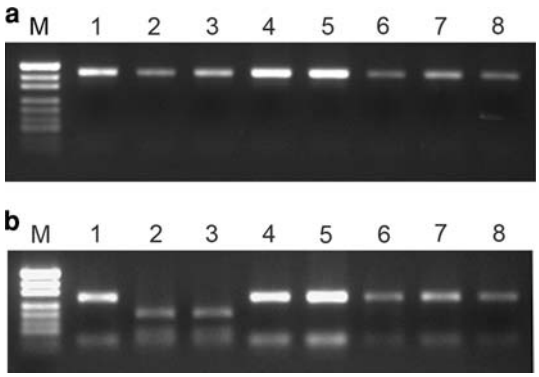

Fig. 2 **a** Yr5STS-9/10 PCR amplification products obtained from *Yr5* (*lanes 1*, *4–8*) and *Yr7* (*lanes 2* and *3*) homozygous resistant F_3 lines in cross Avocet S Yr5/Yr7. All plants amplified a 439 bp fragment. M—pUC19 DNA/*Hpa* II molecular weight marker. **b** PCR products from (**a**) were digested with *Dpn*II; the sizes of the top bands are 289 bp for *Yr5* (*lanes 1*, *4–8*) and approximately 200 bp for *Yr7* (*lanes 2* and *3*) lines

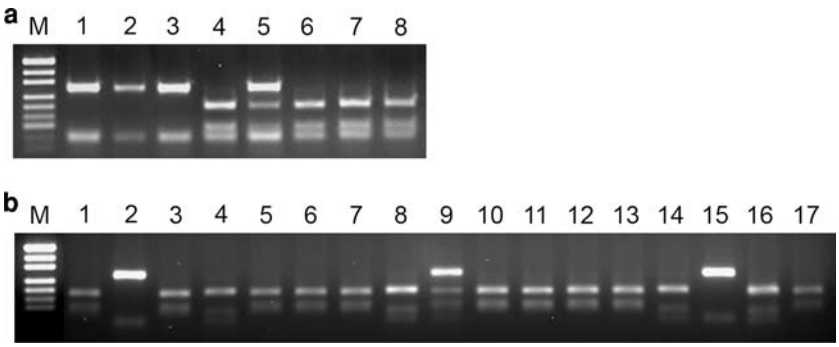

Fig. 3 *Dpn*II digestion of Yr5STS-9/10 PCR amplification products obtained from **a** cultivars and **b** Avocet S (AVS), and AVS NILs. M—pUC19 DNA/*Hpa* II molecular weight marker. **a** (*1*) AVS + Yr5, (*2*) TSA, (*3*) Spaldings Prolific (SP), (*4*) AVS + Yr7, (*5*) Lee, (*6*) Thatcher, (*7*) Celebration, and (*8*) Cranbrook. The gel patterns of TSA and SP were the same as that of AVS + Yr5. Thatcher, Celebration, and Cranbrook had the same gel pattern as AVS + Yr7. The first and second bands in Lee had the same size as the AVS + Yr5 and AVS + Yr7 bands, respectively. **b** (*1*) AVS + Yr1, (*2*) AVS + Yr5, (*3*) AVS + Yr6, (*4*) AVS + Yr7, (*5*) AVS + Yr8, (*6*) AVS + Yr9, (*7*) AVS + Yr10, (*8*) AVS + Yr15, (*9*) AVS + Yr17, (*10*) AVS + Yr18, (*11*) AVS + Yr24, (*12*) AVS + Yr26, (*13*) AVS + Yr27, (*14*) AVS + Yr32, (*15*) AVS + YrSp, (*16*) Avocet R (AVR) (*YrA*), (*17*) AVS. AVS + YrSp (*15*) had the same gel pattern as AVS + Yr5 (*2*). AVS + Yr15 (*8*), AVS + Yr32 (*14*), and AVR (*16*) had the same pattern as AVS + Yr7 (*4*). AVS + Yr17 (*9*) had a unique pattern different from all the other NILs; The rest of NILs all had the same pattern as that of AVS (*17*)

Sequencing results on approximately 350 bp clear traces from Lee showed seven sites with mixed nucleotides. After comparing this sequence with that of the PCR products from AVS + Yr5 and AVS + Yr7, it was evident that this primer set most likely amplified two fragments in Lee; one was the same as that from AVS + Yr7, the other was different from those in both AVS + Yr5 and AVS + Yr7. AVS + YrSp had the same gel pattern as AVS + Yr5; AVS + Yr15, AVS + Yr32, and AVR had the same pattern as AVS + Yr7; AVS + Yr17 had a unique pattern different from all the other NILs; the rest of NILs all had the same pattern as that of AVS (Fig. 3b).

Discussion

In wheat, genes with different specificities and failing to recombine in recombination tests involving a minimum of about 200 gametes are considered to be allelic and are designated accordingly (http://shigen.lab.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp). In some instances genes thought to be allelic, such as those designated *Lr14a* and *Lr14b*, were later shown to recombine (Dyck and Samborski 1970); in others, genes thought to be at separate loci, such as *Lr2* and *Lr15* (McIntosh RA, unpublished) and in the present example of *Yr5* and *Yr7*, are probably allelic. Although the similar genomic locations of *Yr5* and *Yr7* have been known for three decades following Law (1976), further attempts to confirm allelism by intercrosses have proved unsuccessful (Johnson and Dyck 1984; McDonald 1996) because of unexpected phenotypic complexities in segregating populations. This confounding variation was attributed to suppressor genes and genetic backgrounds. In the present study involving segregation in the uniform genetic background of Avocet S, each gene retained its unique infection type and segregated normally. The two genes behaved as alleles.

Johnson and Dyck (1984) found that F_1 plants from Thatcher/TSA were susceptible to a *Yr7*-virulent/*Yr5*-avirulent pathotype, whereas F_1 plants from Lee/TSA were resistant to the same pathotype. They proposed that Thatcher carried a dominant inhibitor of *Yr5* and that linkage between *Yr5* and *Yr7* should be tested in the absence of any such inhibitor gene. Genetic background effects on the effectiveness and the expression of resistance genes have been reported in many studies on rust resistance in wheat (e.g., The 1973; McIntosh and Dyck 1975; Williams et al. 1992; Kema et al. 1995; Ma et al. 1995). In the present work we intercrossed NILs with *Yr5* and *Yr7* in the genetic background of AVS and found distinct segregation for each gene, and therefore, strong evidence for allelism. This knowledge should now serve as a sound platform for gaining an understanding of the genetic basis of the problems encountered in the studies of Johnson and Dyck (1984) and McDonald (1996).

In addition, linkage studies on the stripe rust resistance gene *YrSp* (Guan et al. 2005), which is also located on the long arm of chromosome 2B, with *Yr5* and *Yr7* demonstrated that *YrSp* is also allelic to both *Yr5* and *Yr7* (McIntosh RA and Zhang P, unpublished). This provides additional evidence that *Yr5* and *Yr7* are allelic. The recorded pedigree of Lee is Timstein/Hope, but on the basis of the presence of *Yr7* and the closely linked *Sr9g*, the pedigree was more likely Timstein/Thatcher (McIntosh et al. 1981). The reason that Lee appeared to amplify both the '*Yr5*' and '*Yr7*' bands was due to the fact that the primer

set actually amplified two fragments, one being the same as that amplified from AVS + Yr7, the other, a paralogue of the band amplified from AVS + Yr7 coincidently having a similar size to the AVS + Yr5 band.

Thirty-six of the 37 homozygous resistant F_3 lines showed a perfect match between the respective marker bands and the host response genotypes, except line 2152, which was shown to be homozygous for *Yr7*, but amplified both the '*Yr5*' and '*Yr7*' bands. Although Chen et al. (2003) did not find recombination between the marker and *Yr5* and showed that the 95% confidence interval for the distance between the marker and *Yr5* was ±0.7 cM, this CAPS marker is actually not within the gene (XM Chen, personal communication). This conclusion was also supported by the recombinant found in the present study. Given that the origins of *Yr5* (TSA), *Yr7* (Iumillo durum to Double Cross (or Thatcher)), and *YrSp* (Spaldings Prolific) in wheat cultivars worldwide were likely limited to single or very few sources this diagnostic, but not perfect, marker for *Yr5* alleles should be useful in gene postulation and association studies.

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