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H. S. Bariana · N. Parry · I. R. Barclay
R. Loughman · R. J. McLean · M. Shankar
R. E. Wilson · N. J. Willey · M. Francki

Identification and characterization of stripe rust resistance gene *Yr34* in common wheat

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Abstract An uncharacterized source of seedling resistance to *Puccinia striiformis* f.sp. *tritici* was identified in an advanced wheat breeding line WAWHT2046. Genetic analysis based on a WAWHT2046/Carnamah-derived double haploid (DH) population demonstrated monogenic inheritance of seedling stripe rust resistance in WAWHT2046. The gene controlling stripe rust resistance in line WAWHT2046 was tentatively designated *YrWA*. The chromosome 5AL located awn inhibitor gene *B1*, possessed by WAWHT2046, also showed monogenic inheritance when the DH population was scored for the presence and absence of awns. Joint segregation analysis at the *B1* and *YrWA* loci indicated genetic linkage between the two loci. A recombination value of 12.2 cM was computed using Mapmanager. This association located *YrWA* in the chromosome arm 5AL. Molecular mapping using microsatellite markers placed *YrWA* distal to *B1*. All molecular markers mapped proximal to the awn inhibitor locus *B1*. As no other stripe rust resistance gene is reported to be located in the chromosome arm 5AL, *YrWA* was permanently designated as *Yr34*. *Yr34* produced an intermediate (23C) seedling infection type and expressed very low stripe rust response (10R-MR) on adult plants in the field, similar to the resistance gene *Yr17*. In addition to *Yr34*, this mapping population segregated for three genetically independent adult plant stripe rust resistance genes. The detection of DH lines with completely susceptible response, higher than that shown by the *Yr34*-lacking parent Carnamah, suggested that both parents contributed adult plant resistance. The use of WAWHT2046 as a parent in breeding programs would also contribute APR in addition to *Yr34*.

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H. S. Bariana (✉) · N. J. Willey
Faculty of Agriculture, Food and Natural Resources,
University of Sydney Plant Breeding Institute-Cobbitty, PMB11,
Camden, NSW 2570, Australia
E-mail: harbansb@camden.usyd.edu.au
Tel.: +61-2-93518809
Fax: +61-2-93518875

N. Parry · M. Francki
Value Added Wheat CRC, North Ryde, NSW 2113, Australia

I. R. Barclay · R. Loughman · R. J. McLean · M. Shankar
R. E. Wilson · M. Francki
Department of Agriculture Western Australia,
3 Baron-Hay Court, South Perth, WA 6151, Australia

Introduction

Pathotypic variation in highly evolving rust pathogens is one of the important factors influencing deployment of resistance sources. Likewise, exotic introductions of new pathogen genotypes have a significant bearing on resistance breeding. While the exotic introductions of pathotypes pose a great threat to prevailing resistance sources, they also enable identification of some uncharacterized sources of resistance. Stripe rust caused by *Puccinia striiformis* f.sp. *tritici* (Pst) was first detected in Australia in 1979 (O'Brien et al. 1980). Stepwise development of virulence in Australian Pst pathotypes (pts) was reported by Wellings and McIntosh et al. (1990). The Pst pathotype 110 E143A+ carried virulence for commonly present genes (*Yr6*, *Yr7* and *YrA*) in Australian germplasm. The detection of a new exotic Pst pathotype 134 E16A+ in Western Australia during the 2002 crop season spread to the eastern Australian wheatbelt during the 2003 crop season (Wellings et al. 2003). This pathotype rendered seedling stripe rust resistance genes *Yr6*, *Yr7* and *YrA*, *Yr8* and *Yr9* ineffective and was avirulent on stripe rust resistance genes *Yr3* and *Yr4*. While pathotype 134 E16A+ produced higher responses on several Australian cultivars known to carry acceptable levels of resistance, the line WAWHT2046 from the wheat breeding program of the Department of Agriculture Western Australia, known to produce intermediate terminal stripe rust response against Pst pt 110 E143A+, showed a highly resistant response (10 R-MR) in the field. This observation

suggested the presence of a stripe rust resistance gene in line WAWHT2046 that was ineffective against Pst pt 110 E143A+. This paper reports results on the inheritance of seedling stripe rust resistance in line WAWHT2046, its chromosomal location and its genetic association with other traits located in the target genomic region. Results on the genetics of adult plant resistance (APR) in this population are also reported.

Materials and methods

Plant materials

Field studies established that advanced breeding line WAWHT2046 (tip awn) and cv Carnamah (awned) were resistant and susceptible to the Pst pt. 134 E16A+, respectively. A doubled haploid (DH) population from the cross WAWHT2046/Carnamah was produced to determine the genetic basis of resistance to stripe rust in WAWHT2046 according to Laurie and Bennett (1986). The DH population of 116 individuals was used for both seedling and adult plant stripe rust response phenotyping and molecular marker mapping. The population was also classified for segregation at the awn inhibitor locus *B1*.

Rust response tests

Pathogen inoculum

The Pst pathotype 134 E16A+ was used for screening parental lines and the DH population. This pathotype was first detected in Western Australia during the 2002 crop season (Wellings et al. 2003). Prior to the detection of this pathotype, stripe rust was not detected in Western Australia. An eastern Australian Pst pt 110 E143A+ was also used to test parental genotypes.

Greenhouse screening

Ten seeds of each DH line and four lines per pot were planted in 9 cm pots filled with mixture of pinebark and river sand. Plants were allowed to grow in a temperature-controlled greenhouse at 20°C. Aquasol fertiliser was applied at the time of sowing and 1 week after the sowing. Twelve day old seedlings (two leaf stage) were inoculated with Pst pathotype 134 E16A+ according to the procedure explained in Bariana and McIntosh (1993). Inoculated plants were incubated at 9–12°C for 24 h under 100% relative humidity. Pots were then moved to a greenhouse maintained at 17 ± 2°C for disease development. Infection type responses were scored 14 days after inoculation on a 0–4 scale as described in Bariana and McIntosh (1993). Parental lines were tested against Pst pathotype 110 E143A+ as well.

Field screening

The double haploid population, parents and control cultivars representing a range of responses to stripe rust were sown as four replicate 1 m rows in a randomized block design at Manjimup, Western Australia, on 30 June 2004. A spore suspension of pt. 134 E16A+ was applied to adjacent susceptible spreader plots (cv. Harrismith, sown 27 May 2004) using an ultra low volume applicator (Micron-ULVA) during August. Subsequent spread and development of infection throughout the experiment occurred naturally. This population was grown as 60 cm unreplicated rows at the University of Sydney Plant Breeding Institute, Cobbitty together with parental genotypes.

Percent rust severity (Peterson et al. 1948) and awn phenotype were assessed when the most susceptible lines approached maximum stripe rust severity.

Genetic mapping

Isolation of DNA from DH individuals was performed according to Francki et al. (1997). Primer sequences for SSR markers located on the chromosome arm 5AL were obtained from Graingene (website). Markers polymorphic between Carnamah and WAWHT2046 were screened on DNA from individuals of the DH population. A linkage map was constructed using Mapmanager version QTXb20 (Manly et al. 2001).

Statistical analyses

Field stripe rust severity was subject to analysis of variance and treatment means were compared using least significant difference criteria ($P < 0.05$). Angular transformation was used to stabilise variance for severity assessments. These procedures used GenStat7© (2003, Lawes Agricultural Trust).

Chi-squared analyses were performed to check the goodness of fit of observed segregations for stripe rust response and awn phenotype with the expected ratios.

Results

Inheritance studies

Resistance to stripe rust

The advanced breeding line WAWHT2046 and cultivar Carnamah produced susceptible infection types in the seedling stage when tested against Pst pathotype 110 E143A+. Line WAWHT2046 produced infection type 23C, when tested against Pst pathotype 134 E16A+ (Table 1) under greenhouse conditions. No change in the seedling response of cultivar Carnamah was

Table 1 Seedling infection types produced by parental genotypes and the susceptible control when inoculated with Pst pathotypes134 E16A+ and 110 E143A+

Genotype	Infection type	
	134 E16A+	110 E143A+
WAWHT2046	23C	4
Carnamah	4	4
AUS22857	3C	4
Kulin	4	4
Blade	4	4
Avocet S (susceptible control)	4	4

observed when it was tested against pt 134 E16A+. This observation indicated that the line WAWHT2046 carried seedling resistance that was effective against Pst pt 134 E16A+ but not against 110 E143A+.

Chi-squared analysis of data indicated the monogenic inheritance of resistance among 116 individuals screened against Pst pt 134 E16A+ under greenhouse conditions (Table 2). The resistance gene in line WAWHT2046 was tentatively designated as *YrWA*.

Analysis of segregation for awns

Line WAWHT2046 carries the awn inhibitor gene *B1* and therefore is phenotypically tip awned. Cultivar Carnamah is fully awned. The mapping population was scored for segregation at the awn inhibitor locus *B1*. Chi-squared analysis indicated the involvement of a single gene (Table 2).

Joint segregation analysis at the B1 and YrWA loci

Joint segregation analysis of data for the *YrWA* and the *B1* loci was performed. The significant chi-squared value (Table 2) for the test of independence suggested genetic linkage between the two loci. A recombination fraction of 12.2 cM was computed using Mapmanger. The observed genetic association with previously reported location of gene *B1* in the chromosome arm 5AL of wheat (McIntosh et al. 1998) suggested that the stripe rust resistance gene *YrWA* was likely to be located in this chromosome.

Table 2 Frequency distribution of WAWHT2046/Carnamah-derived DH lines with respect to segregation at the *B1* and *YrWA* loci

Genotype	B1B1	b1b1	Total
YrWAYrWA	53	2	55
yrWAyrWA	10	51	61
Total	63	53	116

$\chi^2_{1:1}$(*B1* vs. *b1*) = 0.86, (non significant at $P = 0.05$ and 1 d.f.)
$\chi^2_{1:1}$(*YrWA* vs. *yrWA*) = 0.31 (non significant at $P = 0.05$ and 1 d.f.)
χ^2_{joint}(*B1* vs. *YrWA*) = 74.13 (significant at $P = 0.05$ and 0.01 at 3 d.f.)

Molecular mapping and confirmation of the chromosomal location of YrWA

Simple sequence repeat (SSR) loci mapped on the chromosome arm 5AL were used to determine the precise genomic position of *YrWA* in the chromosome arm 5AL. SSR markers gwm595, gwm6a, gwm291 and gwm410.2 showed polymorphism when DNA from parents Carnamah and WAWHT2046 were assayed for each marker locus. These markers mapped to the chromosome arm 5AL proximal to the *B1* locus with a gene order of *Xgwm595-Xgwm6a-Xgwm291-Xgwm410.2-B1-YrWA* (Fig. 1). The chromosomal location of two markers gwm595 and gwm410.2 was confirmed using nullisomic-tetrasomic analysis. These results confirmed the location of *YrWA* in the chromosome arm 5AL distal to *B1*.

Inheritance of APR and comparison with other cultivars

Inheritance

Parental genotypes WAWHT2046 and Carnamah exhibited 10 and 60% stripe rust severity, respectively, when tested under field conditions at Cobbitty against Pst pathotype 134 E16A+. Necrotic areas (resistant-moderately; R-MR response) without active sporulation on WAWHT2046 were observed. In contrast cultivar Carnamah showed moderately susceptible response (MS). Chi-squared analyses of data presented in Table 3 indicated segregation at four genetically independent

Table 3 Frequency distribution of WAWHT2046/Carnamah-derived DH lines based on adult plant stripe rust response segregation, when tested against Pst pt 134 E16A+

Response class	No. of lines	$\chi^2_{7:1}$	$\chi^2_{15:1}$
Resistant (0–80)	108	0.39	0.01
Susceptible (90–100)	8	5.28	0.07
Total	116	5.67*	0.08

*Significant $P = 0.05$ at 1 d.f.; **significant at $P = 0.01$ at 1 d.f.

Table 4 Frequency distribution of WAWHT2046/Carnamah-derived *YrWA*-lacking DH lines based on adult plant stripe rust response segregation, when tested against Pst pt 134 E16A+

Response class	No. of lines	$\chi^2_{7:1}$	$\chi^2_{15:1}$
Resistant (0–80)	53	0.00	0.33
Susceptible (90–100)	8	0.02	2.19
Total	61	0.02	2.52

*Significant $P = 0.05$ at 1 d.f.; **significant at $P = 0.01$ at 1 d.f.

loci. These results suggested the involvement of three APR genes in addition to *YrWA* in controlling stripe rust resistance in this population under field conditions. Similar analysis was conducted on 61 *YrWA*-lacking genotypes. Data presented in Table 4 were a good fit for segregation at both three and four independent loci. The Chi-squared value for four gene model was higher than the three gene model. Detection of completely susceptible lines (a higher response than parent Carnamah) among the doubled population indicated that both parents contributed APR and the APR contributed by two parents was genetically independent.

Comparison with other cultivars

The resistant cultivars Camm and Trident, carrying *Yr17*, which is effective against Pst pt 134 E16A+, developed less than 10% stripe rust (Table 5). Cultivars Carnamah, EGA Bonnie Rock, Ajana and Westonia developed high (64–93%) stripe rust severity, whereas the remaining seven cultivars possessing commercially acceptable levels of adult plant stripe rust resistance developed a relatively much lower terminal stripe rust severity (9–27%). The average terminal stripe rust severities of tip-awned DH lines (8%) and the resistant parent WAWHT2046 (9%) were very low. *YrWA* carrying genotypes produced a resistant to moderately resistant (R-MR) response with a very low level of tissue damage (< 10%). The adult plant stripe rust response of *YrWA* lines was similar to the *Yr17* carrying cultivars Camm and Trident (Table 5). The tip awned DH lines showed a significant reduction in average stripe rust severity when compared with awned lines.

Pedigree analysis

Line WAWHT2046 was derived from crosses involving three parents (AUS22857/Kulin//Blade). AUS22857 was derived from three parents (Mildress/Mochis 73//Pallow) and was originally obtained from ICARDA, Syria. All three parents of WAWHT2046 were tested as seedlings against Pst pathotypes 110 E143A+ and 134 E16A+. Parents Kulin and Blade showed susceptible responses against both pathotypes, whereas wheat accession AUS22857 exhibited an infection type 3C similar to line WAWHT2046 against Pst pt 134 E16A+. This observation suggested that the resistance gene *YrWA* was derived from AUS22857.

Discussion

Genetic analysis of stripe rust resistance carried by line WAWHT2046 suggested monogenic inheritance of *YrWA* and its genetic association with *B1* placed it in the chromosome arm 5AL. Molecular mapping located *YrWA* distal to *B1*. All SSR markers screened on the DH population mapped proximal to both *YrWA* and *B1* with a gene order of *Xgwm595-Xgwm6a-Xgwm291-Xgwm410.2-B1-YrWA*. Despite an exhaustive search, we failed to map any available SSR marker distal to gwm410.2. This observation is in agreement with recently published consensus map (Somers et al. 2004). None of the 5,000 diversity array technology (DArT) clones mapped distal to gwm410. Stripe rust resistance genes characterized so far are located in chromosomes 1B, 2A, 2B, 4D, 5B, 6B, 7B and 7D (McIntosh et al. 1998).

Table 5 Stripe rust severity of parental, control and doubled haploid lines (grouped on the basis of tip awn phenotype linked to *YrWA*) assessed in the field at Manjimup, Western Australia, 2004

Genotype	Effective resistance	Terminal stripe rust severity (%)	
		Arcsin	Detrans
Parents			
WAWHT2046 (tip awn)	*Yr34*	17	9
Carnamah (normal awn)		52	62
Doubled haploids (WAWHT2046/Carnamah)			
Tip awned		16	8
Normal awned		37	37
Control lines (ranked by severity)			
Camm	*Yr17*	14	6
Trident	*Yr17*	16	7
Kukri	APR	18	9
Sunco	APR	22	14
Janz	APR	23	15
Annuello	APR	26	19
Sunstate	APR	28	22
Frame	APR	31	27
Wyalkatchem	APR	31	27
EGA Bonnie Rock		57	70
Ajana		64	81
Westonia		74	93
P		< 0.001	
lsd (P < 0.05)		11	
cv%		41	

APR adult plant resistance

Chromosome 5A has not yet been reported to carry any stripe rust resistance gene. Thirty-three loci for resistance to stripe rust were already characterized and named by various workers prior to the identification and characterization of *YrWA*. *YrWA* was named *Yr34*. Line WAWHT2046 was submitted to the Australian Winter Cereal Collection, Tamworth as the source for *Yr34* and it was given the accession number AUS91389. Susceptibility of two parents Kulin and Blade and the relatively low infection similar to that of WAWHT2046 produced by the third parent AUS22857 against Pst pt 134 E16A+, indicated that *Yr34* was derived from the parent AUS22857.

Yr34 is a useful source of resistance against Pst pathotype 134 E16A+. Resistance expression in field grown plants was at a similar level to *Yr17*. Grouping on the basis of awn type significantly reduced the mean stripe rust severity of a tip awned DH sub-population. It is important not to use *Yr34* alone in susceptible genetic backgrounds as the pathogen would require only a single step increase in virulence to render it ineffective. Molecular markers closely linked with stripe rust resistance genes *Yr9*, *Yr10*, *Yr15*, *Yr24*, *YrH52*, *Yr17* have been identified (Bariana et al. 2002; Frick et al. 1998; Nadella et al. 2002; Peng et al. 1999; Robert et al. 1999; Seah et al. 2001; Sun et al. 1997; Zakeri et al. 2003). Metzger and Silbaugh (1970) also reported the close genetic association of *Yr10* with brown glume colour. It would be feasible to combine *Yr34* with some of these genes to achieve durability for stripe rust resistance in new cultivars through gene combinations. A future objective of our research group is to identify markers closely linked with *Yr34*, however, in the meantime the loose linkage of *Yr34* with *B1* (12.2 cM) can be exploited together with stripe rust response tests to transfer this resistance to commercial genotypes.

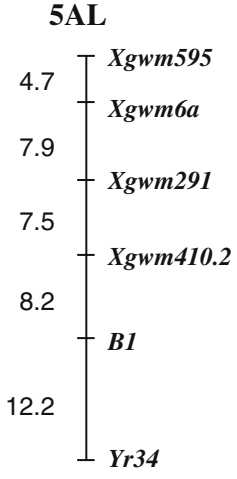

Fig. 1 Genetic map derived from WAWHT2046/Carnamah population showing the location of *Yr34* at the distal end of the chromosome arm 5AL. Genetic distances (in cM) are presented to the *left* of the genetic map

Genetic analysis of APR on *Yr34*-lacking lines (61) indicated the presence of at least three genes. The higher responses of some lines than that exhibited by both parents indicated that APR was contributed by both parents and resistance genes are genetically independent. The results presented here demonstrated that the line WAWHT2046 (AUS91389) if used as a resistance donor in breeding programs for stripe rust improvement, will also provide APR to stripe rust in addition to *Yr34*. This population is currently being screened at different locations against stripe rust under field conditions. A comprehensive genetic map is also being developed to identify APR controlling genomic regions.

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