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MOLECULAR MAPPING OF RUST RESISTANCE GENES IN A DOUBLED HAPLOID POPULATION OF WHEAT

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SUMMARY

Molecular mapping of rust resistance genes was investigated in the doubled haploid (DH) population derived from a hybrid between a line DM5637*B8 and an Australian wheat variety H45. The DH population was grown at the University of Sydney's Plant Breeding Institute site at Cobbitty in 2006 and 2007. The Diversity Array Technology (DArT) approach was employed to genotype the population. Resistance genes *Yr7*, *Sr36* and *Lr13* mapped on chromosome 2B, while linked stem rust and leaf rust resistance genes *Sr24* and *Lr24* were placed on the long arm of chromosome 3D. Rust resistant loci *Sr36*, *Lr13* and *Yr7* were flanked by the marker loci wPt-0395 and wPt-0981. The rust resistance gene combination, *Sr24/Lr24*, was flanked by DArT markers wPt-7752 and wPt-8845 on chromosome 3D. QTL analysis for adult plant stripe rust responses indicated the involvement of chromosomes 2B, 3B, 4B and 5B. QTL on chromosome 4B (*QYr.sun-4B*) can be revealed as the second component of *YrA*. Two QTLs (*QLr.sun-2B* and *QLr.sun-3D*) detected on chromosomes 2B and 3D, controlling low leaf rust response, corresponded to genomic locations of seedling leaf rust resistance genes *Lr13* and *Lr24*. DM/H45 population possessed low Polyphenoloxidase (PPO) activity, good YAN colour stability and sprouting tolerance. The lines of this population were identified having high levels of adult plant rust (APR) to rust diseases and having good end-use quality. Genetic associations of DArT markers with rust resistance genes *Lr13, Sr36, Yr7* and *Lr24/Sr24* would be useful to identify PCR-based markers for tracing these genes in diverse wheat germplasm.

Key words: Adult plant resistance, DArT markers, molecular mapping, quantitative trait loci, rust resistance

Key findings: A breeding effort that aims to combine different desirable colour attributes and rust resistance in a single genotype is required to generate improved noodle wheat cultivars. Genetic associations of DArT markers with rust resistance genes *Lr13, Sr36, Yr7* and *Lr24/Sr24* would be useful to identify PCR-based markers for tracing these genes in diverse wheat germplasm.

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INTRODUCTION

A sustainable Australian wheat industry is dependent on the continuing development of high yielding wheat varieties that possess desired quality attributes. To be economically viable for the producer, such varieties need to perform well in the presence of various biotic and abiotic stresses. Among the biotic stresses, wheat rust diseases are of major importance because of their ability to cause substantial yield losses (McIntosh *et al.,* 1995). The three rust diseases of wheat are leaf rust caused by *Puccinia triticina* (Pt), stripe rust, caused by *Puccinia striiformis* f. sp. tritici (Pst) and stem rust, caused by *Puccinia graminis* f. sp. tritici (Pgt). Deployment of resistance genes in combinations has been an effective and economic strategy used over the decades (Watson and Singh, 1952; McIntosh and Brown, 1997; Bariana *et al.,* 2007a).

Resistance can be categorised as either seedling or adult plant depending on the growth stage it is expressed. Seedling resistance is effective throughout the life of the plant while adult plant resistance is expressed at the later-stages of plant development (Bariana and McIntosh, 1995; Chen, 2005). Adult plant resistance is regarded as potentially durable because combinations of more than two genes are required to achieve acceptable levels of resistance (Bariana and McIntosh, 1995; Bariana *et al.,* 2010). The attraction of employing several adult plant resistance genes in combination is that in the event of pathogen change causing one of the resistance genes ineffective other gens will remain effective.

Various molecular marker systems are available to determine genomic regions controlling seedling and adult plant resistance in wheat (Bansal *et al.,* 2009; Liu *et al.,* 2010). These include microsatellite markers, RFLP, AFLP and Diversity Array Technology (DArT). The latter marker system is an efficient approach of whole genome analyses (Bariana *et al.,* 2007a; Jing *et al*. 2009, Wenzl *et al*. 2010). In this study QTL mapping of Molecular mapping of rust resistance genes was performed on a doubled haploid (DH) population derived from DM5637*B8 (low PPO line) x H45 (high PPO line) because it will provide information that is relevant to prime hard wheat breeding in Northern NSW and QLD. The objective of this investigation was to study inheritance of resistance to rust diseases at both seedling and adult plant stages, and to find quantitative trait loci (QTL) for rust resistance using DArT markers.

MATERIALS AND METHODS

Plant materials

A doubled haploid (DH) population derived from the cross DM/H45 was used to study inheritance of resistance to rust diseases at both seedling and adult plant stages. Genetic mapping of rust resistance using the DArT molecular markers was performed to identify genomic regions that controlled adult plant resistance in this population. A set of 187 doubled haploid (DH) lines was generated from a hybrid between DM5637*B8 (DM) and H45. The experimental material was sown in 2 replications at the experimental area of the Plant Breeding Institute, Cobbitty during the 2006 and 2007 growing seasons. Ninety-two randomly chosen lines and parents were used for molecular mapping studies.

Seedling resistance assessment

Four lines were sown as clumps of 10-12 seedlings per line in 9cm diameter plastic pot filled with standard potting mix (mixture was composed of 80% pine bark and 20% coarse sand). Fourteen-day old seedlings were inoculated with *Pgt*, *Pt* and *Pst* pathotypes separately as listed in Table 1. Seedlings inoculated with *Pst* pathotypes were incubated in a dark room at 10^{O}C for 24 hours, while seedlings inoculated with *Pgt* pathotypes were placed in a microclimate room with natural light at 18^{O}C for 48 hours. Seedlings inoculated with *Pt* pathotypes were incubated in a dark room at ambient temperatures of about 15^{O}C for 24 hours, in which mist (100% humidity) was generated by an ultrasonic humidifier. Inoculated plants were then transferred from an incubation room to microclimate rooms where temperature and irrigation were programmed.

Table 1. Pathotypes used for seedling rust tests and in field studies.

Rust pathotype	Green house	Field	
		Crop season 2006	Crop season 2007
Puccinia graminis	34-1,2,3,6,7,8,9 [=205]		
	98-1,2,3,5,6 [=279]		
Puccinia striiformis	108 E141 A+ [=420]	134E16A+ [=572]	134E16 A+, Yr17+ [=599]
		104E137A-Yr17+ [=544]	110E143A+ [=444]
			150E16A+ [=598]
Puccinia triticina	104-1,2,3,(6),(7),11 [=521]	104-1,2,3,(6),(7),9,11 [=521]	104-1,2,3,(6),(7),9,11 [=521]
	104-1,2,3,(6),(7),11,13 [=547]	104-1,2,3,(6),(7),11,13[=547]	104-1,2,3,(6),(7),11,13 [=547]
		10-1,3,7,9,10,12 [=592]	10-1,3,7,9,10,12 [=592]
		76-3,5,9,10 +Lr37 [=594]	76-3,5,9,10 +Lr37 [=594]

Rust reactions were recorded 10 days after inoculation for leaf rust and 14 days after inoculation for stem rust and stripe rust. Infection types (IT) of stripe, leaf and stem rust were assessed based on the scale described by McIntosh *et al.,* (1995).

Adult plant rust assessment

DH lines (15-20 seeds) were sown in 60 cm long rows along with parents. The experimental area was inoculated with the pathotypes listed in Table 1. The adult plant rust responses were scored on 1 to 9 scales (Bariana *et al.,* 2007b).

DArT marker analyses

Genomic DNA was extracted from 92 DH lines derived from the cross DM/H45 using a modified CTAB protocol (Doyle *et al.,* 1990). The 92 lines were randomly selected from the population. Fifty µL of restriction quality DNA of each DH genotype and the parents were sent to Triticarte Pty. Ltd., Canberra, Australia (http://www.triticarte.com.au) for whole genome profiling. The marker loci were scored as present (1) or absent (0). Markers with 'P' values >80, 77-80, and 75-77 were termed as extremely reliable, usually scored, and provide useful information, respectively. The 'P' value reflected how well the two phases (present = 1 vs absent = 0) of the marker were separated in the sample and P was based on ANOVA, which is an estimate of marker quality. DArT markers consisted of the prefix 'wPt', followed by a number corresponding to a particular clone in the genomic representation, where 'w' stands for wheat, 'P' for *Pst*I (primary restriction enzyme used) and 'T' for *Taq*I (secondary restriction enzyme) (Wenzl *et al.,* 2004; Huttner *et al.,* 2006).

Linkage map construction and QTL mapping

The initial linkage mapping was performed with Cartablanche software, version 1.5.0(111), Keygene Products B.V. Linkage groups were further reassessed and reconstructed with Map Manager (QTXb20). Interval mapping was performed at $P = 0.01$ for marker-trait association using Map Manager QTXb20 (Manly *et al.,* 2001). The analysis was carried out for the two individual environments (years) and as well as the pooled data from both environments. The logarithm of the odds (LOD) thresholds in the regression analyses $P < 0.01$ and $P < 0.001$ were used for declaring significant and highly significant levels, respectively. QTL effects were calculated by using the permutation function. The average contribution of each QTL was calculated by comparing means of phenotypic scores of genotypes carrying the positive and negative alleles. The MapChart version 2.2 (Voorrips, 2002) was used to present chromosomes and QTL figures.

Statistical analyses

At seedling stage, DH lines were grouped according to specific infection types produced by rust resistance genes segregating in the population. Chi-squared (χ^2) analyses were performed to identify the goodness of fit of observed segregations with the expected genetic ratios (Mather 1951).

RESULTS

Seedling resistance

Stem rust

Parents DM and H45 showed infection types (IT) 0; and IT X-, respectively, against *Pgt* pathotype 34-1,2,3,6,7,8,9. H45 produced IT 22+, when tested against pathotype 98-1,2,3,5,6 and no change in infection type of DM was observed. IT 0;, IT 2 =, IT X- and IT 22+ are conditioned by stem rust resistance genes *Sr36, Sr24, Sr17* and *Sr30*, respectively (McIntosh *et al*., 1995). DM, a Sunco-derivative, carries *Sr24* and *Sr36* (unpublished data), and H45 possesses *Sr17* and *Sr30* (Bariana *et al.,* 2007b). Seedling stem rust response segregation among DH lines is summarised in Table 2. χ^2 analyses of data indicated the involvement of four stem rust resistance genes against both *Pgt* pathotypes. The population was partitioned based on distinct infection types (0 to 0; vs. >;, 2- to 23 vs. 3+, X vs. 3+ and 2 = vs. 3+). Digenic inheritance of resistance was observed when χ^2 analysis for the subgroup IT 0 to 0; vs. IT >; was performed. Monogenic inheritance was observed for the other subgroups expressing IT 2- to 23 vs. IT 3+, IT X vs. IT 3+ and IT 2 = vs. IT 3+. Detection of IT0; and IT2 =, which are characteristic of *Sr36* and *Sr24*, respectively, among DH lines confirmed the presence of these genes in the parent DM. Similarly, the presence of infection types 2 to 23 and X conditioned by *Sr30* and *Sr17*, respectively, indicated the genes carried by the other parent H45. These results validated the identities of both parents.

Stripe rust

Results of seedling stripe rust response segregation among the DH population are summarised in Table 2. Parents DM and H45 showed IT 33+ and; respectively, when tested against *Pst* pathotype 108E141A+.Cultivar H45 carries *Yr7* (Bariana *et al.,* 2007b) and IT; N is characteristic of this gene. A very high proportion of susceptible lines were observed. Statistical analysis showed the deviation of segregation from the monogenic ratio (Table 2).

Leaf rust

Parents DM and H45, carry leaf rust resistance genes *Lr24* and *Lr13*, respectively, and produced infection types 4 and 3- against *Lr24*-virulent and *Lr13*-avirulent *Pt* pathotype 104-1,2,3,(6),(7),11,13. χ^2 analysis of leaf rust response data did not conform to the monogenic segregation due to a high proportion of lines lacking *Lr13* (Table 3). DM and H45 produced infection types; and 3-, respectively, against the *Lr24*-avirulent *Pt* pathotype 104-1,2,3,(6),(7),11. Chi-squared analysis of seedling leaf rust response segregation data (Table 2) suggested monogenic inheritance of resistance (IT; vs. IT >;) against this *Pt* pathotype 104-1,2,3,(6),(7),11. These results demonstrated the segregation of *Lr13* and *Lr24* in the DM/H45 DH population.

Adult plant resistance

Stripe rust

DM consistently produced a low adult plant stripe rust response score of 2 and scores for H45 varied from 6 to 7. Two replications of DH lines and parents were scored for adult plant stripe rust response variation during the 2006 and 2007 crop seasons. The frequency distribution of rust responses (scored four times at 10 days intervals) observed during the crop season 2006 are summarised in Figure 1. In 2007, only a selected set of lines that were used for molecular mapping were studied for their responses (Figure 2). An almost normal distribution for adult plant stripe rust response segregation was observed, however, it was slightly skewed towards resistance in both seasons.

Table 2. Chi-square analyses of seedling stem rust, stripe rust and leaf rust response variation among DM/H45 DH population when tested against different pathotypes under greenhouse conditions.

Rust pathogen and classification	Phenotype	Frequency					χ^2			
		Observed	Expected							
		-	1:1[a]	3:1[b]	7:1[c]	15:1[d]	1:1[a]	3:1[b]	7:1[c]	15:1[d]
Stem rust *Pgt* 34-1,2,3,6,7,8,9										
1	Resistant (IT<3)	175			163.62	175.31			0.791	0.001
	Susceptible (IT>3)	12			23.38	11.69			5.535	0.008
	Total	187			187	187			6.326	0.009
2	Resistant (IT0 to ;)	143	93.5	140.25			26.21	0.054		
	Susceptible (IT>;)	44	93.5	46.75			26.21	0.162		
	Total	187	187	187			52.42	0.216		
3	Resistant (ITX)	13	14.5				0.155			
	Susceptible (IT3+)	16	14.5				0.155			
	Total	29	29				0.310			
4	Resistant (IT2=)	18	17				0.059			
	Susceptible (IT3+)	16	17				0.059			
	Total	34	34				0.118			
Stem rust *Pgt* 98-1,2,3,5,6										
1	Resistant (IT< 3)	171			159.25	170.62			0.867	0.001
	Susceptible (IT>3)	11			22.75	11.38			6.069	0.012
	Total	182			182	182			6.936	0.013
2	Resistant (IT0 to ;)	137	91	136.5			23.253	0.002		
	Susceptible (IT>;)	45	91	45.5			23.253	0.005		
	Total	182	182	182			46.506	0.007		
Stripe rust *Pst* 108E141A+										
	Resistant (IT;NN)	54	93.5				16.69			
	Susceptible (IT>;NN)	133	93.5				16.69			
	Total	187	187				33.38			
Leaf rust *Pt* 104-1,2,3,(6),(7) 11,13										
1	Resistant (ITX to 3-)	59	93.5				12.73			
	Susceptible (IT4)	128	93.5				12.73			
	Total	187	187				25.46			
2	Resistant (IT;)	96	93.5				0.067			
	Susceptible (IT>;)	91	93.5				0.067			
	Total	187	187				0.134			

Table value of χ^2 at 1 d,f.,*P* = 5% is 3.84; *P* = 1% is 6.64. [a]Monogenic segregation ratio, [b]Digenic segregation ratio, [c]Trigenic segregation ratio, [d]Tetragenic segregation ratio.

Table 3. List of QTLs explaining APR to stripe and leaf rust in the DM/H45 doubled haploid population.

Site-Year/Traits	Chromosome	QTLs	Closest marker	LOD[1] value	R^2 (%)	Source parent
Year-2006						
Stripe rust	2B	*QYr.sun-2B*	*Lr13/Sr36/Yr7*	1.02	5	DM
	3B	*QYr.sun-3B*	wPt-2416	2.06*	11	H45
	4B	*QYr.sun-4B*	wPt-7569	5.90**	30	DM
	5B	*QYr.sun-5B*	wPt-3457	1.28	7	DM
Leaf rust	2B	*QLr.sun-2B*	Wpt-0395; *Lr13/Sr36/Yr7*	6.81**	32	H45
	3D	*QLr.sun-3D*	wPt-8845; *Lr24/Sr24*	1.89*	10	DM
Year-2007						
Stripe rust	2B	*QYr.sun-2B*	*Lr13/Sr36/Yr7*	2.54*	12	DM
	3B	*QYr.sun-3B*	wPt-2416	1.17	6	H45
	4B	*QYr.sun-4B*	wPt-7569	5.4**	28	DM
Leaf rust	2B	*QLr.sun-2B*	Wpt-0395; *Lr13/Sr36/Yr7*	1.58	9	H45
	3D	*QLr.sun-3D*	wPt-8845; *Lr24/Sr24*	3.88**	20	DM

[1]Logarithm of odds, [2]Phenotypic variation explained. Asterisk (*) and (**) indicate significant level at 0.05 and 0.01, respectively and non-asterisk LOD values indicate the suggestive level (determined at 5cM interval and 1000 permutations).

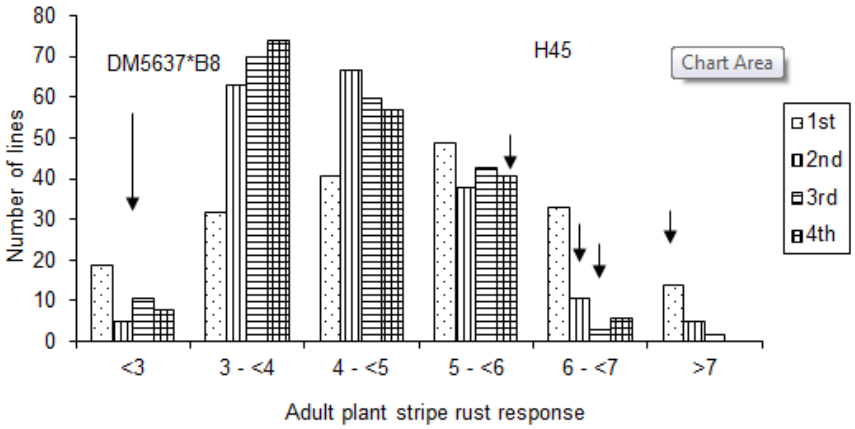

Figure 1: Frequency distribution of adult plant stripe rust responses of DM/H45 doubled haploid lines during 2006 (stripe rust response variations were assessed four times at 10 days intervals).

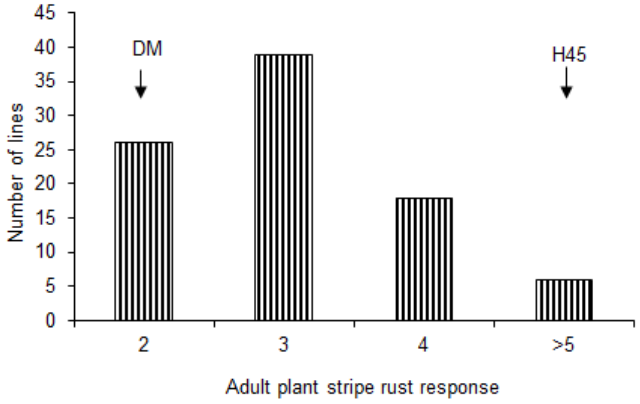

Figure 2: Frequency distribution of adult plant stripe rust responses of a sub set of DM/H45 doubled haploid lines during 2007 crop season.

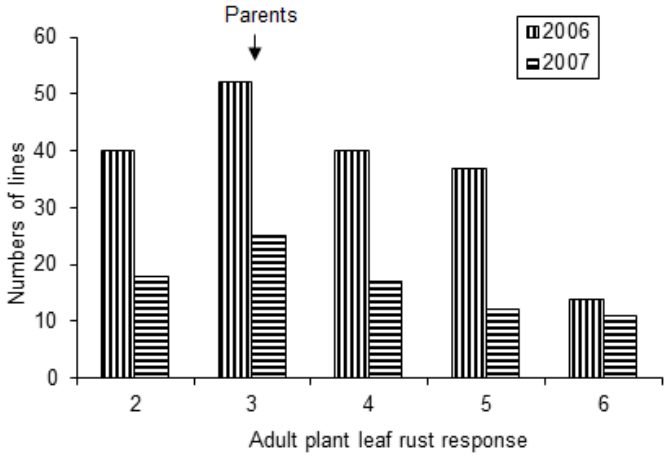

Figure 3: Frequency distributions of adult plant leaf rust responses of DM/H45 DH population during 2006 and 2007 crop seasons (a sub set of 92 lines and parents that were used in molecular mapping were sown in 2007).

Leaf rust

The DH population was tested for adult plant leaf rust response variation in 2006 using a mixture of *Pt* pathotypes 104-1,2,3,(6),(7),9,11; 104-1,2,3,(6),(7),11,13; 10-1,3,7,9,10,12 and 76-3,5,9,10+*Lr37*(numbers in parentheses indicate an intermediate response on the corresponding differential). Both parents produced a similar field response of 3. Adult plant leaf rust response variation among DH lines is presented in Figure 3. Only 92 DH lines were tested in 2007. DH lines that exhibited lower leaf rust responses carried seedling leaf rust resistance genes *Lr13* and *Lr24* in combination. This combination is effective against the Australian *Pt* pathotypes used in this study. Absence of completely susceptible segregates among DH lines at the adult plant stage indicated that both parents carried a gene in common (Figure 3). The leaf rust response data for both crop seasons (2006 and 2007) was slightly skewed towards resistance. Transgressive segregation for leaf rust response was observed in this DH population (Figure 3).

Molecular mapping

Linkage groups construction

The DNA of 92 lines and the two parents of the DM/H45 population were analysed with DArT. Three hundred and eighty-four DArT markers with an overall call rate of 94.28% were polymorphic between parents. The overall marker P values ranged between 73.87 and 98.84 with a mean of 89.63. Seventeen markers had P values below 77. Of the 384 polymorphic markers, 317 markers were assembled into 30 linkage groups using Cartablanche software, version 1.5.0(111). Sixty-seven markers could not be assigned to any linkage groups. Eight linkage groups were assigned to chromosomes in the A genome, 13 linkage groups were assigned to chromosomes in the B genome, and 7 linkage groups were assigned to the D genome. Two linkage groups could not be assigned to chromosomes and were designated mixed groups. The marker number in each linkage group varied considerably. A total map distance of 882.9cM was developed (Sadeque 2008).

Seedling rust resistance genes

The seedling rust response data against *Pgt* pathotype 34-1,2,3,6,7,8,9; *Pt* pathotypes 104-1,2,3,(6),(7),11 and 104-1,2,3,(6),(7),11,13; and *Pst* pathotype 108E141A+ were converted to genotypes (numbers in parentheses indicate an intermediate response on the corresponding differential). Segregations at rust resistance loci *Sr36*, *Sr24/Lr24*, *Lr13* and *Yr7* enabled incorporation of these loci into the respective map location. Resistance genes *Yr7, Sr36* and *Lr13* mapped to their previously reported locations on chromosome 2B (McIntosh *et al.*, 1995; Figure 4). Linked stem rust and leaf rust resistance genes *Sr24* and *Lr24* were also correctly placed on the long arm of chromosome 3D (Figure 4). Rust resistance loci *Sr36, Lr13* and *Yr7* showed no recombination due to the absence of pairing in this region. These genes were flanked by the marker loci wPt-0395 and wPt-0981. The rust resistance gene combination, *Sr24/Lr24*, was flanked by DArT markers wPt-7752 and wPt-8845 on chromosome 3D at 18.6cM and 15.2cM, respectively.

Adult plant resistance genes

Composite interval mapping analysis of adult plant stripe rust response data detected four QTLs viz., *QYr.sun-2B*, *QYr.sun-3B, QYr.sun-4B* and *QYr.sun-5B* in the 2006 experiment (Table 3 and Figure 5). *QYr.sun-2B* (LOD = 1.02) explained 5% of phenotypic variation and was detected only at the suggestive level. *Sr36* showed the closest genetic association with this QTL and it was contributed by the parent DM. The chromosome 3B located QTL (*QYr.sun-3B*) explained 11% of phenotypic variation (LOD = 2.06) and was contributed by the H45 parent. DArT marker wPt-2416 was the closest marker. The highly significant QTL *QYr.sun-4B*, explained 30% of phenotypic variation (LOD = 5.90), mapped close to the DArT marker wPt-7569 and was contributed by the parent DM. The fourth QTL, *QYr.sun-5B* contributed 7% towards stripe rust severity reduction. Marker wPt-3457 mapped closest to *QYr.sun-5B.* This QTL was also detected only at the suggestive level.

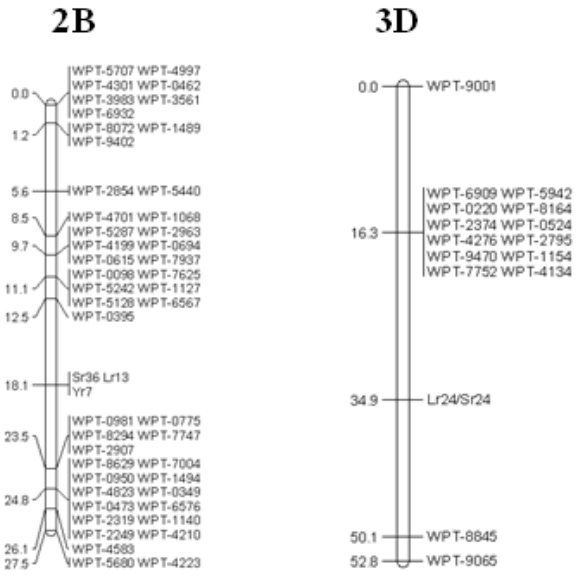

Figure 4: A partial genetic map of chromosomes 2B and 3D showing location of seedling rust resistance genes.

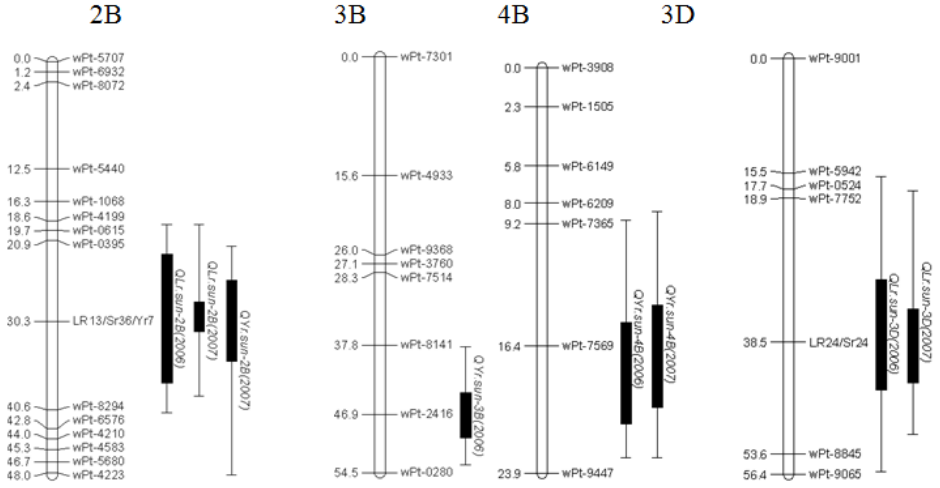

Figure 5: QTL explaining involvement of genomic regions in controlling adult plant leaf and stripe rust response variation in the DM/H45 DH population. Rust response data from 2006 and 2007 cropping seasons were used to generate QTL figures.

In 2007, QTLs were identified on chromosomes 2B, 3B and 4B. The major QTL (*QYr.sun-4B*) was located on chromosome 4B (LOD = 5.40) and explained 28% of phenotypic variation. Marker wPt-7569 was closely associated with this QTL. The other two QTLs *QYr.sun-2B* and *QYr.sun-3B* explained 12% (LOD = 2.54) and 6% (LOD = 1.17) of phenotypic variation, respectively. These three QTLs together accounted for 46% of phenotypic variation in stripe rust response in both the seasons.

The QTL analyses for APR to leaf rust identified genomic regions on chromosomes 2B and 3D contributed towards low leaf rust response in 2006 and 2007 (Table 3 and Figure

5). The QTLs *QLr.sun-2B* and *QLr.sun-3D* corresponded to genomic regions carrying leaf rust resistance genes *Lr13* and *Lr24*, respectively. In 2006, a major QTL identified on chromosome 2B explained 32% of phenotypic variation (LOD = 6.81) and DArT marker wPt-0395 was closely associated. The second QTL that was detected on chromosome 3D (LOD value of 1.89) explained 10% of observed phenotypic variation. Marker wPt-8845 was closely associated with this QTL. In 2007, *QLr.sun-2B* explained 9% of phenotypic variation with a LOD value of 1.58, whereas *QLr.sun-3D* contributed 20% towards reduction in leaf rust response alone (LOD = 3.88). The DArT markers wPt-0395 and wPt-8845 were significantly associated with the QTLs on chromosomes 2B and 3D, respectively.

DISCUSSION

DM carries rust resistance genes *Lr24/Sr24* and *Sr36* and H45 possesses *Yr7, Sr17, Sr30* and *Lr13*. *Pgt* pathotypes 34-1,2,3,6,7,8,9 and 98-1,2,3,5,6 differed with respect to their pathogenic specificities for *Sr30* and *Sr17*, respectively. Genetic analysis results using these pathotypes indicated the involvement of four independent genes in controlling low seedling stem rust responses among the DM/H45 DH population. The *Pgt* pathotype 34-1,2,3,6,7,8,9 detected the presence of *Sr24*, *Sr36* and *Sr17*. While the pathotype 98-1,2,3,5,6 identified segregation at the *Sr24*, *Sr36* and *Sr30* stem rust resistance loci. The genetic population was categorised into different classes based on distinct infection type expressions (Table 3). Resistance expressed by stem rust resistance genes *Sr17*, *Sr24* and *Sr30* showed monogenic inheritance, whereas resistance conditioned by *Sr36* locus exhibited digenic inheritance. *Sr36* is located on a large *Triticum timopheevii* translocation (Friebe *et al.,* 1996). Over-transmission of *Sr36* in segregating populations was reported by Nyquist (1962). In a molecular mapping study involving *Sr36*, Bariana *et al.* (2001) also observed preferential transmission of gametes carrying *Sr36* over those lacking it. In light of preferential transmission of *Sr36*, it was concluded that only three genes each were involved in conditioning low stem rust responses in tests with two *Pgt* pathotypes. The preferential transmission of *Triticum timopheevii* translocation was noticed in experiments involving segregation at the 2BL located stripe rust resistance gene *Yr7* and 2BS located leaf rust resistance gene *Lr13*. The number of genotypes carrying *Yr7* and *Lr13* was almost 50% less than expected. In contrast, segregation at the *Lr24* locus followed monogenic inheritance.

Rust resistance genes *Sr24/Lr24*, *Sr36*, *Lr13* and *Yr7* mapped to their known locations. The absence of recombination between *Triticum timopheevii* and wheat chromosome 2B was evident from clustering of *Sr36*, *Lr13* and *Yr7*. The *Lr24/Sr24* locus was successfully incorporated into the chromosome 3D map. The map locations of these genes were in agreement with McIntosh *et al.* (1995) and Akbari *et al.* (2006).

QTL analysis for adult plant stripe rust responses indicated the involvement of chromosomes 2B, 3B, 4B and 5B. *QYr.sun-3B* was contributed by the parent H45, whereas *QYr.sun-2B*, *QYr.sun-4B* and *QYr.sun-5B* were contributed by DM. *QYr.sun-3B* (closely linked DArt marker wPt-2416) may correspond to *Yr30* which was reported by Singh *et al.* (2000). Working with an Avocet/Pavon mapping population, William *et al.* (2006) reported the involvement of chromosome 4BL in reducing stripe rust severity and the positive effect was contributed by Avocet. *QYr.sun-4B* (closely linked DArt marker wPt-7569) could be the same as that reported by William *et al.* (2006). Suenaga *et. al.* (2003) also detected a QTL that had effect on stripe rust severity on chromosome 4BL. As different marker technologies were used in this study and a direct comparison was not possible.

Bariana (1991) located one component of the temporarily designated dominant complementary stripe rust resistance *YrA* on chromosome 3D. *YrA* occurs alone or in combination with other genes in Australian wheats that are derivatives of WW15 (McIntosh *et al.,* 1995). Sarker (2006) located the second component of *YrA* in chromosome 4B using SSR markers. *QYr.sun-4B* could be the second component of *YrA*. The presence of this

resistance gene in DM can be further substantiated from the pedigree analysis of this parental line. DM is derived from the cross AUS1408/Sunco. The parent Sunco is a derivative of WW15 (Pedigree: SUN 9E-27*4/3Ag14//WW15/3/3*Cook) (Wrigley *et al.*, 2001). Reconstitution of the *YrA* resistance by crossing DM with the 3D component of *YrA* would be necessary to confirm this observation.

Bariana *et al.* (2010) detected a QTL on chromosome 2B of Sunco, a parent of DM. *QYr.sun-2B,* detected in this study, corresponded to the same genomic region. This QTL appeared to be located on the *Triticum timopheevii* translocation. Kaur (2007) detected a QTL on chromosome 5B of the Indian wheat cultivar HD2009. *QYr.sun-5B,* detected in this study, corresponded to the same genomic region. The associated DArT marker wPt-3457 was common to both of these studies where the QTL was detected only at the suggestive level. Two QTLs (*QLr.sun-2B* and *QLr.sun-3D,* closely linked DArt markers wPt-0395 and wPt-8845 respectively) detected on chromosomes 2B and 3D, controlling low leaf rust response, corresponded to genomic locations of seedling leaf rust resistance genes *Lr13* and *Lr24*. The higher contributions of chromosomes 2B and 3D in 2006 and 2007, respectively, could be attributed to the predominance of the *Lr13*-avirulence and *Lr24*-avirulence in *Pt* pathotypes in the respective seasons.

This study explained the genetic bases of seedling resistance and APR to rust diseases in the DM/H45 population. Lines carrying high levels of APR to rust diseases and having good end-use quality were identified. These genotypes possessed low PPO activity, good YAN colour stability and sprouting tolerance. Interval mapping analysis detected two major QTLs (*QLr.dmh45-2B and QLr.dmh45-3D*) on chromosomes 2B and 3D, that control APR to leaf rust and these genomic locations corresponded to seedling leaf rust resistance genes *Lr13* and *Lr24*, respectively. The limitation identified with the DArT marker system in this study is low marker coverage in the areas of marker loci linked with QTLs. Despite this limitation, the genetic information and marker trait associations of detected QTLs in the traits of interest will complement further studies in this area. Genetic associations of DArT markers with rust resistance genes *Lr13, Sr36, Yr7* and *Lr24/Sr24* would be useful to identify PCR-based markers for tracing these genes in diverse wheat germplasm.

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