Theor Appl Genet (2011) 122:395–403
DOI 10.1007/s00122-010-1455-x

ORIGINAL PAPER

Identification and validation of quantitative trait loci conferring tan spot resistance in the bread wheat variety Ernie

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Received: 30 June 2010 / Accepted: 8 September 2010 / Published online: 24 September 2010

Abstract Tan spot, caused by *Pyrenophora tritici-repentis*, is a foliar disease of wheat, and it can inflict serious reduction in grain yield and quality. The bread wheat variety Ernie was found to be immune to this disease in Australia, and its genetic control was investigated by quantitative trait loci (QTL) analysis using a doubled haploid population. Eight QTL were identified in this population from three independent trials, and four of them were derived from the parent Ernie. The most significant QTL was located on chromosome arm 2BS, explaining 38.2, 29.8 and 36.2% of the phenotypic variance, respectively, in these trials. The effects of the 2BS QTL were further validated in four additional populations. The presence of this single QTL reduced disease severity by between 29.2 and 67.1% with an average of 50.5%. The significant effects of this QTL and its consistent detection across all the trials with different genetic backgrounds make it an ideal target for breeding programmes as well as for its further characterization. Data from this study also showed that neither plant height nor heading date significantly affects tan spot resistance.

Communicated by A. Graner.

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Introduction

Tan spot or yellow leaf spot, caused by *Pyrenophora tritici-repentis*, is a major foliar disease of wheat that affects grain quality and yield (de Wolf et al. 1998; Ciuffetti and Tuori 1999). In Australia, tan spot is widespread throughout the north-eastern wheat areas, and this single disease causes an estimated annual loss of A$212 M with a potential of A$676 M (Murray and Brennan 2010). Tan spot is also one of the most destructive cereal diseases in many other major wheat-growing regions worldwide. Yield losses caused by tan spot amounting up to 48% have been reported (Hosford 1982; Mehta and Gaudencio 1991). Of the various options in managing this disease, the use of resistant varieties is considered the most effective and economical control measure (de Wolf et al. 1998).

As part of the effort in breeding resistant varieties, the genetics of tan spot resistance has been intensively investigated. These studies showed that resistance to this disease can be inherited either qualitatively (Anderson et al. 1999; Singh and Hughes 2005) or quantitatively (Nagle et al. 1982; Elias et al. 1989; Faris et al. 1997). Loci conferring resistance to tan spot have been identified on several wheat chromosomes (Faris et al. 1996, 1997; Faris and Friesen 2005; Anderson et al. 1999; Singh and Hughes 2006; Tadesse et al. 2006a, b; Effertz et al. 2002, Friesen and Faris 2004; Sun et al. 2010).

Genotypes used in previous studies showed different levels of resistance, ranging from modest resistance (Friesen and Faris 2004; Chu et al. 2010) to immunity (Tadesse et al. 2006b). However, genotypes immune to tan spot reported up to date seem to be confined to those of artificial synthetic wheat genotypes. Monosomic analyses revealed that resistance in some of these synthetic wheat genotypes immune to tan spot was controlled by single

genes (Tadesse et al. 2006b). Synthetic wheats have been used for the introgression of a wide range of traits into adapted germplasm breeding programmes, but these genotypes can be difficult to manipulate due to linkage drags between targeted traits and genes deleterious to yield or quality (Peña et al. 1995; Mares and Mrva 2007; Mrva et al. 2009).

We identified a commercial bread wheat variety showing no tan spot symptoms in several consecutive crop cycles in Queensland, Australia. We are interested in knowing whether the resistance of this commercial variety is also controlled by a single gene as reported for some synthetic wheat genotypes (Tadesse et al. 2006b). It is also of interest to clarify if the reported relationships in durum wheat between tan spot resistance and either plant height (Fernandez et al. 2002) or heading date (Elias et al. 1989) also exist in bread wheat.

Materials and methods

Plant materials

A doubled haploid (DH) population consisting of 153 lines derived from a cross between two bread wheat varieties, Batavia and Ernie, was used for mapping QTL conferring tan spot resistance. Ernie is an American soft red winter wheat variety immune to tan spot infection, and Batavia is an Australian variety highly susceptible to this disease (Fig. 1). Four additional populations were developed for validating the effect of QTL identified from the mapping population. They included 67 F5 lines from a cross of 27868/Ernie, 120 F5 lines from a cross of 13832/Ernie, 55 DH lines from a cross of Kennedy/Ernie and 120 F3 lines from a cross of 10903/Ernie. All of the populations used in this study were produced in this laboratory. Both of the DH populations were generated by microspore-culture, and the other three populations were all generated by single seed descent.

Linkage map used and molecular marker analysis of the validation populations

A linkage map consisted of 899 DArT and SSR markers spanning a genetic distance of 1,865.9 cM with an average distance of 2.1 cM between markers had been constructed for the population of Batavia/Ernie (Li et al. 2010). The genotypic data, together with the phenotypic data of tan spot severity, plant height and heading date of the DH lines, were used for QTL analyses.

During the assessment of tan spot resistance, DNA was isolated from all individual lines of the four validation populations. For each line, a small section of a young leaf

Fig. 1 The two parental varieties of the Batavia/Ernie doubled haploid population used for QTL mapping, showing their contrasting difference in tan spot severities

was ground with liquid nitrogen, and DNA was isolated using a standard CTAB protocol (Doyle and Doyle 1990). DNA was diluted to a final concentration of 25 ng/µl prior to PCR analysis.

Individual lines of the four validation populations were genotyped using a microsatellite marker closely linked with the major locus conferring tan spot resistance (see "Results"). PCR reactions for the SSR analysis were carried out using [α-^{33}P]CTP following manufacturer's protocol (Multiplex-Ready Marker User Handbook, version 2.0), and PCR products were separated on 4% polyacrylamide gel containing 7 M urea.

Tan spot assay

An initial tan spot epidemic environment in a glasshouse was established by artificially inoculating two highly susceptible durum wheat varieties (Wallaroi and Bellaroi) with field-infected plants. Both of these two durum varieties were bred by New South Wales Department of Primary Industries at Tamworth. The former was released in 1993 and the latter in 2002. The main consideration for using the durum varieties for establishing the initial epidemic environment was that they would produce more ascocarps than with the use of more resistant bread wheat genotypes. This artificial epidemic environment was maintained by repeatedly planting these two susceptible varieties in each corner of the glasshouse maintained at 25/18 (±5)°C

day/night temperature and 65/80 (±10)% day/night relative humidity with natural illumination.

The method used for tan spot assessment was based on that described by Adee and Pfender (1989). Briefly, infected straws of the two susceptible durum varieties were collected, and the amount of inoculum on the straws was estimated by counting the number of mature ascocarps. Straws with similar number of mature ascocarps were cut into 10 cm pieces, and five pieces of the infected straws were placed on the surface of each pot when seedlings reached the three-leaf stage. To promote tan spot infection, water was sprayed with a backpack sprayer six times a day for seven consecutive days following inoculation. Tan spot severity was assessed 5 weeks post-inoculation using a ranking system between 0 and 5 as described by Lamari and Bernier (1991), where 0 = no visible symptom of either necrosis or chlorosis; 1 = small (1 mm) dark brown or black spots without chlorosis and necrosis; 2 = small lesions with very little chlorosis and necrosis; 3 = medium-sized lesions surrounded with distinct chlorotic and necrotic rings generally not coalescing; 4 = lesions surrounded by chlorotic and necrotic zones, some lesions coalescing and 5 = large lesions consisting of coalescing chlorotic and necrotic zones.

The DH mapping population was assessed in three independent trials, and the four validation populations were assessed twice. Two replicates were used for each of the trials, each containing four plants in four separate pots (1 l each) filled with California potting mix. A randomised complete block design was applied for each of these trials.

Statistical analysis

All statistical analyses were performed using GenStat for Windows in the 10th edition (copy right Lawes Agricultural Trust, Rothamsted Experimental Station, UK). An analysis of variance (ANOVA) was conducted to detect if there were significant genetic effects for tan spot in the DH population. Within each trial, the following mixed-effects model was used: $Yij = \mu + ri + gj + wij$, where Yij observation on the jth genotype in the ith replication; μ general mean; ri effect due to ith replication; gj effect due to the jth genotype; wij error or genotype by replication interaction, where genotype was treated as a random effect and that of replicates as fixed. Heritability was estimated from the ANOVA using the formula: $h^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_e^2/r)]$, with σ_G^2 represents the genetic variance, σ_e^2 stands for the residual variance, and r the number of replicates per genotype (Nyquist 1991). The Pearson correlation coefficients were estimated between different trials. Means of each DH line within each trial were used for QTL analysis.

QTL analysis

The QTL analysis was preformed using MapQTL® 5.0 (van Ooijen 2004). The Kruskal–Wallis test (a non-parametric equivalent of the one-way ANOVA) was used in a preliminary analysis to detect associations between markers and individual traits. This was followed by interval mapping (IM) to identify the major QTL. Automatic cofactor selection was used to fit the multiple QTL model (MQM) [backward elimination ($P > 0.02$)] and to detect significantly associated markers as cofactors. For each trait, a permutation test was performed to identify the LOD threshold corresponding to a genome-wide false discovery rate of 5% ($P < 0.05$). Based on the permutation tests (1,000 permutations), a threshold LOD value was used to declare the presence of a QTL in the composite interval analyses. QTL identified in more than one trial which mapped closely to one another on the same linkage group and with alleles derived from the same parent were considered to represent the same QTL. Linkage maps were drawn using MAPCHART (Voorrips 2002).

Results

Distribution of the phenotypic data for tan spot, heading date and plant height

As having been observed previously, Ernie showed no symptom of tan spot disease, but Batavia was heavily infected in each of the trials conducted (Fig. 1). The average severities among the 153 DH lines were similar between two (TS-1 and TS-3) of the three trials with a mean value of about 1.95, while the average severity in the other trial (TS-2) was lower (1.25). The segregations of tan spot severity from all the three trials were slightly skewed towards the immune parent Ernie. A small one-direction transgressive segregation was observed in this mapping population for each of the three trials (Table 1). Transgressive segregation was apparent for both plant height and heading date in each of the trials (Table 1). Plant height was slightly skewed towards tall plants, while heading date was slightly skewed towards earliness. One-way ANOVA analysis showed significant differences among the 153 DH lines for tan spot severity, heading date and plant height in each of the trials (not shown). Strong correlations were found for tan spot severities between different trials with coefficient values ranging from 0.50 to 0.60 (Table 2). Both heading date and plant height showed strong correlations between different trials with coefficient values of 0.91 for heading date and 0.89 for plant height. Significant correlations were not detected between tan spot severity with either heading date (r ranging from −0.07 to −0.23)

Table 1 Phenotypic description of tan spot severity, heading date and plant height in different trials

Traits	Mean of parents		DH lines				
	Batavia	Ernie	Minimum	Maximum	Mean	SD	h^2
TS-1	4.1	0.0	0.0	5.0	1.9	1.4	0.9
TS-2	3.7	0.0	0.0	5.0	1.3	1.4	0.6
TS-3	4.0	0.0	0.0	5.0	2.0	1.4	0.8
PH-1	56.5	65.8	58.0	95.7	78.6	8.2	0.9
PH-2	58.3	59.5	45.7	89.2	73.0	8.7	0.8
HD-1	69.4	81.0	67.0	83.0	72.8	3.4	0.7
HD-2	71.0	83.5	67.0	85.0	73.6	3.8	0.7

The numeric numbers following the TS-, PH- and HD- are serial numbers of trials conducted for each of these traits

TS tan spot which was measured using a ranking system between 0 and 5, *PH* plant height which was measured in centimetres, *HD* heading data which was the number of days from sowing to heading

Table 2 Correlation coefficients between tan spot severity, heading date and plant height

	TS-1	TS-2	TS-3	HD-1	HD-2	PH-1
TS-1						
TS-2	0.56					
TS-3	0.5	0.6				
HD-1	−0.14	−0.23	−0.2			
HD-2	−0.07	−0.22	−0.15	0.91		
PH-1	0.07	0.12	0.06	−0.15	−0.17	
PH-2	0.06	0.1	0.05	−0.1	−0.14	0.89

The numeric numbers following the TS-, PH- and HD- are serial numbers of trials conducted for each of these traits

TS tan spot, *HD* heading date, *PH* plant height

Table 3 QTL identified for tan spot resistance, heading date and plant height in the population of Batavia/Ernie

Traits	Chromosomes	LOD	Closest markers	R^2	Origin
TS-1	1A	3.02	wPt-5776	18.4	Batavia
	7A	3.63	wpt-6447	16.9	Ernie
	2BS	7.36	wpt-0289	38.2	Ernie
	3BS	5.37	wPt-0571	25.3	Ernie
TS-2	6A	2.74	rpt-9065	13.2	Batavia
	2BS	6.11	wpt-0289	29.8	Ernie
	5BL	2.94	wpt-3076	11.9	Ernie
	3D	3.74	wpt-7705	23.2	Batavia
	7D	3.75	wpt-6769	21.1	Batavia
TS-3	1A	2.72	wpt-5776	15.8	Batavia
	6A	3.42	rpt-9065	16.3	Batavia
	2BS	6.35	wpt-0289	36.2	Ernie
	5BL	3.92	3p7-3076	24.4	Ernie
PH-1	2A	3.25	wpt-0568	15.4	Batavia
	2B	8.96	wpt-9889	36.8	Ernie
	5B	2.72	wpt-3661	13.3	Ernie
	7B	2.83	wpt-0276	14.4	Ernie
PH-2	2A	4.34	wpt-0568	20.1	Batavia
	2B	6.21	wpt-9889	27.5	Ernie
	5B	2.74	wpt-3661	14	Ernie
HD-1	1B	5.61	wpt-9857	25.9	Batavia
	2BL	3.3	wpt-4917	16.2	Batavia
	5B	2.7	wpt-3076	13.4	Ernie
HD-2	1B	3.6	wpt-9857	17.7	Batavia
	2BL	3.5	wpt-4917	17.3	Batavia
	5B	3.0	wpt-3076	12.1	Ernie

The numeric numbers following the TS-, PH- and HD- are serial numbers of trials conducted for each of these traits

TS tan spot, *HD* heading date, *PH* plant height

or plant height (r ranging from −0.04 to 0.12 and $P > 0.05$).

QTL for tan spot resistance

Four QTL were identified for tan spot resistance in the first trial. They were mapped on chromosomes 1A, 7A, 2BS and 3BS, respectively. Three of these QTL were derived from the resistant parent Ernie, and the other one (on chromosome 1A) was derived from the susceptible parent Batavia. The percentage of phenotypic variance explained by these QTL varied from 16.9 to 38.2%, and all together the four QTL explained 98.8% of the phenotypic variance (Table 3).

Five QTL were identified for tan spot resistance in the second trial. Two (on chromosomes 2BS and 5BL, respectively) were derived from the resistant parent Ernie, and the other three (on chromosomes 6A, 3D and 7D, respectively) from the susceptible parent Batavia. The percentage of phenotypic variance explained by these QTL varied from 11.9 to 29.8%, and they together explained 99.7% of the phenotypic variance (Table 3).

Four QTL (located on chromosomes 1A, 6A, 2BS and 5BL, respectively) were detected in the third trial. Two of them (on 2BS and 5BL, respectively) were derived from the resistant parent Ernie, and the other two (on 1A and 6A, respectively) from the susceptible parent Batavia. The effects of these QTL varied from 15.8 to 36.2%, and together they explained 92.7% of the phenotypic variance (Table 3).

Eight significant QTL in total were identified from the three independent trials. Of these, the one on 2BS was detected in each of the three trials (Fig. 2). One of the SSR markers, *Barc0035*, was located about 2.7 cM away from the peak of this major locus (Fig. 2). Not surprisingly, this

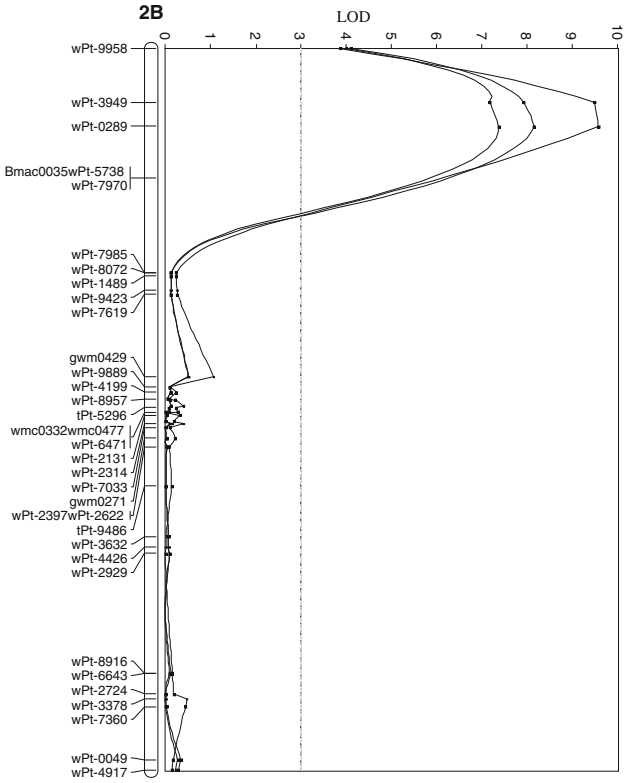

Fig. 2 A major QTL conferring tan spot resistance in the doubled haploid population of Batavia/Ernie identified using composite interval mapping. The LOD values from each centimorgan of the chromosome were plotted against the chromosome

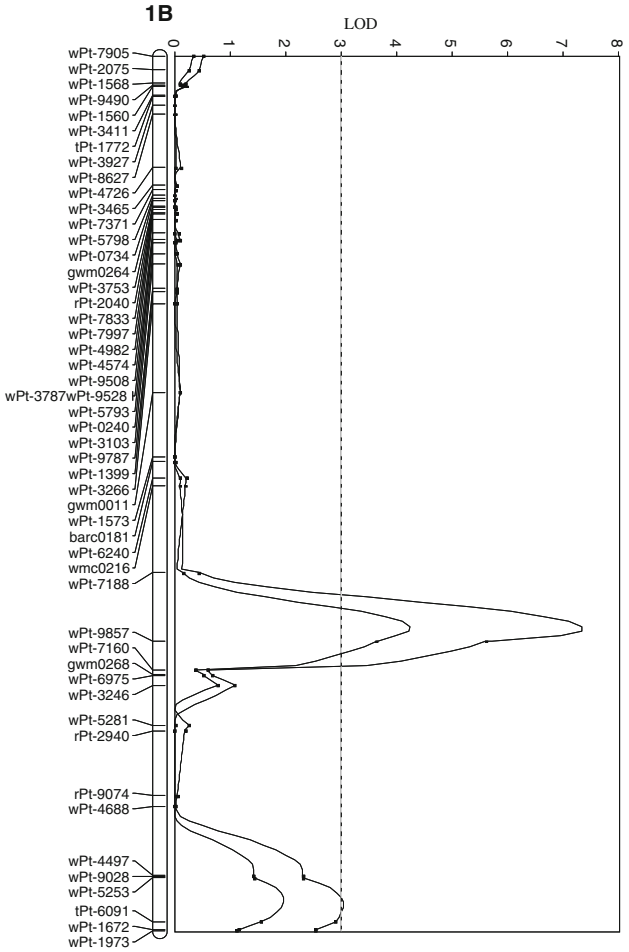

Fig. 3 A major QTL identified for heading date in the doubled haploid population of Batavia/Ernie. The LOD values from each centimorgan of the chromosome were plotted against the chromosome

QTL was also the one showing the largest effects, explaining up to 38.2% of the phenotypic variance. Three of the other seven loci (on 1A, 6A and 5BL, respectively) were each detected in two of the three trials, and the remaining four (on 7A, 3BS, 3D and 7D, respectively) were each detected in only one of the trials (Table 3).

QTL for plant height and heading date

Four significant QTL were identified for plant height in the first trial, and they were mapped to chromosomes 2A, 2BL, 5B and 7B, respectively. The one on 2A was derived from the parent Batavia, and the other three were derived from the parent Ernie. In the second trial, the QTL on 2A, 2BL and 5B remained significant, but the one on 7B became undetectable. Among the four QTL identified for plant height, the one on 2BL gave the largest effect, explaining up to 36.8% of the phenotypic variance (Fig. 3; Table 3).

The same three significant QTL were detected in each of the two trials for heading date, and they were located on chromosomes 1B, 2B and 5B, respectively (Table 3). The QTL on chromosome 1B was the most significant one, explaining 46.6 and 34.1% of the phenotypic variance, respectively, in the two trials (Table 3; Fig. 4). The 2B QTL explained 16.2 and 17.3% of the phenotypic variance, respectively, and the one on 5B explained 13.4 and 12.1% of the phenotypic variance, respectively, in the two trials (Table 3).

Effects of the major QTL conferring tan spot resistance on 2BS in different genetic backgrounds

The SSR marker closely linked with the major tan spot resistance locus, *Barc0035*, was used to identify homozygous individuals with (RR) or without (rr) the resistant locus on 2BS from the immune parent Ernie. The segregation ratios of this marker fit the expected ratio of 1:1 in three of the four validation populations. The exception is the F4 population of 13832/Ernie in which the allelic ratio of the SSR marker was 1:2, favouring the immune parent Ernie. The average scores of tan spot severity differed among the four populations, with the F5 population of 10903/Ernie giving the lowest scores for both of the RR

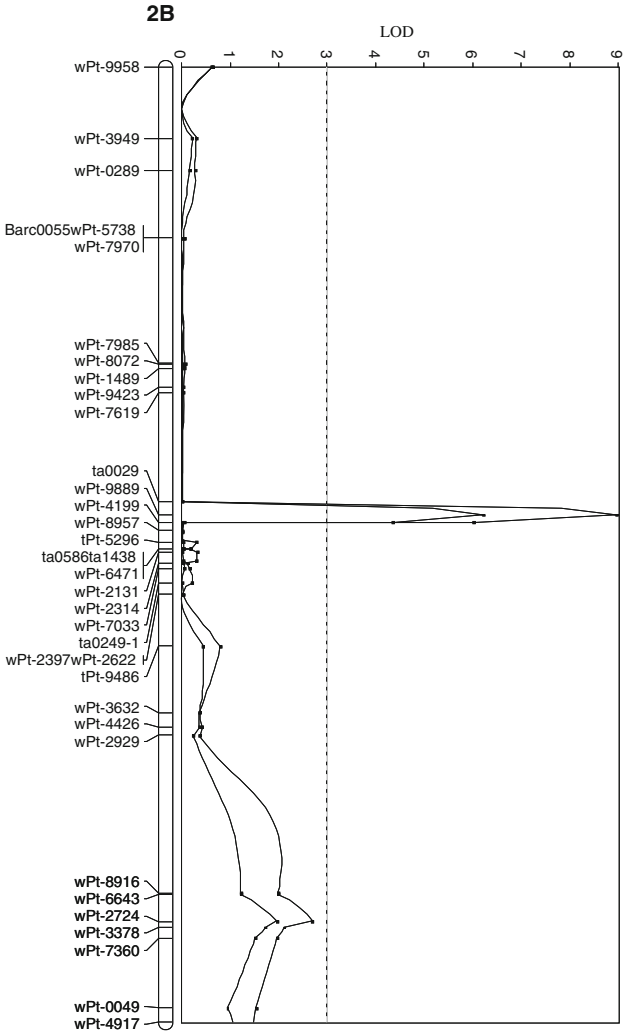

Fig. 4 A major QTL identified for plant height in the doubled haploid population of Batavia/Ernie identified using composite interval mapping. The LOD values from each centimorgan of the chromosome were plotted against the chromosome

(0.50) and rr (0.71) classes (Table 4), and the F5 population of 13832/Ernie giving the highest scores (1.24 for RR and 2.32 for rr, respectively). The effects of the 2BS locus, based on the differences in the average severities between the homozygous RR and rr individuals, varied from 29.2 to 67.1% with an average of 50.5% among the four validation populations (Table 4).

Discussion

The genetics of resistance to tan spot in the immune variety Ernie and the effects of the major QTL identified in different genetic backgrounds

Of the numerous reports on tan spot resistance in bread wheat, there is no description of commercial varieties

Table 4 Effects of the 2B QTL conferring tan spot resistance in four validation populations

Populations	RR	rr	Differences (%)	*P* value
Kennedy/Ernie DH	0.48	1.18	59.3	<0.01
13832/Ernie F5	1.24	2.32	47.0	<0.01
10903/Ernie F3	0.50	0.71	29.3	<0.05
27868/Ernie F5	0.50	1.52	67.1	<0.01

RR homozygous alleles from Ernie, *rr* homozygous alleles from non-Ernie parents

immune to this disease. One of the possible reasons why Ernie showed immunity to tan spot infection in Australia is due to the limited pathogen variation in this country. Although this disease was first detected about six decades ago (Valder and Shaw 1952), there is no known differentiation among the existing isolates in Australia, and they all belong to a single race (Antoni et al. 2010). It is also not clear whether the immunity in Ernie was due to the evaluation method used and what would happen if concentrated *P. tritici-repentis conidia* was used for inoculation. Nevertheless, results from this study showed that, different from those synthetic wheat genotypes (Tadesse et al. 2006b), the immunity of the bread wheat variety Ernie to this disease is controlled by several different QTL. These results suggest that gene pyramiding would not only prolong life-span of resistance but could also generate genotypes with high levels of resistance. For the same reason, it could be predicted that the changes of resistance of Ernie to this disease, if occurs, is likely to be progressive.

Of the eight different QTL detected in this study, the one on 2BS was significant in each of the three independent trials. This major QTL seems to have a similar chromosomal location as the one identified by Friesen and Faris (2004) which was resistant to races 5 and insensitive to Ptr ToxB. It is known that race 5 does not produce Ptr ToxA (Orolaza et al. 1995), but the Australian race does (Antoni et al. 2010). Another minor QTL, conferring race 1 resistance and accounting for only 4% of the phenotypic variation, was recently described by Sun et al. (2010) on the same chromosome arm. Different from the Australian pathogens which produce Ptr ToxA only (Antoni et al. 2010), race 1 produces two host-selective toxins, Ptr ToxA and Ptr ToxC (Sun et al. 2010). Thus, the 2BS locus detected in the current study may represent a novel gene different from those reported by Friesen and Faris (2004) or Sun et al. (2010), or the same gene conferring resistance to different races of *P. tritici-repentis*. The effects of the locus detected in the current study were further confirmed in the four validation populations. The presence of this single QTL across the four populations reduced tan spot severity by an average of 50.7%, showing its great potential in breeding resistant varieties.

Of the QTL which were each detected in two of the three trials (Table 3), the one on 1A is likely to be the same as that reported previously (Faris et al. 1997, 1999; Effertz et al. 2001, 2002) which showed resistance to races 1 and 3 and was insensitive to Ptr ToxC. The locus on 5BL identified in this study is likely to be the same as the one identified by the use of race2/Ptr ToxA (Faris et al. 1996; Stock et al. 1996; Anderson et al. 1999; Friesen et al. 2003; Cheong et al. 2004). The locus on 6A detected in this study, however, seems to represent a novel locus as this chromosome region has never been reported to harbour a tan spot resistance locus.

The remaining four QTL (located on 3D, 3BS, 7A and 7D respectively) were each detected in only one of the three independent trials; thus, their existence can only be speculative at this stage. Chromosome 3D has been inferred to harbour at least three tan spot resistance loci based on monosomic analysis (Tadesse et al. 2006b), and the available data cannot ascertain which one of them is allelic with the 3D locus identified in this study. None of the previous studies detected tan spot resistance loci on 3BS, 7A or 7D where the other three putative QTL were detected in this study.

Relationships between tan spot resistance and heading date

An association between heading date and tan spot severity was noticed in durum wheat, with late maturing lines giving better resistance (Elias et al. 1989). The existence of such a relationship could have serious effect on the selection of tan spot resistance sources for breeding. However, there is no report on their possible relationship in bread wheat. A significant correlation between tan spot severity and heading date was not detected in this study, and none of the tan spot loci was co-located with any of the QTL for heading date (Table 3). Thus, a causal relationship between these two traits does not seem to exist in bread wheat.

Of the three QTL conferring heading date, the ones on 2BL and 5B are likely to be equivalent to those reported by Shindo et al. (2003). However, the 1B QTL detected in this study may represent a new locus as no gene conferring heading date on this chromosome has been reported.

Relationships between tan spot resistance and plant height

There are two reports on the possible relationship between tan spot severity and plant height in durum wheat with contradictory results: one indicating that plant height affected tan spot severity (Fernandez et al. 2002), but the other showing that these two traits did not interfere with each other (Elias et al. 1989). Clearly, the different germplasm used in these studies might explain the different results obtained. In this first study in bread wheat we detected no significant correlation between these two traits, and the QTL identified for them also do not co-locate (Table 3; Figs. 2, 4). These results provide further evidence indicating that a causal relationship between plant height and tan spot resistance may not exist.

Of the three plant height QTL identified in this study, the major one on 2B seems to be novel. Chromosome 2B has been reported as harbouring a reduced height gene *Rht4* (Gale et al. 1985; Börner et al. 1996). However, the location of *Rht4* at the distal end of the long arm (Ellis et al. 2005) seems to be different from the one identified in this study which was located near the centromere (Fig. 4). As there are at least two reduced height genes on chromosome 2A (*Rht7* (Worland et al. 1980) and *Rht21* (Yang et al. 1995)) and their relative locations are still unknown, it is not clear whether the 2A locus detected in this study represents either of them. The plant height locus on 5B has not been reported previously. Although this locus explains only about 13% of the phenotypic variance, it was consistently detected in both of the trials. It is known that Batavia carries *Rht-B1b* (*Rht1*) (Brennan et al. 1994) which is located on the short arm of chromosome 4B. However, no effect of the *Rht-B1b* locus was detected in this study, suggesting that the DH population may not segregate for this locus; thus, Ernie likely possesses *Rht-B1b* as well.

Conclusions

We have identified Ernie as the first commercial bread wheat variety immune to tan spot infection in Australia. In contrast to that of some synthetic wheat genotypes immune to this disease, the resistance of Ernie is not controlled by a single gene. The significant genetic effects of the major locus on the short arm of chromosome 2B were consistently detected across different genetic backgrounds, and the lack of associations between its resistance and either plant height or heading date in this variety all makes it an ideal source of resistance for breeding programmes.

Acknowledgments The visit of WY to CSIRO Plant Industry was supported through a China-Australia collaborative project funded by the Chinese government (No. 2006DFA32850) and a High Tech Program of China (No. 2006AA100102). WY also acknowledges the National Key Technology R&D Program of China (No. 2006BAD13B02-11) and the National Key Basic Research Program of China (No.2006CB101701-4) for financial supports. GRL and SMW thank the Service Centre for Experts and Scholars of Hebei Province, China for financially supporting their visit to CSIRO Plant Industry.

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