New Quantitative Trait Loci in Wheat for Flag Leaf Resistance to Stagonospora nodorum Blotch

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ABSTRACT

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Stagonospora nodorum blotch (SNB) is a significant disease in some wheat-growing regions of the world. Resistance in wheat to *Stagonospora nodorum* is complex, whereby genes for seedling, flag leaf, and glume resistance are independent. The aims of this study were to identify alternative genes for flag leaf resistance, to compare and contrast with known quantitative trait loci (QTL) for SNB resistance, and to determine the potential role of host-specific toxins for SNB QTL. Novel QTL for flag leaf resistance were identified on chromosome 2AS inherited from winter wheat parent 'P92201D5' and chromosome 1BS from spring wheat parent 'EGA Blanco'. The chromosomal map position of markers associated with QTL on 1BS and 2AS indicated that they were unlikely to be associated with known host–toxin insensitivity loci. A QTL on chromosome 5BL inherited from EGA Blanco had highly significant association with markers *fcp001* and *fcp620* based on disease evaluation in 2007 and, therefore, is likely to be associated with *Tsn1*-ToxA insensitivity for flag leaf resistance. However, *fcp001* and *fcp620* were not associated with a QTL detected based on disease evaluation in 2008, indicating two linked QTL for flag leaf resistance with multiple genes residing on 5BL. This study identified novel QTL and their effects in controlling flag leaf SNB resistance.

Additional keywords: DArT, *Phaeosphaeria nodorum*, pleiotropy, SSR.

Necrotrophic fungal pathogens play a significant role in disease of wheat worldwide. *Phaeosphaeria nodorum* (E. Müll.) Hedjar., anamorph *Stagonospora nodorum* (Berk.) E. Castell. & Germano (= syn. *Septoria nodorum* (Berk) Berk.) causes Stagonospora nodorum blotch (SNB) disease on leaves and glumes. In Australia, SNB contributed average annual wheat yield losses of 12.9% in the Western region in the first decade of the 21st century (19). Therefore, breeding resistant cultivars could significantly reduce yield losses during disease epidemics.

An in-depth understanding of the genetics of host–pathogen interactions provides much needed information for efficiently deploying resistance genes in commercial wheat cultivars. The genetics of host resistance to *S. nodorum* in seedling and adult plants is complex. Polygenic inheritance with minor effects and interacting in a additive, dominance, or epistatic manner has been reported for seedling resistance (9,18,24,31), whereas adult plant resistance is similarly inherited but controlled by independent genes for flag leaf and glume resistance (6,12,20,23,30). The reduced number of lesions in seedlings does not unequivocally correlate with field resistance or tolerance (23,27), and it is not often possible or practical to predict SNB reaction in adult plants based on seedling tests (5). Therefore, breeding effective resistance to SNB relies on combining multiple genes and selecting progeny for specific and independent resistance in flag leaf, glume, and seedling (6,24).

Identification of molecular markers linked to quantitative trait loci (QTL) is becoming increasingly important to track SNB resistance genes. A number of QTL for seedling resistance have been identified using biparental mapping populations as residing on wheat chromosomes 1B, 2B, 2D, 4B, 5A, 5B, 5D, and 6A (1,4,8,14,17,22). QTL for seedling resistance on chromosomes 6A and 7A were recently identified in association mapping studies (2). QTL for flag leaf resistance were identified on chromosomes 1B, 2A, 2D, 5A, 5B, and 7A (3,13,27) and glume resistance on 2D, 3B, 4B, and 5A (3,25,27,28). The majority of QTL for seedling, flag leaf, and glume resistance from these studies contributed a small proportion (usually <20%) of phenotypic variation. In some instances, QTL on similar chromosomal positions in separate studies indicated different genes or alleles contributing to seedling and adult plant resistance (17,25,27).

Studies have provided evidence that host-specific toxins SnTox2 and SnToxA are secreted from *Stagonospora nodorum* and corresponding host sensitivity QTL reside on wheat chromosomes 2DS (*Snn2*) and 5BL (*Tsn1*), respectively (16,17). Both *Snn2* and *Tsn1* co-locate with a QTL for flag leaf resistance from a Brazilian genotype, BR34, indicating a role for toxin–host interactions. In other studies, however, QTL for flag leaf and glume resistance were not associated with any known loci for toxin insensitivity (25,27,28), indicating that either different genes or alleles control toxin insensitivity or mechanisms different from the compatible toxin–host interactions are responsible for adult plant resistance in spring and winter wheat accessions.

SNB is prominent in high-rainfall regions and is most damaging in warm, moist conditions at the time when plants reach physiological maturity. Therefore, there is a greater emphasis on developing flag leaf and glume resistance in commercial cultivars. Two QTL for glume resistance on chromosomes 2D and 4B have shown to be effective in Western Australian environments (27,28) and are suitable for deploying in genetic backgrounds. However, only one QTL for flag leaf resistance has been identified on

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chromosome 2D in spring wheat (27). Therefore, it is important to identify alternative QTL to increase genetic gain for flag leaf resistance to SNB in Western Australian environments. The aim of this study is to identify alternative genes for flag leaf resistance, to compare and contrast with known QTL for SNB resistance, and to determine the potential role of host-specific toxins for SNB QTL. The study focuses on a winter wheat recombinant inbred (RI) line population and a spring wheat doubled-haploid mapping population, whereby winter wheat breeding line 'P92201D5' and spring wheat 'EGA Blanco' are parents in two populations, respectively, and both have moderate levels of flag leaf resistance effective in Western Australian environments.

MATERIALS AND METHODS

Plant material and homozygous populations. Parents were chosen because of their suitable disease reaction and also having the *Rht1* allele to avoid major plant height segregation in the homozygous populations. Parental winter wheat genotypes 'P92201D5' and 'P91193D1', were obtained from the wheat breeding program collection at Purdue University, West Lafayette, IN. An RI population denoted as 'P9819RB1' was developed at Purdue University by single-seed descent from a random population of 254 F_2 plants from a cross of the two parental winter wheat lines, P92291D5 and P91193D1 (28). The spring wheat EGA Blanco and 'Millewa' were obtained from the collection at the Department of Agriculture and Food Western Australia. The spring wheat doubled-haploid (DH) population, '05Y001', consisted of 235 individuals developed from an F_1 cross of parental genotypes (11).

Genetic map development. P9819RB1 and 05Y001 populations were used to generate high-resolution genetic linkage maps consisting of simple-sequence repeat (SSR) and diversity array technology (DArT) markers. Details of genetic marker linkage map construction are reported by Francki et al. (11). Briefly, P9819RB1 consisted of 385 markers distributed on all 21 chromosomes with an average distance of 7.8 centimorgans (cM) between markers and total coverage of 3,013 cM. Similarly, a genetic map for the 05Y001 population consisted of 468 SSR and DArT markers, with an average resolution of 6.5 cM between markers and a genome coverage of 3,058 cM. Additional markers *fcp001* and *fcp620* described by Zhang et al (34) were mapped to chromosome 5BL in the 05Y001 population.

Flag leaf phenotyping. Populations were sown in an irrigated field nursery at South Perth, Western Australia in 2004 and 2005 for the P9819RB1 population and 2007 and 2008 for the 05Y001 population. Prior to sowing, individuals of the P9819RB1 population and parents were germinated on petri dishes for 24 h and vernalized for 7 to 9 weeks at 2°C with 8 h of light per day before transplanting in the field. There were three replications each in randomized split block design and sown as paired 10-cm rows of up to 15 seeds per row sown 10 cm apart and separated by 35 cm of adjacent rows. Plots were fertilized with superphosphate, urea, and potash and protected from powdery mildew infection with Quinoxyfen at 250 g/ha and Bupirimate at 125 g/ha applied 2 weeks after sowing and at 4 weekly intervals for 12 weeks.

Flag leaves of individual plots were sprayed with a conidial suspension (10^6 conidia/ml with 0.5% gelatine) of a mixture of 10 isolates of *S. nodorum* (WAC13068, WAC13069, WAC13070, WAC13071, WAC13072, WAC13073, WAC13074, WAC13075, WAC13076, and WAC13077). These isolates were obtained from the culture collection at the Department of Agriculture and Food Western Australia. Flag leaves of individuals from both populations and parental genotypes were inoculated at Feekes stage 10.0 to 10.3. Immediately following inoculation, each plot was enclosed in a humidity chamber consisting of a plastic bag misted internally with water, secured with a PVC ring (15 cm high and 30 cm in diameter) at the base of the plot, and shaded from direct sunlight with a shade cloth bag (84 to 90% cover factor). Humidity chambers were removed 48 h after inoculation.

Percent leaf area diseased for the P9819RB1 population was assessed on flag leaves at 240°C thermal days (sum of average daily temperatures) in 2004 and 280°C in 2008, whereas the 05Y001 population was assessed at 220°C in both 2007 and 2008. Rating scales for leaf infection were based on a percentage scale from 0% (highly resistant) to 100% (highly susceptible) as previously described (15). Disease ratings on flag leaf were scored for three to six individual plants and an average for each plot was calculated and used for analysis.

Measurements of plant height were taken as the distance from the soil surface to the top of the spike (awns excluded) for each plot. Heading date in 2004, 2005, and 2007 was measured as days to full head emergence from date of planting and, in 2008, as days to awn peep.

Statistical analysis. All statistical analysis was done using Genstat version 8.1. Pearson's correlation coefficient was estimated between plant height, heading date, and flag leaf disease scores. Significant differences between genotypes for all traits were assessed using one-way analysis of variance (ANOVA) in randomized blocks. Broad-sense heritability estimates were calculated using the formula $H^2 = \sigma_g^2/\sigma_g^2+\sigma_e^2/r$, where σ_g^2 and σ_e^2 are the genotypic and error variance, respectively, and r is the number of replications.

QTL analysis. The mean disease scores for flag leaf disease scores, plant height, and heading date were used for composite interval mapping (CIM). CIM model one of Windows QTL Cartographer version 2.5 (29) was used with conditional settings of 10-cM control intervals, five control markers (determined by QTL Cartographer to account for the genetic background variation), and forward regression (33). Experiment-wise critical thresholds for significance of potential QTL for each year were determined using CIM to conduct permutation tests as previously described (7). Highly significant (P = 0.01) and significant (P = 0.05) QTL thresholds were calculated from 1,000 permutations.

RESULTS

Phenotypic analysis for flag leaf disease, plant height, and morphology. Highly significant difference (t test, $P < 0.01$) was observed between parental means of EGA Blanco and Millewa, with the former showing consistently lower flag leaf disease scores in 2007 and 2008 (Table 1). Although a significant difference (t test, $P < 0.05$) was observed between means of P92201D5 and P91193D1 in 2004, the lower flag leaf score varied between parents did not show significant difference ($P > 0.05$) in 2005 (Table 1). Both populations P9819RB1 and 05Y001 showed continuous distribution in each year (data not shown), indicating polygenic inheritance, and ANOVA showed highly significant difference between individual genotypes for flag leaf disease in each year (Table 1).

There was a significant difference between mean values for EGA Blanco and Millewa and DH genotypes with the lowest and highest flag leaf score in 2007, respectively. A significant difference between Millewa and the most susceptible DH individual was observed in 2008. Broad-sense heritability (H^2) for flag leaf resistance in the P9819RB1 and 05Y001 populations were moderate to high across successive years, at 0.53 to 0.70, respectively (Table 1), indicating that the genotypic value of each homozygous individual is suitable for QTL analysis.

Highly significant differences between mean values of parents and individuals with extreme phenotypes were observed in successive years for both plant height and heading date in each population (Table 1). Individuals for each population showed a continuous distribution for plant height and heading date in each year (data not shown) and ANOVA showed highly significant differences between genotypes. Ranges for broad-sense heritabil-

ity for plant height and heading date were moderate to high (Table 1).

Highly significant but moderate correlation between consecutive years was observed for flag leaf disease scores for individuals in each population (Tables 2 and 3). Although correlations between years for plant height scores and heading date scores were generally large and highly significant, *r* values between flag leaf scores, plant height, and heading date for each year were generally low to moderate but significant (Tables 2 and 3).

QTL analysis for flag leaf disease, plant height, and heading date. QTL analysis identified a number of different chromosomal regions controlling flag leaf resistance in P9819RB1 and 05Y001 populations. In the former population, one highly significant QTL on the distal end of the short arm of chromosome 2A was detected in each successive year and accounted for 11.2 to 21.8% of the phenotypic variation (Table 4). Resistance at this QTL was contributed by the P92201D5 allele, and details of marker interval and log of the likelihood ration (LOD) scores are provided in Table 4. Disease evaluation in 2004 identified the QTL between marker loci *wPt-2448* and *wPt-7056* whereas *QSnl05.daw-2A* spanned an overlapping region between markers *gwm614a* and *wPt-9432* based on 2005 disease scores (Table 4; Fig. 1). No other significant QTL for flag leaf resistance were detected in this population in either year.

The 05Y001 population detected two QTL for flag leaf resistance on chromosomes 1B and 5B in 2007 and 2008. The highly significant QTL *QSnl07.daw-1B* and *QSnl08.daw-1B* co-located within a marker interval of 14.8 cM between *wPt-2988* and *gwm264* and accounted for ≈16% of the phenotypic variation (Fig. 1; Table 4). A second QTL for flag leaf resistance was detected on the long arm of chromosome 5B, accounting for 8 to 14% of the total phenotypic variation. The QTL detected using mean data values from phenotypic evaluation in 2007 spanned a marker interval between *wPt-4628* and *wPt-1733* where the LOD

TABLE 1. Summary of mean (± standard error) values for flag leaf disease score, plant height, and heading date for the P9819RB1 and 05Y001 homozygous populations[a]

Population[b]	Flag leaf (% infection)	Height (cm)	Heading date (days)	Flag leaf (% infection)	Height (cm)	Heading date (days)
P9819RB1						
P92201D5	42.9 (±5.7)	84.2 (±2.4)	92.3 (±4.6)	33.8 (±5.1)	64.2 (±2.0)	74.3 (±1.3)
P91193D1	25.8 (±2.2)	79.0 (±14.58)	113.5 (±4.3)	37.7 (±4.3)	70.0 (±2.9)	80.0 (±0.0)
Population						
Minimum	7.7 (±2.7)	60.0 (±4.1)	78.3 (±1.7)	15.6 (±7.9)	50.8 (±3.0)	67.0 (±0.0)
Maximum	90.4 (±0.4)	108.3 (±3.1)	144.0 (±0.0)	77.8 (±1.4)	112.5 (±5.1)	150.0 (±0.0)
Mean	41.3 (±0.8)	79.8 (±0.4)	105.5 (±0.7)	41.5 (±0.7)	79.8 (±0.36)	95.3 (±14.6)
LSD ($P < 0.05$)	16.4	6.6	9.6	10.1	6.2	8.5
CV%	23.7	2.0	1.7	15.4	8.9	6.9
ANOVA (*P*)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
H^2	0.70	0.66	0.79	0.64	0.75	0.93
05Y001						
EGA Blanco	35.0 (±6.3)	79.4 (±1.8)	91.7 (±0.9)	27.0 (±1.8)	80.3 (±1.0)	92.0 (±0.0)
Millewa	56.1 (±1.3)	79.1 (±1.5)	88.0 (±1.4)	54.7 (±2.8)	75.8 (±1.3)	88.4 (±1.1)
Population						
Minimum	22.1 (±1.1)	61.6 (±2.1)	74.5 (±1.5)	24.7 (±2.0)	62.5 (±2.1)	77.7 (±1.7)
Maximum	64.1 (±3.7)	95.0 (±1.3)	99.0 (±0.0)	56.5 (±9.2)	91.6 (±1.7)	101.7 (±1.7)
Mean	42.7 (±1.5)	78.0 (±0.1)	86.9 (±0.4)	37.2 (±0.4)	76.1 (±0.2)	89.6 (±0.2)
LSD ($P < 0.05$)	8.0	4.8	3.7	7.1	4.0	3.4
CV%	14.2	6.6	3.3	14.5	5.6	2.8
ANOVA (*P*)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
H^2	0.61	0.56	0.77	0.53	0.73	0.78

[a] For population P9819RB1, first and second years were 2004 and 2005; and, for 05Y001, first and second years were 2007 and 2008, respectively.
[b] LSD = least significant difference, CV = coefficient of variation, ANOVA = analysis of variance, and H^2 = broad-sense heritability.

TABLE 2. Pearson's correlation coefficient (*r*) matrix for flag leaf disease score, plant height, and maturity in the 'P92201D5'/'P91193D1' recombinant inbred population (P9819RB1)[a]

Trait	Flag leaf 2004	Heading date 2004	Height 2004	Flag leaf 2005	Heading date 2005	Height 2005
Flag leaf 2004	…	…	…	…	…	…
Heading date 2004	0.150**	…	…	…	…	…
Height 2004	0.182**	0.337***	…	…	…	…
Flag leaf 2005	0.65***	N/A	N/A	…	…	…
Heading date 2005	N/A	0.861***	N/A	0.523***	…	…
Height 2005	N/A	N/A	0.675***	0.411***	0.768***	…

[a] Symbols: ** = significant ($P < 0.05$), *** = highly significant ($P < 0.001$), and N/A = not applicable.

TABLE 3. Pearson's correlation coefficient (*r*) matrix for flag leaf disease score, plant height, and heading date in the 'EGA Blanco'/'Millewa' doubled-haploid population (05Y001)[a]

Trait	Flag leaf 2007	Heading date 2007	Height 2007	Flag leaf 2008	Heading date 2008	Height 2008
Flag leaf 2007	…	…	…	…	…	…
Heading date 2007	–0.041*	…	…	…	…	…
Height 2007	–0.189***	0.361***	…	…	…	…
Flag leaf 2008	0.381***	N/A	N/A	…	…	…
Heading date 2008	N/A	0.868***	N/A	–0.189**	…	…
Height 2008	N/A	N/A	0.744***	–0.301***	0.405***	…

[a] Symbols: * = not significant ($P > 0.05$), ** = significant ($P < 0.05$), *** = highly significant ($P < 0.001$), and N/A = not applicable.

score of 8.8 peaked at markers *fcp001* and *fcp620* (Table 4; Fig. 2). Interestingly, *fcp001* and *fcp620* are reported to be tightly linked markers to the *Tsn* locus (34) and shown to have highly significant association and positioned centrally within *QSnl07.daw-5B*. However, analysis of flag leaf resistance using mean values from 2008 disease evaluation detected a highly significant but overlapping locus, *QSnl08.daw-5B*, between marker loci fcp620 and wPt9598 (Table 4; Fig. 2). *Qsnl08.daw-5B* peaked with an LOD score of 4.4 at marker locus *wPt1733* and ≈18 cM from *fcp001* and *fcp620*. The markers tightly linked to *Tsn1*, *fcp001*, and *fcp620* showed no significant ($P > 0.05$) association with *QSnl08.daw-5B* (Table 4; Fig. 2).

The analysis of mean data for plant height and heading date identified a number of QTL for each year. A locus for heading date was detected near *QSnl04.daw-2A* and *QSnl05.daw-2A*, with small effects accounting for <16% of the phenotypic variation (Table 4). A comparison of genetic map positions of QTL for heading date identified some overlap with QTL for flag leaf resistance on chromosome 2A in 2004 but only in a portion of the 4.5-cM *barc124b–gwm512* interval (Fig. 1). Therefore, flag leaf disease and heading date were linked and the small correlation between phenotypic values (Table 2) confirmed that QTL for disease resistance is unlikely to be a pleiotropic effect. The remaining QTL for heading date were detected on other chromosomes but accounted for smaller proportion of the total phenotypic variation (Table 4). QTL for plant height in the 9819RB1 population were also detected on chromosomes 2D, 4D, and 5D and each accounted for ≤20% of the phenotypic variation (Table 4).

The analysis of heading date detected QTL co-located on chromosome 5B in a similar region for flag leaf resistance (Fig. 2), with the *Vrn2* marker locus identified as the major determinant controlling heading date on wheat chromosome 5B (32), positioned within only *QSnl.daw-5B* (Fig. 2). The weak or absent correlation observed between flag leaf resistance and heading date from individuals of the 05Y001 population (Table 3) confirmed the likelihood of linkage between resistance and heading date loci on chromosome 5B rather than pleiotropy. Other significant QTL for flag leaf resistance and heading date that account for minor portions of the total phenotypic variation are summarized in Table 4. No significant QTL were detected for plant height in the 05Y001 population in 2007 or 2008.

DISCUSSION

The identification of alternative sources of genetic variation and corresponding QTL provides an opportunity to manipulate chromosomal regions for desirable trait variation in plant improvement. This study identified a new QTL for flag leaf resistance to SNB on chromosomes 1BS in a spring wheat mapping population 05Y001 (contributed by EGA Blanco). Previous reports have identified a QTL with seedling and flag leaf resistance to SNB and a toxin-sensitive locus, *Snn1*, between markers *KsuD14* and *mwg938* and distal to 1BS-9 breakpoint (13,16,17). Markers *wPt-8948, wPt-1116*, and *gwm264* associated with *QSnl04.daw-1B* and *QSnl05.daw-1B* in this study have been assigned proximal to the 1BS-10 breakpoint and within the centromeric region of 1BS (11). Therefore, it is reasonable to assume that *QSnl04.daw-1B* and *QSnl05.daw-1B* are different from those previously reported on 1BS (13,16,17) based on comparative chromosomal map position for markers associated with QTL. If toxin insensitivity is associated with QTL on 1BS in this study, then a gene or genes

TABLE 4. Quantitative trait loci (QTL) summary for flag leaf resistance, heading, date, and height based on field evaluation in successive years for the ‘P9819RB1’ and ‘05Y001’ homozygous populations

Population, trait[a]	Chromosome	Marker interval	Distance (cM)[b]	Composite interval mapping		Additive[e]	R^2
				LOD threshold[c]	Maximum LOD[d]		
P9819RB1							
Flag leaf resistance (2004)	*QSnl.daw-2A*	wPt2448-wPt7056	24.4	2.8	6.4 (*wPt9432*)	5.25	0.112
Heading date (2004)	*QHd.daw-2A*	gwm512-wPt7056	27.4	2.8	5.5 (*wPt1112*)	7.83	0.162
	QHd.daw-2D	barc230-wPt6003	8.9	2.2	2.5 (*barc230*)	4.55	0.054
	QHd.daw-5A	gwm415-gwm186	12.2	2.3	2.9 (*gwm186*)	5.63	0.084
Height (2004)	*QHt.daw-5D*	cfd102b-gwm371	25.4	1.9	11.2 (*cfd102*)	5.39	0.204
Flag leaf resistance (2005)	*QSnl.daw-2A*	gwm614a-wPt9432	29.5	2.7	13.3 (*barc124*)	3.91	0.218
Heading date (2005)	*QHd.daw-2A*	wPt1657-wPt7626	19.7	3.2	4.0 (*wPt7056*)	6.85	0.072
	QHd.daw-2D	barc230-wPt6003	12.2	2.4	3.3 (*wPt6003*)	6.95	0.074
	QHd.daw-4D	wmc331-barc217	5.0	2.4	2.4 (*barc217*)	6.70	0.069
Height (2005)	*QHt.daw-2D*	barc124a-wPt6003	13.5	2.3	3.8 (*wPt6003*)	3.85	0.085
	QHt.daw-4D	wmc331-barc217	5.1	2.4	2.5 (*barc217*)	6.72	0.069
	QHt.daw-5D	cfd102b-gwm371	8.7	2.2	2.7 (*cfd102*)	3.45	0.054
05Y001							
Flag leaf resistance (2007)	*QSnl.daw-1B*	wPt8949-gwm264	8.4	2.6	10.8 (*wPt8949*)	3.16	0.158
	QSnl.daw-4B	barc0163-wPt0391	29.6	2.4	5.1 (*wmc349*)	2.31	0.082
	QSnl.daw-5B	wPt4628-wPt1733	32.2	2.7	8.8 (*fcp001*)	3.00	0.139
Heading date (2007)	*QHd.daw-3A*	gwm0002-cfa2193	56.0	2.6	4.2 (*wmc264*)	1.37	0.059
	QHd.daw-4A	wmc0258-gwm0162	47.8	2.7	4.3 (*barc1047*)	–1.47	0.068
	QHd.daw-4B	wmc0657-wmc0249	11.1	2.3	3.0 (*barc163*)	1.15	0.040
	QHd.daw-5A	barc0319-wPt5231	24.3	2.8	3.0 (*wPt5231*)	0.89	0.025
	QHd.daw-5B	fcp620-wPt9598	39.5	2.7	23.6 (*Vrn2*)	–3.45	0.333
Flag leaf resistance (2008)	*QSnl.daw-1B*	wPt8949-wPt2575	8.4	2.6	10.1 (*wPt8949*)	2.31	0.162
	QSnl.daw-5B	fcp620-wPt9598	39.5	2.7	4.4 (*wPt1733*)	1.72	0.080
Heading date (2008)	*QHd.daw-3A*	gwm0002-cfa2193	56.0	2.5	5.4 (*wmc264*)	1.45	0.078
	QHd.daw-4A	wmc0258-gwm0162	47.8	2.6	5.0 (*barc1047*)	–1.40	0.073
	QHd.daw-4B	wmc0657-barc0163	11.5	2.4	2.6 (*gwm149*)	0.88	0.029
	QHd.daw-5A	barc0319-wPt5231	24.3	2.6	3.7 (*wPt5231*)	0.71	0.017
	QHd.daw-5B	fcp620-wPt9598	39.5	2.7	21.5 (*Vrn2*)	–2.99	0.341

[a] P9819RB1 = P92201D5/P91193D1 and 05Y001 = EGA Blanco/Millewa.
[b] Centimorgan (cM) distance above threshold level at $P = 0.01$.
[c] Log of the likelihood ration (LOD) threshold at $P = 0.01$determined by permutation testing for 1,000 reiterations.
[d] Maximum LOD score for QTL peak with nearest marker in parenthesis.
[e] Positive and negative effects indicate the allele was inherited from the female and male parent, respectively.

alternative to *Snn1* may reside in the centromeric region. Further work is required to investigate whether toxins are secreted from the fungal isolates used in this study and interactions with toxin insensitivity loci are associated with *QSnl04.daw-1B* and *QSnl05.daw-1B*.

There have been no previous reports of QTL for flag leaf or glume resistance to SNB on chromosome 2A; therefore, *QSnl04.daw-2A* and *QSnl05.daw-2A* identified in the P9819RB1 population represent a unique QTL for flag leaf resistance. Previous reports of seedling QTL have been reported in a similar region on 2AS (1,21) but the lack of common markers between genetic maps did not allow unambiguous co-location for seedling and flag leaf resistance at this locus. It was expected that *QSnl04.daw-2A* and *QSnl05.daw-2A* would reside in the same marker interval; however, analysis using data from successive years showed overlapping QTL. The QTL share a chromosomal region of ≈5 cM and, therefore, it is assumed that a single QTL has been detected in the 05Y001 population. We cannot exclude the possibility of multiple linked genes residing on 2AS, and disease evaluation in multi-environments would provide further evidence for either a single or linked QTL at the 2AS locus.

This study identified QTL for flag leaf resistance on the long arm of chromosome 5B in the 05Y001 population contributed by the EGA Blanco. In previous studies, QTL for flag leaf resistance under field conditions were identified on chromosome 5BL, accounted for ≤20% of the total phenotypic variation in a BR34/Grandin RI line population, and co-located with the *Tsn1* locus for sensitivity and insensitivity to the host-specific toxin ToxA (13). In this study, markers *fcp001* and *fcp620,* linked within 0.5 cM of *Tsn1* (34), were significantly associated with flag leaf resistance on chromosome 5BL in at least one environment. Therefore, it is reasonable to assume that *Tsn1*–ToxA interactions for toxin insensitivity contribute to flag leaf resistance identified by *QSnl07.daw-5BL* in the 05Y001 population. However, *fcp001* and *fcp620* did not have a significant association with flag leaf resistance when evaluated in 2008. Therefore, an alternative QTL, *QSnl08.daw-5B*, linked to *Tsn1* was identified in the 05Y001 population. Methods for evaluation of 05Y001 populations were near identical in 2007 and 2008, showing co-location of QTL within a 14.8-cM marker interval on chromosome 1B; therefore, it is unlikely that different QTL positions on 5BL reflect inconsistent disease evaluation between years. Moreover, QTL for seedling resistance have been identified in the same tetraploid wheat mapping RI population in different studies where one appears to be controlled by the *Tsn1* locus (10) and the other is linked to *Tsn1* (14), providing evidence of linked genes for resistance to SNB residing on chromosome 5BL. The reasons for different QTL on 5BL are unknown but environmental conditions may have favored differences in disease development between successive years, causing distinct host gene responses. Indeed, further work is warranted to study the interaction of disease development with different genes on 5BL that contribute to flag leaf resistance in multi-environments.

Morphological traits can have a profound effect on accurate evaluation of disease resistance and subsequent QTL analysis (26). Therefore, phenotyping methods developed to discriminate pleiotropy from linkage (25,27) were implemented in this study for disease evaluation and QTL analysis. Small correlations were observed between trait values in each year for the 05Y001 population, indicating that phenotyping methodologies were effective in reducing or eliminating pleiotropic effects. The *Vrn2* gene identified on chromosome 5BL, accounting for a significant

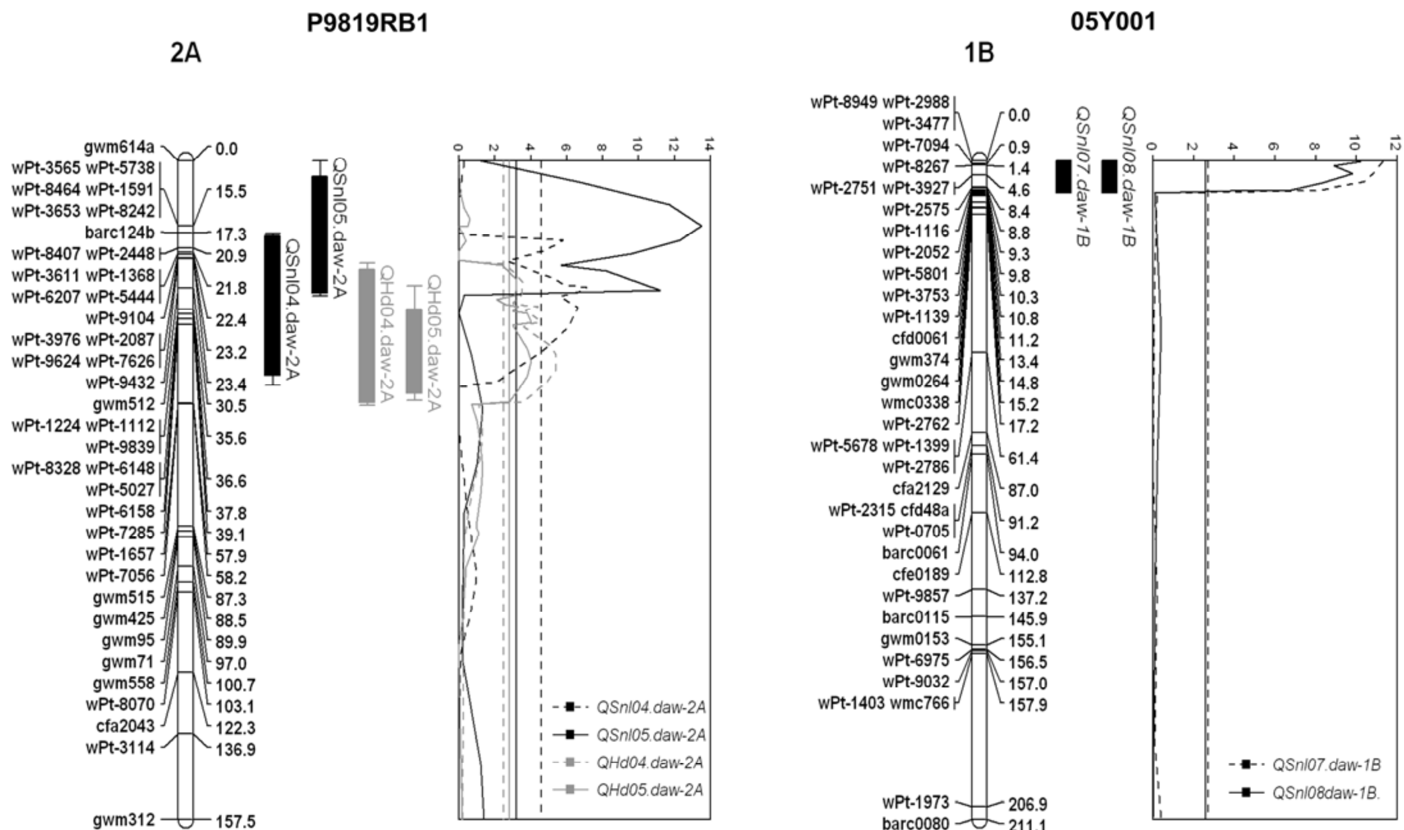

Fig. 1. Quantitative trait loci (QTL) analysis for flag leaf resistance to *Stagonospora nodorum* in the recombinant inbred line population 'P9819RB1' ('P92201D5'/'P91193D1') and doubled-haploid population '05Y001' ('EGA Blanco'/'Millewa'). QTL on chromosome 2A for flag leaf resistance and heading date from P9819RB1 evaluated in Western Australia in 2004 and 2005 are denoted *QSnl04.daw-2A*, *QSnl05.daw-2A*, *QHd04.daw-2A*, and *QHdl05.daw-2A*, respectively. QTL on chromosome 1B for flag leaf resistance and heading date from 05Y001 evaluated in Western Australia in 2007 and 2008 are denoted *QSnl07.daw-1B*, *QSnl08.daw-1B*, *QHd07.daw-1B*, and *QHdl08.daw-1B*, respectively. Black and gray solid bars represent QTL regions. Numbers on the y-axis of the graph represent log of the likelihood ratio (LOD) scores with highly significant ($P < 0.01$) LOD thresholds for each trait represented by solid and dashed lines.

proportion of phenotypic variation for heading date (32), including the 05Y001 population in both years, had no significant association with *QSnl07.daw-5B* or *QSnl08.daw-5B*, providing further evidence that heading date was unlikely to have pleiotropic effects on flag leaf resistance. Similarly, low to moderate correlation between heading date and disease scores and inconsistent co-location 2A in the P9819RB1 population indicated that flag leaf resistance and heading date QTL are linked. Therefore, methodology used in this study successfully discriminated linkage from pleiotropy between QTL for heading date and flag leaf resistance on chromosomes 2A and 5B.

Generally, wheat breeding programs have not been able to make significant improvements in genetic gain for SNB resistance. This has been largely due to the complex quantitative genetic control of adult plant resistance and effects of environmental interactions on expression of resistance, making phenotypic selection in breeding an arduous task. The key to developing germplasm with adult plant resistance will be to assess which QTL from different sources are consistently detected across multiple and relevant field environments, deploy them in appropriate genetic backgrounds, and identify which QTL combinations provide significant improvements in SNB resistance. The QTL for flag leaf resistance and associated markers identified in this study can be combined with other QTL (3,13,27,28) to develop new germplasm with improved flag leaf resistance to SNB. The use of molecular markers will be an important tool to track and select QTL combinations in germplasm development and commercial breeding. The QTL on chromosome 1B, 2A, and 5B identified in this study are obvious choices for increasing genetic gain for SNB resistance.

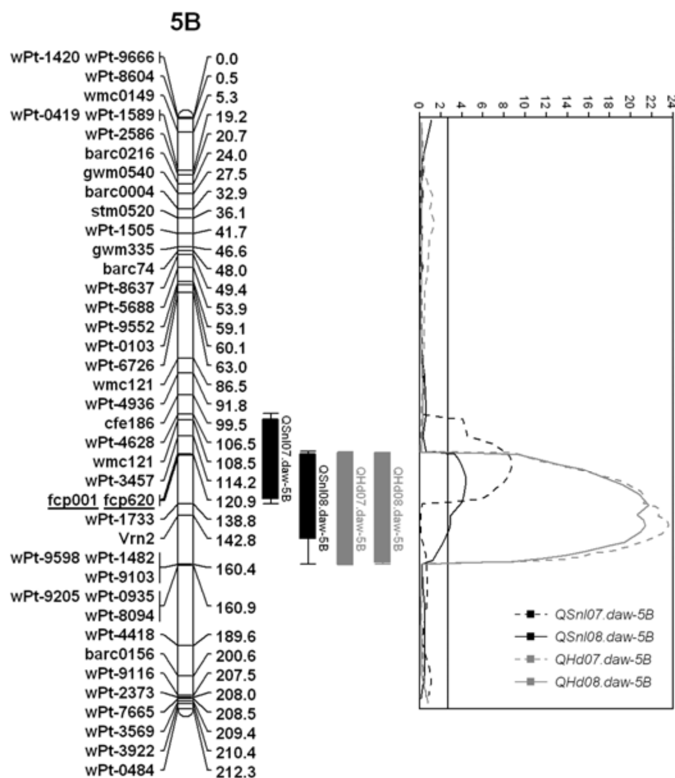

Fig. 2. Quantitative trait loci (QTL) analysis for flag leaf resistance to *Stagonospora nodorum* on chromosome 5B in the doubled-haploid population 05Y001 ('EGA Blanco'/'Millewa'). QTL for flag leaf resistance and heading date evaluated in Western Australia in 2007 and 2008 are denoted *QSnl07.daw-5B*, *QSnl08.daw-5B*, *QHd07.daw-5B*, and *QHdl08.daw-5B*, respectively. Markers *fcp001* and *fcp620*, closely linked to *Tsn1* (34) are underlined. Black and gray solid bars represent QTL regions. Numbers on the y-axis of the graph represent log of the likelihood ratio (LOD) scores with highly significant ($P < 0.01$) LOD thresholds for each trait represented by solid and dashed lines.

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