

Quantitative Trait Loci for Seedling and Adult Plant Resistance to *Stagonospora nodorum* in Wheat

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ABSTRACT

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Stagonospora nodorum blotch (SNB) caused by *Stagonospora nodorum* is a severe disease of wheat (*Triticum aestivum*) in many areas of the world. *S. nodorum* affects both seedling and adult plants causing necrosis of leaf and glume tissue, inhibiting photosynthetic capabilities, and reducing grain yield. The aims of this study were to evaluate disease response of 280 doubled haploid (DH) individuals derived from a cross between resistant (6HRWSN125) and susceptible (WAWHT2074) genotypes, compare quantitative trait loci (QTL) for seedling and adult plant resistance in two consecutive years, and assess the contribution of QTL on grain weight. Flag leaves and glumes of individuals from the DH population were inoculated with mixed isolates of *S. nodorum* at similar maturity time to provide accurate disease evaluation independent of

morphological traits and identify true resistance for QTL analysis. Fungicide protected and inoculated plots were used to measure relative grain weight (RGW) as a yield-related trait under pathogen infection. The lack of similar QTL and little or no correlation in disease scores indicate different genes control seedling and adult plant disease and independent genes control flag leaf and glume resistance. This study consistently identified a QTL on chromosome 2DL for flag leaf resistance (*QSn1.daw-2D*) and 4BL for glume resistance (*QSn2.daw-4B*) from the resistant parent, 6HRWSN125, explaining 4 to 19% of the phenotypic variation at each locus. A total of 5 QTL for RGW were consistently detected, where two were in the same marker interval for *QSn1.daw-2D* and *QSn2.daw-4B* indicating the contribution of these QTL to yield related traits. Therefore, RGW measurement in QTL analysis could be used as a reliable indicator of grain yield affected by *S. nodorum* infection.

Additional keywords: *Phaeosphaeria nodorum*, pleiotropy.

Phaeosphaeria nodorum (E. Muller) Hefjaroude, anamorph *Stagonospora nodorum* (Berk.) Castellani & Germano (=Syn. *Septoria nodorum* (Berk) Berk. in Berk. & Broome) is a major fungal pathogen of wheat and other cereals in many parts of the world. It induces *Stagonospora nodorum* blotch (SNB) of leaves and glumes and is one of the most severe fungal diseases affecting wheat production in Western Australia. A 30 to 50% reduction in grain yield has been reported (3,19) with an estimated loss to grain growers in excess of 58 million dollars annually in wheat growing regions with high disease pressure (5). Only partial control of the disease can be achieved in the field with fungicides, adding substantial cost to wheat production. An alternative is to breed wheat cultivars with high level leaf and glume resistance.

A strategic approach to breed wheat cultivars relies on the knowledge underpinning the inheritance of resistance. Although single genes have been identified in some *Triticum* accessions (11,20,23,26), resistance in seedlings and adult plants is largely under polygenic control (4,38). Several independent genes control glume and flag leaf resistance (4,14) and their effects are mainly additive in adult plants and seedlings (8,31). The complex genetic control of independent genes for seedling, flag leaf, and glume resistance (24,28) indicates that selection for transgressive expression of resistance is probably the most effective strategy for developing resistant wheat cultivars (25,37).

A significant factor that needs to be considered when analyzing the genetic basis controlling disease response is the effect of plant architecture on disease ratings. Morphological characteristics including variation in heading date and plant height of host plants can have a major influence on *S. nodorum* infection causing inaccuracy in assessing disease resistance under natural infection. Therefore, populations segregating for height and heading date indicated pleiotropy as a significant genetic factor influencing SNB resistance in wheat (31). However, it is difficult to exclude the possibility that resistance may be linked to genes controlling height and heading date. Similar studies have shown that plant height and heading date play a significant role in accurately assessing leaf resistance to *Septoria tritici* blotch in wheat (32) and Type II resistance to *Fusarium* head blight in barley (41). The authors in those studies were unable to determine pleiotropy or linked resistance as the causal effect of disease severity. An immediate implication is that morphological traits need to be considered when identifying quantitative trait loci (QTL) for disease resistance affecting adult plants and the application to plant breeding.

Several studies have identified QTL for resistance to SNB infection at the seedling and adult plant stages using either pathogen inoculation or natural infection. QTL for latent and incubation periods and disease severity for seedling resistance were identified on chromosomes 2B, 3B, 5B, and 5D in winter wheat (7). However, the effect of these QTL in adult plant resistance is unknown. Similarly, QTL for glume blotch resistance were identified on 2B, 3B, 5B, and 5D in addition to nine other chromosomal regions (30) in another winter wheat population, but most were not consistently detected across different environ-

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ments and their effect in seedling resistance has not been determined. More recently, the same QTL for glume blotch resistance on the long arm of chromosome 2D (2DL) was detected in a winter wheat accession in the United States and Australia even though extreme environmental conditions and genetically distinct isolates are likely to exist across continents (35). However, the lack of disease rating for seedlings in the same populations precluded the identification of alternative or similar QTL interacting between seedling and adult plant resistance.

The relationship between glume blotch resistance, height, and heading date has been previously reported (30). Although 13 QTL were reported for resistance in a winter wheat recombinant inbred line population, six of these on chromosomes 2A, 3B, 5A, 5B, 6B, and 7D shared the same marker interval with QTL for either height, heading date, or both traits (30). In that study, it is unknown whether glume blotch resistance is linked to genes controlling height and heading date or pleiotropic effects are detecting "pseudo" resistance QTL. Similarly, seven QTL common for glume blotch resistance and heading date in a wheat/spelt cross, indicated possible pleiotropic effects on disease resistance (1). Therefore, it is necessary to consider variation in morphological characteristics when assessing disease severity of adult plants under natural infection for QTL analysis.

The independent and complex genetic inheritance of SNB resistance, coupled with pleiotropic effects caused by morphological characteristics at the adult plant stage, makes the identification of QTL for resistance a challenging task. In all previous reports on QTL identification for SNB resistance, there have been no comparisons made between seedling, flag leaf, and glume resistance, and their effects on yield related traits. Therefore, the aim of this study was to deploy a phenotyping strategy to reduce confounding effects of height and heading date on disease severity in genotypes of a doubled haploid (DH) population segregating for flag leaf and glume resistance and increase the likelihood of assessing resistance QTL. Also, this study aimed to compare QTL for seedling and adult plant (flag leaf and glume blotch) resistance in order to discriminate QTL suitable for breeding resistance to *S. nodorum*. Fungicide protected and inoculated plots were used as a means to measure relative grain weight (RGW) and compare QTL for a yield-related trait with disease evaluation based on visual scores but independent of variation in morphological characteristics.

MATERIALS AND METHODS

Parents and DH population. A resistant genotype from Centro Internacional De Mejoramiento De Maiz Y Trigo (CIMMYT), 6HRWSN125 and a susceptible inbred genotype, WAWHT2074, from the Department of Agriculture and Food Western Australia, were crossed. An F₁ derived DH mapping population was constructed using the wheat/maize pollination system, from which 280 homozygous genotypes were randomly selected. The DH population was designated 98W816. Genotypes of the population, parents, and 14 additional control genotypes (with varying heading date and plant height) were assessed for seedling resistance in the glasshouse and for flag leaf and glume necrosis in the field in 2003 and 2004.

Maintenance of *S. nodorum* cultures. Fungal cultures were produced by growing pathogen isolates on sterile wheat grains for 3 months to induce formation of pycnidia and pycnidiospores (13). Fungal cultures were then air-dried and ground into a coarse powder. Equal amounts of the dried powder of each isolate were mixed and the inoculum mixture stored at 4°C. Fungal cultures in this form remained viable for 2 or more years. The pathogen isolate mixture was rehydrated prior to inoculation.

Seedling phenotyping. Plants were grown with 22/18°C day/night temperatures and natural lighting in 120-mm-diameter pots containing a sand-loam mix with 1 g of Osmocote (slow release

fertilizer). Six seeds per genotype were planted in each pot. The experiment was conducted with three replicate pots per genotype placed in a randomized block design on the glasshouse bench. Plants were spray inoculated to run-off at the two-and-a-half-leaf stage with a conidial suspension (10⁶ conidia/ml with 0.1 ml/liter of Tween 80) of *S. nodorum* produced from a mixture of four isolates. Disease was assessed 8 days after inoculation on the two lowest leaves that were fully emerged at inoculation using a 0 to 5 scale (0 = no infection, 5 = severe infection).

Field screening and adult plant phenotyping. The population was sown in an irrigated field nursery at South Perth, Western Australia in 2003 and 2004. There were two treatments in the experiment. One treatment consisted of plots infected with mixed isolates of *S. nodorum* whilst the other treatment consisted of plots protected against *S. nodorum* infection using fungicide. There were three replications each in a randomized split-plot design for the infected and uninfected treatments. Genotypes were sown in the second week of May as paired 10-cm rows of up to 10 seeds per row sown 10 cm apart, separated by 30-cm centers from adjacent rows. Infected and fungicide protected main plots were separated by 1-m wide rows of barley. Plots were fertilized with a mixture of superphosphate, urea, and potash (6:4:1) at a rate of 100 kg/ha at planting and at 8 weeks after sowing. Plots with fungicide treatment were protected from *S. nodorum* infection by spraying with 125 g/ha Tebuconazole. Disease plots were protected from powdery mildew infection with 250 g/ha of Quinoxifen and 125 g/ha Bupirimate. All fungicides were applied 2 weeks after sowing and at 4 weekly intervals for 12 weeks.

To promote a high humidity environment for fungal infection, plots were watered prior to inoculation. Flag leaves and glumes of individual plots in the infected treatment were sprayed with a conidial suspension (10⁶ conidia/ml with 0.5% gelatin) consisting of four isolates of *S. nodorum* in 2003 and an additional six isolates in 2004. The conidial suspension was sprayed to run-off at half spike emergence (Feekes stage 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; 30-cm 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.

Disease was scored on flag leaves and glumes in infected plots. Percentage leaf area diseased was assessed on flag leaves 220°C thermal days (sum of average daily temperatures) and percentage glume infection 600°C thermal days (in 2003) and 370°C thermal days (in 2004) after inoculation. Rating scales for glume and leaf infection was based on a percentage scale from 0 (highly resistant) to 100% (highly susceptible) as previously described (16). Disease ratings on flag leaf and glume were scored for individual plants and an average for each plot was calculated and used for analysis.

In addition to visual disease scores, RGW of genotypes were calculated from infected and uninfected plots. Glumes from infected and uninfected plots were hand harvested and 500 kernel weight measured for each plot and converted to 100 grain weight. RGW for genotypes was calculated as the percentage of grain weight from infected plots relative to a mean average of three uninfected plots.

Measurements of plant height were taken as the distance from the soil surface to the top of the spike (awns excluded) for each plot. Time to half-ear emergence from date of planting were taken for all plots (measured as single readings per plot).

Statistical analysis. All statistical analysis was done using Genstat version 8.1. Ratings for flag leaf and glume disease severity for data sets that showed a nonrandom distribution in scatter value plots (residual versus fitted value) were transformed using the arcsine (angular) function. Pearson's correlation coefficient was estimated between plant height, flag leaf, and glume

scores using all the DH genotypes. Significant differences between genotypes for all traits and within plant height groups was assessed using one-way analysis of variance (ANOVA) in randomized blocks. Narrow sense heritability estimates were calculated using the formula $h^2 = \sigma_A^2/\sigma_P^2$ where σ_A^2 and σ_P^2 are the additive and phenotypic variance, respectively.

Genetic map construction and QTL analysis. Molecular markers polymorphic between parents, 6HRWSN125 and WAWHT2074, were selected based on distribution across the wheat genome. A genetic map consisting of 492 molecular markers including simple sequence repeats (SSR), DaRT (2,15), and expressed sequence tags (EST) was constructed. In addition “perfect” markers for two reduced height genes, *Rht-B1b* and *Rht-D1b* (9) were genotyped in the DH population. Linkage analysis was done using Mapmanager QTXb20 (21) using the “Distribute” command, linkage criterion of $P = 0.001$ and Kosambi function to calculate genetic distances from recombination fractions. The “Ripple” command was used to find the best order of markers within linkage groups. Linked markers had a minimum logarithm of odds (LOD) score of 2.8 and the order of SSR markers were supported using consensus genetic maps and bin map locations where markers are assigned to specific regions of the wheat genome (33). The linked markers comprised an average of 23.4 markers per chromosome covering a total genetic map distance of 4309 cM with a mean average of 9.7 cM between linked marker loci.

The mean disease scores for seedling, flag leaf and glume, and RGW were used for composite interval mapping (CIM). CIM model one of Windows QTL Cartographer version 2.5 (36) 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 (40). Experiment-wise critical thresholds for significance of potential QTL for each year were determined using CIM to conduct permutation tests as previously described (6). Highly significant ($P = 0.01$) and significant ($P = 0.05$) QTL thresholds were calculated from 1,000 permutations.

RESULTS

Seedling, flag leaf, and glume response to SNB infection.

Table 1 shows the mean disease scores and RGW for the parents 6HRWSN125 and WAWHT2074. A significant ($P \leq 0.05$) difference in seedling resistance was observed between the genotypes. Similarly, a significant difference in flag leaf and highly significant ($P \leq 0.01$) difference in glume resistance and RGW were observed between 6HRWSN125 and WAWHT2074 in 2003 and 2004. The 98W816 population showed a continuous distribution (Fig. 1) and individual genotypes showed high significant differences from each other for seedling, flag leaf and glume resistance, and RGW (Table 1). The mean values of DH genotypes had

higher or lower values than the resistant or susceptible parent, respectively (Table 1), indicating transgressive segregation for seedling, flag leaf and glume resistance, and RGW.

Narrow sense heritability for seedling resistance was moderate whereas adult plant resistance was low to moderate for leaf and glume resistance (Table 1). Narrow sense heritability for RGW was consistently high in 2003 and 2004.

Pearson’s correlation co-efficient showed a small or no significant ($P > 0.05$) correlation between seedling and flag leaf, seedling and glume disease scores, or seedling and RGW (Table 2). In adult plants, moderate but highly significant correlation between years for flag leaf resistance and glume resistance was observed. Similarly, low to moderate but highly significant correlation between flag leaf and glume resistance was observed in each year. RGW showed a moderate but highly significant correlation between years and a moderate negative but highly significant correlation between flag leaf and glume resistance in each year (Table 2).

Morphological characteristics. A continuous distribution for plant height and heading date (data not shown) and ANOVA showed a highly significant difference between DH genotypes of the population (Table 1). In addition, DH genotypes were identified with mean values beyond those of the parents, indicating transgressive segregation and moderate heritability for heading date and plant height (Table 1). There was a large and highly significant correlation for plant height and heading date between years for genotypes of the DH population.

QTL analysis for disease resistance. The number of QTL detected for seedling, flag leaf and glume resistance, and RGW ranged from three for flag leaf resistance in 2003 and 2004 to seven for RGW in 2004 (Table 3). The proportion of variation contributed by each QTL varied from 3 to 21% (Table 3). For seedling resistance, a total of 5 QTL were detected and each accounted between 5 to 13% of total phenotypic variation. Three QTL were detected for flag leaf resistance in 2003 and 2004 and each accounted between 4 and 21% of the total phenotypic variation. However, only one common QTL (*QSn1.daw-2D*) was detected in 2003 and 2004 (Table 3). The markers corresponding to the maximum LOD score was *gwm157* in 2003 and *wPt-2544* in 2004, separated by a genetic distance of 19.9 cM, indicating two alleles in this marker interval. The remaining QTL were detected either in 2003 or 2004. None of the QTL for flag leaf resistance co-located with QTL for seedling resistance.

Each QTL detected for glume resistance also accounted for a small proportion of the total phenotypic variation, ranging from 8 to 19% (Table 3). However, *QSng.daw-4B* detected glume resistance in 2003 and 2004. The remaining QTL had smaller effects on the total phenotypic variation and were detected either in 2003 or 2004. Similar to flag leaf resistance, no QTL for glume resistance were co-located with seedling resistance (Table 3).

Five QTL for RGW were detected in 2003 and 2004 and the proportion of the total variation accounting for each QTL ranged

TABLE 1. Summary of *Stagonospora nodorum* infection on seedling, flag leaf, and glume in the spring wheat doubled haploid population, 98W816, and the resistant (6HRWSN125) and susceptible (WAWHT2074) parents evaluated in South Perth, Western Australia, in 2003 and 2004

Trait	6HRWSN125 (resistant)	WAWHT2074 (susceptible)	Population mean	Population min.	Population max.	ANOVA ^a	h^2	LSD (0.05)
Seedling glasshouse 2003 (scale 0–5)	1.2	2.6	2.2	0.1	5.0	6.83*	0.59	0.7
Glume infection 2003 (%)	25.5	87.3	55.1	12.0	90.0	12.83*	0.67	16.0
Glume infection 2004 (%)	38.0	68.7	55.6	26.0	89.0	6.56*	0.34	13.0
Flag leaf infection 2003 (%)	20.1	59.1	43.0	17.0	67.0	12.45*	0.40	9.0
Flag leaf infection 2004 (%)	25.8	78.0	50.9	15.0	95.0	11.13*	0.54	14.0
Relative grain weight 2003 (%)	77.0	36.0	61.0	19.6	135.0	9.31*	0.95	16.3
Relative grain weight 2004 (%)	51.0	36.2	50.3	23.0	93.0	6.49*	0.94	16.2
Heading date 2003 (days)	86.0	89.7	97.3	63.0	128.0	84.90*	0.35	4.1
Heading date 2004 (days)	83.7	88.3	101.5	67.0	148.0	105.70*	0.54	3.9
Plant height 2003 (cm)	96.7	88.3	84.1	48.0	124.0	61.51*	0.62	5.5
Plant height 2004 (cm)	84.2	70.8	77.3	45.0	124.0	74.90*	0.56	5.1

^a Significant difference ($P \leq 0.01$) for F values is indicated by an asterisk.

from 3 to 21% (Table 3). Two QTL detected for flag leaf (*QSnl.daw-2D*) and glume (*QSnng.daw-4B*) spanned the same marker intervals for RGW in both years (Table 3; Figs. 2 and 3). A second linked QTL on chromosome 2D was consistently detected for RGW that was not detected for flag leaf resistance (Table 3 and Fig. 2). In some instances, QTL for flag leaf or glume resistance detected in 1 year but not in another co-located with QTL for RGW detected in both years. For example, the QTL for flag leaf resistance *QSnl.daw-5A* was detected in 2003 only whereas *QSnr.daw-5A* for RGW and in the same marker interval was detected in 2003 and 2004 (Table 3).

QTL analysis for morphological characteristics. Analysis for heading date identified a major QTL on chromosome 6B in 2003 (*QHd.daw-6B*) accounting for 48% of the phenotypic variation (Table 3). This QTL did not co-locate with any QTL for seedling, flag leaf and glume resistance, or RGW in 2003 or 2004. Three QTL accounting for large proportions of phenotypic variation for heading date were detected in 2004 on chromosomes 2A, 5A, and 5B (Table 3). However, two of these QTL, *QHd.daw-5A* and

QHd.daw-5B, co-located with RGW in 2004. QTL for plant height were detected on chromosomes 4B in 2003 and 2004 (Table 3) where *Rht-B1b* is shown to be linked to glume resistance *QSnng.daw-4B* and *QSnr.daw-4B* in 2003 and 2004 (Fig. 3).

DISCUSSION

In adult plants, resistance to pathogens can be divided into true resistance or escape (26). Disease escape results from reduced contact between host and pathogen. Variation in morphological traits such as heading date and plant height can provide good escape mechanisms. Disease severity may be influenced by plant height because the flag leaves and glumes are infected with rain-splashed pycnidiospores and shorter plants are less likely to escape than taller plants (10,31). Also, infection may be more severe in earlier maturing plants allowing the pathogen to develop disease prior to senescence (39). Therefore, stringent phenotyping methodologies were employed to reduce escape mechanisms caused by variation in morphological characteristics and assess SNB

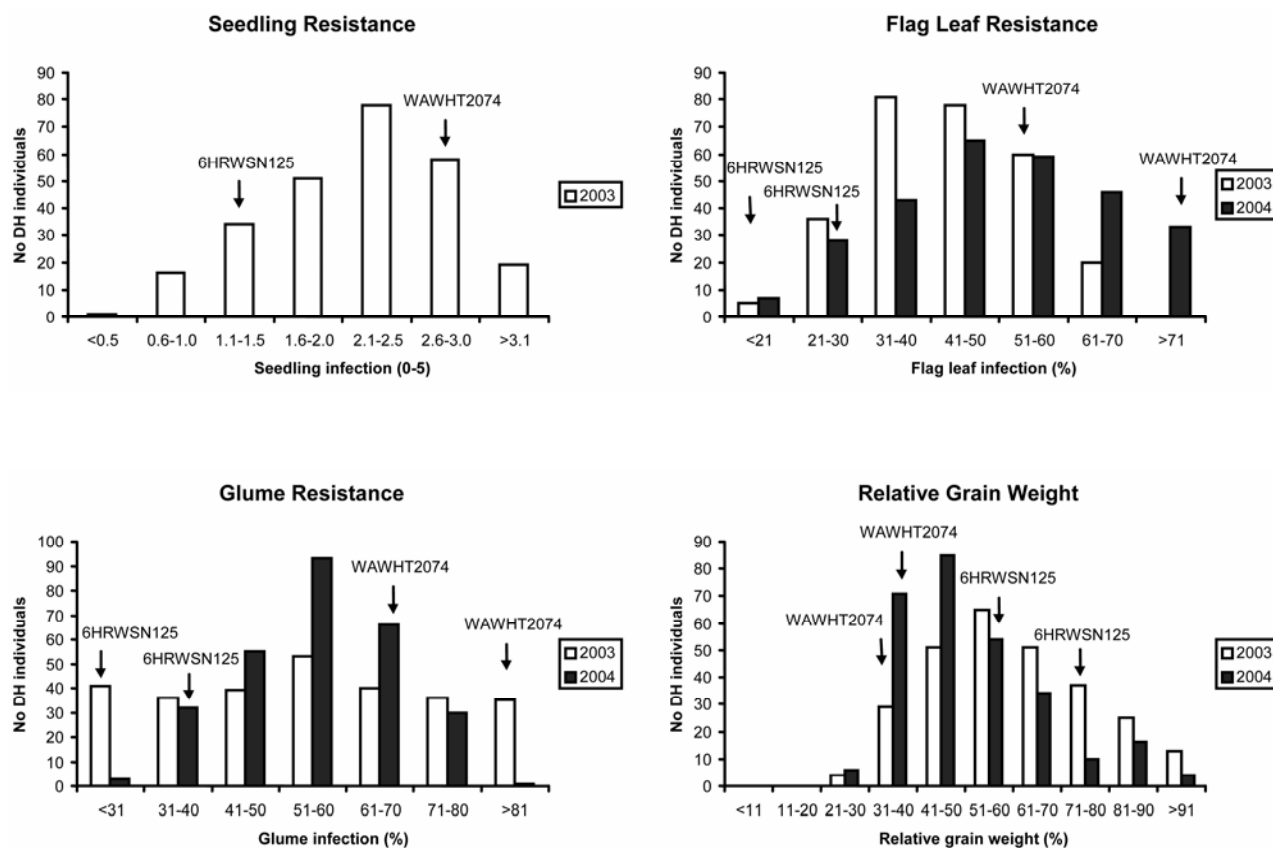


Fig. 1. Phenotypic distribution of seedling, flag leaf, and glume response to infection with *Stagonospora nodorum* and relative grain weight (RGW) for the doubled haploid population, 98W816, from field assessments in 2003 and 2004. Arrows show the relative position of the resistant (6HRWSN125) and susceptible (WAWHT2074) parents.

TABLE 2. Matrix of Pearson's correlation coefficient between seedling, flag leaf and glume resistance to *Stagonospora nodorum* for the spring wheat doubled haploid population, 98W816, and the resistant (6HRWSN125) and susceptible (WAWHT2074) parents evaluated in South Perth, Western Australia, in 2003 and 2004^a

	Seedling	Flag leaf 2003	Glume 2003	RGW 2003	Flag leaf 2004	Glume 2004	RGW 2004
Seedling	—						
Flag leaf 2003	0.31**	—					
Glume 2003	0.09ns	0.60**	—				
RGW 2003	-0.19*	-0.47**	-0.64**	—			
Flag leaf 2004	0.24**	0.66**	0.60**	-0.58**	—		
Glume 2004	0.16*	0.30**	0.46**	-0.62**	0.36**	—	
RGW 2004	-0.09ns	-0.56**	-0.64**	0.63**	-0.46**	-0.52**	—

^a RGW = relative grain weight. Values are significant at $P \leq 0.05$ (*), highly significant at $P \leq 0.01$ (**), or not significant at $P > 0.05$ (ns).

resistance. This was achieved by inoculating both flag leaves and glumes of DH genotypes with conidial suspensions at similar maturity. If segregation of morphological traits confounded disease assessment, then QTL for plant height and heading date would be co-located with either flag leaf or glume resistance QTL, as observed in previous studies (1). The phenotyping strategy employed in this study reduced pleiotropic effects of morphological characteristics on disease evaluation and QTL for flag leaf and glume resistance were independent of heading date and plant height. Although a QTL for glume resistance was identified on chromosome 4B, the location of the *Rht-B1b* gene on the same chromosome (9) provided evidence that the QTL *QSng.daw-4B* was linked to the *Rht-B1b* gene and not a pleiotropic effect of plant height.

The disease evaluations and QTL analysis in this study showed that SNB resistance has been identified. This study also indicated that seedling, flag leaf, and glume resistance are controlled by independent QTL. The majority of each QTL for seedling, flag

leaf, and glume resistance contributed a small proportion of the total phenotypic variation, similar to previous reports for seedling (7) and adult plant resistance (1,30,35). For seedling resistance, the QTL on chromosome 5B shared common regions with seedling resistance previously reported (7), but the QTL on chromosomes 2D, 4D, and 6D are unique. Several studies have reported insensitivity to toxin produced by *S. nodorum* as a factor in seedling disease resistance (17,18), but none of the QTL for seedling resistance detected in this study aligned with QTL for toxin insensitivity. This indicated that either different genes confer toxin insensitivity in 6HRWSN125 or different toxins are produced in Western Australian isolates of *S. nodorum* that are not effective against known genes for insensitivity. Further studies are required to determine whether proteinaceous toxins are produced in Western Australian isolates of *S. nodorum* and whether they have a biological role in conferring insensitivity and seedling resistance.

Heritabilities for adult plant resistance were inconsistent with other reports where higher heritability values of 0.90 and 0.92

TABLE 3. Summary of quantitative trait loci (QTL) analysis for *Stagonospora nodorum* resistance using composite interval mapping for seedling, glume and flag resistance, relative grain weight, and morphological attributes in the doubled haploid mapping population, 98W816

Trait	Chromosome	Marker interval	Distance	Composite interval mapping			<i>R</i> ²
				LOD threshold ^a	Max. LOD ^b	Additive ^c	
Seedling (Glasshouse 2003)	QSns.daw-2D	<i>cfid50-gwm382</i>	20.2 cM	2.7	3.12 (<i>wPt-8524</i>)	-0.16	0.05
	QSns.daw-4D	<i>cfid54-wPt-7009</i>	10.7 cM	2.1	3.45 (<i>cfid54</i>)	0.15	0.05
	QSns.daw-5B.1	<i>wPt-6136-wPt-1420</i>	39.9 cM	2.7	5.40 (<i>gwm234</i>)	-0.21	0.08
	QSns.daw-5B.2	<i>wPt-9613-wPt-5896</i>	37.7 cM	2.7	7.43 (<i>wPt-0498</i>)	-0.25	0.13
	QSns.daw-6D	<i>gdm46-edm148</i>	14.1 cM	2.3	4.22 (<i>cfid76a</i>)	0.16	0.06
Glume resistance (2003)	QSng.daw-2A	<i>wPt-7026-gwm71c</i>	40.0 cM	2.4	4.71 (<i>wmc258</i>)	4.78	0.05
	QSng.daw-2B	<i>gwm148-wPt-8072</i>	63.4 cM	2.6	3.01 (<i>gwm319</i>)	-3.63	0.03
	QSng.daw-4B	<i>Rht1-gwm495</i>	28.4 cM	2.2	15.83 (<i>barc340</i>)	-9.17	0.19
	QSng.daw-5A	<i>gwm617a-wPt0373</i>	45.0 cM	2.4	10.43 (<i>wPt-0373</i>)	-6.30	0.08
	QSng.daw-5B	<i>wPt-5896-gwm604</i>	12.7 cM	2.7	3.01 (<i>wPt-5896</i>)	3.69	0.03
	QSng.daw-7B	<i>wPt-4025-wPt-6498</i>	25.9 cM	2.4	4.17 (<i>wPt-2572</i>)	-4.53	0.05
	QSng.daw-1B	<i>cfid59b-wPt-3566</i>	15.3 cM	2.2	7.51 (<i>wPt-0170</i>)	3.86	0.10
Glume resistance (2004)	QSng.daw-2B	<i>wPt-4199-wPt-9336</i>	77.1 cM	3.1	7.15 (<i>gwm191c</i>)	-4.35	0.13
	QSng.daw-2D	<i>cfid11-gwm157</i>	52.3 cM	2.9	3.50 (<i>wPt2544</i>)	-2.53	0.04
	QSng.daw-4B	<i>Rht1-gwm149</i>	33.7 cM	2.4	6.35 (<i>barc340</i>)	-3.52	0.08
	QSnl.daw-1B	<i>wPt-3566-cfa2129b</i>	17.1 cM	2.3	4.00 (<i>wPt-8279</i>)	2.34	0.05
Flag leaf resistance (2003)	QSnl.daw-2D	<i>wPt-0638-gwm30</i>	39.8 cM	2.6	5.46 (<i>gwm157</i>)	-3.26	0.08
	QSnl.daw-5A	<i>gwm617a-wPt-0373</i>	45.0 cM	2.4	10.39 (<i>wPt-0373</i>)	-5.35	0.21
	QSnl.daw-2D	<i>cfid11-gwm157</i>	52.3 cM	2.7	8.69 (<i>wPt-2544</i>)	-7.01	0.17
Flag leaf resistance (2004)	QSnl.daw-4B	<i>wPt-3991-barc340</i>	7.2 cM	2.5	2.94 (<i>wPt-7569</i>)	-3.42	0.04
	QSnl.daw-5B	<i>wPt-6136-wPt-1420</i>	39.9 cM	2.7	2.72 (<i>gwm234</i>)	-3.37	0.04
	QSnr.daw-2A	<i>wPt-7026-wmc296</i>	23.1 cM	2.7	6.56 (<i>wmc453</i>)	-4.51	0.06
Relative grain weight (2003)	QSnr.daw-2D.1	<i>cfid11-wPt-8330</i>	27.3 cM	2.5	3.06 (<i>gwm157</i>)	4.03	0.05
	QSnr.daw-2D.2	<i>wPt-8524-wPt-8319</i>	1.9 cM	2.5	2.60 (<i>wPt-3757</i>)	3.11	0.03
	QSnr.daw-4B	<i>Rht1-gwm149</i>	33.7 cM	2.4	8.74 (<i>barc340</i>)	5.34	0.08
	QSnr.daw-5A	<i>gwm617a-wPt-0373</i>	45.0 cM	2.5	17.39 (<i>wPt-0373</i>)	8.65	0.21
	QSnr.daw-5B	<i>wPt-0498-gwm604</i>	41.3 cM	2.5	13.06 (<i>wPt-5896</i>)	-6.60	0.13
	QSnr.daw-2A	<i>wPt-7026-wmc296</i>	23.1 cM	2.5	2.70 (<i>wmc453</i>)	-2.72	0.03
	QSnr.daw-2B	<i>wPt-4199-wPt-9336</i>	77.1 cM	2.8	3.40 (<i>gwm191c</i>)	4.54	0.08
Relative grain weight (2004)	QSnr.daw-2D.1	<i>cfid11-wPt-8330</i>	27.3 cM	1.8*	2.18 (<i>wgm157</i>)	2.64	0.03
	QSnr.daw-2D.2	<i>gwm311-barc197</i>	69.0 cM	1.8*	4.95 (<i>cfid44</i>)	4.29	0.08
	QSnl.daw-4B	<i>Rht1-gwm149</i>	33.7 cM	2.3	8.80 (<i>barc340</i>)	5.26	0.11
	QSnr.daw-5A	<i>gwm617a-wPt-0373</i>	45.0 cM	2.7	5.06 (<i>wPt-0373</i>)	5.11	0.09
	QSnr.daw-5B	<i>wPt-0498-gwm604</i>	41.3 cM	2.4	8.44 (<i>wPt-5896</i>)	-5.02	0.10
	QHd.daw-5B	<i>wPt-0498-gwm604</i>	41.3 cM	1.4	1.95 (<i>wPt-5896</i>)	-11.93	0.03
	QHd.daw-6B	<i>wPt-4858-wPt7642</i>	24.4 cM	3.0	25.22 (<i>wPt-3774</i>)	260.65	0.48
Heading date (2004)	QHd.daw-2A	<i>wPt-5498-wPt-7024</i>	25.2 cM	2.5	20.61 (<i>wPt-3114</i>)	-7.97	0.15
	QHd.daw-5A	<i>gwm617a-wPt0373</i>	45.0 cM	2.3	58.59 (<i>wPt-0373</i>)	14.66	0.47
	QHd.daw-5B	<i>wPt-0498-gwm604</i>	41.3 cM	2.8	39.13 (<i>wPt-5896</i>)	-10.77	0.27
Plant height (2003)	QHgt.daw-4B	<i>barc193-gwm340</i>	39.6 cM	2.3	18.48 (<i>Rht1</i>)	12.04	0.14
	QHgt.daw-4D	<i>wPt-4572-barc217</i>	64.8 cM	2.3	40.39 (<i>Rht2</i>)	-12.58	0.36
	QHgt.daw-5A	<i>gwm617a-wPt0373</i>	45.0 cM	2.3	3.59 (<i>wPt-0373</i>)	3.93	0.03
Plant height (2004)	QHgt.daw-4B	<i>barc193-gwm340</i>	39.6 cM	2.7	10.57 (<i>Rht1</i>)	9.46	0.11
	QHgt.daw-4D	<i>wPt-4572-barc217</i>	64.8 cM	2.4	9.40 (<i>Rht2</i>)	-7.81	0.11
	QHgt.daw-5A	<i>gwm617a-wPt0373</i>	45.0 cM	2.7	9.61 (<i>wPt-0373</i>)	6.39	0.11
	QHgt.daw-5B	<i>wPt-0498-gwm604</i>	41.3 cM	2.5	5.38 (<i>wPt-2707</i>)	-3.44	0.03
	QHgt.daw-7A	<i>wmc247-cfa2123</i>	40.4 cM	2.7	5.31 (<i>wPt-8897</i>)	3.51	0.04

^a Logarithm of odds (LOD) threshold values calculated using 1,000 permutations and significant at *P* = 0.01 or *P* = 0.05 (*).

^b Maximum LOD score for QTL with corresponding marker in parentheses.

^c Positive and negative values indicate that 6HRWSN125 alleles increase and decrease for each trait, respectively.

were reported based on visual assessment of leaf and glume resistance, respectively (1). The heritability for glume scores in 2003 and 2004 were lower than those based on similar disease evaluations in a winter wheat population (35), where both populations were assessed simultaneously using the same isolates, environment, and phenotyping strategy. This indicated likely differences between spring and winter wheat genotypes in response to *S. nodorum* infection in Western Australia. However, this study showed that RGW consistently produced higher heritability.

Therefore, breeding for suitable levels of SNB resistance may benefit from using RGW as a more reliable indicator on the effects of disease infection.

This study consistently detected QTL for flag leaf (*QSnL.daw-2D*) and glume resistance (*QSnG.daw-4B*) in consecutive years, identifying them as stable QTL and potential candidates for improving wheat germplasm for adult plant resistance to SNB. However, QTL for flag leaf and glume resistance detected in one year but not in another may be unreliable for improving genetic

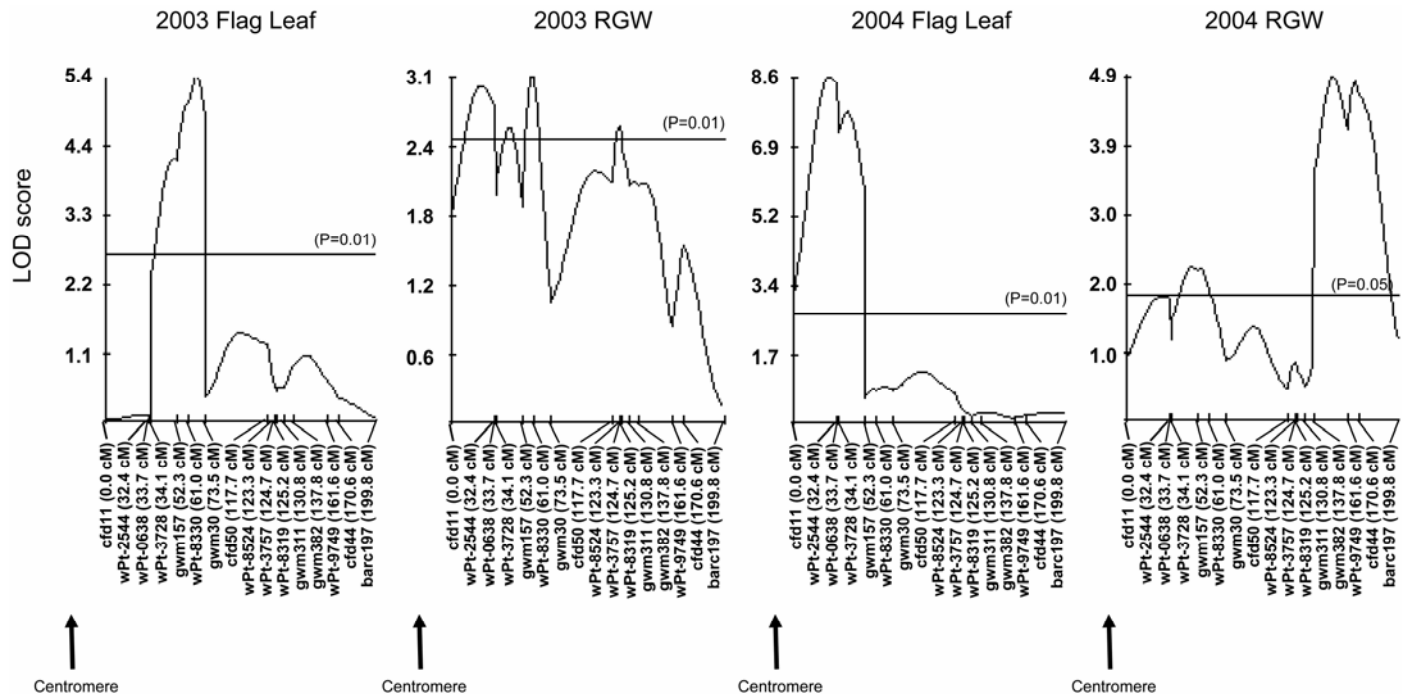


Fig. 2. Composite interval mapping and quantitative trait loci (QTL) on chromosome 2D for percentage flag leaf infection with *Stagonospora nodorum* and relative grain weight (RGW) in 2003 and 2004 for the doubled haploid population, 98W816. The threshold values for significant QTL at $P = 0.05$ or 0.01 is shown as horizontal line and calculated by permutation testing using 1,000 reiterations. Marker location (cM) and approximate position of centromere is shown.

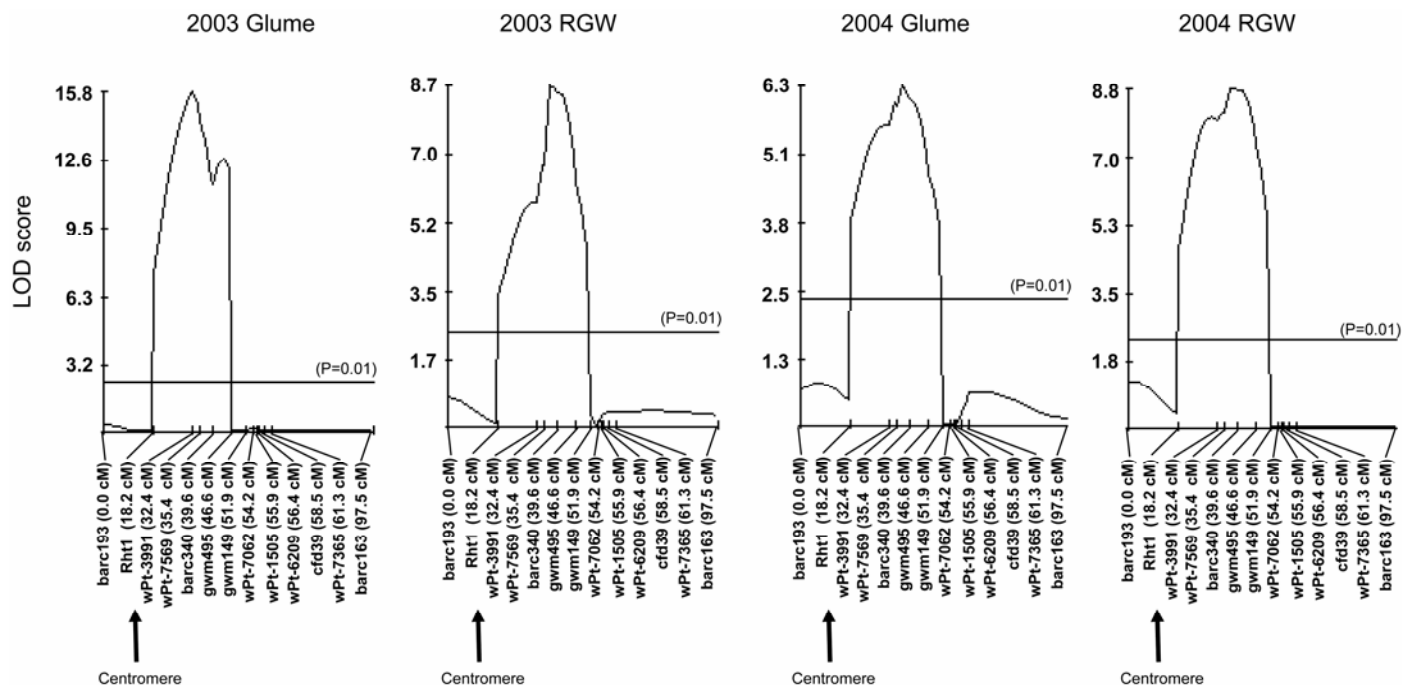


Fig. 3. Composite interval mapping and quantitative trait loci (QTL) on chromosome 4B for percentage glume infection with *Stagonospora nodorum* and relative grain weight (RGW) in 2003 and 2004 for the doubled haploid population, 98W816. The threshold values for significant QTL at $P = 0.01$ is shown as horizontal line and calculated by permutation testing using 1,000 reiterations. Marker location in centimorgans (cM) and approximate position of centromere is shown.

gain. *QSn1.daw-2D* is in a similar chromosomal position to flag leaf resistance, *QSn1.eth-2D*, identified in winter wheat and detected in European environments (1). Similarly, a glume resistance QTL in a winter wheat, *QSn1.pur-2DL* was also located in the same position on chromosome 2DL and consistently detected across a range of environments (35). However, this study did not identify a QTL for glume resistance in the same marker interval on 2DL even under similar phenotyping conditions using the same isolates in Australian environments. This indicated that independent QTL, at least two for flag leaf and one for glume resistance, reside in the same region on 2DL in winter and spring wheats.

The markers delineating QTL for insensitivity to *S. nodorum* toxins in seedling tests (17,18) were aligned with markers for flag leaf and glume resistance, predicting whether toxin insensitivity may play a biological role in adult plant resistance. A comparison of marker intervals revealed only *QSn1.daw-4B* coincided with the same QTL for toxin seedling insensitivity on 4BL inherited from the synthetic genotype W7984 (18). Therefore, toxin insensitivity may play a role in glume resistance. However, *QSn1.daw-4B* did not coincide with any QTL for seedling resistance in this study. If QTL conferring toxin insensitivity on 4BL are involved in disease resistance, then at least two independent QTL from spring and winter wheat accessions reside in this region, similar to the QTL for flag leaf and glume resistance on chromosome 2DL. Further clarification of insensitivity to purified toxin preparations on seedlings and glumes in the 98W816 population is required.

The DH population was evaluated against mixed isolates of *S. nodorum* as previous studies have indicated no specificity in the *S. nodorum*-wheat pathosystem (12,22,29). In the 2004 field evaluation, additional isolates were used to take advantage of the probable differences in aggressiveness amongst isolates and to evaluate the stability of QTL identified in 2003. It was expected that the QTL detected in 2003 would be detected in 2004 when an additional six isolates were used in the inoculation. Although two QTL identified by visual assessment were identified in successive years, the varying magnitude of resistance response to four isolates used in 2003 and 2004 may account for the low reproducibility of other QTL.

Although disease ratings provide a visual assessment of resistance or susceptibility, the effects of QTL for flag leaf and glume resistance on yield potential is largely unknown. Previous studies (25,27,34) used RGW measurements as a means to determine the effect of flag leaf and glume disease on grain yield traits. In this study, QTL for RGW was used as indicators of yield potential under pathogen infection and data indicated that flag leaf and glume resistance contributed to increasing grain weight. However, in some instances, QTL detected for flag leaf and glume resistance indicated that visual disease score alone may not be a reliable indicator of yield performance. Therefore, RGW is an important consideration for assessing the impact of SNB on yield potential and future QTL analysis for SNB resistance could use RGW as a measure for pathogen effects on grain yield. However, the strategy will be at the expense of increasing plot numbers to include inoculated and fungicide protected treatments.

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