Evaluation of Septoria nodorum blotch (SNB) resistance in glumes of wheat (*Triticum aestivum* L.) in multiple field environments and the genetic relationship with foliar disease response

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Abstract

Septoria nodorum blotch (SNB) is a necrotrophic disease of wheat prominent in some parts of the world, including Western Australia (WA) causing significant losses in grain yield. The genetic mechanisms for resistance are complex involving multiple quantitative trait loci. In order to decipher comparable or independent regulation, this study identified the genetic control for glume compared to foliar resistance across four environments in WA against 37 different isolates. High proportion of the phenotypic variation across environments was contributed by genotype (84.0% for glume response and 82.7% for foliar response) with genotype-by-environment interactions accounting for a proportion of the variation for both glume and foliar response (14.7% and 16.2%, respectively). Despite high phenotypic correlation across environments, most of the eight and 14 QTL detected for glume and foliar resistance, respectively, were identified as environment-specific. QTL for glume and foliar resistance neither co-located nor were in LD in any particular environment indicating autonomous genetic mechanisms control SNB response in adult plants, regulated by independent biological mechanisms and influenced by significant genotype-by-isolate-by environment interactions. Known *Snn* and *Tsn* loci and QTL were compared with 22 environment-specific QTL. None of the eight QTL for glume or the 14 for foliar response were co-located or in linkage disequilibrium with *Snn* and only one foliar QTL was in LD with *Tsn* loci on the physical map. Therefore, known NE-*Snn* interactions are of limited relevance to glume and foliar SNB response in WA environments and other biological mechanisms are likely to prevail for host resistance and susceptibility.

Key Message

Glume and foliar response to Septoria nodorum blotch (SNB) in wheat is regulated by multiple environment-specific loci which function independently, with limited relevance to known NE-Snn interactions in Western Australian environments.

Introduction

Parastagonospora (syn. ana, *Stagonospora*; teleo, *Phaeosphaeria*) *nodorum* (Berk.) Quaedvlieg, Verkley & Crous is the causal pathogen of Septoria nodorum blotch (SNB) of wheat that infects the lower leaves of the canopy and is identified by dark brown round or lens shaped spots that coalesce and develop black pycnidia as lesions mature. Early foliar symptoms in Western Australia (WA) are seen at tillering (Feekes 5) and is a precursor to glume infection. Rain splash disperses spores whereby foliar disease symptoms proliferate under high humidity and infection continues up the canopy through to stem elongation and ripening. Infected heads will turn dark brown often with a purple tint and black pycnidia evident as typical glume blotch symptoms. Yield losses are estimated to be approximately 12% where SNB is considered to be a major necrotrophic disease affecting grain yield in Western Australian production environments (Murray and Brennan et al. 2009) as well as other regions of the world, particularly as a recurrent disease of wheat in several geographical areas of the USA (Cowger et al. 2020). Management practices provide strategies for controlling the pathogen, but the use of resistant cultivars can significantly reduce on-farm costs. However, breeding for leaf and glume blotch resistance is challenging due to the inherent genetic complexity controlling SNB response when the disease is most damaging (reviewed in Francki, 2013).

Similar to leaf blotch, glume blotch response is under quantitative control having additive-dominance effects for resistance with some interactions with non-allelic genes (Wicke et al. 1999). Dominance for glume blotch susceptibility is common (Freid and Meister 1987; Wicki et al 1999) whereas dominance for resistance also exists in specific crosses (Wicki et al 1999). Moreover, morphological characteristics can have a profound effect on disease response so it is important to discriminate between pleiotropy and linkage with resistance in genetic analysis (Francki, 2013). There have been at least 20 QTL associated with glume resistance identified across the wheat genome with each accounting for up to 24% of the phenotypic variation indicating small effects on resistance phenotypes (Czembor et al. 2019; Lin et al. 2020; Schnurbusch et al. 2003; Shankar et al. 2008; Uphaus et al. 2007). Similarly, at least 18 QTL have been identified for foliar resistance (reviewed in Francki, 2013; Ruud and Lillemo, 2018) with subsequent reports of others that may represent existing or, indeed, new QTL (Czembor et al. 2019; Ruud et al. 2019; Francki et al. 2020; Lin et al. 2020). Recent quantitative genetic analysis detected QTL for either glume or foliar SNB response in different field environments whereby some shared the same marker interval (Schnurbusch et al. 2003; Lin et al 2020) indicating similar genes may have an effect on disease resistance or susceptibility in both organs. On the contrary, some studies did not detect the same QTL for glume and foliar resistance (Czembour et al. 2019; Shankar et al. 2008) confirming that alternative genes are seemingly under independent control and in agreement with earlier studies (Fried and Meister, 1987; Wicki et al. 1999). However, comparison between the genetic control of glume and foliar response to SNB in those studies were based on bi- or multi-parental populations where diversity is limited and the extent of alleles and effects on either resistance, susceptibility or both is not broadly exploited in global germplasm pools. Evaluation of a wider gene pool coupled with high marker density genetic mapping would further extrapolate allelic diversity and gene interactions to expand our knowledge on similar and/or independent genetic mechanisms controlling both glume and foliar SNB response in WA environments.

P. nodorum expresses a range of necrotrophic effectors (NE) that interact with corresponding sensitivity loci (*Snn*) that induce necrosis in wheat. There were nine NE-*Snn* interactions identified in wheat on chromosomes 1A, 1B, 2A, 4B, 5B and 6A (Friesen et al. 2009; Phan et al. 2016; Ruud et al. 2017; Downie et al. 2018; Abeysekara et al. 2009; Friesen et al. 2012; Gao et al. 2015; Shi et al. 2015). Reports have shown that some *Snn* loci may play a role in foliar disease progression under SNB infection in multiple field environments (Friesen et al. 2009) whilst other studies indicated known NE-*Snn* interactions were either inconsistent or not associated with QTL in controlling disease development in different environments when inoculated with single or a mixture of isolates (Czembor et al. 2019; Francki et al. 2020; Lin et al. 2020; Ruud and Lillemo, 2018; Ruud et al, 2019). Interestingly, it has been suggested that known NE-*Snn* interactions are not a significant determinant for foliar response in eastern soft red winter wheat germplasm but the effect of unknown *Snn* loci cannot be excluded (Cowger et al. 2020). Similar observations and conclusions were drawn when an extensive collection of wheat germplasm from different regions of the world were evaluated in multi-environments using mixture of isolates from Western Australia (Francki et al. 2020). Despite the increased knowledge of NE-*Snn* interactions controlling foliar response to SNB in relevant production environments, the role of characterized NE-*Snn* interactions for glume susceptibility and resistance is largely unknown.

Genome-wide association studies (GWAS) provide an opportunity to simultaneously evaluate wheat accessions and identify the genetic basis of trait variation through marker-trait associations (MTA). GWAS is used increasingly to identify the genetic control of foliar response to SNB using germplasm representing a wider representation of alleles from different regions of the world (Ruud et al. 2019; Francki et al. 2020). High-density single nucleotide polymorphic (SNP) markers using the iSelect Infinium 90K SNP genotyping array (Wang et al, 2014) have provided a finer resolution of QTL and their association with previous QTL and *Snn* loci. The majority of QTL for SNB response were detected as environment specific (Francki et al. 2020; Ruud et al. 2019) with few exceptions of loci detected across multiple environments (Ruud et al. 2019). The relationship between NE-*Snn* interactions and foliar disease response in adult plant in GWAS was largely inconsistent across multiple environments (Cowger et al 2020; Francki et al. 2020; Ruud et al. 2019). To date, GWAS has neither been applied to investigate the genetic control for glume blotch resistance nor its association with known NE-*Snn* loci from a wider representation of alleles in global germplasm. Finer mapping resolution using GWAS and the iSelect Infinium 90K SNP genotyping array (Wang et al, 2014) will provide an in-depth analysis and increase our knowledge on the relationship between glume and foliar response and known NE-*Snn* interactions when adult plants are infected with different isolates across multiple field environments in WA.

Although consistent and high disease pressure enabled a reliable evaluation of foliar resistance to SNB across six WA environments in 2016–2018 (Francki et al. 2020), the lack of sustained disease progression during the grain filling period at most sites precluded reliable analysis for glume resistance. The aim of this study, therefore, was to evaluate glume response to SNB for 232 wheat lines in successive year field trials at sites where sustained glume blotch disease progression was consistent during the grain fill period in Western Australia. Moreover, the study aimed to identify genotype-by-isolate-by-environment interactions, compare and contrast the genetic control of glume with foliar response using GWAS and ascertain the relevance of NE-*Snn* interactions in WA environments. The outcome of the study will provide knowledge on shared or independent genetic determinants regulating glume and foliar resistance to SNB in global wheat germplasm when evaluated in multiple field environments under different isolates.

Materials And Methods

Plant material

The GWAS panel consisted of 232 wheat lines including 71 lines from Australia, 72 inbred and commercial lines from Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), 78 inbred lines from International Center for Agricultural Research in the Dry Areas (ICARDA), and 11 landraces from various origins. Description of lines, pedigrees and their origins for the GWAS population used in this study was reported in Francki et al. (2020).

Field trial design

Trials were sown at Department of Primary Industries and Regional Development (DPIRD) Manjimup Research Station and DPIRD South Perth Nursery (Western Australia) in 2018–2020 and 2020, respectively. All trials were sown as completely randomized designs with three replications for each genotype. Plots in each trial at Manjimup were sown as two-rows of 1.9 m length and 0.2 m row spacing. Each row contained ~ 100 seeds. The susceptible cultivar “Amery” was sown as two-row plots of 1.9 m length adjacent to each treatment plot. In the 2020 South Perth trial, plots were sown as two-rows of 0.5 m length and 0.2 m spacing with a spreader two-row plot (“Amery”) of 0.5 m length adjacent to each treatment. Each row contained ~ 25 seeds. The susceptible genotypes for glume and leaf blotch (three replications) included “Millewa”, “Arrino”, “Scout” and the landrace, 040HAT10, were sown in each trial at Manjimup and South Perth and used to monitor disease progression.

Isolates, culture preparation and inoculation of field trials.

Isolates of *P. nodorum* were sourced from the culture collection at DPIRD and were representative of different regions of WA. A total of 19, 17 and 12 isolates were selected as mixed inoculum for trials in 2018, 2019 and 2020, respectively (Supplementary Table 1). At least 40% of the isolates used in each year were represented in the mixed inoculum for trial inoculation in the following year with three common isolates, WAC13077, WAC13206 and WAC13872 used in inoculum of all trials (Supplementary Table 1). Fungal cultures and mixed inoculum (10^6 spores/ml) were prepared with field trials inoculated at a rate of 28.5 m^2/L as previously described (Francki et al. 2020). First trial inoculation in each trial commenced at tillering (Feekes 5) with three subsequent inoculations at 14-day intervals.

Environment characterization, SNB disease and agronomic measurements

Trials at DPIRD Manjimup research station and South Perth nursery were in close proximity to weather stations for recording of climatic conditions including air temperature, relative humidity, rainfall, solar exposure and pan evaporation. Climate data was recorded daily and accessed through DPIRD weather and radar database (https://weather.agric.wa.gov.au/). Thermal time (^oCd) for the duration of disease progression was calculated using the sum of average daily minimum and maximum air temperature as $\sum (\min temp + \max temp/2)$ from the day of first inoculation to the day of disease measurement.

Susceptible check varieties were monitored weekly for disease progression and visually assessed on a percent leaf area disease (PLAD) and percent glume area disease (PGAD) scale as described by James (1971). Each plot scored five individual random plants from the middle of the row closest to the spreader susceptible plot. PLAD on the flag leaf represented foliar disease whereas PGAD on the head represented glume disease for each replicate. All plots in the trial were assessed when at least two check susceptible varieties had PLAD > 70% and PGAD > 50%. Foliar and glume disease scores for each replicate was determined by mean plot value.

Heading date was measured from the number of days from sowing for each replicate to reach 50% full head emergence. Plant height was measured for three random plants from the middle of the row closest to the spreader susceptible plot. Height (cm) was taken from the soil level to the top of the head (excluding awns) and mean plot values for each replicate was used for statistical analysis.

Statistical analysis

All statistical analyses for phenotypic evaluation were done using Genstat, 19th edition (https://genstat.kb.vsni.co.uk). Generalized linear models and linear mixed models were used in phenotypic analysis of trait data. Treatment factors and co-variates were fitted to fixed models to estimate main effects and interactions. Finlay-Wilkinson joint regression analysis was used to compare genotypes for SNB response and agronomic traits across four environments. 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.

Genome-wide association analysis

As the same wheat lines in this study were used previously, detailed methodology for genotyping, analysis of population structure and genome wide association was previously described by Francki et al. (2020). Briefly, the 232 wheat lines were genotyped using the 90K Infinium SNP chip array (Wang et al. 2014) and SNP markers with < 80% call rate and < 5% minor allele frequencies were removed resulting in a total of 20,563 SNPs used for analysis. TASSEL v.5.2.52 was used to identify marker-trait associations (MTAs) (Bradbury et al. 2007). A mixed linear model (MLM) was determined to be the most appropriate to account for both structure and cryptic relatedness for this population (Francki et al. 2020). The genotypic kinship matrix (K) was estimated by selecting the "Centered_IBS" method and population structure (Q) was corrected using principal component (PC) analysis. The suitable number of PCs for each trait was determined by testing one through 15 PCs with visual assessment of quantile-quantile plots (Q-Q plots). The option "P3D" was not selected during the MLM analysis with the variance component re-estimated after each marker. The R programs 'qqman' and 'Rcolorbrewer' were used to draw Manhattan plots (R Core Team. 2018; Turner, 2017). A genome-wide significance threshold for MTAs was set at $p < 2.43\text{x}10^{-6}$ ($-\log_{10}(p) > 5.61$) using Bonferroni correction with $\alpha = 0.05$. To estimate the number of independent tests the tagger function in Haploview was implemented as described in Maccaferri et al. (2016) with a r^2 of ≤ 0.1. This returned a genome-wide moderate threshold significance of $p < 7.65\text{x}10^{-5}$ ($-\log_{10}(p) > 4.12$). A suggestive threshold of significance of $p < 1\text{x}10^{-3}$ ($-\log_{10}(p) > 3.00$) was also included as previously reported (Gao et al., 2016: Alomari et al. 2017; Muqaddasi et al. 2019).

Marker pairwise r^2 values were calculated in PLINK 1.9 (Purcell et al. 2007) with a sliding window of 50 and LD decay curves fitted by non-linear regression for each genome (A, B and D) as described by Marroni et al. (2011) with decay of r^2 against distance. LD decay plots were drawn in R with a critical threshold of $r^2 = 0.2$ (R core Team 2013). MTA for QTL were defined to be in LD when their physical distance was within the linkage decay value for their respective sub-genomes.

Assignment of QTL, Snn **and** Tsn1 **to the physical map**

Physical locations of SNP markers were obtained using Pretzel v2.2.6, an interactive, web-based platform for navigating multi-dimensional wheat datasets, including genetic maps and chromosome-scale physical assemblies (Keeble-Gagnère et al. 2019). *Snn* and *Tsn1* loci were anchored to the physical map using SNP markers, or the closest linked SSR markers, as described in Francki et al. (2020). For markers not available in Pretzel v2.2.6, putative locations were obtained using the IWGSC RefSeq v1.0 and the BLAST tool at URGI INRA (https://urgi.versailles.inra.fr/).

Results

Environment characterization

Daily average climate measurements during disease progression at DPIRD Manjimup research station in 2018–2020 were consistent in successive years for air temperature, relative humidity, rainfall, solar exposure and pan evaporation (Supplementary Table 2). Similarly, the total rainfall recorded was 500 mm, 411 mm and 441 mm in 2018, 2019 and 2020, respectively. The climatic conditions at Manjimup WA, therefore, were consistent in 2018–2020. However, the site at South Perth WA was higher in average daily air temperature, solar exposure and pan evaporation but lower for relative humidity and rainfall compared to any year at Manjimup (Supplementary Table 2), with considerable less total rainfall of 313 mm in the period from first inoculation to final disease score. The trial at South Perth in 2020, therefore, was different in climatic conditions and provided an opportunity to compare the response of 232 wheat lines to glume and foliar SNB infection under a different environment.

Assessment of glume response to SNB

A total of 232 wheat lines were evaluated for glume and leaf response to SNB in each year at Manjimup (2018–2020) and at South Perth in 2020. Thermal times for disease evaluation when PGAD was > 50% for at least two susceptible check varieties at Manjimup was 1117 ^{o}Cd -1238^{o}Cd in 2018–2020 but higher (1589^{o}Cd) at South Perth (Supplementary Table 3) indicating climate differences affected rate of progression of glume blotch symptoms. Nevertheless, glume response showed consistently high heritability across all sites (H^2 = 0.79 to 0.89; Table 1) indicating that a significant proportion of phenotypic difference within each environment is controlled by genetic variation. The mean and median of the population for glume response were similar (29.0 to 33.0 and 27.0 to 30.0, respectively; Table 1) indicating comparable disease pressure for glume response across environments within and between years. There was a high and significant linear relationship for PGAD scores between successive trials at Manjimup (r = 0.76 to 0.82; P < 0.001) and between the South Perth and Manjimup trials (r = 0.73 to 0.78; P < 0.001) indicating consistent glume response of genotypes across all environments (Table 2). There was moderate negative correlation between heading date and PGAD in each trial (r=-0.46 to -0.70; P < 0.001) and low to moderate negative correlation between plant height and PGAD (r=-0.34 to -0.64; P < 0.001) in each environment (Table 3) indicating potential pleiotropic effects between morphological traits and glume response. The genotype, environment and their interactions were fitted as terms in a linear mixed model and the significant proportion of glume response was attributed to genotype (84%) followed by genotype-by-environment interactions (14.7%) with only small proportion of the variation (1.3%) attributed by the environment (Table 4).

Table 1
Summary of percent glume area disease (PGAD), percent leaf area disease (PLAD), heading date (HD) and plant height (PH) for 232 global wheat lines evalua
in four environments in Western Australia in 2018–2020.

	Manjimup 2018				Manjimup 2019				Manjimup 2020				South Perth 2020			
	PGAD	PLAD[a]	HD[a]	PH[a]	PGAD	PLAD	HD	PH	PGAD	PLAD	HD	PH	PGAD	PLAD	HD	PH
Minimum	4.0	2.0	86.0	73.0	1.0	4.0	89.0	71.0	0.0	4.0	95.0	63.0	3.0	3.0	81.0	68.0
Maximum	78.0	97.0	133.0	123.0	83.0	90.0	126.0	117.0	72.0	95.0	131.0	117.0	75.0	97.0	113.0	107.
Grand Mean	29.0	38.0	111.0	94.0	29.0	40.0	109.0	94.0	30.0	44.0	113.0	89.0	33.0	43.0	93.0	83.0
Median	27.0	36.0	110.0	94.0	27.0	38.0	108.0	93.0	30.0	43.0	113.0	89.0	30.0	42.0	92.0	82.0
Mode	17.0	53.0	115.0	94.0	45.0	52.0	106.0	94.0	45.0	60.0	117.0	91.0	53.0	50.0	89.0	82.0
ANOVA (*P*)	*P*< 0.001	*P*< 0.001	*P*< 0.001	*P*< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.00
LSD (*P*< 0.05)	15.3	19.6	4.9	6.8	14.8	24.4	4.2	6.2	15.3	21.9	5.7	8.6	17.9	21.8	6.6	6.9
CV (%)	31.7	30.6	2.8	4.5	32.4	15.6	2.4	4.2	31.5	31.3	3.1	6.0	34.1	31.5	4.4	5.2
H^2	0.79	0.88	0.94	0.90	0.89	0.91	0.92	0.96	0.86	0.79	0.88	0.78	0.86	0.89	0.81	0.80

[a]PLAD and agronomic scores reported in Francki et al. (2020)

Table 2
Phenotypic correlation between four trials at Manjimup (MJ) and South Perth (SP) Western Australia in 2018–2020 of 232 wheat lines for percent glume and leaf area diseased (PGAD and PLAD, respectively).

	PGAD				PLAD			
	MJ2018	MJ2019	MJ2020	SP2020	MJ2018	MJ2019	MJ2020	SP2020
MJ2018	-				-			
MJ2019	0.78**	-			0.82**	-		
MJ2020	0.76**	0.82**	-		0.71**	0.75**	-	
SP2020	0.73**	0.78**	0.75**	-	0.75**	0.77**	0.68**	-

** P < 0.001

Table 3
Phenotypic correlations (*r*) between percent glume and leaf area disease (PGAD and PLAD, respectively), heading date (HD) and plant height (PH) at four environments in Western Australia in 2018–2020.

	Manjimup 2018				Manjimup 2019				Manjimup 2020				South Perth 2020			
	PGAD	PLAD	HD	PH	PGAD	PLAD	HD	PH	PGAD	PLAD	HD	PH	PGAD	PLAD	HD	PH
HD	-0.53**	-0.66**			-0.58**	-0.70**	-		-0.46**	-0.54**	-		-0.63**	-0.59**	-	
PH	-0.64**	-0.59**	0.40**		-0.48**	-0.52**	0.33**	-	-0.34**	-0.40**	0.37**	-	-0.45**	-0.37**	0.21*	-

** *P*< 0.001; * *P*< 0.01

Table 4
Linear mixed model analysis for genotypes, environments and their interactions with respect to percent glume and leaf area diseased (PGAD and PLAD, respectively) for 232 wheat lines evaluated in four environments in Western Australia in 2018–2020.

	PGAD				PLAD			
Source of Variation	Wald Statistic	F	P[a]	%Var[b]	Wald Statistic	F	P[a]	%Var[b]
Genotype (G)	6189.21	19.46	< 0.001	84.0	6245.22	19.04	< 0.001	82.7
Environment (E)	96.83	32.28	< 0.001	1.3	80.37	26.79	< 0.001	1.1
GxE	1079.16	1.77	< 0.001	14.7	1222.59	2.04	< 0.001	16.2

[a] *F*-test probability of Wald statistic.

[b]Percentage of variation associated with each term or interaction.

Assessment of foliar response to SNB

Similar to glume response, thermal times (when PLAD was > 70% for at least two susceptible check varieties) were comparable between years at Manjimup but lower than at South Perth (Supplementary Table 3), indicating climate affected rate of foliar disease progression between geographical locations. PLAD on flag leaves representing foliar response to SNB showed consistently high broad-sense heritability (H^2 = 0.79–0.91) and comparable population mean, median and mode between environments (Table 1). High Pearson's correlation was evident (r = 0.68 to 0.82; P < 0.001) indicating comparable foliar response of genotypes across four environments (Table 2). As with glume response, a moderate but significant negative correlation was observed between foliar response and morphological traits including heading date (r=-0.54 to -0.70; P < 0.001) and plant height (r=-0.37 to -0.59; P < 0.001) (Table 3). The phenotypic variation for foliar response contributed by genotype, environment and their interactions was similar to glume response with genotype and genotype-by environment interactions accounting for most of the variation (82.7% and 16.2%, respectively) whilst environmental effects (1.1%) contributed the smallest proportion of variation across environments (Table 4).

Comparison of glume and foliar response to SNB

The moderate to high Pearson's correlation (r = 0.71 to 0.82; P < 0.001) observed between PGAD and PLAD across environments (Table 5) indicated that a higher proportion of wheat lines have similar SNB response for glume and foliar disease when evaluated in a given environment regardless of the same or different isolates used as inoculum. A Finlay and Wilkinson joint regression model identified 35 lines as glume resistant (PGAD < 20%) across four environments in 2018–2020 ranked in ascending order based on sensitivity to SNB response compared to susceptible control lines with similar heading date and plant height (Table 6). Furthermore, 21 lines identified as resistant to glume infection also had resistance to foliar disease with PLAD < 30% (Table 6). The remaining 14 lines identified as glume resistant were identified as moderately susceptible or susceptible to foliar disease (PLAD > 30%) similar to the susceptible control lines (Table 6). Therefore, similarities and differences in glume and foliar SNB response of individual genotypes evaluated across multiple environments indicated that either comparable or alternative genetic loci play a role in controlling resistance and susceptibility in different organs of adult plants.

Table 5
Pearson's correlation coefficient between four trials at Manjimup (MJ) and South Perth (SP) Western Australia in 2018–2020 of 232 wheat lines for percent glume and leaf area diseased (PGAD and PLAD, respectively).

	PGAD MJ2018	PGAD MJ2019	PGAD MJ2020	PGAD SP2020	PLAD MJ2018	PLAD MJ2019	PLAD MJ2020	PLAD SP2020
PGAD MJ2018	-							
PGAD MJ2019	0.78**	-						
PGAD MJ2020	0.76**	0.82**	-					
PGAD SP2020	0.73**	0.78**	0.75**	-				
PLAD MJ2018	0.81**	0.67**	0.65**	0.62**	-			
PLAD MJ2019	0.73**	0.82**	0.71**	0.66**	0.82**	-		
PLAD MJ2020	0.63**	0.66**	0.76**	0.56**	0.71**	0.76**	-	
PLAD SP2020	0.65**	0.68**	0.63**	0.71**	0.75**	0.77**	0.68**	-
** P < 0.001								

Table 6

Selection of wheat lines for low mean PGAD scores (< 20%) with corresponding PLAD scores, heading date and plant height across four environments in Western Australia in 2018–2020 using Finlay-Wilkinson joint regression analysis compared with control lines susceptible to glume and foliar SNB. Wheat lines are ordered accordingly to phenotype sensitivity. Standard error is denoted by s.e.. Wheat lines with low PLAD scores evaluated in 2016–2018 (Francki et al. 2020) are shown with an asterix.

	PGAD			PLAD			Heading Date	Plant Height
Varieties/Inbreds	Mean (s.e.)	Sensitivity (s.e.)	Mean square deviation	Mean (s.e.)	Sensitivity (s.e.)	Mean square deviation	Mean (s.e.)	Mean (s.e.)
PGAD Resistant								
EGA Bonnie Rock	9.95 (9.7)	-17.33 (7.3)	89.3	35.42 (6.8)	-4.78 (4.1)	68.2	104.3 (2.9)	91.4 (2.5)
ZWW09Qno177	13.11 (9.4)	-12.53 (6.2)	210.0	49.02 (6.8)	-5.07 (2.9)	171.7	103.1 (2.8)	90.7 (2.5)
EGA Blanco	6.01 (9.7)	-3.75 (7.3)	32.8	11.60 (6.8)*	-4.25 (4.0)	43.9	108.6 (2.9)	92.1 (2.5)
53:ZIZ12	15.30 (9.4)	-3.75 (6.2)	212.8	34.83 (6.8)	-1.60 (3.0)	261.6	111.7 (2.8)	96.9 (2.5)
ZEE10Qno133	13.62 (9.4)	-3.68 (6.2)	192.6	41.8 (6.8)	1.96 (2.9)	60.2	107.7 (2.8)	84.8 (2.5)
ZVS07Qno227	7.44 (9.4)	-2.94 (6.2)	19.2	41.44 (6.8)	5.70 (2.9)	208.3	104.5 (2.8)	94.7 (2.5)
ZWW09Qno72	16.22 (9.4)	-2.94 (6.2)	58.0	48.2 (6.8)	0.89 (2.9)	83.5	111.2 (2.8)	91.5 (2.5)
ZWB11Qno95	3.88 (9.4)	-2.15 (6.2)	13.4	26.49 (6.8)	5.27 (2.9)	56.5	110.4 (2.8)	91.3 (2.5)
WAWHT2046	16.67 (3.0)	-1.77 (1.2)	71.1	15.33 (3.8)*	-1.57 (1.1)	85.2	103.6 (1.0)	96.7 (1.4)
ZWW10Qno139	6.91 (9.4)	-1.25 (6.2)	64.8	10.99 (6.8)	-1.26 (2.9)	69.9	110.0 (2.5)	100.5 (2.5)
ZEE10Qno77	9.78 (11.3)	-0.23 (7.2)	24.1	24.49 (8.2)*	5.40 (3.3)	34.4	109.8 (2.9)	100.4 (3.0)
ZWW10Qno60	13.51 (9.4)	-0.19 (6.2)	23.9	40.78 (6.8)	-0.25 (2.9)	262.9	111.4 (2.8)	89.3 (2.5)
Pfau	14.83 (3.0)	-0.09 (1.2)	63.5	22.68 (3.8)	-1.51 (1.1)	69.3	110.4 (1.0)	90.8 (1.4)
Yandanooka	17.5 (3.0)	0.11 (1.2)	52.4	37.00 (3.8)	-2.67 ((1.1)	77.8	108.2 (1.0)	95.6 (1.4)
54:ZIZ13	9.00 (3.0)	0.26 (1.2)	38.3	19.50 (3.8)	0.36 (1.1)	151.6	111.2 (1.0)	97.2 (1.4)
159:ZIZ13	19.67 (3.0)	0.30 (1.2)	50.0	36.67 (3.8)	1.27 (1.1)	81.8	111.6 (1.0)	91.7 (1.4)
75:ZIZ13	17.50 (3.0)	0.34 (1.2)	27.1	22.58 (3.8)	1.07 (1.1)	177.5	111.3 (1.0)	85.6 (1.4)
6HRWSN125	5.83 (3.0)	0.38 (1.2)	21.9	13.92 (3.8)*	-0.23 (1.1)	248.2	105.5 (1.0)	97.9 (1.4)
Brookton	14.92 (3.0)	0.47 (1.2)	13.1	27.02 (3.8)	3.59 (1.1)	202.2	109.8 (1.0)	94.2 (1.4)
Bumper	15.83 (3.0)	0.71 (1.2)	33.3	24.67 (3.8)	-2.31 (1.1)	162.5	107.8 (1.0)	94.9 (1.4)
Lang	16.67(3.0)	0.73 (1.2)	86.0	26.17 (3.8)	1.18 (1.2)	132.8	109.7 (1.0)	90.1 (1.4)
ZJN10Qno12	11.08 (3.0)	0.92 (1.2)	55.1	16.62 (3.8)	1.78 (1.1)	82.7	109.9 (1.0)	99.2 (1.4)
88:ZIZ13	15.17 (3.0)	1.06 (1.2)	111.0	32.17 (3.8)	-0.00 (1.1)	60.0	109.9 (1.0)	89.7 (1.4)
110:ZIZ13	16.83 (3.0)	1.18 (1.2)	17.0	26.08 (3.8)	0.89 (1.1)	243.4	110.0 (1.0)	94.0 (1.4)
Excalibur	16.00 (3.0)	1.21 (1.2)	26.9	33.08 (3.8)	1.50 (1.1)	251.9	108.5 (1.0)	90.6 (1.4)

	PGAD			PLAD			Heading Date	Plant Height
ZWW10Qno31	12.61 (9.4)	1.23 (6.2)	106.1	29.04 (6.8)	6.34 (3.0)	201.5	112.2 (2.8)	96.3 (2.5)
Sokoll	12.00 (3.0)	1.26 (1.2)	62.1	51.02 (3.8)	3.93 (1.1)	202.8	108.5 (1.0)	91.8 (1.4)
56:ZIZ13	18.33 (3.0)	1.32 (1.2)	38.0	36.08 (3.8)	-0.09 (1.1)	183.8	106.8 (1.0)	93.6 (1.4)
EGA Castle Rock	15.51 (4.5)	1.39 (1.4)	93.7	10.03 (5.5)*	-0.19 (1.3)	9.2	101.8 (1.6)	96.6 (2.0)
Suntop	17.58 (3.0)	1.45 (1.2)	70.5	28.05 (3.8)	3.78 (1.1)	352.1	111.0 (1.0)	91.1 (1.4)
30ZJN09	8.17 (3.0)	1.81 (1.2)	8.2	22.30 (3.8)*	-1.01 (1.1)	98.2	106.8 (1.0)	94.7 (1.4)
Tammin	13.33 (3.0)	2.88 (1.2)	48.3	12.85 (3.8)*	-0.544 (1.1)	61.9	112.2 (1.0)	89.9 (1.4)
Ajana	17.92 (3.0)	3.20 (1.2)	100.9	42.63 (3.8)*	4.77 (1.1)	219.9	106.3 (1.0)	90.4 (1.4)
ZWW09Qno157	19.93 (9.4)	4.79 (6.2)	13.5	27.81 (6.8)	3.22 (2.9)	29.3	110.4 (2.8)	102.6 (2.5)
ZVS09Qno133	18.64 (9.4)	5.85 (6.2)	19.1	16.38 (6.8)*	0.47 (2.9)	19.7	110.1 (2.8)	93.7 (2.5)
PGAD Susceptible								
Millewa	60.83 (3.0)	1.88 (1.2)	113.1	81.25 (3.8)	-1.44 (1.1)	73.0	105.1 (1.0)	84.2 (1.4)
Arrino	36.92 (3.0)	2.69 (1.2)	86.8	52.08 (3.8)	1.70 (1.1)	88.6	101.8 (1.0)	86.5 (1.4)
Scout	31.08 (3.0)	2.95 (1.2)	157.2	47.92 (3.8)	3.31 (1.1)	116.4	111.6 (1.0)	86.4 (1.4)
040HAT10	47.52 (3.2)	3.30 (1.4)	84.0	59.86 (4.0)	1.21 (1.3)	294.0	107.7 (1.1)	91.0 (1.4)

GWAS for glume and foliar response to SNB and relationship with known NE-Snn interactions

The genetic relatedness of the GWAS panel was previously reported to have low population structure with 15.6% of the genetic variance accounted for in the first three principal components using the 20,563 filtered SNP markers (Francki et al. 2020). Linkage decay for physical distance was estimated by non-linear regression at 9.6 Mbp, 14.9 Mbp and 21.0 Mbp for the A, B and D sub-genomes, respectively, for threshold $R^2 = 0.2$ (Supplementary Fig. 1). The linkage decay values were used as estimates for markers in LD when multiple significant MTA were identified in similar genomic regions on the physical map.

GWAS was used to identify shared and independent genomic regions that control glume and foliar response to SNB in different environments. Heading date and plant height were fitted as co-variates in a general linear model to reduce confounding pleiotropic effects of plant morphology on disease scores in each environment. Adjusted mean PGAD and PLAD values were subsequently used for MTA in GWAS analysis. Q-Q plots showed deviations of the observed associations compared to the null hypothesis indicating SNP markers are associated with glume and foliar SNB response with QTL detected for at least a moderate level of significance of $p < 7.65 \times 10^{-5}$ ($-\log_{10}(p) > 4.12$) in each environment (Supplementary Figs. 2&3). There were eight QTL detected on chromosomes 1D, 2A, 3A and 7B having at least moderate threshold significance of $-\log_{10}(p) \geq 4.12$ for glume response to SNB from four environments (Table 7). The estimated allelic effects ranged from 7.72–20.93% (Table 7) indicating the difference in average phenotypic values for each MTA between contrasting homozygous genotypes. Interestingly, only one region at 423.20 Mbp on chromosome 2A was associated with QTL in more than one environment (*QSng.MJ18.daw-2A.2* and QSng.MJ19.daw-2A) possibly representing a similar gene at this locus controlling glume response to SNB in two environments. The remaining were environment-specific as they did not co-locate or were in LD with QTL for glume response detected from other sites (Table 7). QTL for heading date and plant height with small allelic effects (4.61–12.35% and 4.67 to 10.75%, respectively) were detected in some environments in 2018–2020 (Supplementary Table 4) but none were co-located or in LD with QTL for glume resistance (Table 7). Therefore, QTL for glume resistance was unlikely to be associated with morphological characteristics.

Table 7
Summary of marker trait associations for adjusted PGAD (glume) and adjusted PLAD (foliar) scores from four Western Australian environments in

Environment	Trait	QTL	Chromosome	SNP id	SNP name	SNP[a]	IWGSC-bp	R^2	MAF[c]	Allele effect estima %[d]
Manjimup 2018	Glume	*QSng.MJ18.daw-2A.1*	2A	IWB59332	RAC875_c57998_165	[T/C]	202,872,663	0.08	0.42	20.25
		QSng.MJ18.daw-2A.2	2A	IWB35263	IAAV6884	[T/C]	423,204,105	0.10	0.45	-20.93
		QSng.MJ18.daw-2A.3	2A	IWB908	BobWhite_c1634_563	[A/G]	453,520,296	0.07	0.46	16.77
			2A	IWB51426	Ra_c21219_505	[A/G]	461,417,569	0.08	0.45	-18.86
Manjimup 2019	Glume	*QSng.MJ19.daw-2A*	2A	IWB35263	IAAV6884	[T/C]	423,204,105	0.09	0.45	-20.93
Manjimup 2020	Glume	*QSng.MJ20.daw-1D*	1D	IWB35174	IAAV6247	[A/G]	10,661,637	0.08	0.41	-7.72
			1D	IWB26984	Excalibur_c4876_832	[A/G]	10,662,717	0.10	0.43	8.31
			1D	IWB18376	D_GBF1XID01C7T2Q_63	[T/C]	10,668,578	0.09	0.40	7.92
			1D	IWA7533	wsnp_Ra_c1020_2062200	[A/G]	10,719,634	0.09	0.45	-7.75
South Perth 2020	Glume	*QSng.SP20.daw-1D*	1D	IWB8605	BS00051826_51	[A/G]	56,751,122	0.08	0.12	-13.79
		QSng.SP20.daw-3A	3A	IWB14389	CAP7_rep_c12940_130	[T/C]	646,272,690	0.07	0.07	-15.26
		QSng.SP20.daw-7B	7B	IWB30294	Excalibur_rep_c107796_229	[T/C]	105,559,208	0.09	0.15	-12.80
Manjimup 2018 †	Foliar	*QSnl.MJ18.daw-1B*	1B	IWB49491	Kukri_rep_c111213_148	[A/G]	300,949,280	0.08	0.13	-18.24
			1B	IWB72968	Tdurum_contig63991_404	[T/C]	301,257,710	0.10	0.14	-19.32
			1B	IWB40986	Kukri_c13156_129	[T/C]	301,257,922	0.08	0.14	17.51
			1B	IWB55131	RAC875_c21131_3615	[T/C]	302,206,634	0.08	0.15	17.80
			1B	IWB23446	Excalibur_c20228_135	[T/C]	305,270,049	0.09	0.14	-19.09
			1B	IWB71062	Tdurum_contig42289_1857	[A/C]	306,072,514	0.08	0.14	-18.34
			1B	IWB74187	tplb0024i16_800	[A/G]	307,427,828	0.08	0.14	-17.51
			1B	IWB72756	Tdurum_contig60809_268	[T/G]	308,587,768	0.08	0.14	17.08
			1B	IWB72755	Tdurum_contig60809_255	[T/C]	308,587,781	0.08	0.14	-17.95
			1B	IWB37294	JD_c2834_381	[T/C]	309,387,695	0.09	0.13	-18.57
			1B	IWB71413	Tdurum_contig43346_108	[T/C]	309,491,071	0.08	0.13	17.33

[a]Desirable SNP for reduced disease, based on the allele effect estimate, is in bold and underlined.

[b]IWGSC: IWGSC RefSeq v1.0, bp: base pairs.

[c]MAF: minor allele frequency.

[d]The effect estimates the difference between the average phenotypic values of the homozygous A genotype relative to the homozygous B genotype.

Environment	Trait	QTL	Chromosome	SNP id	SNP name	SNP[a]	IWGSC-bp	R^2	MAF[c]	Allele effect estima % [d]
			1B	IWB63613	RFL_Contig1354_484	[A/G]	315,383,705	0.08	0.17	-15.85
			1B	IWB64056	RFL_Contig2784_641	[A/G]	317,320,498	0.08	0.18	-15.57
		QSnl.MJ18.daw-5A	5A	IWB35961	IACX448	[T/C]	588,377,301	0.08	0.39	-12.57
		QSnl.MJ18.daw-5B	5B	IWB43679	Kukri_c29267_215	[T/C]	539,460,125	0.09	0.07	18.24
Manjimup 2019	Foliar	*QSnl.MJ19.daw-1A*	1A	IWB6426	BS00011521_51	[T/C]	579,830,542	0.07	0.49	9.83
		QSnl.MJ19.daw-2B	2B	IWB9450	BS00065105_51	[T/C]	69,648,943	0.07	0.08	-19.36
		QSnl.MJ19.daw-4B.1	4B	IWB57527	RAC875_c39524_181	[A/G]	126,323,033	0.10	0.06	-21.86
		QSnl.MJ19.daw-4B.2	4B	IWB41569	Kukri_c16392_1468	[T/C]	558,051,887	0.08	0.12	-20.16
			4B	IWB38540	Ku_c16392_2687	[A/C]	558,053,253	0.08	0.11	19.30
			4B	IWB52053	Ra_c41921_1056	[T/G]	558,053,925	0.09	0.11	-20.63
			4B	IWB35570	IAAV8975	[T/C]	558,057,833	0.09	0.12	-22.16
			4B	IWB63337	RAC875_rep_c95493_490	[T/C]	558,059,510	0.08	0.10	-20.31
			4B	IWB53588	RAC875_c12762_791	[T/C]	558,580,422	0.08	0.07	20.59
		QSnl.MJ19.daw-5A	5A	IWB7820	BS00031117_51	[T/C]	588,375,856	0.07	0.42	-13.46
Manjimup 2020	Foliar	*QSnl.MJ20.daw-1A.1*	1A	IWB45604	Kukri_c46010_872	[T/G]	32,894,182	0.08	0.12	11.12
			1A	IWB26996	Excalibur_c4887_1814	[T/C]	35,533,570	0.07	0.16	9.45
		QSnl.MJ20.daw-1A.2	1A	IWB10491	BS00070695_51	[T/C]	586,914,453	0.11	0.45	8.95
		QSnl.MJ20.daw-5B	5B	IWA7227	wsnp_Ku_c6464_11320381	[T/C]	402,843,711	0.09	0.34	8.53
			5B	IWB8558	BS00049793_51	[T/G]	402,843,834	0.08	0.37	8.39
South Perth 2020	Foliar	*QSnl.SP20.daw-3D*	3D	IWB17658	D_F5XZDLF02HWOJZ_227	[A/G]	31,764,661	0.07	0.05	-24.50
		QSnl.SP20.daw-6D	6D	IWB70297	Tdurum_contig31718_229	[T/C]	307,881,449	0.08	0.36	12.53
			6D	IWB36455	Jagger_c1746_113	[T/C]	307,882,817	0.08	0.36	-12.00
		QSnl.SP20.daw-7A	7A	IWB52779	Ra_c8937_191	[A/G]	81,498,302	0.08	0.27	13.66
			7A	IWB64358	RFL_Contig3447_1177	[A/G]	81,498,578	0.07	0.27	13.46

[a]Desirable SNP for reduced disease, based on the allele effect estimate, is in bold and underlined.

[b]IWGSC: IWGSC RefSeq v1.0, bp: base pairs.

[c]MAF: minor allele frequency.

[d]The effect estimates the difference between the average phenotypic values of the homozygous A genotype relative to the homozygous B genotype.

At total of 14 QTL were detected for foliar response in trials at Manjimup and South Perth in 2018–2020 (Table 7). There were SNP markers 1445 bp apart that detected QTL at Manjimup in 2018 and 2019, *QSnl.MJ18.daw-5A* and *QSnl.MJ19.daw-5A* (Table 7), indicating QTL are co-located on chromosome 5A. The remaining QTL for foliar response were detected in only one environment and, therefore, were determined as environment-specific (Table 7). The estimated allelic effects ranged from 8.39–24.50% (Table 7). The physical position of SNP markers associated with heading date and plant height (Supplementary Table 4) were not co-located or in LD and, therefore, were not considered to be associated with foliar response.

A genetic relationship between glume and foliar response was recognized if QTL for each trait were either co-located or were in LD. A comparison based on the physical map position of associated SNP markers indicated that QTL for glume and foliar response neither co-located nor were in LD within or between environments in 2018–2020 (Table 7; Fig. 1). Furthermore, QTL detected in this study were not in LD with other QTL for foliar response detected in other WA environments (Francki et al. 2020) (Fig. 1). It is reasonable to assume, therefore, that glume and foliar responses to SNB are controlled by multiple but independent genes that respond in specific environments.

Snn loci were positioned on physical chromosome maps with QTL for glume and foliar response detected in 2018–2020. *Snn4*, *Snn1*, *Snn5* were mapped on chromosomes 1A, 1B, and 4B, respectively whilst both *Snn3-B1* and *Tsn1* mapped to chromosome 5B (Fig. 1). Neither QTL for glume nor foliar response detected across four environments in 2018–2020 were in LD to the *Snn* loci based on physical map position, indicating that interactions with known NE were not evident in any field environments in 2018–2020. The exception was *QSnl.MJ18.daw-5B* in LD with *Tsn1* (Fig. 1) previously reported in Francki et al. (2020).

Discussion

There is increasing evidence that disease response to glume and foliar SNB in the field is controlled by many independent and mostly environmental-specific QTL (Czembor et al. 2019; Francki et al. 2020; Lin et al. 2020; Ruud and Lillemo, 2018; Ruud et al, 2019) exacerbating the complexity of genetic resistance and susceptibility to SNB in wheat. The majority of the QTL detected for either glume or foliar response to SNB in this study were detected at one location but not another, confirming the inherent and convoluted genetic mechanisms for resistance and susceptibility in field assessment. The outcome of this study also confirms an independent genetic relationship between glume and foliar response when wheat lines were evaluated at any particular location, evident by the lack of SNP markers associated with QTL that were neither co-located nor in LD. It is assumed, therefore, corresponding genes for biological mechanisms underpinning resistance and susceptibility to pathogen infection and disease progression are dissimilar in glumes and foliage whereby several host genes may be influenced by developmental stages and host-isolates-environmental interactions.

Environment-isolate interactions can have a significant effect on host genes responding to SNB in WA (Francki et al. 2020). This study monitored climatic conditions in successive years at Manjimup in 2018–2020 and showed similar daily average air temperature, relative humidity, rainfall, solar exposure and pan evaporation. On the contrary, South Perth in 2020 had higher daily average air temperature, solar exposure and pan evaporation but lower rainfall and relative humidity than any of the Manjimup environments. Therefore, it was expected that SNB response across 232 wheat lines would be consistent across Manjimup environments but variable to South Perth. Although climate impacted disease progression between Manjimup and South Perth sites, there was insubstantial effects in disease response in 2018–2020 evident through high phenotypic correlations and low environment interactions. However, this conclusion is in contrast to moderate correlation reported for foliar response of wheat genotypes across six WA environments in 2016–2018 (Francki et al. 2020). Differences in aggressiveness due to isolate-by-environment interactions (Pariaud et al. 2009; Sharma and Verma, 2019) can partly explain the variable SNB response across environments in 2016–2018 (Francki et al. 2020). Since isolates in this study were different to those reported in Francki et al (2020) it is plausible, therefore, aggressiveness of isolates selected for this study could be less affected by environmental variables in 2018–2020. Alternatively, several but different host loci from diverse germplasm may respond independently to varying levels of aggressiveness of the isolates used in this study and may account for higher phenotypic correlations between environments. There is a need, therefore, for increased knowledge on the significance of environment-by-isolate interactions and their effects on host quantitative resistance to provide a holistic perception of the tripartite interaction central for glume and foliar SNB disease response in different field environments.

Evaluation of 232 wheat lines for glume response to SNB across four environments identified 35 lines that showed PGAD scores < 20% and are resistance donors for breeding glume blotch resistance. EGA Bonnie Rock and ZWW09Qno177 are of interest because of their high stability and predictability for glume resistance across multiple field environments, where the former showed consistently low PGAD and PLAD scores across multiple environments. Included in the panel were eight lines with low foliar response when evaluated against 42 different isolates across six environments in 2016–2018 (Francki et al. 2020) which indicated sustained foliar resistance when evaluated in multiple environments and exposed to different isolates. The phenotypic correlation between glume and foliar response in the GWAS population was generally higher within each environment to those previously reported for bi- or multi-parental populations evaluated in Australia (Shankar et al. 2008), Europe (Wicki et al. 1996; Aguilar et al. 2005) and Nordic regions (Lin et al. 2020).

We further explored the genetic relationship between glume and foliar response in any particular environment by projecting SNP markers associated with QTL on the physical map and identifying those co-located or in LD to assess if there was common genetic control for these traits. Despite eight QTL for glume and 14 for foliar resistance detected, none were either co-located or in LD within and between four environments. Therefore, GWAS using higher resolution genetic mapping confirmed that genetic control for glume and foliar response is independent even though high phenotypic correlation was observed across environments. The increased number of loci detected for both traits and better precision in mapping of alleles using GWAS gives particular credence to this conclusion. Independent loci controlling glume and foliar response is in agreement with previous studies using bi-parental and multi-parental mapping populations (Czembor et al, 2019; Bostwick et al. 1993; Fried and Meister, 1987; Lin et al. 2020; Shankar et al. 2008; Wicki et al. 1993).

QTL for heading date and height were not co-located or in LD with any QTL for glume response so it is reasonable to assume that resistance was not a consequence of pleiotropy from morphological characteristics. The majority of QTL for glume resistance in this study were detected in one environment only.

The exception was a QTL on chromosome 2A at 423.20 Mbp detected at Manjimup in 2018 and 2019 (*QSng.MJ18.daw-2A.2* and *QSng.MJ19.daw-2A*, respectively) indicating the same QTL is effective in different environments. Interestingly, the nature of QTL for glume resistance in this study was in agreement with previous reports in that some were detected in only one environment (Czembor et al. 2019; Lin et al 2020; Shankar et al. 2008) whilst only a few QTL in the same genomic region are detected across multiple environments (Lin et al 2020; Schnurbusch et al. 2003; Shankar et al. 2008; Uphaus et al, 2007). QTL for glume resistance has not been previously identified on chromosome 1D, so it appears that *QSng.MJ20.daw-1D* and *QSng.SP20.daw-1D* are novel and accentuates the importance of evaluating wider germplasm pools to identify new sources of variation suitable for breeding glume blotch resistance. A comparison of the physical position of SNP markers associated with QTL for glume response on chromosome 2A, 3A and 7B were neither co-located or in LD with QTL for glume resistance reported by Lin et al. (2020). The physical co-location of QTL for glume resistance previously reported on chromosomes 2A (Schnurbusch et al. 2003), 3A (Schnurbusch et al. 2003; Aguilar et al 2005) and 7B (Schnurbusch et al. 2003) was not readily discernible due to ambiguous positioning of markers and, consequently, identity of same genomic regions controlling glume response between studies was inconclusive.

Similar to glume response, QTL for morphological traits did not co-locate or were in LD with QTL for foliar resistance so it appears that loci detected are specific to SNB disease. We used SNP markers associated with foliar resistance to SNB in adult plants from other studies, wherever possible, to anchor QTL and compare their location on the physical map. Foliar QTL detected in 2018–2020 other than *QSnl.MJ18.daw-1B* neither co-located nor were in LD with previous QTL detected when the population was evaluated in WA environments (Francki et al. 2018; Francki et al. 2020). However, some QTL including *QSnl.MJ18.daw-1A, QSnl*.MJ20.daw-1A.2 and *QSnl.MJ19.daw-2B* were either co-located or in LD with similar genomic regions controlling foliar resistance on chromosomes 1A, and 2B reported by Ruud et al (2019). It is reasonable to assume, therefore, that these QTL are within common genomic regions that harbour genes controlling SNB response in different regions of the world and presumably genetically different isolates. Similar to the comparison for glume resistance, it was not discernible to accurately compare existing or identify novel QTL for some foliar SNB resistance on the physical map from earlier studies (Aguilar et al. 2005; Schnurbsuch et al. 2003; Czembor et al. 2019; Friesen et al. 2009) mainly due to low resolution genetic mapping and ambiguous anchoring of markers other than SNPs. Nevertheless, a myriad of loci responded to foliar SNB infection in an environmental-specific manner and/or as a result of variability in pathogen isolates.

High abundance of SNP markers discriminated co-located QTL from low resolution genetic mapping into separate but closely accompanying QTL containing clusters of concomitant disease-related genes for glume and foliar SNB resistance (Francki et al. 2018) with increasing evidence from recent GWAS studies that clusters of individual genes respond to pathogen infection in a genotype-by-environment-by isolate manner (Francki et al. 2020). This study identified accompanying QTL for glume resistance separated by a physical distance of ~ 30 Mbp on chromosome 2A, *QSng.MJ18.daw-2A.2* and *QSng.MJ18.daw-2A.3*, providing further evidence that some genes responding to SNB are within distinct clusters on chromosomes. Likewise, a pair of QTL for foliar response in LD were detected on chromosome 1A in regions 579.83 Mbp to 586.91 Mbp (*QSnl.MJ19.daw-1A* and *QSnl.MJ20.daw-1A.2*, respectively) and within a 1,445 bp region on 5A around 588.37 Mbp (*QSnl.MJ18.daw-5A* and *QSnl.MJ19.daw-5A*) is credence that clusters of genes reside within a small physical distance and respond to different environments and/or isolates. Sequence analysis will reveal whether the region on 1D and 1A contain related disease resistance gene classes and whether the QTL on 5A has one or tandem genes.

The physical map position for the *Tsn1* locus and four *Snn* loci were located on the physical map and compared with QTL location for their potential role in controlling glume and foliar response. Although the physical location of *Snn4*, *Snn1*, *Snn5* were located on chromosomes 1A, 1B and 4B respectively and *Snn3-B1* and *Tsn1* mapping to chromosome 5B, the QTL for glume and foliar response were not in LD with *Snn* loci. The only exception was *QSnl.MJ18.daw-5B* previously identified to be in LD with *Tsn1* on 5B (Francki et al. 2020). Therefore, it does not appear that known NE-*Snn* interactions have a prominent effect on glume or foliar disease in any of the four environments in 2018–2020. Taken collectively with multiple field evaluation in Francki et al (2020), this study validated that known NE-*Snn* interactions are of limited relevance for quantitative glume and foliar resistance in WA environments, a supposition shared for wheat in the eastern region of the USA (Cowger et al. 2020). We cannot exclude the possibility that undetected NE-*Snn* interactions may serve a role in SNB response in wheat. If so, a myriad of interactions would be assumed given that multiple and environment-specific loci contributing to glume and foliar response. The importance of increasing our knowledge on the genetic diversity of isolates, the interaction of environmental effects on pathogenicity and aggressiveness and on host genes would play a critical role in deciphering the biological mechanisms underpinning glume and foliar response to SNB. In the meantime, breeding for improved SNB resistance in wheat remains a challenging task. Developing a genomic selection breeding strategy would be a worthwhile proposition but would require multi-environment trial, biological and biophysical environmental information for modelling and deriving accurate prediction equations.

Conclusions

The majority of QTL for glume resistance to SNB were environmentally-specific in four environments and provided further understanding of genotype-by-environment interactions. Moreover, QTL for glume resistance did not coincide with foliar resistance confirming the added complexity of different genotype-pathogen-environment interactions and underpinning biological pathways leading to alternative SNB responses in adult plants. It appears that none of the known NE-*Snn* or *Tsn1* loci are particularly relevant for controlling glume or foliar response to SNB and it is important to consider further research on other disease resistance pathways to gain a better understanding on fundamental biology underpinning resistance and susceptibility. In the meantime, strategies for breeding will rely on recurrent phenotypic evaluation to capture and retain favourable alleles for both glume and foliar resistance relevant to the particular environment.

Declarations

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Author contribution statement

MGF acquired research funding, designed experiments, collated, analysed and interpreted data and wrote the manuscript. EW contributed to data acquisition, analysis and interpretation, and contributed to writing the manuscript. CM and WM contributed to trial designs, planting, maintenance, and data acquisition. All authors read and approved the final manuscript.

Availability of data and material

Data for 232 wheat lines are publicly available at https://www.agric.wa.gov.au/grains-research-development/improving-resistance-septoria-nodorum-blotch-snb-wheat.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest

Ethical standards

We declare that these experiments complied with the ethical standards in Australia.

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Figures

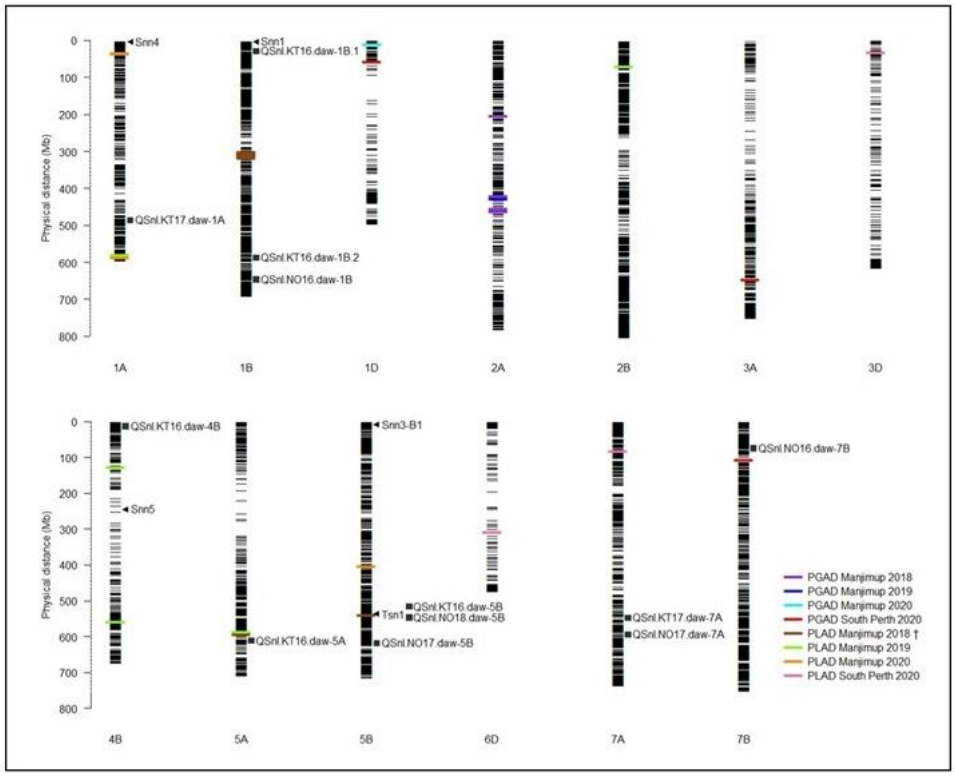

Figure 1

Comparison of QTL for PGAD and PLAD resistance. Assignment of known Tsn and Snn loci and position of MTA detected in multiple environments in 2018–2020 on the Chinese Spring physical map (IGWSC RefSeq v1.0). Black horizontal lines represent the physical locations (Mb) of SNP markers used in GWAS analysis. Coloured bars represent the MTA detected in different environments in 2018–2020. Arrows indicate putative location of known Snn and Tsn1 loci. Squares indicate QTL for foliar SNB resistance detected in 2016-2018 and reported in Francki et al. 2020. † PLAD Manjimup 2018 also reported in Francki et al 2020.

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