RESEARCH

Novel resistance loci for quantitative resistance to Septoria tritici blotch in Asian wheat (*Triticum aestivum*) via genome-wide association study

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Abstract

Background Septoria tritici blotch (STB) disease causes yield losses of up to 50 per cent in susceptible wheat cultivars and can reduce wheat production. In this study, genomic architecture for adult-plant STB resistance in a Septoria Association Mapping Panel (SAMP) having 181 accessions and genomic regions governing STB resistance in a South Asian wheat panel were looked for.

Results Field experiments during the period from 2019 to 2021 revealed those certain accessions, namely BGD52 (CHIR7/ANB//CHIR1), BGD54 (CHIR7/ANB//CHIR1), IND92 (WH 1218), IND8 (DBW 168), and IND75 (PBW 800), exhibited a high level of resistance. Genetic analysis revealed the presence of 21 stable quantitative trait nucleotides (QTNs) associated with resistance to STB (Septoria tritici blotch) on all wheat chromosomes, except for 2D, 3A, 3D, 4A, 4D, 5D, 6B, 6D, and 7A. These QTNs were predominantly located in chromosome regions previously identified as associated with STB resistance. Three Quantitative Trait Loci (QTNs) were found to have significant phenotypic effects in field evaluations. These QTNs are *Q.STB.5A.1*, *Q.STB.5B.1*, and *Q.STB.5B.3*. Furthermore, it is possible that the QTNs located on chromosomes 1A (*Q.STB.1A.1*), 2A (*Q.STB_DH.2A.1*, *Q.STB.2A.3*), 2B (*Q.STB.2B.4*), 5A (*Q.STB.5A.1*, *Q.STB.5A.2*), and 7B (*Q.STB.7B.2*) could potentially be new genetic regions associated with resistance.

Conclusion Our findings demonstrate the importance of Asian bread wheat as a source of STB resistance alleles and novel stable QTNs for wheat breeding programs aiming to develop long-lasting and wide-ranging resistance to *Zymoseptoria tritici* in wheat cultivars.

Keywords Wheat, *Zymoseptoria tritici*, Quantitative inheritance, QTN, STB resistance

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Introduction

Wheat (*Triticum aestivum* L.), an important foodgrain with economic impact, reaped significant production gains during the Green Revolution. However, the spectacle in distended wheat production is drastically restrained and challenged by discrete biotic stresses, amongst which prominent fungal pathogens severely decrease the yield and quality of wheat crops [1, 2]. Among these biotic factors, wheat production is limited by a foliar disease caused by the haploid ascomycete pathogenic fungus *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola;* anamorph: *Septoria tritici*), the causal agent of Septoria tritici blotch (STB) disease. The reduction in photosynthetic capacity due to chlorotic and necrotic blotches symptomized by STB causes yield losses of up to 50 per cent in susceptible wheat cultivars [1–3]. The disease causes persistent challenges to world wheat growers of temperate climate regimes like Europe, the Mediterranean, Africa, the Americas, and Australia [4, 5]. The changing climate favours moderate rainfall and temperature conditions (20–25 ℃ with germination at temperatures ranging from 2 ℃ to 37 ℃), increasing STB incidences and yield losses [6–8]. Amongst multifaceted disease control strategies, resistance genes/loci discovery and their utilization in resistance breeding are an appealing perspective to achieve an economical, durable, and environmentally friendly control of STB in wheat.

Breeding for qualitative resistance is considerably easier but is subjected to breakdowns periled by the emergence of new virulence in pathogen populations. Such "boom-to-bust" resistances to STB have been observed and reported by different researchers [9–14]. A classic example was exhibited by the US cultivar "Gene" with the *Stb4* gene, which displayed exceptional resistance to STB in the early 1990s [15]. Still, it became susceptible by the mid-1990s [16] due to the evolution of virulence amongst local *Z. tritici* populations. Similarly, the wheat variety "Tadinia", having a single dominant resistance gene, *Stb4,* was used in the California wheat breeding programme and remained effective for 15 years [17] but later became ineffective. Therefore, breeding for STB resistance encompassing quantitative resistance is the most effective, durable, and preferred method [3, 18].

Up to now, 23 resistance genes: *Stb1* to *Stb20, StbSm3, StbWW,* and *TmStb1* [19–21] have been designated, with two of these, *Stb6* and *Stb18q* have been cloned, encoding a wall-associated receptor kinase-like protein and a plasma membrane cysteine-rich receptor-like kinase, respectively [11, 14]. Additionally, several quantitative trait loci (QTLs) and many marker-trait associations (MTAs) for resistance to *Z. tritici* have been identified and mapped on the different wheat chromosomes [20]. However, 3BL, 6BS and 7DL chromosome arms seem especially involved in quantitative resistance, highlighting the remarkable complexity and diversity of the genetic underpinnings of STB resistance [19].

So far, STB has not yet been reported in South Asia, and no reports on resistant sources in South Asian wheat germplasm and the underlying resistance mechanism are currently available. Therefore, characterization of the genomic regions governing STB resistance in a South Asian wheat panel is important to supplement the germplasm resources of wheat against STB. Here, we present a genome-wide association study for quantitative resistance to STB under field conditions in a diverse panel of 181 bread-wheat lines. We anticipate that this study provides breeders with a rich basis for improving durable STB resistances in future wheat cultivars.

Results

Resistance spectra of wheat accessions to *Zymoseptoria tritici*

The 181 wheat genotypes tested for STB severity showed different AUDPC scores, with the mean AUDPC scores ranging from 356.58 (BGD 52) to 1384.84 (IND 51) (Table S1). The frequency distribution curve indicated a near-normal distribution for the STB severity (Fig. 1a). Among the 181 SAMP accessions, BGD52 (CHIR7/ANB//CHIR1), BGD54 (CHIR7/ANB//CHIR1), IND92 (WH 1218), IND8 (DBW 168) and IND75 (PBW 800) showed high levels of resistance to STB, with mean AUDPC score of 356.58, 369.86, 370.27, 372.43 and 484.16, respectively (Table S1, Fig. 1b), as compared to susceptible check "Huirivis #1" (1185.49), wheat lines from the cross CHIR7/ANB//CHIR1 showed increased resistance.

Descriptive statistical analysis revealed that the mean STB severity showed seasonal fluctuation and was higher during the 2019 growing season (918.74) (Table S2). The genotypic effect was significant ($p < 0.01$) for STB resistance in individual years, and moderate to high heritability estimates were obtained (Table S3). The combined analysis of variance over years highlighted that the effect of genotype, year, and their two-way interaction (genotype × years) were all highly significant for STB severity (Table 1). The analysis of pooled data revealed that the genotypic variance (35,613.20) was higher than the environmental effect (1353.32) for STB. The broad sense heritability of STB severity was high (H = 88%).

Pearson's correlation analysis of the means over all environments revealed that STB severity was significantly negatively associated with days to heading (r = -0.61) and plant height (r = -0.46) traits (Table 2).

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 3 of 18

Fig. 1 Septoria tritici blotch severity in individual years (2019–2021) and across the years (**a**) Weather data during the cropping season (standard metrological week 21 to 34) (**b**) area under the disease progress curve (AUDPC) of top ten wheat genotypes showing the least Septoria tritici blotch severity

Population structure, diversity, and linkage disequilibrium (LD) analysis

Initially, 13,191 SNPs were scored, of which 9924 (75.23%) were mapped to known chromosomal positions and were well distributed along all the chromosomes of the reference wheat genome (Fig. 2a and b). Maintaining SNPs with higher call rate (> 70%) and MAF > 0.05 resulted in 8353 SNP markers, among which 78% (6524) had a known chromosome position, of which 2,501 were distributed on the A genome, 3,034 on the B genome, and 989 on the D genome. These 8,353 SNPs were used

in downstream analyses. Among the 21 wheat chromosomes, the highest and the lowest numbers of SNPs were mapped to chromosomes 2B (594) and 4D (47), respectively (Fig. 2c).

The SAMP was divided into two sub-populations, as confirmed by hierarchical clustering and constellation plots (Fig. S1a and S1b). The phenotyping division of the population based on STB AUDPC is shown in a hierarchical cluster. The wheat lines were randomly distributed across two groups, splitting into nine sub-clusters. Group I contained 51 wheat genotypes, while Group II

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 4 of 18

Table 1 Analysis of variance, genotypic variance and broad sense heritability (H) for Septoria severity (STB), days to heading (DH) and plant height (PH) traits in 181 wheat genotypes combined over the years 2019–2021 and individual years of testing

Statistics	Overall BLUP_STB	BLUP_STB 2019	BLUP_STB 2020	BLUP_STB 2021
Heritability	0.88	0.88	0.88	0.75
Genotypic variance	35,613.20**	67,044.84**	35,371.61**	26,427.29**
Environment variance	1353.32**	-	-	-
Residual variance	14,718.44	17,429.45	9211.17	17,514.70
Grand mean	883.78	918.74	893.55	839.06
LSD	185.04	245.06	178.14	226.32
CV	13.72	14.36	10.74	15.77
Genotype significance	8.73E-64	-	-	-
Genotype x Environment significance	3.28E-13	-	-	-
Source of variation	**DF**	**Mean Square**		
Year	2	600,414.96**		
Replication within environment	3	95,842.58**		
Genotype	180	243,067.14**		
Genotype x year	360	29,387.83**		
Phenotypic variance		44,301.21**		
Genotype x year interaction variance		7334.69**		

STB- septoria tritici blotch

** Significant at $p < 0.01$

Table 2 Pearson correlation coefficients between Septoria tritici blotch (STB) and days to heading (DH) or plant height (PH)

Environment	Trait	DH	PH
STB 19	PH	0.030**	
	STB	-0.60**	-0.40**
STB 20	PH	0.17**	
	STB	-0.47**	-0.40**
STB 21	PH	0.06**	
	STB	-0.53**	-0.26**
Overall	PH	0.13**	
(pooled of three years)	STB	-0.61**	-0.46**

** Significant at $p < 0.01$

STB19 = year 2019; STB 20 = year 2020; STB 21 = year 2021

comprised 130 accessions. All five resistant genotypes having low AUDPC scores for STB (BGD52, BGD54, IND92, IND8, IND75) were reported from Group I, and four of them fell under the same sub-cluster (BGD52, BGD54, IND92, IND8-light green). The Kinship and neighbour-joining cluster analysis also verified the presence of two clusters (Fig. 3). The kinship (Fig. 3C) showed a large proportion of yellow, representing greater diversity among genotypes.
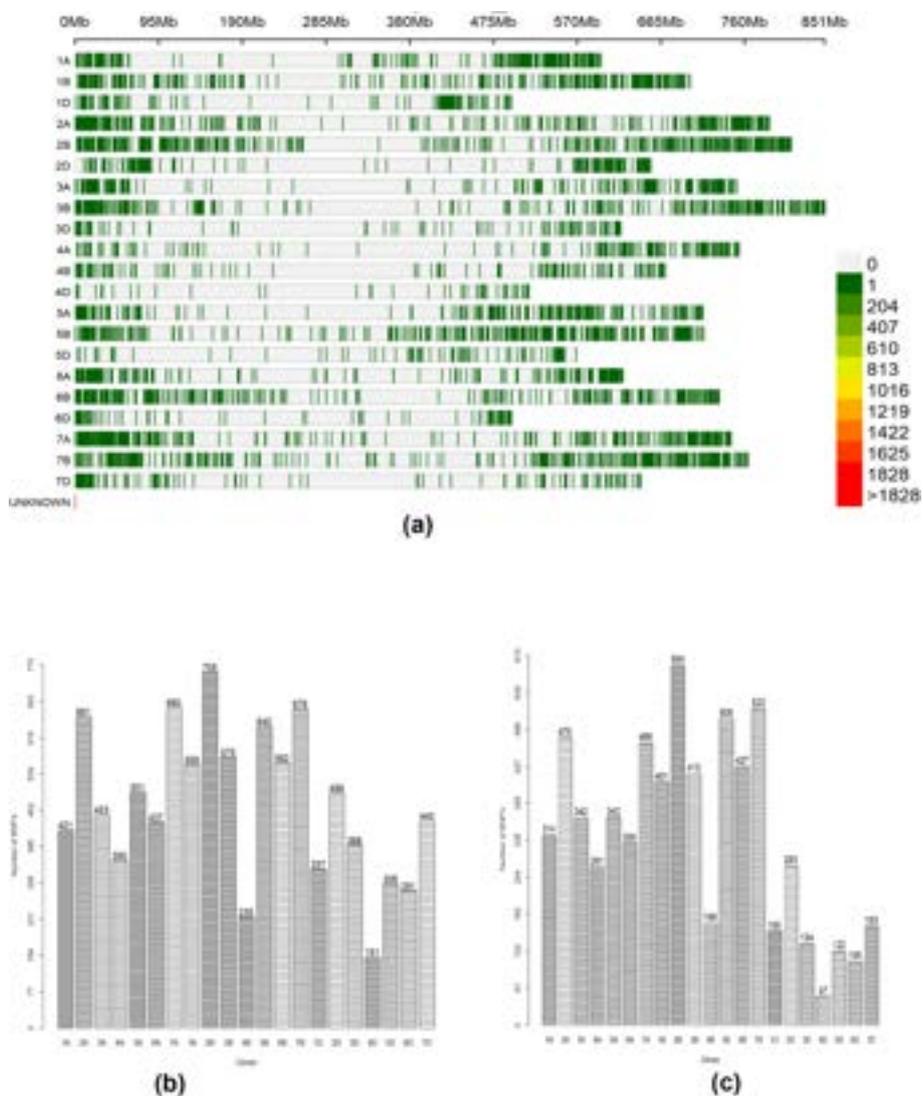
Linkage disequilibrium varied considerably along individual chromosomes, among chromosomes and sub-genomes (Table S4). Genome A showed a higher proportion of significant marker pairs (38.25%) in

comparison to genome B (37.15%) and genome D (23.86%). However, the SNPs on the A genome showed the strongest LD, with a mean value of $r^2 = 0.14$. The marker pairs for chromosomes 4D ($r^2 = 0.04\%$) and 2D ($r^2 = 0.24\%$) showed the weakest and strongest LD values, respectively. Genome-wide LD decayed to its half at 4.91 Mb, and for A genome at 4.38 Mb, B genome at 5.08 Mb, and D genome at 7.13 Mb (Fig. S2).

Marker-trait association for STB, Pleiotropic regions, and stable QTNs

Across all experiments, a total of 99 quantitative traits SNP's (QTNs) were identified with significant -log10(p) value of ≥ 3 (Fig. 4, Table S5). Among them, 21 QTNs showing repeatability across years were located on all wheat chromosomes except 2D, 3A, 3D, 4A, 4D, 5D, 6B, 6D and 7A (Table 3). The most stable QTNs include *Q.STB.5A.1* on 5AS associated with SNP 995502, *Q.STB.5B.1* on 5BS associated with SNP 1122319, and *Q.STB.5B.3* on 5BL associated with SNP 3222429, they were significant in all experiments as well as the overall mean (Table 3).

In addition to QTNs for STB only, there were a few QTNs associated with both STB and DH, like *Q.STB_DH.2A.1* and *Q.STB_DH.3B.2* that were significant in two years and *Q.STB_DH.5A.3* and *Q.STB_DH.5D.4* that were significant in one year only (Table S6, Fig. S3). The same applies to *Q.STB_DH.UN.5,* but its chromosome location

Fig. 2 Distribution of DArT Seq SNPs on 181 bread wheat chromosomes (**a**) SNPs density across the genome; The number of SNPs identified on each chromosome is described at the top of the chromosome. (**b**): Display of initial 13,191 SNPs used (**c**): Display of 8353 SNP markers maintained after higher call rate (> 70%) and MAF > 0.05
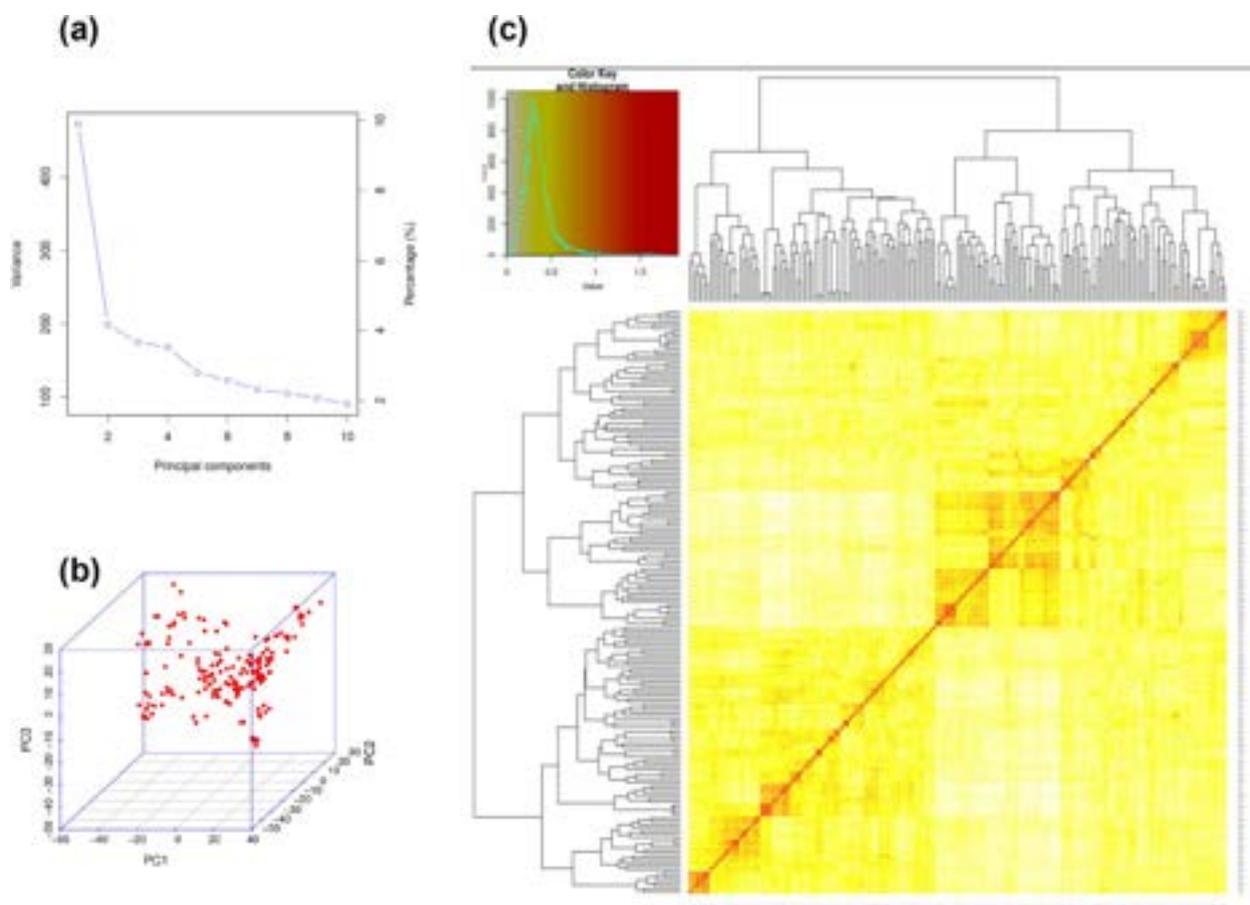
is unknown. No common QTN between STB and PH was observed.

In silico *analysis*
Most SNPs were close to transcripts or candidate genes (CGs) associated with disease resistance. For repetitive QTNs, 42 CGs (Table 3) were sorted, while for unique QTNs, 62 CGs (Table S7) were sorted in the IWGSC Ref-Seq v1.1 reference genome. The linked genes were associated with pathogen resistance in wheat and other crops, encoding ADP binding, protein-coding/binding, metal ion binding, chromosome binding or lipid binding activities. (Table 3).

Haplotype analysis and staking of R alleles
The 21 significant QTNs in Table 3 were used to understand the frequency of favourable alleles and their effects on STB resistance. The grand mean frequency of favourable alleles in the panel was 43.88%, with the highest percentage of favourable alleles identified in BGD52 (78.57%), followed by IND 75 (71.43) (Table S8). Haplotype analysis conducted on different allele combinations showed a significant reduction in STB severity with increased resistant alleles (Fig. 5). For example, the accession BGD 52 has 11 resistant alleles and exhibited the lowest mean AUDPC score of 356.58, and IND 75, BGD 53, BGD55, and IND 72,

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 6 of 18

Fig. 3 Principal component and familial relatedness analyses of 181 bread wheat genotypes based on 8353 genome-wide scanned high-quality SNPs (**a**) A screen plot displaying the first 10 principal components with their corresponding fraction of variation explained, (**b**) 3D plot of the three principal components to depict the response of genotypes to STB (2019,2020 & 2021), days to heading and plant height, and (**c**) Heat map showing the kinship analysis. The kinship values showed a normal distribution (turquoise curve), and yellow and orange colours represent weak and high kinship relations in the panel, respectively. The resulting clustering tree is indicated outside of the matrix

having 10 resistant alleles, had low values of 484.16, 579.01, 567.39, 495.88 respectively (Table S8). On the contrary, the most susceptible genotypes, like IND 51 with an AUDPC score of 1384.84, were associated with only one resistant allele.

Seven significant QTNs i.e., *Q.STB.2A.3*, *Q.STB.2B.4*, *Q.STB_DH.3B.2*, *Q.STB.5A.1*, *Q.STB.5B.1*, *Q.STB.5B.3*, and *Q.STB.6A.1*, were used in haplotype analysis. As expected, accessions with the resistance alleles exhibited significant lower AUDPC than those with the susceptibility alleles (Fig. 6). The alleles C, G and T alleles of SNP markers 995,502, 1,122,319 and 3,222,429 associated with QTNs- *Q.STB.5A.1*, *Q.STB.5B.1* and *Q.STB.5B.3* displayed a significant reduction in STB severity (Fig. 6). The allele G associated with the SNP 1122319 on chromosome 5B (*Q.STB.5B.1*) had the highest allele frequency (94.77%), followed by allele A associated with SNP 1059080 (88.70%) on chromosome 3B (*Q.STB_DH.3B.2*)

and contributed significantly for reduction in the pycnidia development (Table S8).

Discussion

Finding resistance sources and genetic loci for marker-assisted breeding is crucial because most temperate wheat cultivars are susceptible to STB. Bread wheat accessions from India and Bangladesh have not been screened for STB, so this study identifies new sources of STB resistance. Our findings revealed complex genotypic and environmental interactions, quantitative STB resistance inheritance, and novel QTNs for STB breeding programs.

Genetic variation for STB resistance

This study found polygenic control of STB severity due to its near-normal distribution over three years. For future wheat breeding and improvement, phenotypic evaluation

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 7 of 18

Table 3 The repetitive quantitative trait SNPs (QTNs) controlling the Septoria tritici blotch resistance and the putative candidate genes covering the flanking region of repetitive QTNs

Sr	QTNs	SNP ID	Position	P.Value	Marker R^2	Experiment	Transcript ID	Biological Function
1	Q.STB.1A.1	997942	370228607	2.25E-04 to 3.52E-04	0.08291 to 0.07845	STB19 and STBm	TraesCS1A02G208700	Guanyl-nucleotide exchange factor activity
							TraesCS1A02G208500	L-valine transaminase activity, L-isoleucine transaminase activity, catalytic activity, branched-chain-amino-acid transaminase activity, catalytic activity
2	Q.STB.1B.2	1093490	572985684	6.30E-04 to 0.000721	0.07875 to 0.07677	STB19 and STBm	TraesCS1B02G344600	Protein binding
							TraesCS1B02G344900	Inositol tetrakisphosphate 1-kinase activity, metal ion binding, magnesium ion binding
3	Q.STB.1B3	1090302	579824305	0.000358 to 9.47E-04	0.06594 to 0.07557	STB20 and STBm	TraesCS1B02G349300	Translation initiation factor activity, GTPase activity,hydrolase activity, tRNA binding
							TraesCS1B02G349200	Regulation of transcription, positive regulation of long-day photoperiodism, flowering
							TraesCS1B02G349300	hydrolase activity, tRNA binding, nucleotide binding, translation initiation factor activity, GTPase activity,
4	Q.STB.1D.1	3064683	44775232	0.00146 to 0.00049	0.11093 to 0.11724	STB19 and STBm	TraesCS1D02G064200	ADP binding
							TraesCS1D02G064000	Zinc ion binding
5	**Q.STB_DH.2A.1**	**3955868**	106815147	2.07E-07 to 8.76E-04	0.06042-0.14214	STB19, STB20 and STBm	TraesCS2A02G159300	4-alpha-glucanotransferase, Glycoside hydrolase superfamily
6	Q.STB.2A.3	1091069	528700315	6.45E-05 to 8.10E-05	0.08697 to 0.0898	STB19, STB20 and STBm	TraesCS2A02G307700	Metal ion binding
							TraesCS2A02G307700	Metal ion binding
7	Q.STB.2A.6	1060943	763087785	0.000352 to 9.92E-04	0.07873 to 0.09192	STB19 and STBm	TraesCS2A02G561400	Zinc ion binding,sequence-specific DNA binding
							TraesCS2A02G561300	Transcription factor activity, zinc ion binding, sequence-specific DNA binding
s8	Q.STB.2A.7	1101257	764311151	0.00109 to 0.000283	0.07464 to 0.09122	STB19 and STBm	TraesCS2A02G563900	Serine/threonine kinase activity, protein kinase activity, protein, protein binding, ATP binding
							TraesCS2A02G563700	Carbohydrate binding
9	Q.STB.2B.4	3026452	812292519	0.00108 to 0.000000655	0.07663 to 0.17215	STB19, STB21 and STBm	TraesCS2B02G630000	Protein kinase activity, ATP binding, protein phosphorylation

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 8 of 18

Table 3 (continued)

Sr	QTNs	SNP ID	Position	P. Value	Marker R²	Experiment	Transcript ID	Biological Function
10	**Q.STB_DH.3B.2**	**1059080**	680s375512	0.000397 to 0.000793	sssssssss0.0631 to 0.07068	*STB19 and STB21*	*TraesCS2B02G630100*	Protein binding, protein ubiquitination
							TraesCS2B02G629800	ADP binding
							TraesCS3B02G440800	Protein kinase activity, protein tyrosine kinase activity, transmembrane receptor protein tyrosine kinase activity, ATP binding
							TraesCS3B02G440700	
11	*Q.STB.3B.1*	1037234	681714448	0.000598 to 0.0000782	0.06694 to 0.08853	*STB19 and STB21*	*TraesCS3B02G442303*	Zinc ion binding, transferase activity, metal ion binding
							TraesCS3B02G442400	Cysteine-kinase, deubiquitinase activity, ubiquitin-dependent protein catabolic process, protein deubiquitination
							TraesCS3B02G442600	Protein coding
							TraesCS3B02G442100	Catalytic activity, aldose 1-epimerase activity, isomerase activity, carbohydrate binding, carbohydrate metabolic process, glucose metabolic process, hexose metabolic process, galactose catabolic process via UDP-galactose
12	*Q.STB.4B.1*	1092528	22704276	0.00143 to 0.00149	0.06384 to 0.06498	*STB 21 and STBm*	*TraesCS4B02G030300*	DNA binding
13	**Q.STB.5A.1**	**995502**	3653824	0.0000209 to 0.000551	0.07149 to 0.10462	*STB19, STB20, STB21 and STBm*	*TraesCS5A02G040600*	Protein binding
							TraesCS5A02G040500	Protein coding
14	*Q.STB.5A.2*	1228444	37896699	3.32E-04 to 0.0000691	0.07483 to 0.09046	*STB20 and STBm*	*TraesCS5A02G042200*	Protein coding
s15	*Q.STB.5A.5*	1023146	417225903	2.21E-04 to 3.95E-05	0.15921 to 0.20859	*STB21 and STBm*	*TraesCS5A02G206700*	Protein kinase activity, ATP binding
16	**Q.STB.5B.1**	**1122319**	49925060	0.00111 to 5.07E0.5	0.06974 to 0.11234	*STB19, STM20, STN21 and STBm*	*TraesCS5B02G044500*	Protein coding
17	**Q.STB.5B.3**	**3222429**	244491166	0.000176 to 7.75E-05	0.07687 to 0.10041	*STB19, STM20, STN21 and STBm*	*TraesCSSB02G131500*	Protein coding
18	*Q.STB.6A.1*	2258509	s600737360	0.00015 to 0.00000698	0.09489 to 0.14017	*STB19, STB21 and STBm*	*TraesCS6A02G380300*	Metal ion binding
							TraesCS6A02G380200	Chromatin binding, methyltransferase activity
							TraesCS6A02G380500	Protein binding
19	*Q.STB.6A.3*	1089240	614688143	0.00103 to 0.000314	0.06105 to 0.07382	*STB21 and STBm*	*TraesCS6A02G41400*	ADP binding
							TraesCS6A02G414200	ADP binding
							TraesCS6A02G413700	Lipid binding

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 9 of 18

Table 3 (continued)

Sr	QTNs	SNP ID	Position	P. Value	Marker R^2	Experiment	Transcript ID	Biological Function
20	Q.STB.7B.2	1228174	68307597	0.00102 to 0.00038	0.06196 to 0.07229	STB19 and STBm	TraesCS7B02G063400	Sucrose synthase activity, transferase activity, glycosyltransferase activity
							TraesCS7B02G063500	Phosphatase activity, phosphoric ester hydrolase activity, phosphatidylinositol-3,5-bisphosphate 5-phosphatase activity
							TraesCS7B02G063500	Phosphatase activity, phosphoric ester hydrolase activity, phosphatidylinositol-3,5-bisphosphate 5-phosphatase activity
s21	Q.STB.7D.5	2273491	556224991	0.000414	0.09115	STB19 and STBm	TraesCS7D02G437000	Lipid binding
							TraesCS7D02G436800	DNA binding, protein binding

*Bold red colour represents QTNs associated with STB and DH

*Bold black colour represents stable QTNs

Patial *et al. BMC Plant Biology*   (2024) 24:846
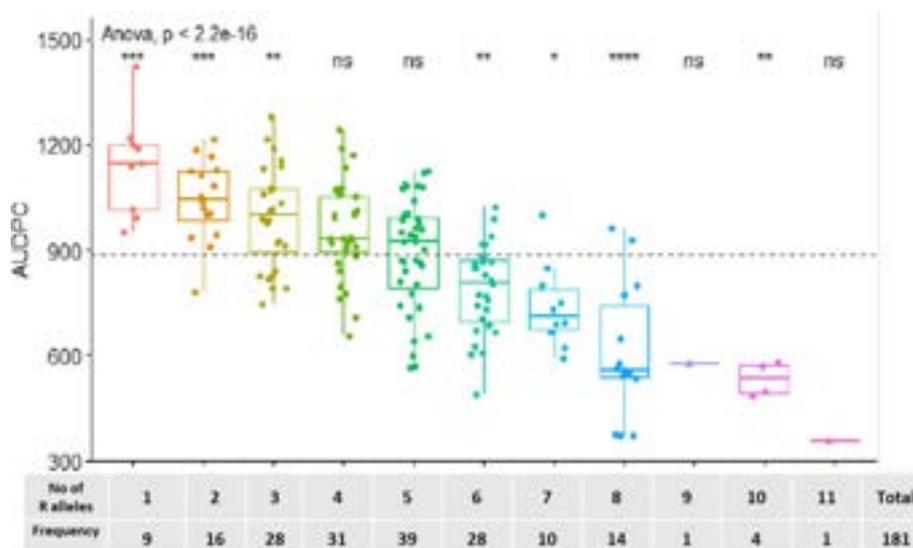
Page 10 of 18

Fig. 4 Manhattan plot for GWA scan for Septoria progress coefficient measured in different years. Each dot represents a single SNP. On the X-axis is the genomic position of the SNPs on the corresponding chromosomes indicated in different colours. On the Y-axis is the −log10 of the P-value, signifying the significance of the association test. The horizontal dotted line is the Bonferroni correction significance (α = 0.05) threshold used in the association study of STB. Figure 4a-d indicates the significant makers for the STB resistance during 2019, 2020, and 2021 and the mean AUDPC

showed significant genetic variability among tested wheat genotypes for STB resistance. The broad sense heritability estimated across environments was high, as suggested by previous studies [18, 22–25]. However, there were significant year- and genotype-by-year effects, supporting the need for multiple-year germplasm evaluation to identify stable STB-resistant genotypes [25, 26].

Many lines from the cross CHIR7/ANB//CHIR1 showed increased resistance, highlighting the significance of the parents in this cross for the STB resistance breeding programme. STB resistance is negatively correlated with PH and DH, among the most confounding factors. Several studies reported increased disease severity in earlier heading and dwarf genotypes [22, 27]. The relationship suggests that genotypes with tall and late phenology may escape STB with reduced infection or that their genes may have pleiotropism or be tightly linked to STB genes. Many GWAS and QTL mapping studies

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 11 of 18

Fig. 5 The decreasing trend of Septoria tritici blotch infection with the stacking of favourable alleles of QTNs and allele frequency of resistant alleles. The Y-axis indicates the AUDPC of the Septoria tritici blotch, while the X-axis indicates the number of resistant alleles and their frequency in the population of the 181 genotypes

have found genetic linkage to cause the relationship [28, 29]. We found genetic factors supporting the association between STB and DH in QTNs *Q.STB_DH.2A.1*, *Q.STB_DH.3B2*, *Q.STB_DH.5A.3*, and *Q.STB_DH.5D.4*.

Association analysis and candidate genes for STB resistance

In the current study, the 'year' effect significantly influenced disease expression, as observed in other studies on STB resistance, justifying the importance of multiple field trials. The temperature varied between 17 °C to 28 °C and rainfall between 36 to 94 cm during 3 different cropping seasons. The experiments were analyzed individually using pooled data to find the most stable QTNs. Of the 99 identified QTNs, only 21 were significant in more than one environment, and most were of minor effects. Three loci, *Q.STB.5A.1*, *Q.STB.5B.1*, and *Q.STB.5B.3*, were the most stable QTN consistent among all 3 years irrespective of the environmental variations. This agrees well with the idea that resistance against STB in field experiments is a quantitatively inherited trait [19, 30]. It is possible to develop germplasm resistant to STB by combining beneficial alleles from multiple loci. This can be achieved by adding the alleles from their respective donors to the current SAMP.

Out of the 21 repeatable STB QTNs that were identified, the presence of allele A on *Q.STB.2A.3, Q.STB.2B.4, Q.STB_DH.3B.2, Q.STB.6A* resulted in a decrease in STB severity. Additionally, the alleles C, G, and T on QTNs *Q.STB.5A.1*, *Q.STB.5B.1*, and *Q.STB.5B.3* were also

associated with significantly reduced STB severity. The seven QTNs mentioned above are located within the previously identified QTLs for STB resistance, specifically in the regions of *MQTL14* on chromosome 3B (identified at both seedling and adult stages) and QTL10 on chromosome 5B (identified at the seedling stage) [19].

Some tagged QTNs concurred with the known mapping locations for STB resistance genes (Fig. 7). For example, the markers of *Q.STB.1D.1* were positioned at similar chromosomal region as STB resistance genes *QStb.ipk-1D* and close to *Stb10*; *Q.STB_DH.3B.2* and *Q.STB.3B.1* was positioned at *MQTL14*; *Q.STB.5B.1*, *Q.STB.5B.3* at *QTL10*; *Q.STB.6A.1*, *Q.STB.6A.3* at *MQTL20;* and *Q.STB.7D.5* at *MQTL26* region of previously published QTLs [19]. The QTNs, *Q.STB.1B.2* and *Q.STB.1B3* on chromosome 1B were found close to SSR *wmc206*, which was linked to *MQTL3*. Similarly, QTNs, *Q.STB.2A.6* and *Q.STB.2A.7* were close (3-4 Mb) to marker *gwm294*, conferring *MQTL5* for STB resistance. The *Q.STB_DH2A.1* was located close to *MQTL4* and is responsible for adult plant resistance [19]. On chromosome 5A, we detected QTNs, *Q.STB.5A.5* positioned at 417.22 Mb and *Q.STB_DH.5A.3* positioned at 588.45 Mb, which are in the region of a previously published *MQTL19* and *QTL9* [3] respectively, conferring resistance to STB. However, both *QTL9* and *MQTL19* provide STB resistance at the seedling stage, while in our study, the QTNs were associated with adult plant resistance. Moreover, the QTN, *Q.STB_DH.5A.3* also coincided with the *Stb17* gene having a quantitative effect on disease at
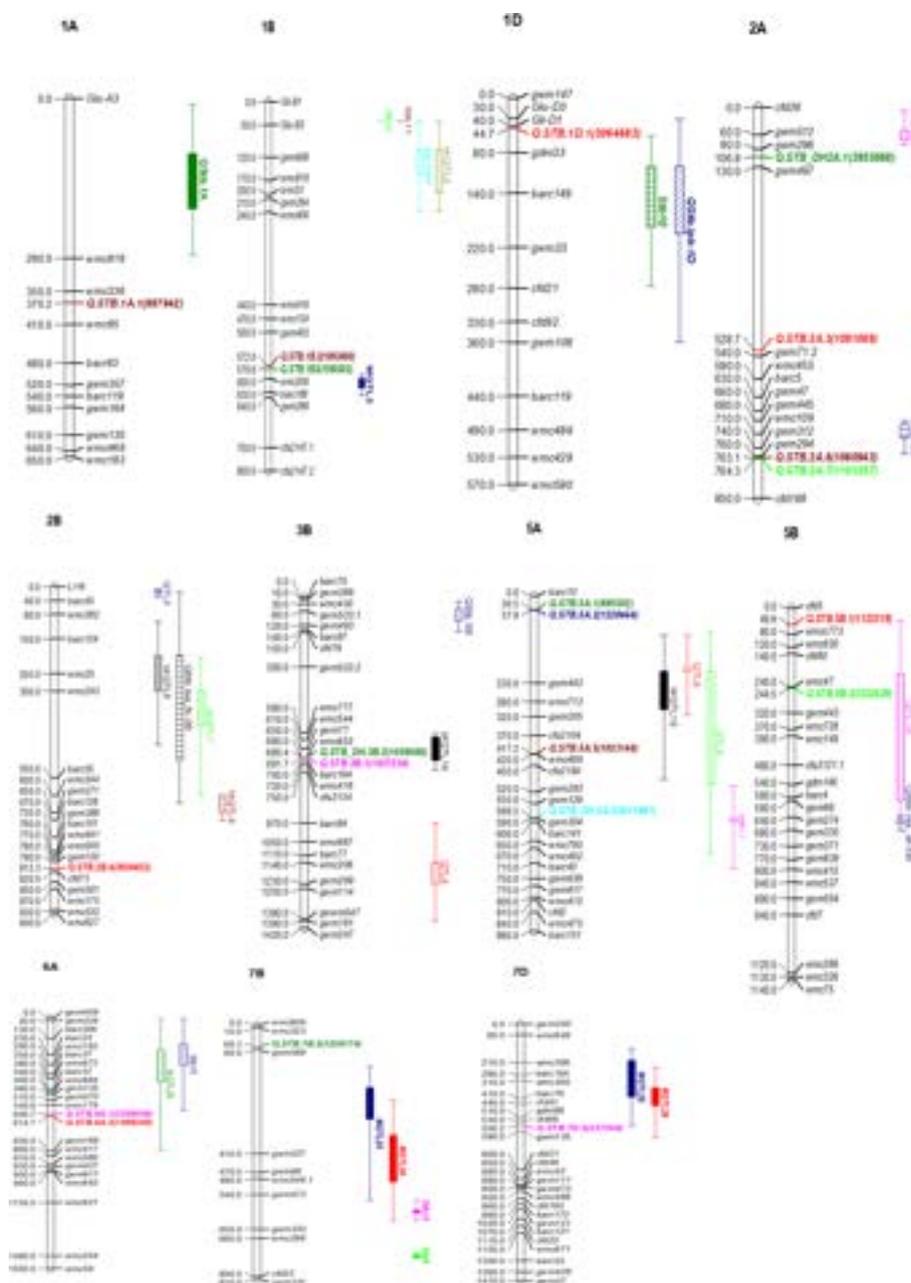
Fig. 6 The haplotypes of highly significant QTNS linked to Septoria tritici blotch resistance, showing significant differences in AUDPC due to resistance and susceptible alleles. The X-axis indicates the response of the individual allele in QTN regarding the AUDPC. The QTN locus with the corresponding marker on the respective chromosome is named *Q.STB.*, e.g. *Q.STB.6A*

the adult plants stage [31]. *Stb10* in chromosome 1D is a qualitative resistance gene imparting durable resistance to STB [32]. The *QTL10* and *MQTL9* are responsible for seedling resistance, while *MQTL14*, *MQTL20* and *MQTL19* are effective at seedling and adult plant stages. Interestingly, four SNPs synchronizing with QTLs: *MQTL3* at 1B chromosome, *MQTL5* and *MQTL4* on 2A chromosome and *MQTL26* at 7D chromosome imparting adult plant resistance were also mapped. Brown et al. [19] also reported the involvement of QTLs on chromosomes 1B, 2A, and 7D, imparting quantitative resistance to STB, which is also true in our findings. Hence, strategic use of the identified major and minor genes/QTNs can be used to deploy STB-resistant varieties.

Q.STB.1A.1, *Q.STB_DH 2A.1*, *Q.STB.2A.3*, *Q.STB.2B.4*, *Q.STB.5A.1*, *Q.STB.5A.2* and *Q.STB.7B.2* didn't coincide the location on the previously reported STB resistance loci in respective chromosomes. At the 7B chromosome,

the *Q.STB.7B.2* didn't coincide with *Stb13*, *Stb3* or *Stb 8* genes and any of the previously reported QTLs (*MQTL25*, *MQTL26*). Similar was the case with other QTNs. We have found novel QTNs on chromosomes 1A, 2A, 2B,5A and 7B, which can be good candidates for MAS for the STB resistance wheat breeding programme.

Examining the putative CG associated with significant SNP markers and STB resistance is crucial. *Q.STB_DH 2A.1*, *2A.3*, *2A.6*, and *2A.7* were mapped on chromosome 2A. SNP 3955868 at 106.81 Mb is associated with the putative candidate gene linked to this marker, *TraesCS2A02G159300* (glycoside hydrolase superfamily). The loci covered glycosyl group manipulation genes. Molecular modifications by glycosylation change protein properties, activity, and target location. Glycosylation of metabolites and hormones occurs during biotic and abiotic stress. He et al. [33] found that wheat *Glycosyl transferases* convert *Fusarium graminearum* toxin DON

Fig. 7 Location in the wheat genome of major genes, QTL, meta-QTL and QTNs involved in resistance to Septoria tritici blotch in 181 wheat genotypes

into non-toxic DON-3-glucoside. *QTNs Q.STB.2A.3* encodes CG *TraesCS2A02G307700, Q.STB.2A.6* encodes *TraesCS2A02G561400* and *TraesCS2A02G561300,* and *Q.STB.2A.7* encodes *TraesCS2A02G563700* and *TraesC-S2A02G563900,* which are associated with metal ion binding, zinc ion binding, serine/threonine kinase activity. These proteins aid plant-pathogen interactions [34]. Saintenac et al. [11] cloned a major STB resistance gene

(*Stb6*) that encodes a conserved wall-associated recep-tor kinase-like protein. In wheat, the inactivating serine/threonine kinase gene *TaPsIPK1* confers broad-spec-trum resistance to Chinese *Ps*t races endemic in 2020 and 2021 [35]. Additionally, we found that chromosome 5A harboured protein binding, protein-coding and pro-tein kinase domain (Peak marker 995,502 of *Q.STB.5A.1*; 1,228,444 of *Q.STB.5A.2*; 1,023,146 of *Q.STB.5A.5* and

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 14 of 18

Q.STB_DH.5A.3 (5,411,867). In wheat, Wang et al. [35] reported that the inactivation of a wheat protein kinase gene, *TaPsIPK1* confers broad-spectrum resistance to rust fungi.

Further, *TraesCS3B02G440700 (Q.STB_DH.3B.2)* encodes protein kinase activity, while *TraesCS3B02G442100* encodes aldose 1-epimerase and *TraesCS3B02G442400* encodes cysteine-kinase. The major STB resistance gene *Stb16q* encodes a cysteine-rich receptor-like kinase and resists *Z. tritici* broadly [14]. Most R genes already identified have the ATPase domain, which is involved in biotic stress response and found in our CGs. The present study outlined the CG-encoded proteins that confer plant disease resistance (Table 3).

Overlapping regions for STB resistance and disease-escape traits

One of the most perplexing factors affecting the selection for STB resistance could be the reported interaction between resistance and plant height (PH) or heading date (DH) [36, 37]. In the present study, a few loci were identified to be associated with both STB and DH on chromosomes 2A, 3B, 5A, 5D, and unknown location *Q.STB_DH.UN.5.* Louriki et al. [30] also reported QTLs for heading days in wheat on chromosome 2A. Previous studies confirmed chromosome 5 for flowering and the presence of the vernalization gene, *VRN-1* having three homoeologous loci *VRN-A1*, *VRN-B1*, and *VRN-D1,* which are reported on the long arm of homologous chromosomes 5A, 5B, and 5D, respectively [38, 39]. The identified QTN in our study at 5A was close to *VRN-A1*. Also, for flower induction, the photoperiod response in wheat is mainly controlled by the *PHOTOPERIOD1* (*PPD1)* loci located on the short arms of chromosomes 2A, 2B, and 2D. *PPD1* genes identified in wheat are members of the pseudo-response regulator family [40]. The fact that in our panel, days to heading provide putative chromosome locations with QTNs for disease resistance at 2A, 5A and 5D chromosomes hypothesizes the presence of a common genetic base for plant structure, phenology, and disease susceptibility in the studied material. The present study also revealed that some of the significant QTNs for DH, co-mapped with previously identified STB-resistant regions like *MQTL4* (*Q.STB_DH.2A.1/3955868*), *MQTL14* (*Q. STB_DH.3B.2/1059080*), *QTL9 and Stb17* gene (*Q. STB_DH.5A.3/5411867*) [19] thereby showed strong pleiotropism. QTN such as *Q.STB_DH.3B.2/1059080* located near transcript *TraesCS3B02G440800* related to magnesium ion transmembrane transporter activity has been reported to play a significant role in powdery mildew and stripe rust resistance in wheat [41]. The putative-associated regions identified in the present study need further

study to confound indirect selection for STB resistance via late heading.

Conclusion

Septoria tritici blotch resistance breeding will improve wheat yield. Thus, genetic dissection of the STB-resistant genomic region is highly desired. The study found STB resistance sources, genomic regions, QTLs, haplotypes, pleiotropic SNPs, and candidate genes in Asian bread wheat genotypes. The SAMP had many STB resistance alleles for wheat improvement. BGD52, BGD54, IND92, IND8, and IND75, which are more resistant than "Huirivis#1", can be used to generate STB-resistant varieties and mapping populations to find STB resistance genes/QTLs. This study revealed 21 high-confidence STB resistance markers. Many MTAs were near the candidate gene and protein-coding transcript, which may affect desirable traits. Five of the discovered QTNs were potentially unique, one on each chromosome 1B (*Q.STB.1A*), 2A (*Q.STB_DH2A.1*, *Q.STB.2A.3*), *2B.4*, 5A, and 7B. Our findings demonstrate the value of Asian bread wheat as a source of STB resistance alleles and novel stable QTNs for wheat breeding initiatives to generate durable and broad-spectrum *Z. tritici*-resistant wheat cultivars.

Materials and methods
Association mapping panel
The Septoria Association Mapping Panel (SAMP) was constituted of 181 bread wheat (*T. aestivum* L.) accessions (Table S1) from wheat breeding programs in India (88 genotypes) and Bangladesh (93 genotypes).

Adult plant screening for STB resistance
The field screening experiments were conducted at CIMMYT's Sanjaya Rajaram Experimental Station, Toluca, Mexico. This location experiences warm and humid wheat seasons from May to September, with frequent rainfall, which is ideal for creating both artificial and natural epiphytotic conditions for STB infection. The SAMP panel was evaluated during the planting seasons of 2019, 2020, and 2021, and the corresponding experiments were designated STB19, STB20, and STB21, respectively. The accessions were planted by a randomized complete block design (RCBD), with two replications using double rows spaced 1 m apart and 25 cm apart within each row. Wheat genotypes "Murga" and "Huirivis#1" were sown as resistant and susceptible checks, respectively. A combination of six aggressive isolates of *Z. tritici*, namely St1 (B1), St2 (P8), St5 (OT), St6 (KK), 64 (St 81.1), and 86 (St 133.4), was sprayed at a concentration of 1×10^7 spores/ml for artificial inoculation in the field [42]. The first inoculation was done at the Zadoks growth stage (ZGS) 29, i.e. pseudo stem erect stage [43], using an ultra-low

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 15 of 18

volume sprayer succeeded by two more applications at ZGS 33 (third node detectable) and ZGS 39 (flag leaf fully visible) to maintain sufficient inoculum load under the field conditions. STB severity was evaluated on leaves with necrotic lesions bearing pycnidia, using a double-digit scale (00 to 99) [44], wherein the first digit (D1) represents the relative height of the disease spread vertically, and the second digit (D2) represents the severity based on the diseased leaf area using the following formula:

$$STBSeverity(\%) = (D1/9 \times D2/9) \times 100$$

Disease evaluations were conducted five times at weekly intervals, starting from approximately ZGS 60. The STB severity values were subsequently used to calculate the area under the disease progress curve (AUDPC) using the following equation [45]:

$$AUDPC = \sum_{i=1}^{n}[(STB_{i+1} + STB_i)/2][t_{i+1} - t_i]$$

where STB_i = STB severity on i^{th} day, t_i = time at i^{th} observation, and n is the total number of observations.

Agronomic data scoring
Plant height (PH) and days to heading (DH) were scored in all the field experiments. PH was measured at physiological maturity, from the ground to the average spike tips, excluding awns. DH was scored as days from sowing to the date when approximately 75% of the spikes emerged.

DNA extraction and genotyping
Genomic DNA was extracted from two-week-old seedlings employing the CTAB method [46]. After that, extracted DNA quality and quantity were inspected on a Thermo Scientific™ NanoDrop™ 2000 Spectrophotometer (Thermo Scientific™, USA). Single nucleotide polymorphism (SNP) genotyping was carried out using DArTSeq genotyping-by-sequencing platform [47]. Markers were filtered for minor allele frequency (MAF) higher than 5%, missing data less than 30% and heterozygosity less than 10% for further analysis.

Population structure and linkage disequilibrium
Population structure was visualized by principal component analysis (PCA) using the KDCompute system version 1.0.1 (https://kdcompute.seqart.net/kdcompute/plugins), and kinship among the individuals was computed using the centred identity-by-state method. PCA over the genotypic data was conducted by plotting the PC1 over PC2 using the R software (v. R-4.4.1) [48]. The Linkage disequilibrium (LD) decay at the half-life (R = 0.5) analysis was performed separately for each

sub-genome (A, B and D) and across the genome. The LD was calculated through TASSEL software version 5.2.53 [49] using SNP markers with known physical positions, and the LD decay plots were produced by plotting the R^2 values against physical distance (bp).

Genome-wide association mapping
Association mapping was conducted with the mixed linear model (MLM) in the TASSEL software (v 5.2.53). The analysis for STB resistance was executed for individual years, and the BLUE means across years. In addition, GWAS on PH and DH was also conducted to investigate their potential association with STB resistance.

All marker-trait associations (MTAs) with the logarithm of the odds, LOD ≥ 3 (-log₁₀ of *P* value), were declared to be significant for STB resistance. The Manhattan and Quantile–Quantile plots were recreated in R-studio using the CMplot-R tool. The physical position of the significant markers was further confirmed with the BLAST search tool of the Wheat@URGI portal (http://www.plants.ensembl.org/Triticum_aestivum/Tools/Blast) using marker sequences against the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v1.0 genome [50]. For comparison, QTNs identified in the present study and those already catalogued STB genes/QTLs [19] were projected onto a linkage map developed using MapChart software version 2.3 [51]. A QTN was considered potentially new if the physical distance was ≥ 50 Mb from the reported *STB* gene or QTL.

Putative candidate gene identification
SNP markers for the detected QTLs were used to identify candidate genes in the genomic regions encompassing the SNPs in the PGSB (Plant Genome and Systems Biology) database; the search was focused on genes or domains that are functionally related to disease resistance mechanisms. The SNP sequences were BLASTed against the wheat reference genome sequence IWGSC (RefSeq v1.0) in "Plant Ensembl" (https://plants.ensembl.org/Triticum_aestivum/) to retrieve the corresponding genes and their functional descriptions [52, 53]. For precise identification of candidate genes, the physical starting point of the marker and the chromosome name were entered into Ensembl Plants. An additional 300 bp was added before and after the SNP to increase accuracy. Some markers had SNPs within gene sequences, classified as direct gene hits. However, for markers without SNPs within a gene, potential candidate genes were selected 2 Mb upstream and downstream of the SNPs. These genes were related to the pathogenic process or known to regulate the induction of genes related to pathogenesis. In cases where annotations were unavailable in the *Triticum aestivum* genome, orthologous genes in related species with known

Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 16 of 18

predicted functions were screened using the comparative genomics tool in Ensembl.

Staking resistance alleles

The BLUP-corrected mean disease severity data was used to assess the effects of pyramiding different numbers of resistance alleles at the significant loci detected in the current study. Wilcoxon-test and t-test (p < 0.05) were used to examine the significance of phenotypic differences across groups in the R package *'multcom View'* and *'ggpubr'* [54, 55].

Haplotype analysis

Stable MTAs on chromosomes 2A, 2B, 3B, 5A, 5B, and 6A were chosen for haplotype analysis, for which seven markers that met the criterion of being significant in all environments were selected for analysis: 2A:1,091,069, 2B:3,026,452, 3B:1,059,080, 5A:995,502, 5B:1,122,319, 5B:3,222,429 and 6A:2,258,509. Using the R package, the Wilcoxon test was used to assess the corrected disease severities between haplotypes [55].

Statistical analysis

Data analysis was performed using R software. Analysis of variation (ANOVA) was employed to assess variability in disease severity, and the least significant difference (LSD) was used to compare the significant means. Phenotypic and genotypic coefficients of variation and broad-sense heritability were estimated using a formula described by Singh and Chaudhary [56]:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gy}^2}{y} + \sigma_e^2/ry}$$

where σ_g^2 stands for genetic variance, σ_{gy}^2 for variance associated with genotype-by-year interaction, σ_e^2 the experimental error, and y and r indicate the number of years and replication, respectively. Using SAS software version 9.2 [57], Pearson's correlation between STB and agronomic variables was determined.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05547-x.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Authors' contributions
Madhu Patial and Sudhir Navathe contributed to manuscript writing and data analysis. Xinyao He contributed to conceptualization, disease scoring and data analysis, Umesh Kamble and Manjeet Kumar helped in data analysis and manuscript writing. Pawan Kumar Singh and Arun Kumar Joshi contributed to the conceptualization, project monitoring, fund acquisition and revising. All authors contributed to the article write-up and approved the submitted version.

Availability of data and materials
The original data presented in the study are included in the supplementary materials (phenotypic data) and are accessible via https://hdl.handle.net/11529/10548634 (genotypic data). Further inquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate:
All the methods involving plants, and their materials complied with relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Patial *et al. BMC Plant Biology*   (2024) 24:846

Page 17 of 18

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