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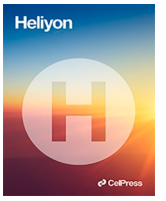

Research article

Association mapping of septoria tritici blotch resistance in bread wheat in Bale and Arsi highlands, Ethiopia

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ABSTRACT

Septoria tritici blotch (STB) caused by the fungal pathogen *Zymoseptoria tritici,* anamorph *Septoria tritici* Rob. ex Desm., is an important wheat pathogen worldwide, reported to be major wheat production threating factor, posing considerable yield loss every year. Developing resistant cultivars is an efficient, economical, environmentally friendly and simple approach for managing STB. This study was carried out to evaluate spring bread wheat lines for their reaction to STB disease under field conditions; to associate phenotypic and genotypic data for identification of STB disease resistance; and to identify genomic region(s) associated with resistance to STB in spring bread wheat lines. Two hundred forty (240) spring bread wheat lines were evaluated under field conditions in non-replicated trials, using an augmented design. The trials were conducted at three locations (Kulumsa Agricultural Research Center, Madda Walabu University Research Site and Sinana Agricultural Research Center) in 2017 main cropping season (July to December). Out of these 240 wheat lines, 123 of them were genotyped with 10263 single nucleotide polymorphism (SNPs) markers and population structure and association mapping analysis was done. The wheat lines showed significant variations in percentage disease severity and area under the disease progress curve at all the three locations they were evaluated. The wheat lines were classified as resistant, moderately resistant, moderately susceptible and susceptible based on the percentage disease severity scored. Five wheat lines were found to be resistant to STB in all the three locations and are recommended for direct release by the national program and parentage purposes in wheat breeding programs. The 123 wheat lines were clustered into 3 subpopulations in which the first cluster contained 99 wheat lines; the second 17 and the last one 7. Among the polymorphic 8127 SNPs markers, 26 markers on chromosomes 7B, 1D, 3A, 2B, 6B and 3D were found to be significantly ($P < 0.001$) associated with STB resistance so that they can be utilized for marker assisted selection and gene pyramiding in resistance breeding programs.

1. Introduction

Wheat (*Triticum aestivum* L.) is an important staple grain produced all over the world and occupies about 220.76 million hectares of land worldwide. In 2021, the worldwide wheat production was about 770.88 million tons, which makes it fourth after sugarcane, maize and rice. China is currently the world's leading wheat producer, accounting for above 17.76 % of the world's total production.

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Other major wheat producing countries are India, Russia, USA, France, Ukraine, Australia, Pakistan, Canada and Germany. These 10 countries together produce about 69.96 % of the world's total wheat [1].

Ethiopia is the 3rd wheat producing country in Africa, after Egypt and Morocco. In Ethiopia, wheat is cultivated on 1.95 million hectares of land, accounting for about 18.06 % of the total cereal crop area, with an annual production of 5.21 million tons, contributing about 17.32 % of the total cereal production [1].

In terms of area of production, wheat ranks third after *tef* (*Eragrostis tef* Zucc.) and maize (*Zea mays* L.) in Ethiopia. In total grain production, wheat ranks second after maize [2]. Wheat is largely grown in the mid and highland areas of the country, spanning at altitudes of 1500–3000 m above sea level (m.a.s.l) [3]. However, it is mainly grown between 1800 and 2500 m.a.s.l [4]. The Arsi-Bale area of Oromia region is the highest wheat producing area of Ethiopia and is considered as "wheat belt" area [5]. The "wheat belt" zones produce about 31.64 % of Ethiopian wheat [2].

In Ethiopia, from 2011 cropping season to 2021, the area of wheat production has increased by about 35.65 % (512,515 ha) and wheat production for the same year range has increased by about 78.79 % (nearly 2.3 million tons) [1]. Nowadays, almost all of bread wheat cultivars grown in Ethiopia are improved varieties, and this has brought improvements in Ethiopian wheat production.

Despite the increase in the area of wheat production and the development and dissemination of improved varieties and production technologies, wheat production in Ethiopia remained much less than the world's average. In 2021, Ethiopia produced nearly 5.21 million tons of wheat and the world's average wheat production of the same year was about 7.15 million tons, with productivity levels of 2.67 and 3.42 t/ha, respectively [1]. According to the 2012 report of the Ethiopian Institute of Agricultural Research (EIAR) [6], more than 6 tons per hectare (t/ha) of wheat was obtained with best management and cultivation practices of model farmers. These evidences indicate that there exist gap between potential yield and the yield at farmers' level in Ethiopia.

The low productivity of wheat in Ethiopia is attributed to biotic (such as diseases, insect pests and weeds) and abiotic (such as waterlogging of vertisol soils, frost, low rainfall and depleted soil fertility) factors [7]. Diseases caused by fungal pathogens are among the most important biotic factors constraining wheat production in the country [8]. Septoria tritici blotch (*Septoria tritici*; perfect (sexual) state: *Zymoseptoria tritici*, formerly called *Mycosphaerella graminicola*) is among the major fungal diseases which threatens wheat production in Ethiopia. The disease is widely distributed all over wheat growing areas of Ethiopia and in most of the areas, its severity is very high [9].

In susceptible wheat varieties grown at hot-spot areas, septoria tritici blotch (STB) resulted in up to 82 % yield losses [10] in Ethiopia. Recent studies also showed that yield loss of up to 41 % and 48 % was recorded due to STB at Holeta [11] and Areka [9] Agricultural Research Centers, respectively [12]. reported the susceptibility to STB of almost all bread wheat varieties under production in the central highlands of Ethiopia where the disease prevailed 100 %.

[13] reviewed the economic importance and management options of STB. Cultural disease management methods such as rotation to non-hosts, field sanitation achieved by deep ploughing of crop debris, late planting and inter-cropping of wheat with other crops can reduce the incidence and severity of STB, by reducing available inoculum to initiate infection. This, however, is less effective due to prolonged survival and long-distance dispersal ability of ascospores [14,15]. Despite the commercial accessibility of several biological pesticides for many disease [16–19], none is yet commercially available for biological control of STB, although several biocontrol agents are evaluated and some have shown promise towards combating the causative pathogen at laboratory levels [14]. Fungicide application, either seed treatment or foliar sprays, can reduce STB disease infection and its spread. A major problem for chemical control is that many populations of *S. tritici* have rapidly evolved resistance to fungicides [14,15,20]. Planting of resistant varieties is the most economical and simple approach for managing STB [20]. This in turn suffered from the evolution of new pathogen races and environmental challenges; and hence, complete resistance remained difficult to achieve [14].

To overcome these challenges, effective resistance breeding should be implemented; and, it requires extensive information on the genetics of host-pathogen interaction and search for new sources of resistance which are potentially durable. To do so, rigorous screening of bread wheat lines is needed so that those which have the capacity to cope up and perform well against the disease can be selected and rationally and effectively utilized in breeding programs. Molecular markers are powerful tools for introgressing and pyramiding resistance to STB in wheat breeding through marker-assisted selection as they can be detected at all plant growth stages and are not affected by environment [21,22]. Single nucleotide polymorphisms (SNPs) are recent type of molecular markers that are becoming popular to be widely used for mapping of associated resistance [23]. Nowadays, about 21 major genes conferring qualitative resistance to STB have been identified and mapped; all genes are effective against avirulent races of *S. tritici* as resistance can be overcome through the evolution of pathogen virulence [24].

Thus, searching for new source of resistance from new bread wheat lines with the help of molecular markers is necessary to cope up with the emerging virulent races of the pathogen. This study was carried out with the objectives to evaluate spring bread wheat lines for their reaction to STB disease under field conditions; to associate phenotypic and genotypic data for identification of STB disease resistance; and to identify genomic region(s) associated with resistance to STB in spring bread wheat lines.

2. Material and methods

2.1. Description of study areas

The trials were conducted in 2017 main cropping season (July to December) at three locations; Kulumsa Agricultural Research Center (KARC), Madda Walabu University Research Site (MWU) and Sinana Agricultural Research Center (SARC). The description of the experimental sites is presentd in Table 1 below.

3. Phenotyping

3.1. Plant materials and experimental design

Two hundred forty (240) spring bread wheat lines of International Center for Agricultural Research in the Dry Areas (ICARDA) origin and 7 check varieties of known and varying host response to STB were used in the study (Supplementary Table 1; Supplementary Table 2). The wheat lines were obtained from ICARDA through KARC and were evaluated for their reaction to STB disease under field conditions in non-replicated trials, using an augmented design. The total experimental area was partitioned into 5 blocks and each block had contained 48 wheat lines and all the check varieties so that only the checks were replicated in each block. The response of wheat lines to the disease was assessed in field plots comprised of two rows with 1 m (m) long, spaced 20 cm (cm) apart, sown at seed rate of 125 kg/ha. To facilitate uniform disease build-up within the nursery, continuous septoria spreader rows (using a mixture of susceptible cultivars Maddawalabu and Morocco in 2:1 proportion) were planted perpendicular to all entries on both sides of the plots. The pathways between plots and blocks, respectively, were 40 cm and 1.1 m. A total of 11.4 m × 32.6 m, 371.64 m^2, land was used in each of the three locations. The trials were fertilized with full dose of NPS (Nitrogen–Phosphorus-Sulphur blended) fertilizer (100 kg/ha) at sowing and Urea fertilizer (100 kg/ha) was applied in split applications (1/3 at sowing and 2/3 at 30–40 days after sowing). Weed control and harvesting were done manually.

3.2. Agronomic data collected

Days to 50 % heading was recorded from each plot as days from sowing to approximately 50 % of the plants in a plot displayed fully emerged heads (Zadoks scale 59) [27].

Days to 90 % maturity was recorded as days from sowing to 90 % of the caryopsis held finger nail impression (Zadoks scale 87).

Grain filling period was calculated as the period between days to heading and maturity.

Effective tillers that produced spike (post completion of inflorescence emergence; Zadoks scale 59) were counted from 5 tagged plants in each plot and the average was used for analysis.

The height (cm) of 5 tagged matured plants (Zadoks scale 87) was measured from the ground to the tip of spike excluding the awn.

Spike length (cm) of the 5 tagged matured plants (Zadoks scale 87) was measured as the distance between the pedicle base and the tip of the spike excluding awns.

Yield from each plot was harvested separately when caryopsis got hard to no longer be dented by thumb nail (Zadoks scale 92), measured in grams and converted into t/ha.

The weight (in grams (g)) of randomly counted 1000 kernels from each wheat line and check variety was measured on electric weighing balance.

3.3. Disease assessment and analysis

Disease severity was recorded using the Saari and Prescott double digit scale, 00–99 [28], which gained acceptance for rapid screening of a large number of entries. The scale applies to the whole plant and the important point in the scale is the disease score of 5, which corresponds to the disease development from the base to the middle of the plant. The use of the middle point serves as a natural standardization in the scale, regardless of whether the plant is tall or dwarf.

In this double digit scale, the first digit (0–9) gives the relative height of the disease. A simple 0 score indicates free from infection whereas a score of 9 is designated for severe infection (pycnidia observed on glumes). The number 1 is assigned for infection of the bottom 1 to 2 leaves; 3 for infection of the bottom 4 to 5 leaves; 5 for infection reaching the plant mid-height; 7 for infection reaching all upper leaves except flag leaf; 8 when pycnidia observed at the flag leaf; and 9 when pycnidia observed on glumes. The numbers jumped here are assigned for corresponding intermediate infections. The second 0–9 digit shows the disease severity as percentage, using the following format:

10 % coverage = 1 60 % coverage = 6.

20 % coverage = 2 70 % coverage = 7.

Table 1
Description of experimental sites.

Site	GPS	Distance from A.A	Altitude (m.a.s.l.)	Rainfall (mean)	Temp (Max)	Temp (Min)	Soil type	Soil PH	Reference
KARC	08°01′07″N 39°09′35″E	168 km	2200	832.00	22.8 °C	10.5 °C	Clay Loam	6.0	[25]
MWU	07°08′33″N 39°59′53″E	430 km	2400	847.30	22.7 °C	8.8 °C			
SARC	07°06′12″N 40°12′40″E	460 km	2400	812.00	21 °C	9 °C	Phaeozems, Cambisols	6.4–7.8	[26]

GPS: Global Positioning System; N: North; E: East; A.A: Addis Ababa; km: kilometers; m.a.s.l.: meters above sea level; Max: Maximum; Min: Minimum; KARC: Kulumsa Agricultural Research Center; MWU: Madda Walabu University Research Site; SARC: Sinana Agricultural Research Center.

30 % coverage = 3 80 % coverage = 8.
40 % coverage = 4 90 % coverage = 9.
50 % coverage = 5.

While 0 is used to indicate disease free situations (plant free from infection), the score of 10 is not used.

Disease severity using the double-digit scale was visually estimated as the percentage of infected leaf area covered with pycnidia; and estimations were made over the whole canopy, instead of specific leaves. Starting from 50 % infection of susceptible check 'Maddawalabu', four (4) disease scores (records) were made in each location at an interval of 7 days. Thereafter, disease scoring was stopped since there was no disease progress, as the uppermost four infected leaves were dying. Each double-digit score was converted into percentage disease severity (PDS) based on the formula of [29]:

$$\%\ \text{severity} = \left(D1/9\right)\left(D2/9\right)\ 100$$

where, D1 and D2 are the double digits scored.

For further analysis, the terminal PDS at the soft-dough stage (Zadoks scale 85), in coincidence with the peak of disease severity, was considered as the most informative and was therefore used to carry out analysis of variance (ANOVA) and the molecular-phenotype association tests.

Based on the PDS values calculated from the corresponding double-digit disease severity scores, grouping of the entries was made into the following severity classes, according to Ref. [30]:

VR - highly Resistant: PDS <5 %;
R - Resistant: 5 % < PDS <15 %;
MR - Moderately Resistant: 15 % < PDS <30 %;
MS - Moderately Susceptible: 30 % < PDS <40 %; and.
S - Susceptible: PDS >40 %.

Area under disease progress curve (AUDPC) was calculated for each wheat line and check variety from the PDS values using the formula of [31]:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [\mathbf{0.5}(\mathbf{x_{i+1}} + \mathbf{x_i})(\mathbf{t_{i+1}} - \mathbf{t_i})]$$

where, n is total number of assessments made, t_i is time of the ith assessment in days, x_i is percentage of disease severity at ith assessment.

AUDPC, yield and yield component data were subjected to ANOVA to compare performances of wheat lines with 'Maddawalabu'. Mean comparison was carried out using Dunnett's fixed range test [32] after the data were examined for satisfaction of assumptions of ANOVA (i.e., the normality, constant variance and independence of the error terms were checked prior to data analysis). Correlation analysis was made to determine the relationship among PDS, AUDPC, yield and yield components data. Analysis of all these data was done with SAS statistical software, version 9.0 [33], using the mixed procedure (PROC MIXED) with entries as fixed and blocks as random effects.

3.4. Genotyping

Out of the 240 spring bread wheat lines used in the field evaluation for their reaction to STB, genotyping was done only for 123 of them. These wheat lines were sampled based on pedigree and response to diseases to represent both susceptible and resistant ones.

3.5. DNA extraction

Genomic DNA was extracted from leaves of two week old (14 days old) seedlings from five individual plants per accession. The leaves were bulked and frozen in liquid nitrogen, and stored at −80 °C, until freeze-dry. DNA extraction was carried out following the method described by Ref. [34]. Ten (10) μl of a 100 ng μl^{-1} DNA of each sample was sent to TraitGenetics GmbH, Germany, as a commercial service provider for genotyping using single nucleotide polymorphism (SNPs) markers. The initial 15 K (15000) SNPs markers have been filtered and reduced to 10263 markers. Markers that failed in over 10 % of the samples were removed. Markers having a minor allele frequency below 5 % were also filtered out.

3.6. Population structure

The genetic structure of the 123 wheat lines was investigated using 101 highly polymorphic unlinked markers spread in the whole genome. The discriminant analysis of principal components (DAPC) was performed using the 'adegenet' package 1.4–1 in Rstudio and a Bayesian clustering method was applied to identify clusters of genetically similar individuals using the software STRUCTURE version 2.3 [35]. To infer population structure among the wheat lines, ten runs for each cluster (sub-population) from 2 to 3 was performed based on an admixture model and correlated allele frequency.

Table 2
Summary ANOVA table of spring bread wheat lines evaluated for STB resistance at three locations.

Trait	Location																	
	MWU						SARC						KARC					
	df	MS	EMS	CV	R^2	H^2	df	MS	EMS	CV	R^2	H^2	df	MS	EMS	CV	R^2	H^2
PDS	246	325.29[a]	28.1	12.11	99.18	0.99	246	293.34[a]	58.28	21.9	98.15	0.99	246	137.13[b]	53.2	21.15	96.53	0.96
AUDPC	246	69363.24[a]	6865.93	13.33	99.06	0.99	246	47587.49[a]	8051.35	19.75	98.45	0.99	246	39428.02[a]	10134.56	17.61	97.6	0.99
GY	246	0.78[a]	0.22	23.92	97.36	0.78	246	1.92[c]	0.95	22.46	95.54	0.23	246	3.8[a]	0.53	12.9	98.71	0.43
PLH	246	55.89[a]	11.7	3.63	98.07	0.95	246	46.04[a]	10.37	3.55	97.99	0.91	246	53.25[a]	11.8	4.13	97.94	0.92
TKW	246	48.48[c]	22.91	15.38	95.73	0.95	246	29.02[b]	11.1	10.1	96.43	0.93	246	73.4[c]	39.41	18.22	95.07	0.96
NET	246	0.05[ns]	0.07	18.24	89.74	0.57	246	0.04 [ns]	0.05	17.2	89.46	–	246	0.06 [ns]	0.04	15.48	94.78	0.48
SL	246	0.92[a]	0.26	5.22	97.45	0.71	246	0.8[b]	0.29	5.9	96.86	0.75	246	0.83[b]	0.36	7.71	95.92	0.84

PDS: Percentage disease severity; AUDPC: area under disease progress curve; GY: Grain yield (t/ha); PLH: Plant height (cm); TKW: Thousand kernel weight (g); NET: Number of effective tillers; SL: Spike length (cm); df: Degree of freedom, MS: Means of squares; EMS: mean square of error; CV: Coefficient of variation (%); H^2: broad-sense heritability.
ns: not significant; MWU: Madda Walabu University Research Site; SARC: Sinana Agricultural Research Center; KARC: Kulumsa Agricultural Research Center.
[a] Very highly significant ($P < 0.001$).
[b] Highly significant ($P < 0.01$).
[c] significant ($P < 0.05$).

3.7. Association mapping and analysis

TASSEL software version 5.2.4 [36] was used to perform association mapping analysis using the mixed linear model (MLM) which takes into consideration kinship matrix and population structure to control both Type I and Type II errors due to relatedness and population structure [37]. Separate phenotypic data (PDS values) from three locations and genotypic data were used for association mapping (AM). Markers with P values < 0.001 were declared significantly associated with STB resistance in the associations between markers and phenotypes.

4. Results

4.1. Response of spring bread wheat lines for septoria tritici blotch

The response of the spring bread wheat lines evaluated at three locations was measured using percentage disease severity (PDS) and the Area Under Disease Progress Curve (AUDPC) as disease parameters.

4.2. Percentage disease severity (PDS)

The test for homogeneity of variance of PDS data recorded from the three different locations revealed a significant variation between the locations and therefore PDS data recorded from each location were analyzed separately (Table 2).

At MWU, a highly significant variation ($P < 00.0001$) in PDS was observed among the wheat lines and 'Maddawalabu' (Table 2). While 48 wheat lines showed significantly lower PDS values from 'Maddawalabu' at 0.1 % level of significance, 22 other wheat lines were significant at 1 % level of significance and another 20 wheat lines at 5 % level of significance. The remaining 150 wheat lines scored a high level of PDS which was not significantly different from 'Maddawalabu'. The minimum PDS (9.88 %) was recorded from 14 wheat lines, designated number 14, 29, 45, 49, 81, 91, 148, 172, 207, 234, 235, 237, 248 and 251; and the maximum PDS value (88.89 %) was recorded from the wheat line designated number 2 (Table 5). All the check varieties included in the experiment showed significantly lower PDS values from that of 'Maddawalabu' (65.19 %). The lowest PDS value, from the check varieties, was recorded from 'Shorima' (13.83 %), followed by 'Hidasse' (17.78 %). These two checks weren't significantly different from each other.

At SARC also, a highly significant variation ($p < 00.0001$) in PDS was observed among the wheat lines and 'Maddawalabu' (Table 2). At SARC, 61 wheat lines were found to score significantly lower PDS from 'Maddawalabu' at 0.1 % level of significance. Thirty (30) other wheat lines scored significantly lower PDS from 'Maddawalabu' at 1 % level of significance. Another 24 wheat lines also scored significantly lower PDS at 5 % level of significance. The remaining 125 wheat lines scored a high level of PDS which was not significantly different from 'Maddawalabu'. The minimum PDS value (9.88 %) was recorded from 30 wheat lines and the highest PDS value (88.89 %) was recorded from the wheat line designated number 12 (Table 5). All of the checks showed significantly lower (at 0.1 % level of significance) PDS values from 'Maddawalabu' (69.14 %). From the checks, the minimum PDS value (13.83 %) was scored from 'Shorima' and 'Hidasse'.

At KARC, a significant variation ($Pr > F$ 0.0038) in PDS was observed among the wheat lines and 'Maddawalabu' (Table 2). At KARC, 7 wheat lines with the minimum PDS value (9.88 %), designated number 30, 123, 125, 172, 175, 234 and 251, were found to show significantly lower PDS from 'Maddawalabu' at 5 % level of significance. The highest PDS value (69.14 %) was recorded from 4 wheat lines, designated number 2, 11, 12 and 201. Although none of the check varieties were found to significantly vary from 'Maddawalabu', the minimum PDS value (29.63 %), from the check varieties, was recorded from 'Shorima' and 'Hidasse' (Table 5).

According to Ref. [30], the entries were grouped based on their PDS values (Table 3).

5. Area under disease progress curve (AUDPC)

AUDPC is an efficient instrument to evaluate the epidemic development of the foliar pathogen *S. tritici* [38]. Calculated AUDPC data of each location were analyzed separately as the locations revealed significant variation between them (Table 2).

At MWU, a highly significant variation ($P < 00.0001$) in AUDPC was observed among the wheat lines and 'Maddawalabu' (Table 2). One hundred and twenty eight (128) wheat lines resulted in significantly lower AUDPC values from 'Maddawalabu' at 0.1 % level of significance; 33 other wheat lines at 1 % level of significance and another 26 wheat lines at 5 % level of significance. The

Table 3
Grouping of spring bread wheat lines based on PDS values at three locations.

Disease Severity Class	Number of Lines at Location		
	MWU	SARC	KARC
R - Resistant (5 % < PDS <15 %)	14	30	7
MR - Moderately Resistant (15 % < PDS <30 %)	44	85	–
MS - Moderately Susceptible (30 % < PDS <40 %)	32	–	–
S - Susceptible (PDS >40 %)	150	125	233

PDS: Percentage Disease Severity; MWU: Madda Walabu University Research Site.
SARC: Sinana Agricultural Research Center; KARC: Kulumsa Agricultural Research Center.

Table 4
PDS, AUDPC and Yield obtained from the top five STB resistant spring bread wheat lines at three locations.

NAME/PEDIGREE	Designated Number	PDS				AUDPC				Yield (t/ha)			
		SARC	MWU	KARC	Mean	SARC	MWU	KARC	Mean	SARC	MWU	KARC	Mean
ZERBA-6/FLAG-6/3/TAM200/PASTOR//TOBA97	**30**	19.75	19.75	9.88	16.46	285.18	207.45	181.55	224.72	4.31	2.16	8.35	4.94
NJORO SD - 2/SHIHAB-12	**172**	9.88	9.88	9.88	9.88	121.03	129.68	138.29	129.67	7.19	1.35	7.05	5.2
NJORO SD - 7/3/VEE/TSI//F134.71/CROW	**175**	9.88	19.75	9.88	13.17	121.03	233.31	103.74	152.69	5.4	1.78	6.1	4.43
ATTILA/3*BCN//FLAG-2	**234**	9.88	9.88	9.88	9.88	181.55	207.48	207.48	198.84	3.84	2.07	3.93	3.28
SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PFAU/MILAN	**251**	9.88	9.88	9.88	9.88	181.55	181.55	185.85	182.98	6.37	3.53	6.15	5.35

AUDPC: Area Under Disease Progress Curve; PDS: Percentage Disease Severity; SARC: Sinana Agricultural Research Center; MWU: Madda Walabu University Research Site; KARC: Kulumsa Agricultural Research Center.

remaining 53 wheat lines resulted in higher AUDPC values which were not significantly different from 'Maddawalabu'. The minimum AUDPC value (121.03) was calculated from the wheat line designated number 91 and the maximum (1244.5) from the wheat line designated number 12 (Table 5). All the check varieties included in the experiment resulted in significantly lower (at 0.1 % level of significance) AUDPC values from 'Maddawalabu' (1189.18). From the check varieties, the minimum AUDPC value (283.48) was calculated from 'Shorima', followed by 'Hidasse' (366.39). These two checks weren't significantly different from each other.

At SARC also, highly significant variation ($P < 00.0001$) in AUDPC was observed among the wheat lines and 'Maddawalabu' (Table 2). Eighty six (86) wheat lines resulted in significantly lower AUDPC values from 'Maddawalabu' at 0.1 % level of significance; 39 other wheat lines at 1 % level of significance and another 27 wheat lines at 5 % level of significance. The remaining 88 wheat lines resulted in higher AUDPC values which were not significantly different from 'Maddawalabu'. The minimum AUDPC value (51.87) was calculated from the wheat lines designated number 29 and the maximum (1175.34) from the wheat line designated number 195 (Table 5). All the check varieties resulted in significantly lower AUDPC values from 'Maddawalabu' (926.44). From the checks, the minimum AUDPC value (243.75) was calculated from 'Shorima', followed by 'Hidasse' (248.93). These two checks weren't significantly different from each other.

At KARC, highly significant variation ($Pr > F$ 0.0001) in AUDPC was observed among the wheat lines and 'Maddawalabu' (Table 2). Only 3 wheat lines resulted in significantly lower AUDPC values from 'Maddawalabu' at 0.1 % level of significance; 12 other wheat lines at 1 % level of significance and another 11 wheat lines at 5 % level of significance. The remaining 214 wheat lines resulted in higher AUDPC values which were not significantly different from 'Maddawalabu'. The minimum AUDPC value (103.74) was calculated from the wheat line designated number 175 and the maximum (1244.46) from the wheat line designated number 201 (Table 5). From the check varieties, the minimum AUDPC value (381.1) was calculated from 'Hidasse'. It was with only this check variety that 'Maddawalabu' (739.76) significantly differed in AUDPC value. 'Hidasse' didn't significantly differ from 'Shorima'.

Overall, five wheat lines were found to be resistant to STB in all the three locations (Table 4).

6. Agronomic performances of spring bread wheat lines

6.1. Yield and yield components

Yield and yield component data of each location were analyzed separately as the locations revealed significant variation between them (Table 2).

At KARC, a highly significant variation ($P < 00.0001$) in yield was observed among the wheat lines and 'Maddawalabu' (Table 2). Thirty two wheat lines gave significantly higher yield from 'Maddawalabu' at 0.1 % level of significance; 22 other wheat lines at 1 % level of significance and another 32 wheat lines at 5 % level of significance. The remaining 154 wheat lines produced lower yield which was not significantly different from 'Maddawalabu'. The maximum yield (9.98 t/ha) was obtained from the wheat line designated number 70, followed by 9.83 t/ha, 9.75 t/ha and 9.65 t/ha, from the wheat lines designated number 81, 37 and 117, respectively. The minimum yield (0.58 t/ha) was obtained from the wheat line designated number 254 (Table 5). All the check varieties except 'Kubsa' and 'Digalu' gave significantly higher yield from 'Maddawalabu' (3.36 t/ha, the minimum yield from the checks). 'Hidasse' gave very significantly higher yield (8.8 t/ha) from all the other checks.

At MWU also, highly significant variation ($Pr > F$ 0.0001) in yield was observed among the wheat lines and 'Maddawalabu' (Table 2). Eleven (11) wheat lines produced significantly higher yield from 'Maddawalabu' at 0.1 % level of significance; 17 other wheat lines at 1 % level of significance and another 31 wheat lines at 5 % level of significance. The remaining 181 wheat lines produced lower yield which was not significantly different from 'Maddawalabu'. The maximum yield (4.18 t/ha) was obtained from the wheat line designated number 69, followed by 4.0 t/ha and 3.95 t/ha, from the wheat lines designated number 72 and 37, respectively. The minimum yield (0.11 t/ha, only a quintal and 10 kg from a hectare of land) was obtained from the wheat line designated number 102 (Table 5). The check varieties 'Shorima', 'Hidasse' and 'Honqolo' gave significantly higher yield from 'Maddawalabu' (0.71 t/ha) at MWU. Yield obtained from 'Shorima' and 'Hidasse' was significantly higher from 'Maddawalabu', 'Kubsa' and 'Digalu'. 'Hidasse' was the check variety to give the maximum yield (2.75 t/ha), from the checks, followed by 'Shorima' (2.66 t/ha). The minimum yield (0.64 t/ha) was obtained from 'Kubsa', 'Maddawalabu' being the second minimum check.

At SARC, however, no wheat line gave significantly higher yield from 'Maddawalabu'. Yield obtained in SARC ranged from 0.6 t/ha (from the wheat line designated number 210) to 7.44 t/ha (from the wheat line designated number 37) (Table 5). The only one entry which gave significantly higher yield from 'Maddawalabu' ($Pr > F$ 0.0216) was the check variety 'Shorima' (6.39 t/ha). This check variety also gave significantly higher yield from 'Kubsa' (2.2 t/ha, the lowest yield from the checks) and 'Digalu'.

At MWU, a highly significant variation ($P < 00.0001$) in plant height (PLH) was observed among the wheat lines and 'Maddawalabu' (Table 2). The height of 14 wheat lines was significantly different from 'Maddawalabu' at 0.1 % level of significance; 31 other wheat lines at 1 % level of significance and another 21 wheat lines at 5 % level of significance. The height of the remaining 174 wheat lines was not significantly different from 'Maddawalabu'. The maximum height (113.6 cm) was obtained from the wheat lines designated number 43, followed by 110 cm, 109.2 cm and 108.4 cm, from the wheat line designated number 247, 129 and 251, respectively. The minimum plant height (74.2 cm) was measured from the wheat line designated number 227 (Table 5). The height of the check varieties 'Kubsa', 'Kingbird' and 'Digalu' was significantly different from 'Maddawalabu' (103.72 cm, the maximum height from the checks). 'Kubsa' was the shortest check (86.64 cm).

At SARC also, a highly significant variation ($P < 00.0001$) in PLH was observed among the wheat lines and 'Maddawalabu' (Table 2). The height of 7 wheat lines was significantly different from 'Maddawalabu' at 0.1 % level of significance; 7 other wheat lines at 1 % level of significance and another 24 wheat lines at 5 % level of significance. The height of the remaining 202 wheat lines was not

Table 5
Mean, Minimum and Maximum values of disease and agronomic parameters of spring bread wheat lines evaluated for STB resistance at three locations.

Trait	MWU			SARC			KARC		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
PDS	9.88	88.89	44.73	9.88	88.89	35.42	9.88	69.14	34.49
AUDPC	121.03	1244.5	620.75	51.87	1175.34	454.25	103.74	1244.46	569.03
GY	0.11	4.18	2.02	0.6	7.44	4.36	0.58	9.98	5.67
TKW	12.6	45.75	31.28	12.8	47.54	32.89	18.92	54	34.65
PLH	74.2	113.6	93.83	67.2	107.8	90.32	62.5	102.3	82.71
SL	7.8	12.8	9.82	7	12	9.02	5.8	10.2	7.8
NET	0	7.4	1.57	0	3.4	1.17	0	5.6	1.12
DH	63	96	71.05	62	92	68.68	54	89	68.3
DM	122	149	134.85	118	140	124.36	102	142	123.66
GFP	52	71	63.84	39	71	55.71	37	65	55.36

AUDPC: area under disease progress curve; PDS: Percentage disease severity; GY: Grain yield (t/ha); PLH: Plant height (cm); TKW: Thousand kernel weight (g); SL: Spike length (cm); NET: Number of effective tillers; DH: Days to 50 % heading; DM: Days to 90 % maturity; GFP: Grain filling period; Min: Minimum; Max: Maximum; MWU: Madda Walabu University Research Site; SARC: Sinana Agricultural Research Center; KARC: Kulumsa Agricultural Research Center.

significantly different from 'Maddawalabu'. The maximum height (107.8 cm) was obtained from the wheat line designated number 129, followed by 105.6 cm, from the wheat line designated number 170. The minimum plant height (67.2 cm) was measured from the wheat line designated number 90 (Table 5). Only the height of the check varieties 'Kubsa' (the shortest check, 86.56 cm) was significantly different from 'Maddawalabu' (97.8 cm, the maximum height from the checks).

At KARC also, a highly significant variation ($P < 00.0001$) in PLH was observed among the wheat lines and 'Maddawalabu' (Table 2). The height of 4 wheat lines was significantly different from 'Maddawalabu' at 0.1 % level of significance; 11 other wheat lines at 1 % level of significance and another 21 wheat lines at 5 % level of significance. The height of the remaining 204 wheat lines was not significantly different from 'Maddawalabu'. The maximum height (102.3 cm) was obtained from the wheat line designated number 247, followed by 101.7 cm, from the wheat line designated number 93. The minimum plant height (62.5 cm) was measured from the wheat line designated number 254 (Table 5).

In all the three locations, thousand kernels weight (TKW) and Spike Length (SL) of the wheat lines were not significantly different from that of 'Maddawalabu'. The error terms for the number of effective tillers (NET) data collected from each location were not normally distributed and hence violated the assumptions of ANOVA. Therefore, the data underwent square root transformation as a remedial action thereby the assumptions of ANOVA were satisfied. In all the three locations, however, NET of the wheat lines wasn't significantly different from that of 'Maddawalabu'.

6.2. *Phenology of spring bread wheat lines*

The phenological parameters days to heading (DH), days to maturity (DM) and grain filling period (GFP) failed to satisfy the assumptions of ANOVA and any remedial action was not effective due to the fact that large number of wheat lines had similar values. For these parameters, therefore, descriptive statistics was used.

Seven (7) wheat lines, designated number 36, 41, 47, 58, 69, 74 and 123 were the first to head (63 days after planting, DAP) and 229 & 90 were the last to head (96 DAP) at MWU. 'Honqolo' was the check variety to head first (67 DAP) and 'Digalu' and 'Shorima' headed last (72 DAP) from the check varieties (Table 5). At SARC, 4 wheat lines, designated number 123, 74, 69 and 58 were the first to head (62 DAP) and wheat line 90 headed last (92 DAP). From the check varieties, 'Honqolo' was the first to head (65 DAP) and 'Shorima' headed last (70 DAP) (Table 5). The wheat line designated number 69 headed first (54 DAP) at KARC while 90 was the last to head (89 DAP). DH of the check varieties at KARC ranged from 62 ('Honqolo') to 71 ('Kubsa') DAP (Table 5).

The early maturing wheat lines at MWU were those designated number 1, 12 and 195 (122 DAP) whereas the late maturing one was 229 (149 DAP). Of the checks, 'Kingbird' matured early (130 DAP) and the last matured check was 'Shorima' (140 DAP) (Table 5). At SARC, nine (9) wheat lines designated number 69, 96, 114, 116, 123, 135, 149, 195 and 206, matured earlier (118 DAP) whereas the wheat lines designated number 31, 90 and 209 matured late (140 DAP). The check variety 'Kingbird' matured earlier (120 DAP) and Shorima was the last matured check (130 DAP) (Table 5). Wheat line designated number 37 mature early (102 DAP) at KARC while 90 was the late maturing wheat line (142 DAP). While 'Honqolo' was the check variety to mature early (117 DAP), 'Digalu' matured last (130 DAP) at KARC (Table 5).

The wheat line designated number 105 completed its grain filling with the shortest period of time (39 days) at SARC; whereas, it took the longest 71 days for the wheat line designated number 229 to fill its grain. From the check varieties, it was 'Kingbird' and 'Digalu' that completed their grain filling with the shortest period of time (54 days). 'Shorima' took longer period of time (60 days) to complete its grain filling (Table 5). At KARC, it was the wheat line designated number 59 which completed its grain filling with the shortest period of time (37 days) while the longest 65 days were needed for the wheat line designated number 178 to complete its grain filling. 'Kingbird' was the check variety to complete its grain filling with the shortest period of time (51 days) and it took 60 days for 'Digalu' to complete its grain filling (Table 5). At MWU, the wheat line designated number 90 completed its grain filling with the

shortest period of time (52 days); whereas, it took the longest 71 days for the wheat lines designated number 127 & 271 to fill their grain. It was 'Kingbird' and 'Kubsa' from the check varieties that completed their grain filling with the shortest period of time (62 days). 'Shorima', 'Hidasse' and 'Maddawalabu' took longer period of time (68 days) to complete their grain filling (Table 5).

6.3. Correlation between PDS, AUDPC, yield, thousand kernel weight and plant height

The epidemiological parameters PDS and AUDPC were found to be highly positively correlated in all the three locations, MWU ($P < 0.0001$; R = 0.84), SARC ($P < 0.0001$; R = 0.88) and KARC ($P < 0.0001$; R = 0.87). An increase in the disease severity percentage resulted in higher AUDPC values. PDS and AUDPC were found to be highly ($P < 0.0001$) and negatively correlated to grain yield at MWU and SARC. This indicates the adverse effect that the prevailed STB disease had on grain yield of the spring bread wheat lines evaluated. TKW was also highly negatively correlated with both PDS and AUDPC at MWU and SARC. At KARC, however, grain yield wasn't correlated with PDS and it showed only significant (*P* 0.038) negative (R = −0.13) correlation with AUDPC. In addition, TKW it was not correlated with both PDS and AUDPC at KARC. PLH was found to have highly negative correlation with both PDS and AUDPC at all the three locations. This illustrates the situation that dwarf wheat lines suffer heavy septoria infestation than the corresponding taller entries (Table 6).

Grain yield and TKW were found to be highly positively correlated in all the three locations. An increase in the weight of thousand kernels increased yield. PLH was found to have highly positive correlation with grain yield and TKW at MWU and SARC; but, a significant positive correlation with only grain yield at KARC (Table 6).

7. Association mapping of spring bread wheat lines for septoria tritici blotch resistance

7.1. Population structure

The entire germplasm used in this study (123 wheat lines) was clustered into k = 3 major clusters (Fig. 1). From the first graph (R plot), the number on the X axis that corresponds to the troph point indicates the number of clusters. In the second graph, the three clusters are clearly indicated by 3 distinct colors. The color admixtures indicate the existence of recombinants in addition to the three major clusters.

Cluster 1 was the largest cluster with 99 wheat lines accounting for approximately 80.49 % of the total wheat lines. In this wide group, a large number of susceptible as well as resistant wheat lines were included. The wheat lines from which maximum as well as minimum PDS scores were taken were included in this group. Cluster 2 consisted of 17 wheat lines which accounted about 13.82 % of the total wheat lines. This cluster also consisted of susceptible as well as resistant wheat lines. The third cluster had 7 wheat lines and accounted about 5.69 % of the total wheat lines. The seven wheat lines included in this cluster were all susceptible to STB.

7.2. Marker statistics

Form the 10263 SNPs markers analyzed, only 8127 (79.19 %) were polymorphic and were used for the AM analysis. Of these, 7590 (93.39 %) were distributed on A, B and D chromosomes of known position in which 2915 were located on the A, 3722 on the B and 953

Table 6
Pearson correlation between PDS, AUDPC, Yield and Yield Component Parameters.

Location		PLH	TKW	GY	PDS	AUDPC
MWU	PLH	1				
	TKW	0.4[a]	1			
	GY	0.41[a]	0.69[a]	1		
	PDS	−0.27[a]	−0.21[b]	−0.23[a]	1	
	AUDPC	−0.32[a]	−0.42[a]	−0.48[a]	0.84[a]	1
SARC	PLH	1				
	TKW	0.4[a]	1			
	GY	0.27[a]	0.65[a]	1		
	PDS	−0.23[a]	−0.31[a]	−0.29[a]	1	
	AUDPC	−0.16[b]	−0.39[a]	−0.37[a]	0.88[a]	1
KARC	PLH	1				
	TKW	0.06	1			
	GY	0.14[c]	0.61[a]	1		
	PDS	−0.17[b]	−0.008	0.03	1	
	AUDPC	−0.26[a]	−0.08	−0.13[c]	0.87[a]	1

PDS: Percentage disease severity; AUDPC: area under disease progress curve; GY: Grain yield (t/ha); PLH: Plant height (cm); TKW: Thousand kernel weight (g).
ns: not significant; MWU: Madda Walabu University Research Site; SARC: Sinana Agricultural Research Center; KARC: Kulumsa Agricultural Research Center.
[a] Very highly significant ($P < 0.001$).
[b] highly significant ($P < 0.01$).
[c] significant ($P < 0.05$).

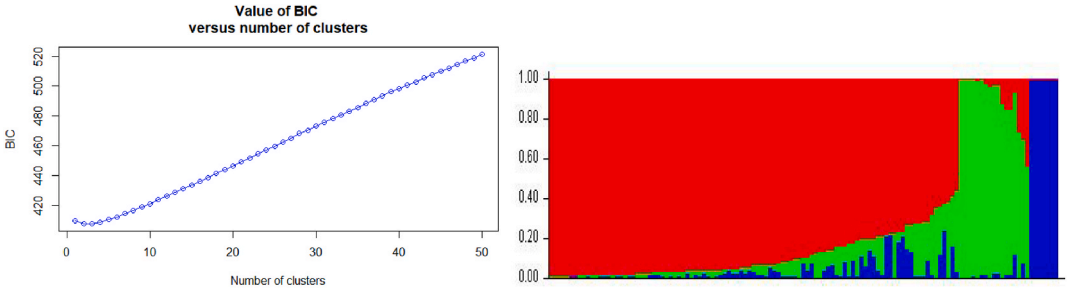

Fig. 1. Population structure among the wheat lines.

on the D genomes. Thirty three (33) other markers were of known chromosomes but have no position. Chromosomes with the largest number of markers are 2B (728 markers) followed by 6B (644 markers) and 5B (615 markers). Chromosomes 4D, 7D and 3D showed the least number of loci, 34, 79 and 82 markers, respectively (Table 7).

7.3. Association analysis for septoria tritici blotch resistance

Twenty six (26) environment-specific markers representing 6 genomic regions were found to be significantly associated ($P < 0.001$) with STB resistance (Table 8; Fig. 2 (A, B)).

While no significant SNPs were detected at SARC, 20 of the 26 markers significantly associated with STB resistance were detected at MWU and the remaining 6 at KARC; and no SNPs markers were common at the two locations. Eleven (11) of the significant markers from MWU were located on chromosome 7B and the other 9 on 1D. Of the six (6) significant markers from KARC, 3 were located on chromosome 3A and the others 3 on chromosomes 2B, 6B and 3D.

Overall, chromosome 7B had the largest number of markers significantly associated with STB resistance (42.31 %) followed by 1D (34.62 %) and 3A (11.54 %). Chromosomes 2B, 6B and 3D had one significant marker each. The markers accounted for 8.8 %–12.02 % of the phenotypic variation (Table 8).

Of the significant markers, the marker 'Excalibur_c17655_467' was present on 111 (90.24 %) wheat lines among which the highly resistant wheat lines designated number 30 and 251 are found. The markers 'GENE-1981_131' and 'GENE-0293_154' were present on 107 (86.99 %) and 102 (82.93 %) wheat lines, respectively. The two markers, 'Kukri_c18420_705' and 'BS00081688_51', on chromosomes 3D and 3A, respectively, were present only on 15 (12.2 %) and 12 (9.76 %) wheat lines, respectively (Table 8).

8. Discussion

The spring bread wheat lines evaluated for their reaction to septoria tritici blotch under natural infection at field conditions at three locations showed variations in their response to the disease as measured in terms of PDS and AUDPC. This could be attributed to diversity in their genetic background. The number of wheat lines that showed significantly lower PDS and AUDPC from the susceptible

Table 7
Marker statistics: number of markers and number of markers with position on specific chromosomes.

Chromosome	Number of markers	Number of positions
1A	364	86
1B	534	90
1D	190	50
2A	429	77
2B	728	100
2D	345	56
3A	355	84
3B	466	90
3D	82	30
4A	305	82
4B	273	69
4D	34	22
5A	532	88
5B	615	119
5D	104	36
6A	475	82
6B	644	85
6D	119	37
7A	455	101
7B	462	100
7D	79	57

Table 8
Chromosome location, *P* values, R^2 and observed proportion of significantly associated single nucleotide polymorphism (SNPs) markers with septoria tritici blotch resistance.

Marker	Chromosome location	Position	*P* value	R^2	Observed Proportion (%)
BobWhite_rep_c63264_629	1D	164	0.000868	8.99	42.28
BS00009738_51	1D	171	0.000814	9.3	41.46
BS00010946_51	1D	164	0.000928	8.89	56.1
BS00059313_51	1D	164	0.000716	9.22	56.1
Excalibur_c500_279	1D	164	0.000928	8.89	56.1
RAC875_c19174_645	1D	164	0.000994	8.8	55.28
RAC875_c37075_53	1D	164	0.000928	8.89	56.1
RAC875_c8503_644	1D	167	0.000928	8.89	56.1
RFL_Contig2961_759	1D	165	0.000716	9.22	56.1
CAP12_c8025_110	7B	73	0.00067	9.54	40.65
CAP12_rep_c4929_51	7B	73	0.00067	9.54	53.66
Excalibur_c49622_60	7B	73	0.000774	9.83	41.46
Excalibur_rep_c103688_258	7B	73	0.000542	9.82	53.66
IAAV2037	7B	73	0.000571	9.77	41.46
IAAV3414	7B	73	0.000705	9.34	43.9
Kukri_c4424_1081	7B	73	0.000752	9.23	55.28
wsnp_CAP11_c1196_692246	7B	73	0.000571	9.77	41.46
wsnp_CD454314B_Ta_2_1	7B	73	0.000571	9.77	53.66
wsnp_Ex_c64815_63464750	7B	73	0.000597	9.71	41.46
wsnp_Ku_c4560_8232439	7B	73	0.000542	9.82	42.28
Excalibur_c48871_625	2B	161	0.000176	12.02	26.02
GENE-1981_131	3A	114	0.000534	10.33	86.99
BS00081688_51	3A	114	0.00058	10.01	9.76
Excalibur_c17655_467	3A	114	0.00058	10.01	90.24
Kukri_c18420_705	3D	148	0.000485	10.43	12.2
GENE-0293_154	6B	37	0.000612	9.92	82.93

check 'Maddawalabu' varied among the locations. This difference could be attributed to variations in environmental factors, especially to rainfall, temperature and relative humidity. At MWU, the maximum and minimum monthly average temperatures were 25.1 °C and 3.8 °C, respectively. The mean annual temperature, precipitation and relative humidity of the site were 15.52 °C, 81.91 mm and 54.42 %, respectively. At SARC on the other hand, the maximum and minimum monthly average temperatures were 23.6 °C and 3.5 °C, respectively. The annual mean temperature and precipitation of SARC were 15.61 °C and 44.24 mm, respectively. These variations in environmental factors might have potentially contributed to the difference in the number of wheat lines found to score significantly lower PDS value from 'Maddawalabu' [38]. also suggested that STB severity depends mainly on climatic conditions and resistance level of the varieties studied.

At KARC, however, even the susceptible check 'Maddawalabu' was not as such heavily infested by the disease (41.48 %) when compared with the other two locations (69.14 % at SARC; 65.19 % at MWU). At MWU and SARC, PDS values recorded from wheat lines reached 88.89 %; but, at KARC, the values reached only 69.14 % (Table 5). This suggests the phenomenon that disease pressure at KARC might have been low and insufficient to screen the wheat lines. This low disease pressure might in turn be attributed to the situation that the site received rainfall insufficient for septoria to progress well in October (much less rainfall than it was on the other two sites in the same month) and no rainfall in the rest months until harvest. That might be why most of the wheat lines, even those from which small PDS value was obtained, were found to be indifferent from the susceptible check 'Maddawalabu' (and hence wrongly decided to be susceptible). This is confirmed by the best overall average yield obtained from the site, although soil fertility variation may also take share (Table 5) [39]. observed a significant variation in septoria severity across locations and concluded that the disease can vary in aggressiveness across locations.

Variations among the wheat lines were observed in their days to heading, days to maturity and grain filling period and differences in these parameters were observed among the locations. This might be attributed to variations in environmental factors [40]. studied the effect of environmental variation on wheat phenology and yield, and reported that the days to heading and maturity of the wheat lines they studied were delayed at one study site than the other. The yield obtained from the wheat lines at KARC and MWU showed variation between 'Maddawalabu' and the wheat lines. At SARC, however, no wheat line gave significantly higher yield from 'Maddawalabu'. This might be attributed to the fertility of the soil which enabled even the susceptible check 'Maddawalabu' to give higher yield and hence became indifferent with the high yielding wheat lines. Soils of SARC contain high organic matter (3.9 %), high surface phosphorus (21–23 ppm[1]), high organic carbon (4.19 %) and are high in their micronutrient content (such as 7.02–39.2 ppm manganese and 6.88–20.5 ppm iron) [26].

In all the three locations, TKW of the wheat lines was not significantly different from that of 'Maddawalabu'. This might be attributed to the phenomenon that the disease hasn't caused head infection (i.e., the disease has stopped its vertical progress once it

[1] ppm = parts per million.

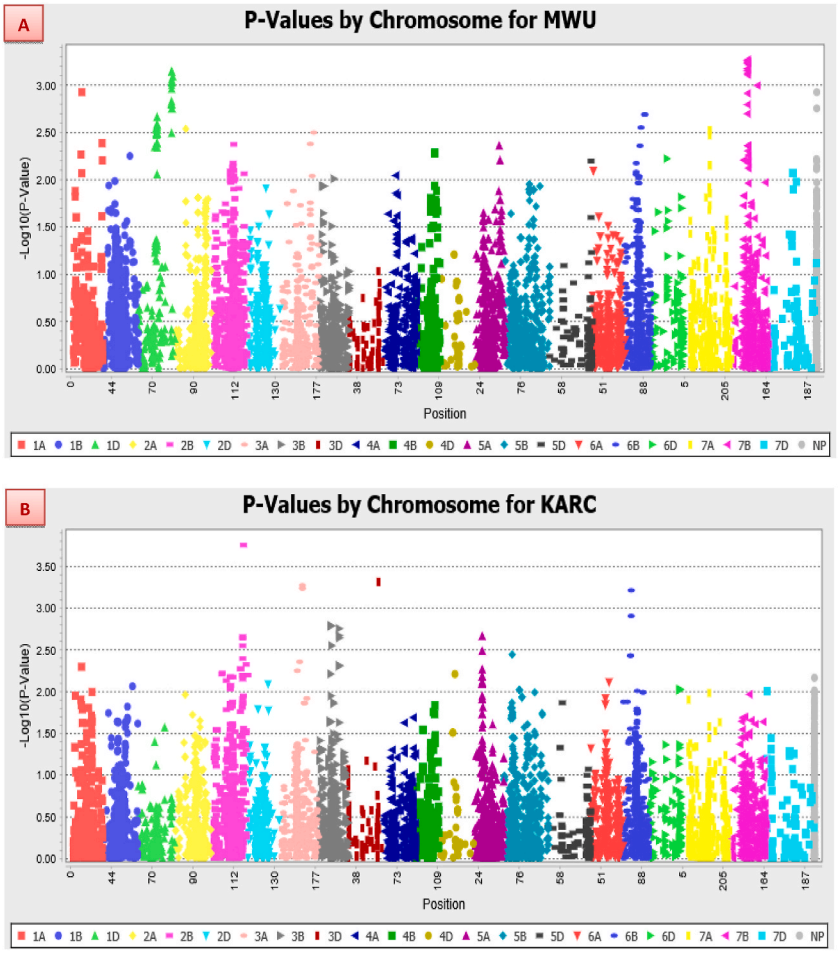

Fig. 2. Manhattan plots of significantly associated SNPs markers with septoria tritici blotch resistance in two different environments (A, Madda Walabu University Research Site; B, Kulumsa Agricultural Research Center) in Ethiopia analyzed using the mixed linear model Markers with -Log10 (*P*-Value) $>$ 3 are significant.

reached the flag leaf of the plant and it failed to climb above the flag leaf and so head infection wasn't observed) in all the three locations. This result is supported by Ref. [41] who reported that *Septoria nodorum* caused reduction in TKW when the percentage of glume blotch severity on wheat heads exceeded 50 %. In other words, the weight of kernels threshed from heads which were free from infection wasn't affected by the disease.

The non-significant difference in NET between 'Maddawalabu' and the wheat lines could be attributed to the good tillering capacity of 'Maddawalabu'. A good evidence for this was that the maximum NET per plant was 10 (not average of five plants, just of a single plant) and it was obtained from 'Maddawalabu' at MWU. SL of the wheat lines was also not significantly different from that of 'Maddawalabu', 'Maddawalabu' had long spike. The maximum SL (14 cm, not average of five plants, just of a single plant) was also measured from 'Maddawalabu' at MWU.

The epidemiological parameters PDS and AUDPC were found to be highly positively correlated in all the three locations. An increase in the disease severity percentage resulted in higher AUDPC values. Similar positive correlations between these two parameters have also been reported in Ref. [38] who stated that there was a correspondence between wheat lines susceptibility and AUDPC; showing, the most susceptible wheat cultivars recorded high AUDPC values (i.e., the varieties which recorded severe necrotic blotches resulted in higher AUDPC values) [11]. also reported a positive significant correlation between STB severity and AUDPC.

PDS and AUDPC were found to be highly and negatively correlated to grain yield at MWU and SARC. This indicates the adverse effect that the prevailed STB disease had on grain yield of the spring bread wheat lines evaluated. This is in agreement with those of [42] who reported a negative correlation between grain yield and STB severity on durum wheat from a research conducted during 2008/9 and 2009/10 cropping seasons in Tunisia [11]. also reported similar significant negative correlation of yield with STB severity and AUDPC. TKW was also highly negatively correlated with both PDS and AUDPC at MWU and SARC. Similar negative correlation was reported by Ref. [43] who reported that the amount of necrosis was highly correlated with reduction in yield and kernel weight. This study is also supported by Ref. [44] who reported a significantly negative correlation of yield and TKW with leaf and stem rusts. At KARC, however, grain yield wasn't correlated with PDS and it showed only significant negative correlation with AUDPC. In addition,

TKW was not correlated with both PDS and AUDPC at KARC. These are also another indication for the phenomenon that disease pressure at KARC might have been low to adversely affect the grain yield and TKW of the wheat lines and hence insufficient to screen the wheat lines.

Plant height was found to have highly negative correlation with both PDS and AUDPC at all the three locations. This illustrates the situation that dwarf wheat lines suffer heavy septoria infestation than the corresponding taller entries. Since STB is a disease with upward climbing behavior, the short internode distances of the dwarf wheat lines will facilitate ease upward progress of the disease by allowing contact between leaves and pycnidiospores splashing and hence, enabling heavy infestation to occur. On the other hand, in tall wheat lines, long internode distance retards ease upward progress of the disease and can reduce the amount of disease to prevail. Similar negative correlation has been reported by different authors. [45], while analyzing data acquired over three years on STB, found a significant negative correlation between STB and PLH. In 2013 [46], reported that STB resistance is associated with increased PLH. According to Ref. [38] also, reduced PLH was usually associated with more necrosis percentage. Paraschivu and co-workers also stated that the greater PLH was strongly associated with reduced AUDPC values and they drew the conclusion that vertical progress of septoria tritici from lower to upper leaves was affected by the distance between consecutive leaves (''the ladder effect''), which is actually in line with the results of this study. Similarly [11], reported a significant negative correlation of PLH with STB severity and AUDPC.

Grain yield and TKW were found to be highly positively correlated in all the three locations. An increase in the weight of thousand kernels increases yield. Similar highly positive correlation results between these two parameters have also been reported by Ref. [44] while evaluating Kenyan wheat lines against leaf rust at adult plant stage [11]. also reported a significant positive correlation between grain yield and TKW. PLH was found to have highly positive correlation with grain yield and TKW at MWU and SARC; but, a significant positive correlation with only grain yield at KARC. For climbing disease like septoria, a tall plant with long internode distance may mean less disease progress and hence low level of disease infestation. In return, less disease infestation is less likely to result in yield reduction and therefore, PLH positively correlates with yield. Inability of STB to climb upward a tall plant with long internode distance may mean a situation less likely for the disease to cause head infection, hence inability to attack the kernels. Kernels free from infection are more likely to weigh better and therefore, PLH positively correlates with TKW. Significant positive correlation results of PLH with yield and TKW have also been reported by Ref. [11].

The one hundred and twenty three (123) wheat lines sequenced for this study were clustered into three subpopulations. The existence of such clusters to imply genetic variation might be attributed to the utilization of diverse parents, originated from different sources, in the breeding program of ICARDA [47]. also attributed the genetic variation among the clusters they identified to the utilization of diverse parents by the wheat breeding program at ICARDA since ICARDA utilizes parents originated from ICARDA, CIMMYT, and from a wide range of genetically unrelated winter wheats from Turkey, Iran, Russia, Ukraine, Romania, Bulgaria, Hungary, and the USA.

SNPs are recent type of molecular markers that are becoming popular to be widely used for mapping of associated resistance in to STB. In this study, 26 SNPs markers located on chromosomes 7B, 1D, 3A, 2B, 3D and 6B were found to be significantly associated with STB resistance. While no significant SNPs were detected at SARC, no SNP markers, among the 26 significant markers, were common at the two locations, KARC and MWU. Differences among the disease environments with regard to races present, temperature, rainfall, humidity, and other environmental factors might have been involved in this lack of consistency. A similar case of markers non-consistency across environments have been observed by Ref. [48] while searching quantitative trait loci (QTL) associated with stem rust resistance at adult plant stage in North American wheat breeding lines evaluated at Njoro, Kenya and Debre Zeit, Ethiopia, and attributed this non-consistency of markers with variations in disease environments.

Previous studies have mapped *Stb8* and *Stb13* to chromosome 7B [21,49], *Stb10* to chromosome 1D [50], *Stb6* and *StbSm*3 to chromosome 3A [23,51], *Stb9* to chromosome 2B [52] and *Stb16q* to chromosome 3D [53]. Thus, the QTL on chromosomes 7B, 1D, 3A, 2B and 3D found in this study could be identical or in very close proximity to the previously mapped ones. In addition to these major genes, several QTL have also been identified on these chromosomes using SNPs markers: on chromosomes 6B and 7B [54], on chromosome 3A [55] and on chromosome 2B [46]. Although the markers found to be significantly associated with STB resistance in this study were located on chromosomes on which septoria tritici blotch resistant genes (*Stb* genes) have been mapped and/or QTL have also been identified previously, localization cannot be compared due to the use of a different type of markers for the reveal or not reveal of sequence homology.

The elite spring bread wheat lines which are identified to be resistant to STB and with very good agronomic performance are recommended for direct release by the national program and parentage purposes in wheat breeding programs. The SNPs markers which are found to be significantly associated with STB resistance can be utilized for marker assisted selection and gene pyramiding in resistance breeding programs.

Data availability

Data will be made available on request.

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CRediT authorship contribution statement

Lakachew Binalf: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hassen Shifa:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Wuletaw Tadesse:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32265.

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