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Genomic dissection of terminal heat tolerance in synthetic hexaploid derived nested introgression libraries of wheat (*Triticum aestivum* L.)

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Abstract

The terminal heat stress during grain filling period is major threat to wheat productivity. Heat tolerant QTLs were harnessed from synthetic hexaploid lines (SHWs) derived from heat tolerant *Ae. tauschii* accessions in present study. SHWs were crossed with two heat susceptible advanced breeding lines to develop nested introgression libraries (S-NILibs). The S-NILibs were evaluated for phenotypic traits and heat tolerance index (HTI) over three years under optimum environment (OE) with sowing at normal date in mid-November and heat stressed environment (HSE) under sown late during mid-December. The panel of 354 S-NILibs was genotyped using the 35 K Axiom® Wheat Breeder's Array. Genome-wide association study (GWAS) identified a total of 61 marker-trait associations (MTAs) across environments (OE and HSE), associated with different traits using multiple models. Of these, two MTAs AX_94985380 for plant height (PH), spikelet number (SN) and AX_94469933 for plant height (PH), thousand grain weight (TGW) were observed for more than one trait across environments and models. Another MTA for HTI (AX-94461626) mapped on 4B chromosome was observed in all four environments and three models. This MTA originated from genes for Ubiquitin carboxyl-terminal hydrolases which is known for maintenance of the circadian clock at high temperature. A subset of MTAs was validated using KASP markers suggested strong association with traits of PH, SN, flag leaf length (FL) and number of productive tillers (pTN). The S-NILib generated, and QTLs identified in this study are potential pre-breeding resource for heat stress tolerance.

Keywords Aegilops tauschii · Synthetic hexaploid wheat · Terminal heat stress tolerance · GWAS · Crop wild relatives

Key message

61 SNPs contribute to trait variation for heat tolerance and associated traits under four environments and three models in synthetic hexaploid lines. 11 S-NILibs screened for terminal heat stress conditions.

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Introduction

Bread wheat (Triticum aestivum L.) is the second most significant cereal crop globally, providing nourishment to over one-third of the world's population (Bhatta et al. 2019). It occupies nearly 30% of the global cereal-growing area with estimated global production of 791 million tonnes in 2023 (USDA 2024). Wheat plays a crucial role in the global economy and society, as it is one of the most widely traded crops, with trade reaching over 199 million tonnes in 2023, representing 40% of all cereal exports (USDA 2024). As a dietary staple for nearly 30% of the global population, wheat contributes up to 60% of daily caloric intake, particularly in developing regions (Arora et al. 2019). Given its substantial role in food security, global trade, and human nutrition, ensuring sustainable wheat production has become increasingly critical amidst rising population demands and changing climatic conditions.

The global population is projected to surpass 9.7 billion by 2050, accompanied by a predicted 70% increase in global wheat demand (Arora et al. 2019; Garcia et al. 2019; Anwar et al. 2024). Simultaneously, the annual global temperature has been rising at a rate of 0.17 °C per decade since 1970, with an increasing frequency of short-term heat stress events (Balla et al. 2019). In temperate regions, heatwaves exceeding 30 °C during the grain-filling period (GFD) are responsible for over 40% of wheat yield variations, affecting approximately 36 million hectares of wheat-growing areas (Zampieri et al. 2016; Birthal et al. 2021). By 2050, heat stress is predicted to impact a significant portion of South Asia's wheat-growing regions, potentially reducing yields by up to 50%, which equates to 7% of the estimated global wheat yield loss (Garcia et al. 2019; Kumar et al. 2024; Ortiz et al. 2008).

India, as both a major wheat producer and consumer, faces significant challenges due to heat stress, which threatens sustainable agricultural development and economic stability. Wheat-growing regions across India experience heat stress at different growth stages. For instance, the northeastern plains are affected by terminal heat stress during the growing season, whereas early heat stress is prominent in central and peninsular regions. In late-sown conditions, major wheat cultivars have shown yield losses of up to 33.6%, highlighting the urgent need to incorporate heat tolerance into wheat varieties for sustainable production (Chatrath 2004; Mishra et al. 2014). Extreme and unpredictable climatic conditions hinder current wheat cultivars from achieving their full yield potential, affecting growth, development, and final yield outcomes (Lesk et al. 2016; Webber et al. 2018; Jagadeesha, et al. 2024).

As thermotolerance is a complex quantitative trait, directly identifying and selecting genes responsible for heat tolerance is challenging. To address this, indirect selection parameters such as the heat tolerance index (HTI) and yieldrelated traits-productive tiller number (pTN), spikelets per spike (SN), and thousand-grain weight (TGW)-are commonly utilized. Additionally, indicators of starch synthesis provide further insights into heat tolerance (Ayeneh et al. 2002; Kumar et al. 2013; Katyal et al. 2022). Advances in genetic research have utilized classical bi-parental QTL mapping to uncover the genetic mechanisms of heat tolerance (Rasheed et al. 2019). Although bi-parental QTL mapping reveals only a fraction of the genetic architecture of traits, numerous OTLs distributed across all wheat chromosomes have been identified (Mason et al. 2010; Kumar et al. 2013; Paliwal et al. 2012; Singh et al. 2024).

To accelerate understanding of complex quantitative traits, high-throughput genotyping combined with genomewide association studies (GWAS) has become the preferred approach. Using high-density SNP markers, GWAS enables the exploration of diverse gene pools, aiding the identification of robust QTLs for traits such as yield components, biotic resistance, and abiotic stress tolerance. Several high-density genome-wide SNP arrays, including the Illumina 9 K iSelect SNP array (Cavanagh et al. 2013), Illumina 90 K iSelect SNP genotyping array (Wang et al. 2014), and Axiom® Wheat Breeder's array (Allen et al. 2017), have been widely used for wheat QTL identification. Although earlier GWAS studies focused on identifying QTLs linked to simply inherited traits, recent efforts have increasingly targeted complex traits such as heat stress tolerance, particularly in multi-environment trials (Rasheed et al. 2014; Abou-Elwafa and Shehzad 2021).

Most Indian wheat cultivars lack sufficient heat tolerance, necessitating a shift toward exploiting genetic diversity in wheat progenitor species such as Aegilops tauschii Coss. and Aegilops speltoides (Jakhu et al. 2021; Seni et al. 2021: Awlachew et al. 2016). As the diploid donor of the D-genome in bread wheat, Aegilops tauschii possesses a rich repository of genes conferring adaptability to environmental stresses, including heat and drought (Waines 1994; Valkoun 2001; Ogbonnaya et al. 2005; Jia et al. 2013; Kishii 2019). The potential of Ae. tauschii for wheat improvement, both directly and through synthetic hexaploid wheat (SHW), is well-documented (Gordon et al. 2019; Truong et al. 2019; Yang et al. 2020; Kaur et al. 2021; Sandhu et al. 2021). SHWs, which are cross-compatible with wheat cultivars, demonstrate heat stress tolerance up to 35-40 °C, with notable resilience during critical anthesis and grain-filling stages (van Ginkel and Ogbonnaya 2007; Pradhan et al. 2012).

Punjab Agricultural University (PAU), Ludhiana, has curated and evaluated a collection of around 250 Ae. tauschii accessions for traits related to abiotic stress, biotic stress, and agronomic performance (Chhuneja et al. 2006; Chhuneja et al. 2008; Chhuneja et al. 2010; Gupta et al. 2010; Arora et al. 2019). Heat-tolerant Ae. tauschii accessions from PAU have been successfully utilized to develop SHWs aimed at improving heat stress tolerance (Kaur et al. 2021, 2022). This study leverages heat-tolerant SHWs to develop nested introgression libraries (S-NILibs) as prebreeding material for targeting heat tolerance and identifying associated genetic factors. The identification and introgression of superior alleles that preserve grain yield under heat stress conditions will pave the way for enhancing wheat cultivars to withstand terminal heat stress, ensuring sustainable production.

Materials and method

Plant materials

The study utilized a set of 354 nested introgression lines (referred to as the Synthetic Wheat-Derived Nested

Introgression Library, S-NILib), developed through crosses between two previously identified heat-tolerant synthetic hexaploid wheats (SHWs) (Syn14135 and Syn14170) (Kaur et al. 2021, 2022, 2023) and two heat-sensitive advanced breeding lines (ABLs) (BWL3531 and BWL4444). The crossing combinations included Syn14135 × BWL3531, Syn14135 × BWL4444, Syn14170 × BWL3531, and Syn14170 × BWL4444. The SHWs (Syn14135 and Syn14170) were selected from a group of seven SHWs evaluated for terminal heat stress tolerance. These SHWs were generated by crossing heat-tolerant *Ae. tauschii* (DD genome) accessions (pau14135 and pau14170) with *Triticum durum* (AABB genome) tetraploid wheat. Detailed information on the SHW development and their heat tolerance screening is documented in Kaur et al. (2021, 2023).

The ABLs utilized in this study represent improved versions of elite wheat cultivars that incorporate stripe rust and leaf rust resistance genes but remain susceptible to terminal heat stress. BWL3531 is an enhanced version of the wheat variety PBW343, containing stripe rust resistance genes Yr17 and Yr70, and leaf rust resistance gene Lr76. Similarly, BWL4444 is an improved version of HD2967, carrying stripe rust resistance gene Yr10. To generate the S-NILib, approximately 1000 florets per synthetic line (used as females) were crossed with the recurrent parents (BWL3531 and BWL4444). The resulting F1 plants were backcrossed to the recurrent parents, and around 100 BC1 F1 plants from each cross were randomly selected and advanced through selfing up to the BC1 F5:7 generation using the single seed descent method. This process resulted in the formation of the S-NILib, consisting of 354 lines distributed as follows: Cross 1 (Syn14135 × BWL4444-77 lines), Cross 2 (Syn14135 × BWL3531–106 lines), Cross 3 (Syn14170 × BWL4444-83 lines), and Cross 4 (Syn14170 ×BWL3531–88 lines) (Table 1).

The development of the S-NILib up to BC1 F3 was facilitated through shuttle breeding, with two cropping seasons per year. The main season (November–May) crops were grown at Punjab Agricultural University (PAU), Ludhiana, Punjab, while the offseason crops (May–October) were cultivated at PAU's Regional Station in Keylong, Himachal Pradesh. From BC1 F4 to BC1 F7, the populations were maintained and assessed during the main wheat-growing season at PAU, Ludhiana, between 2018 and 2021. A summary of the development process for the SHW-derived BC1 F5:7 S-NILib is provided in Fig. 1 and Table 1.

Field trials and trait evaluation

The S-NILibs were evaluated for heat stress tolerance over three consecutive cropping seasons (2018–19, 2019–20, and 2020–21). The trials were conducted in an augmented design under two distinct environments: (1) optimal sowing environment (OE) in mid-November and (2) heat-stressed environment (HSE) in mid-December, at PAU Ludhiana (30°54' N latitude, 75°48' E longitude, 247 m altitude). The late-sown HSE trials subjected plants to terminal heat stress during the grain-filling stage, which adversely affected key economic traits (Farooq et al. 2011).

In both environments, the plant material was grown in four-row plots (1.5 m long, 20 cm row spacing) following standard agronomic practices. Weeds and diseases were controlled with two applications of broad-spectrum herbicides and fungicides, respectively. Seven yield-related traits were recorded: plant height (PH), flag leaf length (FL), spike length including awns (SL), number of productive tillers per plant (pTN), number of spikelets per spike (SN), thousand-grain weight (TGW), and yield per plot (YpP). PH was measured as the mean height (cm) of five plants from soil surface to spike tip (excluding awns). pTN was expressed as the number of spikes per square meter, TGW as the weight of 1,000 grains (g), and YpP as the total grain weight harvested per plot.

Meteorological data, including mean, maximum, and minimum temperatures and rainfall during the grain-filling stage, were obtained from PAU's Department of Climate

Popula- tion Details	Pedigree	Total number of Lines	Experimental Design
Pop1	PDW233-Ae. tauschii acc. pau14135 amphiploid// BWL4444	77	Augmented design in 2018–19, 2019–20, 2020–21; paired row (1.5 m long with 20 cm row to row spacing with
Pop2	PDW233-Ae. tauschii acc. pau14135 amphiploid// BWL3531	106	standard agronomic practices
Pop3	PBW114-Ae. tauschii acc. pau14170 amphiploid// BWL4444	83	
Pop4	PBW114-Ae. tauschii acc. pau14170 amphiploid// BWL3531	88	

Table 1 Details on experimental material evaluated for terminal heat stress tolerance across three cropping seasons (2018–19, 2019–20 and 2020–21)



Trial in Augmented Design across Optimum and Heat Susceptible Environment (2020-21)

<Fig. 1 Schematic strategy followed for the development, and screening of *T. durum-Ae. tauschii* derived synthetic hexaploid wheat (SHW) derived BC₁F₅₋₇ nested introgression libraries for terminal heat stress tolerance. Primary SHWs were crossed with two elite cultivars as recurrent parents (BWL3531 and BWL4444), backcrossed, and selfed till BC₁F₇. At BC₁F₅, nested introgression libraries were sown in augmented design under OE (Optimum environment) and HSE (Heat stress environment) for three subsequent growing seasons. Green plants in the figure represent tolerant plants while brown represent susceptible plants

Change and Agricultural Meteorology (Supplementary Figure S1). On average, the mean temperature during the grainfilling period under HSE was 3–4 °C higher than under OE, confirming the exposure of late-sown plants to terminal heat stress. Temperature profiles remained consistent across the three years, though rainfall patterns varied (Supplementary Figure S1).

Statistical analysis of phenotypic data

Data were recorded for all traits on five randomly selected plants per plot in each environment. Descriptive statistics and variability analyses were conducted using RStudio (Comtois 2020; Aravind et al. 2021). The following model was used:

 $Yik = \mu + Repi + Genk + \epsilon ik(within the environment)$

$Yijk = \mu + Repi(Envj) + Envj \times Genk + Genk + Envj$ $+ \epsilon ijk(acrossenvironments)$

Here, is the observed trait, is the mean, is the replication effect, is the genotype effect, and is the residual error, assumed to follow a normal distribution with homogenous variance. For combined analyses across environments, the model included an environment effect (Env), replication nested within environment, and genotype \times environment interactions. Adjusted phenotypic values were generated as best linear unbiased predictors (BLUPs) while accounting for environmental variations.

$$HTI = \left[1 - \frac{TraitOE - TraitHSE}{TraitOE}\right] \times 100$$

To evaluate heat tolerance, the heat tolerance index (HTI) for YpP was calculated following Dhillon et al. (2021). Trait variability across environments was visualized using the'GGplot2' (Wickham 2016) and'GGpubr' (Kassambara and Mundt 2020) packages in RStudio. Pearson correlation coefficients (r) among traits and sowing conditions were calculated using the cor () function from the'stats'package (R

Core Team 2019) and plotted with the corrplot package (Wei and Simko 2021).

SNP genotyping and population structure analysis

High-density genotyping of the 354 S-NILibs was conducted using the 35 K Axiom® Wheat Breeder's Array (Affymetrix UK Ltd., United Kingdom) (Allen et al. 2017). SNP data were analyzed to assess genetic diversity and identify marker-trait associations (MTAs). Population structure was examined using three clustering methods: (1) STRUCTURE v2.3.4 (Pritchard et al. 2000) for Bayesian model-based analysis, (2) a neighbor-joining tree constructed with TASSEL v5.2.57 (Bradbury et al. 2007) and visualized in MEGA 7.0 (Kumar et al. 2016), and (3) principal component analysis (PCA) performed in GAPIT (Lipka et al. 2012). A kinship matrix (K-matrix) was generated using TASSEL, and linkage disequilibrium (LD) decay was analyzed to determine marker relationships.

Genome-wide association analysis

Genome-wide association studies (GWAS) were performed for all traits using the GAPIT R package (Lipka et al. 2012). Three models—Compressed Mixed Linear Model (CMLM), Multi-Locus Mixed Linear Model (MLMM), and Enriched CMLM (ECMLM)—were employed. Significant MTAs consistent across at least two environments and two models were prioritized for identifying stable heat-tolerant QTLs. Allelic effects were evaluated through Kruskal–Wallis tests (Kruskal and Wallis 1952).

Candidate gene identification

Genomic regions within 5 Mb upstream and downstream of significant MTAs were scanned for candidate genes using the IWGSC RefSeq v2.0 assembly. Functional annotations of genes within these regions were analyzed for their relevance to heat stress tolerance based on literature evidence.

Validation of MTAs using KASP markers

The significant MTAs (Table 3) were converted into KASP markers for validation of GWAS results. The sequence information of KASP markers is provided in supplementary Table S5. The KASP genotyping assay was performed in 384-well plate with a total reaction volume of 4 μ l (0.056 μ l primer mix, 1.944 μ l Kasp mix, 2 μ l DNA template (20

Table 2 heat stre	Summai sss envirc	ry of overall J nment (HSE)	phenotypic pe	rformance (BL	UPs) of synth	aetic derived ne	sted introgress	ion libraries (S	-NILibs) used i	n the present s	study une	ler optimu	m environ	ment (0	E) and
Trait	Env	Syn14135	Syn14170	BWL4444	BWL3531	Pop1	Pop3	Pop2	Pop4	S-NILib	h^2	GV	EV	LSD	CV
Hd	OE	104.7	100.5	9.96	86.2	79.5-107.3	79.7-110.4	70.9–97.3	79.2-119.3	70.9-119.3	0.95	52.4	8.8	4.6	3.2
	HSE	94.0	94.1	91.5	79.7	70.6–97.9	72.6-105.9	71.4-91.4	73.4–99.7	70.6-105.8	0.90	43.5	15.2	5.9	4.6
FL	OE	22.8	22.0	22.2	19.2	19.4 - 26.0	17.9–25.8	17.1-22.9	16.9–25.3	16.9 - 26.0	0.89	3.8	1.4	1.8	5.7
	HSE	21.1	21.3	20.7	17.5	18.4–24.3	17.2-26.6	16.2 - 24.3	15.5-24.8	15.5 - 26.6	0.87	3.5	1.5	1.9	6.1
SL	OE	11.3	11.1	12.5	10.2	10.6 - 14.7	9.6-13.3	8.5-11.8	9.3-13.8	8.5-14.7	0.94	1.4	0.3	0.8	4.7
	HSE	11.5	11.0	12.2	10.1	9.2-13.3	8.8-13.7	7.8-12.0	9.0-13.4	7.8-13.7	0.88	1.1	0.4	1.0	6.1
NS	OE	19.4	18.9	21.2	19.1	17.6-23.2	16.4–22.7	16.1 - 21.8	17.0-23.1	16.2–23.2	0.91	2.1	0.6	1.2	4.0
	HSE	19.1	18.0	20.2	18.6	17.6–22.6	15.3-21.4	16.5 - 21.2	16.4–21.6	15.3-22.6	0.89	1.6	0.6	1.1	4.0
pTN	OE	8.2	8.7	8.2	8.6	6.4–11.3	6.1 - 10.9	7.3-11.1	6.4 - 11.0	6.5-11.3	0.78	1.1	0.9	1.4	11.2
	HSE	T.T	7.9	7.0	7.5	4.9-8.3	5.1 - 9.2	5.2-8.8	4.8-9.4	4.8-9.4	0.77	0.7	0.6	1.1	11.7
TGW	OE	44.8	44.0	35.9	39.4	30.5-47.2	31.8-48.5	32.4-48.9	34.7-48.5	30.5-48.9	06.0	13.8	4.4	3.2	5.3
	HSE	40.1	39.1	31.8	32.3	26.6-38.7	28.9-44.3	26.1 - 39.8	27.1–41.7	26.1-44.3	0.85	10.2	5.5	3.5	6.9
ΥpΡ	OE	101.7	128.7	156.6	130.5	84.7-242.6	78.7–229.2	67.2-221.0	84.8-230.4	67.9–242.6	0.87	1107.9	487.1	33.0	15.1
	HSE	95.3	120.6	113.6	96.4	51.4-155.3	76.8–176.7	56.3-149.5	62.3-138.6	51.4-176.7	0.80	541.2	395.8	28.6	19.2
HTI YpP		94.1	93.8	75.2	76.8	35.5-140.2	48.3–134.5	37.1–177.3	43.3–121.1	35.5–177.3	0.80	576.1	370.8	28.0	25.1



Fig. 2 Distribution of BLUP values for selected traits in the SHW derived nested introgression libraries. 1, 2, 3, 4 in environments indicated the year 2018–19, 2019–20, 2020–21, and BLUPs. OE (green color), HSE (red color), and simple E (yellow color) indicate the optimum environment, heat stress environment, and heat tolerance index,

respectively, across treatments. **a** Plant Height without awns (PH, cm), (**b**) Flag leaf length (FL, cm), (**c**) Spike Length with Awns (SL, cm), (**d**) Spikelet Number (SN), (**e**) Number of Productive Tillers (pTN), (**f**) Thousand Grain Weight (TGW, gm), (**g**) Heat Tolerance Index-Yield Per Plot (HTI_YpP)

ng). Touchdown PCR profile included initial denaturation at temperature of 95 °C for 15 min, 10 cycles of amplification (95 °C temperature for 20 s, touchdown at 65 °C with minus 1 °C per cycle for 25 s) followed by 20 cycles (95 °C temperature for 10 s; 57 °C temperature for 60 s). The fluorescence was captured using Infinite F200 pro microplate reader and it was analyzed by Tecan i-control 1.11 software. The clusters were tagged (XX, XY, YY) on the basis of graphical location using the KlusterCaller software. The genotypic data was tested against phenotypic data of S-NILibs for each trait using Kruskal–Wallis test (Kruskal and Wallis 1952).

Results

Phenotypic trait variation and correlations under two sowing regimes

Seven agronomic traits exhibited significant variations across different S-NILibs under different sowing conditions based on BLUP values. The variation among the treatments was highly significant for all traits (Supplementary Table S1). The information on trial means, least significance difference (LSD), coefficients of variation (CV), and heritability (H²) for all the traits are presented in Table 2. As expected, terminal heat stress (HSE condition) led to



Fig. 3 Principal Component Analysis for different agronomic traits under OE (Optimum environment) and HSE (Heat stress environment). 1, 2, 3, 4 in environments indicated the year 2018–19, 2019– 20, 2020–21, and BLUPs and OE, HSE, indicate the optimum envi-

ronment, heat stress environment across treatments. PH: Plant Height without awns (cm), FL: Flag Leaf Length (cm), SL: Spike Length with Awns (cm), pTN: Number of Productive Tillers, TGW: Thousand Grain Weight (gm)

a significant reduction (P ≤ 0.01) in value of all the traits, indicating the late sown trial was subjected to environmental stress as compared to normal sown trial. There was significant reduction in pTN (6.14–11.28 in OE and 4.89–9.36 in HSE), TGW (30.49–48.97 in OE and 26.13–44.29 in HSE), and YpP (67.18–242.6 in OE and 51.44–176.67 in HSE) (Fig. 2). Evidently, synthetic hexalpoid parental lines had higher HTI (Syn14135 = 94.05, Syn14170 = 93.75, BWL4444 = 75.16, BWL3531 = 76.78) in comparison to ABLs. All the selected traits showed high broad-sense heritability (> 0.70) in both the environments. However, the maximum coefficient of variation (CV) was observed in YpP under the HSE condition (CV = 19.2%) and OE condition (15.1%). Followed by pTN (11.7%) under HSE under OE (11.2%) conditions (Table 2). Transgressive segregants were observed having HTI values higher than the synthetic



Fig. 4 a The appropriate number of the subpopulations determined from the largest delta, K = 2 (b) Population structure within the synthetic derived nested introgression libraries. The population structure plots with each vertical bar representing a breeding line colored according to the particular group to which the breeding line has been assigned. The breeding lines assigned to more than one group represents the degree of their admixed set of the (c) The three-dimensional

view of the PCs explaining the genotypic variation among breeding lines constituting the introgression libraries alleles (**d**) Neighborjoining tree of synthetic derived nested introgression libraries (**e**) The Kinship matrix is displayed as the heat map, where the red indicates the highest correlation between the pairs of breeding lines and yellow indicates the lowest correlation

parent. The detailed information of phenotypic variation of the traits measured in the present study across the treatments are presented as mean values in Supplementary Table S2.

Biplots showing the genetic correlations between different traits were given in Fig. 3 and the correlation plots were given in Supplementary Fig. 2 under OE and HSE conditions. The first two principal components of these biplots explained 54.06% variability in OE and 54.9% variability in HSE conditions. Traits TGW, YpP, PH, FL occupied the same section in both biplots indicating a similar pattern of variability among these traits across different environments (Fig. 3b, d). The noteworthy correlation was a positive correlation of PH with TGW across years and environments (0.18 to 0.38). On the other hand, pTN and TGW showed a weak negative correlation with SN and SL (Supplementary Fig. 2) with pTN negatively correlated with SN in all environments (-0.12 to -0.23).

Based on the performance of the most important traits of pTN, TGW, and YpP affecting the final yield, 55 S-NILibs were outperforming. Thirty-seven of these S-NILibs outperformed checks in at least two OE, while 18 outperformed under HSE conditions (Supplementary Table S3). Most of these outperforming S-NILibs belonged to Cross-3 (27)

S-NILibs) followed by Cross-4 (13 S-NILibs) and Cross-2 (11 S-NILibs). Further, 105 S-NILibs had consistent high heat tolerance index (HTI-YpP) over two or more years (Supplementary table S4) and 52 of these were derived from Syn14135 (16 from Cross-1 and 36 from Cross-2) while 53 were derived from Syn14170 (36 from Cross-3 and 17 from Cross-4). Eleven of these S-NILibs had high pTN, TGW, YpP and HTI.

Statistical analysis of Genotype

The quality pre-processing of 35,143 35 K Axiom® Wheat Breeder's Array, a total of 9,536 single nucleotide polymorphisms (SNPs) were obtained (keeping minor allele frequency (MAF) of > 5%, maximum heterozygote proportion of 0.1, and missing rates < 0.1). These SNPs were distributed on all 21 wheat chromosomes, with 3215, 3700, and 2621 SNPs in the A, B, and D sub-genomes, respectively. Chromosome 1B had the maximum number of SNPs (758), and 4D had the lowest number of SNPs (183) (Supplementary Fig. S3a). Considering the SNP distribution across homoeologous chromosomes, group 1 had the maximum number



◄Fig. 5 Manhattan and QQ-plots of highly associated haplotypes for selected agronomic traits under optimum sown (OE) conditions based on Compressed Mixed Linear Model (C), Enriched CMLM (EC), Multiple Loci Mixed Model (M) models. 1, 2, 3, 4 in environments indicated the year 2018–19, 2019–20, 2020–21, and BLUPs. a Plant Height without awns (cm), (b) Flag leaf length (cm), (c) Spike Length with Awns (cm), (d) Spikelet Number, (e) Number of Productive Tillers, (f) Thousand Grain Weight (gm)

of SNPs (1810), whilst the least SNPs were mapped in group 4 (836). The heterozygosity of all SNPs ranged from 0.0 to 0.11. 2826 SNPs showed heterozygosity above 0.05, and 1243 exhibited 0–0.01 heterozygosity (Supplementary Fig. S3b). The PIC values varied from 0.01 to 0.16 and maximum SNPs (2145) had PIC values between 0.06–0.08 followed by 0.08–0.1.PIC values (1674 SNPs) (Supplementary Fig. S3c). Further, in these high-quality SNPs, MAF ranged from 0.05 to 0.50 with most of SNP markers (3033) having MAF \leq 0.15 (Supplementary Fig. S3 d).

Population structure and LD analysis

The population structure of the 354 S-NILibs was assessed based on 9,536 SNPs. Analysis using STRUCTURE software suggested that the delta K value reached a sharp peak at K = 2 with a sharp decrease afterward (Fig. 4a, b). These two sub-groups clearly comprised of the S-NILibs generated from two different SHW. Similarly, PCA (Fig. 4c) and neighbor-joining tree (Fig. 4d) also classified the whole panel into two clear groups. The kinship heatmap also supported the results of these three grouping approaches and a strong relatedness in the panel was observed (Fig. 4e).

The 9,536 high-quality SNPs selected for association analysis were also used to calculate pair-wise linkage disequilibrium (LD) as squared allele frequency correlation (r^2) between pairs of intra- chromosomal SNP's with the known chromosomal position. The mean r^2 values for the A, B, and D sub genomes were 5.2, 5.7 and 6.9 Mbp (Supplementary Fig. 4) while these values for whole genome gradually decreased with increasing pair wise distance.

GWAS under two sowing conditions

Using the $-\log(P) \ge 0.001$ at a 5% significance level, GWAS identified a total of 61 marker-trait associations (MTAs) across environments (OE and HSE), using multiple models (Figs. 5 and 6, Table 3, Supplementary Fig. S5). Of these, 21 MTAs were under OE, while 36 were under HSE conditions (Table 3). Across environments and models, two MTAs (AX_94985380 for PH, SN and AX_94469933 for PH, TGW) were associated with more than one trait. QQ plots and Manhattan plots for all traits based on BLUP values are shown in Figs. 5 and 6.

Plant height (PH)

17 MTAs were detected for PH (14 in HSE and 3 in OE) with majority concentrated on chromosome 6B (7 MTAs) spanning 56 Mbp and harbouring 50 genes. 4 MTAs were present on chromosome 6 A of which 3 MTAs were closely placed spanning 2.7 Mbp and other were at distance of 9 Mbp. The remaining MTAs for PH were scattered on 2 A, 3 A, 3B, 4B and 7 A chromosomes. The candidates genes involved with these MTAs varies, like Ubiquitin like protein and growth regulating factor related genes present on chromosome 6B. Phosphoinositide phospholipase C and Peroxidase related candidate genes on chromosome 2 A MTAs, Sesquiterpene synthase, Cytochrome P450, Fatty acid oxidation complex alpha subunit, Zinc finger FYVE domain-containing protein 26, senescence-associated family protein on chromosome 3 A MTAs, homeobox protein knotted-1, E2 ubiquitin-conjugating-like enzyme, Peroxidase on chromosome 4B MTAs, cluster of MYB, transmembrane protein (DUF616) on chromosome 6 A MTAs and sulfate transporter candidate genes on chromosome 7 A.

Flag leaf Length (FL)

GWAS for FL identified 12 MTAs (4 MTAs in OE and 8 in HSE), interestingly, all the MTAs for HS (8 MTAs) and one of OE were present in close vicinity of each other on chromosome 5 A. The rest three MTAs were present on chromosomes 3 A and 4D. Some important genes for FL were observed at 5 A chromosome included Nitrate transporter, Ammonium transporter, Beta-glucosidase, Auxin-responsive protein, ABC transporter B family protein. Clavata3/ESR (CLE) gene family member was identified as important candidate gene on chromosome 3 A, while Cellulose synthase and sugar transporter candidate gene were found on chromosome 4D.

Spike Length (SL)

3 MTAs were identified for SL, of which 2 MTAs were present on chromosome 3D and one MTA on 7 A in OE environments. The important candidate genes having potential role in determining length of spike were present in proximity of MTAs were globulin seed storage protein, Pectin acetylesterase on chromosome 3D and Cellulose synthase-like protein on 7 A.

Spikelet Number (SN)

16 MTAs were observed for SN (9 MTAs in HSE and 7 in OE), interestingly three major clusters of MTAs were observed at chromosome 4B, 7 A and 7D harbouring 6, 4, 3 SNPs, respectively. The other MTAs were present on chromosomes 2B, 5 A and 7B. One of the MTA present on chromosome 7D (AX-94440790) was identified in all three models and all four environments of heat stress. The candidate genes in vicimity of MTAs on chromosome 4B includes Amino acid transporter, acetylglucosamine deacetylase, Alpha-L-fucosidase 2. While the important candidate for variation in SN like Flowering-promoting factor 1-like protein 1 was present near MTAs of chromosomes 4B and 5A. Clusters of ABC transporter genes were present in vicinity of MTAs of chromosome 7D and Fructose-1,6-bisphosphatase was observed in mapped region of 7 A chromosome.

Number of productive Tillers (pTN)

7 MTAs were observed for pTN (5 MTAs in OE and 2 in HSE). These MTAs were scattered on chromosomes 2B, 3A, 3B, 6B, 7A. The major candidate genes associated with tiller number were Auxin-responsive protein, Sulfate transporter on chromosome 2B, Starch synthase in vicinity of MTAs of chromosome 3A, major facilitator superfamily protein, LysM domain containing protein on chromosome 3B and Indole-3-acetic acid-amido synthetase on chromosome 6B.

Thousand grain weight (TGW)

TGW is severely affected by heat stress, and two MTAs were observed for TGW on chromosome 5D and 7A in OE and HS, respectively. Major candidates governing TGW directly or indirectly included were found on chromosome 5D were clusters of lipid transfer protein, folate/biopterin transporter family protein and glycosyltransferase responsible for sugar mobilization.

Heat tolerance index

Four MTAs were identified for HTI, an index obtained by comparing yield in OE and HSE. Two of these MTAs were present on chromosome 2B, one on 4B and one on chromosome 4D. The MTA present on 4B chromosome (AX-94461626) was observed in all four environments and three models having associated candidate genes for Ubiquitin carboxyl-terminal hydrolases. This gene has been reported for maintenance of the circadian clock at high temperature. Genes for cyclopropane-fatty-acyl-phospholipid synthase, glycosyltransferase and peptidylprolyl isomerise were present near MTAs of chromosome 2B with potent role against heat stress.

Validation of MTAs using KASP markers

A subset of 28 SNPs associated with different traits (Table 3) were converted into KASP markers for validation. 10 SNPs were polymorphic and only 7 showed association. The SNP AX-94436234 on chromosome 3 A and AX-94985380 on chromosome 4B showed non-significant association with PH in all environments with p-value of 0.06 (Chi-squared = 5.78) and 0.41 (Chi-squared = 1.76), respectively. Another SNP AX-94488051 on chromosome 7 A, associated with SN with donor allele "C" showed non-significant deviation in population with p-value of 0.148 (Chi-squared = 3.81). Two SNPs AX-94491172 on chromosome 4D and AX-94800332 on chromosome 5 A with donor allele "A" were associated with FL indicated by p-value of 0.57 and 0.784, respectively. Non-significant deviation of genotypic data of two SNPs AX-94541597 on chromosome 3 A and AX-94699856 on chromosome 3B associated with pTN was observed indicating linkage of MTAs.

Discussion

Climate change impacts life on earth, this inevitable phenomenon had shifted its gear rapidly since the beginning of this century. The increase in temperature in winter months causes decline in productivity of rabi crops. The terminal heat during grain filling period of wheat reduces its yield by 15 per cent in North India (Dubey et al. 2020; Redhu, et al. 2024, 2025). The cultivated gene pool of hexaploid wheat is highly susceptible to terminal heat stress. Exploring the diversity of wild germplasm is only answer to the declining diversity of cultivated germplasm. Ae. tauschii, the D genome progenitor of wheat reported to be source of heat stress tolerance but this diploid species cannot be used directly in crosses, generating synthetic hexaploid wheat (SHW) matching the ploidy level of cultivated wheat is a better alternative (Kaur et al 2021, 2022, 2023). In the present study, we developed the synthetic hexaploid wheat from two heat-stress tolerant accessions of diploid wild progenitor Ae. tauschii (D) acc pau 14,135 and acc pau 14,170. To understand the genetics of heat stress tolerance transferred from Ae. tauschii in SHW, these SHW were then crossed with two advanced breeding lines (ABL) generating 354 BC₁F_{5:7} S-NILib from four crosses. Each S-NILib generated Fig. 6 Manhattan and QQ-plots of highly associated haplotypes for selected agronomic traits under heat-stressed (HSE) conditions based on Compressed Mixed Linear Model (C), Enriched CMLM (EC), Multiple Loci Mixed Model (M) models. 1, 2, 3, 4 in environments indicated the year 2018-19, 2019-20, 2020-21, and BLUPs. a Plant Height without awns (cm), (b) Flag leaf length (cm), (c) Spike Length with Awns (cm), (d) Spikelet Number, (e) Number of Productive Tillers, (f) Thousand Grain Weight (gm), (g) Heat Tolerance Index-Yield Per Plot



Table 3	Summary of QTLs n	napped Synthetic of	lerived near introg	gression line librai	ries (S-NILib) deriv	ved from the cross of	synthetic wheats and
advance	d breeding lines (ABI	Ls)					

Trait	QTL	SNP	Chr	Pos. (Bp)	Model	Env [#]	Min. P-value	Avg. P-value	Chr
РН	qHSE.PH.SHW.pau.2 A	AX-94417025	2 A	43,091,489	M, C, EC	HSE1, HSE2, HSE3, HSE4	1.64E-06	3.19E-04	2 A
	qHSE.PH.SHW.pau.3 A	AX-94436234	3 A	56,647,304	М	HSE1, HSE2, HSE3, HSE4	6.56E-04	7.48E-04	3 A
	qOE.PH.SHW.pau.3 A	AX-95187760	3 A	395,774,914	M, C, EC	OE1, OE2 ⁻ , OE3, OE4	1.31E-04	4.88E-04	3 A
	qOE.PH.SHW.pau.3B	AX-95652719	3B	788,056,558	C, EC	OE2, OE3, OE4	1.82E-04	2.94E-04	3B
	qOE.PH.SHW.pau.4B	AX-94985380	4B	639,500,075	M, C, EC	OE2, OE3, OE4	3.51E-06	1.08E-04	4B
	qHSE.PH.SHW.pau.6 A.1	AX-95102079	6 A	423,282,969	M, C, EC	HSE1, HSE2, HSE4	3.44E-05	2.77E-04	6 A
	qHSE.PH.SHW.pau.6 A.2	AX-95658579	6 A	514,603,183	M, C, EC	HSE1, HSE2 ⁻ , HSE3, HSE4	2.44E-04	5.66E-04	6 A
	qHSE.PH.SHW.pau.6 A.3	AX-94593646	6 A	542,117,917	C, EC	HSE2, HSE3, HSE4	3.77E-04	5.64E-04	6 A
	qHSE.PH.SHW.pau.6 A.4	AX-94770234	6 A	542,169,136	C, EC	HSE2, HSE3, HSE4	2.04E-04	2.14E-04	6 A
	qHSE.PH.SHW. pau.6B.1	AX-94975377	6B	511,770,004	C, EC	HSE1, HSE3, HSE4	2.09E-04	5.90E-04	6B
	qHSE.PH.SHW. pau.6B.2	AX-94439000	6B	643,335,647	C, EC	HSE2, HSE3, HSE4	1.70E-04	3.56E-04	6B
	qHSE.PH.SHW. pau.6B.3	AX-94821898	6B	644,746,220	C, EC	HSE2, HSE3, HSE4	4.99E-04	5.43E-04	6B
	qHSE.PH.SHW. pau.6B.4	AX-94421041	6B	645,035,982	C, EC	HSE2, HSE3, HSE4	2.05E-04	3.36E-04	6B
	qHSE.PH.SHW. pau.6B.5	AX-94861181	6B	647,363,025	C, EC	HSE2, HSE3, HSE4	1.92E-04	4.21E-04	6B
	qHSE.PH.SHW. pau.6B.6	AX-94397766	6B	649,603,403	M, C, EC	HSE1 ⁺ , HSE2, HSE3, HSE4 ⁻	1.01E-04	1.90E-04	6B
	qHSE.PH.SHW. pau.6B.7	AX-94614625	6B	700,079,454	M, C, EC	HSE1, HSE2, HSE3, HSE4	2.24E-05	1.10E-04	6B
	qHSE.PH.SHW.pau.7 A	AX-94469933	7 A	694,663,286	М	HSE1, HSE2, HSE4	1.27E-04	4.43E-04	7 A
FL	qOE.FL.SHW.pau.3 A.1	AX-94621725	3 A	56,564,102	M, C, EC	OE1, OE3, OE4	8.76E-05	4.61E-04	3 A
	qOE.FL.SHW.pau.3 A.2	AX-95109994	3 A	57,427,233	M, C, EC	OE1, OE3, OE4	1.38E-04	3.03E-04	3 A
	qOE.FL.SHW.pau.4D	AX-94491172	4D	497,676,571	М	OE2, OE3, OE4	5.95E-04	7.23E-04	4D
	qHSE.FL.SHW.pau.5 A.1	AX-94800332	5 A	582,834,762	C, EC	HSE1, HSE2, HSE4	2.62E-05	1.80E-04	5 A
	qHSE.FL.SHW.pau.5 A.2	AX-94398741	5 A	583,976,068	C, EC	HSE1, HSE2, HSE4	1.13E-04	3.79E-04	5 A
	qHSE.FL.SHW.pau.5 A.3	AX-95210025	5 A	587,264,837	C, EC	HSE1, HSE2, HSE4	2.91E-05	1.90E-04	5 A
	qHSE.FL.SHW.pau.5 A.4	AX-94543559	5 A	588,158,813	C, EC	HSE1, HSE2, HSE4	3.07E-05	1.84E-04	5 A
	qHSE.FL.SHW.pau.5 A.5	AX-94445381	5 A	590,582,179	C, EC	HSE1, HSE2, HSE4	1.65E-05	1.02E-04	5 A
	qHSE.FL.SHW.pau.5 A.6	AX-95228006	5 A	592,520,010	M, C, EC	HSE1, HSE2, HSE4	3.86E-06	1.71E-04	5 A
	qHSE.FL.SHW.pau.5 A.7	AX-94473833	5 A	596,045,502	C, EC	HSE1, HSE2, HSE4	5.89E-05	3.73E-04	5 A
	qHSE.FL.SHW.pau.5 A.8	AX-94424065	5 A	596,400,289	C, EC	HSE1, HSE2, HSE4	6.18E-05	4.85E-04	5 A
	qOE.FL.SHW.pau.5 A	AX-95075446	5 A	681,455,450	M, C, EC	OE2, OE3, OE4	1.26E-05	6.01E-05	5 A
SL	qHSE.SL.SHW. pau.3D.1	AX-94493158	3D	615,228,356	M, C, EC	HSE1, HSE2, HSE4	2.14E-04	4.41E-04	3D

 Table 3 (continued)

Trait	QTL	SNP	Chr	Pos. (Bp)	Model	Env [#]	Min. P-value	Avg. P-value	Chr
	qHSE.SL.SHW. pau.3D.2	AX-94642392	3D	615,991,659	M, C, EC	HSE1, HSE2, HSE4	9.44E-05	4.26E-04	3D
	qOE.SL.SHW.pau.7 A	AX-95123768	7 A	733,552,767	M, C, EC	OE2, OE3, OE4	5.33E-04	7.24E-04	7 A
SN	qHSE.SN.SHW.pau.2B	AX-94693825	2B	432,498,930	М	HSE1, HSE2, HSE4	1.71E-04	4.43E-04	2B
	qOE.SN.SHW.pau.4B.1	AX-94985380	4B	639,500,075	M, C, EC	OE2, OE3, OE4	6.24E-05	2.49E-04	4B
	qOE.SN.SHW.pau.4B.2	AX-95084128	4B	642,791,090	M, C, EC	OE2, OE3, OE4	6.56E-05	3.64E-04	4B
	qOE.SN.SHW.pau.4B.3	AX-95241637	4B	642,928,494	M, C, EC	OE1, OE2, OE3, OE4	1.23E-05	3.98E-04	4B
	qOE.SN.SHW.pau.4B.4	AX-94593455	4B	642,928,506	M, C, EC	OE2, OE3, OE4	1.78E-05	3.06E-04	4B
	qOE.SN.SHW.pau.4B.5	AX-94436544	4B	643,914,339	M, C, EC	OE2, OE3, OE4	3.30E-05	2.19E-04	4B
	qOE.SN.SHW.pau.4B.6	AX-94422147	4B	648,789,862	M, C, EC	OE1, OE2, OE3, OE4	1.25E-04	4.44E-04	4B
	qOE.SN.SHW.pau.5 A	AX-94523608	5 A	690,173,776	M, C, EC	OE1, OE2, OE3, OE4	5.34E-05	3.75E-04	5 A
	qHSE.SN.SHW.pau.7 A.1	AX-94488051	7 A	676,621,182	C, EC	HSE1, HSE3, HSE4	4.90E-05	7.26E-05	7 A
	qHSE.SN.SHW.pau.7 A.2	AX-94514616	7 A	678,868,976	C, EC	HSE1, HSE3, HSE4	2.23E-05	5.03E-05	7 A
	qHSE.SN.SHW.pau.7 A.3	AX-94512826	7 A	678,873,842	C, EC	HSE1, HSE3, HSE4	1.98E-05	2.99E-05	7 A
	qHSE.SN.SHW.pau.7 A.4	AX-94567073	7 A	680,690,167	C, EC	HSE1, HSE3, HSE4	4.84E-05	2.93E-04	7 A
	qHSE.SN.SHW.pau.7B	AX-94685291	7B	37,557,148	М	HSE1, HSE3, HSE4	4.41E-04	6.70E-04	7B
	qHSE.SN.SHW. pau.7D.1	AX-95248379	7D	71,647,252	М	HSE1, HSE3, HSE4	1.50E-04	4.16E-04	7D
	qHSE.SN.SHW. pau.7D.2	AX-94440790	7D	583,215,678	M, C, EC	HSE1, HSE2 ⁺ , HSE3, HSE4	1.74E-06	5.76E-05	7D
	qHSE.SN.SHW. pau.7D.3	AX-94474937	7D	588,726,365	C, EC	HSE1, HSE3, HSE4	1.50E-04	3.55E-04	7D
pTN	qOE.pTN.SHW. pau.2B.1	AX-94760904	2B	739,731,339	M, C, EC	OE1, OE2, OE4	4.25E-05	1.82E-04	2B
	qOE.pTN.SHW. pau.2B.2	AX-95248718	2B	739,862,173	M, C, EC	OE1, OE2, OE4	8.71E-05	4.33E-04	2B
	qHSE.pTN.SHW.pau.3 A	AX-94541597	3 A	44,893,437	M, C, EC	HSE1, HSE2, HSE4	1.99E-05	2.04E-04	3 A
	qHSE.pTN.SHW. pau.3B	AX-94699856	3B	74,705,329	М	HSE1, HSE2, HSE4	7.79E-05	5.02E-04	3B
	qOE.pTN.SHW.pau.3B	AX-95203429	3B	576,741,297	C, EC	OE1, OE3, OE4	1.21E-04	3.87E-04	3B
	qOE.pTN.SHW.pau.6B	AX-94549612	6B	719,419,172	C, EC	OE1, OE2, OE4	2.96E-04	4.16E-04	6B
	qOE.pTN.SHW.pau.7 A	AX-94535927	7 A	694,662,220	M, C, EC	OE1, OE2, OE4	1.91E-04	4.60E-04	7 A
TGW	qOE.TGW.SHW.pau.5D	AX-94987657	5D	498,036,030	М	OE1, OE2, OE4	2.56E-04	3.87E-04	5D
	qHSE.TGW.SHW. pau.7 A	AX-94469933	7 A	694,663,286	M, C, EC	HSE1, HSE3, HSE4	4.92E-04	6.50E-04	7 A
HTI_YpP	qHTI_YpP.SHW. pau.2B.1	AX-94907113	2B	38,339,714	M, C, EC	E1, E2, E4	7.39E-07	3.09E-05	2B
	qHTI_YpP.SHW. pau.2B.2	AX-94993577	2B	38,603,508	C, EC	E1, E2, E4	5.00E-05	3.67E-04	2B
	qHTI_YpP.SHW.pau.4B	AX-94461626	4 B	594,251,078	M, C, EC	E1, E2, E3, E4	9.04E-06	2.19E-04	4B
	qHTI_YpP.SHW.pau.4D	AX-94505143	4D	10,740,008	М	E2, E3, E4	5.35E-04	6.95E-04	4D

through backcrosses represents the smaller introgression from synthetic wheat in background of ABLs with whole set of S-NILib represents complete genome of SHW in smaller fragments. As anticipated, the SHWs showed better tolerance towards terminal heat stress as indicated by their high HTI values in comparison to cultivated hexaploid lines. The transgressive segregants were observed for HTI which indicated dispersion of positive and negative alleles. The reduction in trait values for seven agronomic traits in HSE indicated that terminal heat stress was detrimental for overall wheat growth with major impact on TGW. The rise in temperature under HSE initiated early flowering and forced maturity which resulted in significant reduction in grain filling period by 7–10 days. The temperature over three years in the current investigation showed HSE faced significant terminal heat stress.

A genome-wide association study was performed exploiting the phenotypic variability and identification of heat tolerant QTLs in the panel of 354 S-NILibs using 9,536 polymorphic SNPs from the 35 K Axiom® Wheat Breeder's Array. To eliminate the influence of the environmental background as much as possible to explain the true genetic variation components, we performed GWAS for all traits with the three different models (MLMM, CMLM, and ECMLM) in different environments under two sowing conditions and defined significant and repetitive SNPs in multiple environments $(E \ge 2)$ and models as true and reliable association loci. The 9536 polymorphic SNPs were evenly distributed throughout the whole genome and mapped the genome wide QTLs efficiently. 35 K SNP chip have been reported to efficiently map the agro-morphological traits (Wang et al. 2022). The two clear sub-groups of S-NILib were formed by PCA and neighbor-joining tree which represented diversity of two different SHW derived from two diverse Ae. tauschii accessions.

GWAS identified a total of 61 marker-trait associations (MTAs) across environments (OE and HSE) associated with different traits using multiple models. We adhered to MTAs originating from common haplotype region or previously reported. GWAS identified major genomic regions associated with PH on chromosome 6B and 6A. The genome annotations facilitated prediction of candidate genes governing PH. Ubiquitin like protein and growth regulating factor genes observed in QTL region were known to control plant height in wheat (le Roux et al. 2019). Interestingly, the wheat plant height locus RHT25 was also mapped near MTAs on chromosome 6A (Zhang et al. 2023).

The major QTL governing flag leaf length was observed on chromosome 5 A, and previous studies also reported fine mapping FL QTLs on 5 A (Xue et al. 2013; Liu et al. 2018; Singh et al. 2024). Genes related to nitrogen transportation such as nitrate transporter and ammonium transporter were observed in QTL region on 5 A. In rice, it was reported that a gene governing leaf width served as an important factor in nitrate uptake and its transport. Leaf width gene affects grain size and plant architecture by regulating nitrogen transfer (Zhu et al. 2020). Thus, there is a strong association of flag leaf size and nitrogen transportation, ultimately controlling yield. The MTAs for spike length were observed on chromosome 3D. A major candidate gene was identified in this region i.e., Pectin acetylesterase which is directly linked to growth of floral organs. The silencing of this gene in *Nico-tiana tabacum* resulted in plants bearing no fruits, due to distorted growth of pollen tube (Lubini et al. 2019). Thus, Pectin acetylesterase could be essential enzyme governing pectin modifications in floral organs and plant reproduction.

Clusters of genes of Flowering-promoting factor 1-like protein were observed near MTAs associated with spikelet number on chromosome 5A and 4B. The domestication gene Q present on 5 A chromosome in vicinity of MTAs regulates spikelet density (Simons et al. 2006; Zhang et al. 2011; Debernardi et al. 2017; Greenwood et al. 2017). The major candidate gene found near MTAs for pTN was Auxinresponsive protein. Auxin-responsive genes were regulating the tiller number in rice (Li et al. 2021). They play important role in the plant reproductive and the nutritional growth (Pekker et al. 2005). Clusters of Lipid transfer protein were observed in MTAs associated with thousand grain weight on 5D chromosome. Lipid transfer protein (OsLTPL18), is also known to control grain weight in rice (Li et al. 2023). The MTA associated with HTI was present on 4B chromosome (AX-94461626). The underlying gene of MTA was Ubiquitin carboxyl-terminal hydrolases which is known for maintenance of the circadian clock at high temperature (Hayama et al. 2019). It was reported that Arabidopsis de-ubiquitylating enzymes help maintain circadian clock period at elevated temperature, thus sustaining optimum period length at high temperatures. Validation of SNPs associated with traits of plant height (PH), flag leaf length (FL), spikelet number per spike (SN) and number of productive tillers (pTN) showed strong associations which could used for marker assisted transfer of these traits and strong candidate for future work.

Conclusion

Terminal heat stress is major concern in wheat production. We identified 11 S-NILibs as better performing under terminal heat stress conditions on the basis of eight environments (yearly variation, two sowing conditions and BLUPs). Our study identified 61 MTAs for major yield traits (in OE and HSE) and heat tolerance from SHWs developed from heat tolerant *Ae. tauschii* accessions. The SNPs identified for heat stress tolerance mechanism were converted into KASP markers. Core marker set will help to identify trait specific donor with favorable allele from wild donor. The favorable wild alleles for key traits of heat stress tolerance could be transferred to exhausted gene pool of hexaploid wheat through step wise marker assisted techniques. A better understanding of genomic landscape of SHWs would open avenues for its utilization in breeding climate resilient wheat.

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Competing interests The authors declare no competing interests.

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