

Genetic dissection of terminal heat-stress tolerance in wheat revealed by QTL mapping

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Abstract

Terminal heat stress adversely affects wheat development and productivity, but its genetic basis is strongly influenced by environmental variation. A doubled haploid population derived from KSG1222 × KSG1203 was evaluated for 17 phenological, morphological, spike, and yield-related traits under timely, late, and very late sowing across two years. Delayed sowing progressively reduced most traits, with biological yield, flag leaf area, peduncle length, and grain yield showing substantial reductions under severe heat stress. Biological yield, harvest index, thousand-grain weight, and spikelets per spike showed consistent positive associations with grain yield across environments. Genotyping-by-sequencing generated 122,112 polymorphic SNPs, of which 12,297 were used to construct a 2,737.84 cM linkage map. Combined individual- and multi-environment QTL analysis identified 179 QTLs, including loci with relatively stable additive effects and others strongly influenced by QTL × environment interactions. Multi-trait co-localization identified regions on chromosomes 4A and 7D affecting yield-related and developmental traits. Within the QTL intervals, 2,727 putative genes were annotated, of which 286 showed heat responsive expression based on in-silico expression profiling, and 170 were further prioritized based on their functional and biological relevance. These candidates implicated heat signalling, transcriptional regulation, proteostasis, redox homeostasis, photosynthesis, and carbohydrate metabolism in terminal heat-stress adaptation.

Key message

Stable and environment responsive QTLs and heat responsive candidate genes highlight the complex genetic architecture of terminal heat stress adaptation and provide targets for improving wheat resilience.

1. Introduction:

Rising global temperatures are expected to increasingly expose crops to temperatures beyond their optimal growth ranges, with global mean temperature projected to reach 1.5°C above the 1850–1900 baseline during 2021–2040, with the 1.5°C warming level likely to be exceeded under several emissions scenarios (IPCC, 2021; Kompas et al. 2024). In bread wheat (*Triticum aestivum* L.), optimal growth generally occurs within a moderate temperature range of approximately 17–25°C, whereas exposure to temperatures above 30°C during reproductive and grain-filling stages can induce heat stress and adversely affect grain yield and quality. In particular, post-flowering temperatures exceeding 32°C have been associated with reductions in individual grain weight and grain yield (Liu et al. 2026). Heat stress during grain development can impair assimilate availability and starch biosynthesis in the endosperm. Post-anthesis heat stress is particularly detrimental, as elevated temperatures shorten the grain-filling period and alter resource allocation, resulting in yield reductions of 6–51% under controlled conditions and 2–27% under field environments (Fernie et al. 2022). Reproductive development is also highly vulnerable to elevated temperature, with pollen formation, particularly during microsporogenesis and microgametogenesis, being among the most heat-sensitive processes in cereals (Hedhly et al. 2016). In wheat, temperatures above 30°C during meiosis and pollen maturation can reduce pollen viability and fertilization success, while heat-induced abnormalities in floret development and floral organ differentiation can further compromise seed

set and yield (Li et al. 2022; **Bheemanahalli et al.**). Due to the complex and quantitative nature of heat tolerance, elucidating the genomic basis of heat adaptation is essential for identifying and efficiently utilizing favourable alleles in wheat breeding. Quantitative trait locus (QTL) mapping provides a powerful framework for dissecting the genetic architecture of heat tolerance by identifying chromosomal regions associated with agronomic and physiological traits expressed under elevated temperatures (Bhusal et al. 2017; Jia et al. 2024; Manjunath et al. 2024). Closely linked markers can facilitate the selection and introgression of favourable QTL through marker-assisted selection; however, their breeding utility is often limited by QTL × environment interactions, small effects, and loose marker linkage. Therefore, identifying stable QTL with consistent effects across environments and tightly linked markers is essential for their effective deployment (Hu et al. 2021; Benaouda et al. 2022).

Advances in next-generation sequencing have facilitated the generation of genome-wide single-nucleotide polymorphism (SNP) markers, while genotyping-by-sequencing (GBS) has emerged as a cost-effective approach for high-density SNP discovery and genotyping (Elshire et al. 2011). By sequencing a reduced representation of the genome following restriction enzyme digestion, GBS enables the generation of large numbers of markers with broad genome coverage, making it particularly useful for genetic analysis of complex and polyploid genomes such as wheat. The incorporation of GBS-derived SNPs into linkage mapping can substantially increase marker density and improve the resolution of QTL intervals, thereby enhancing the ability to dissect complex traits and identify genomic regions of greater relevance to breeding (**Rayaprolu et al.** 2022). Candidate gene identification can bridge the gap between QTL detection and the biological mechanisms underlying heat tolerance in wheat. While QTL mapping identifies genomic regions associated with heat-responsive traits, integrating these regions with genome annotation, gene expression patterns, and functional information can help prioritize genes with potential roles in stress adaptation (Bag et al. 2026). Investigating these molecular mechanisms alongside QTL analysis can therefore provide deeper insights into the biological processes contributing to heat resilience and support the functional validation of candidate genes, ultimately improving the precision of molecular breeding strategies. The present study aimed to dissect the genetic basis of terminal heat-stress adaptation in wheat by integrating multi-environment phenotypic evaluation, genome-wide SNP genotyping, QTL analysis, and candidate-gene characterization.

2. Material and methods:

2.1. Plant materials:

The Doubled Haploid (DH) population investigated in this experiment was developed using the wheat-maize crossing technique from a cross between KSG1222 and KSG1203 at Washington State University, Pullman, USA (Laurie and Bennett, 1988). Among the two parents, KSG1203 is a heat stress-tolerant genotype, whereas KSG1222 is susceptible to heat stress, particularly under late-sown conditions. The male parent KSG1203 is a semi-dwarf wheat cultivar, known to possess complementary genetic mechanisms contributing to heat stress tolerance and was derived as a single-plant selection from the Egyptian variety *Giza 168* (Climate Resilient Wheat Innovation Lab, team led by Kulvinder Gill). It was originally released in Egypt in 1999, with the pedigree *MRL/BUC/Seri.CM93046-8M-0Y-0M-2Y-0B-0GZ*. The

susceptible parent KSG1222 is a selection from HD2967. The DH population consisted of 189 doubled haploid (DH) lines derived from the cross KSG1222 × KSG1203 and was used for all phenotypic and genotypic analyses.

2.2. Experimental site and establishment of heat stress environments

Field experiments were conducted during the Rabi seasons of 2023-24 and 2024-25 at the G. B. Pant University of Agriculture and Technology (GBPUA&T), Pantnagar, Uttarakhand, India. The experiments were carried out at two research farms, namely the Pantnagar Centre for Plant Genetic Resources (PCPGR) and the Norman E. Borlaug Crop Research Centre (N.E.B. CRC). Pantnagar is located at 29° N latitude and 79.3° E longitude, at an altitude of 243.84 m above mean sea level, in the Tarai region at the foothills of the Shivalik Himalayas. The region has a humid subtropical climate with cool winters and hot summers. The experimental fields are characterized by fertile alluvial soils with silty loam to loam texture, suitable for wheat cultivation. The experiment was laid out in an alpha-lattice design with two replications. Each replication comprised 16 incomplete blocks, each containing 12 plots, with genotypes randomly assigned within each block. Each plot consisted of two rows of 2 m length, maintaining 20 cm inter-row and 10 cm intra-row spacing. Seeds were manually sown at a rate of 20 g per plot (approximately 120 kg ha⁻¹). Recommended agronomic practices, including fertilizer application, irrigation, and weed management, were followed uniformly throughout the experiment.

To evaluate the response of wheat genotypes to different levels of terminal heat stress, three sowing environments were established during each cropping season: timely sown (TS) representing optimum conditions, late sown (LS) representing terminal heat stress, and very late sown (VLS) representing extended terminal heat stress. During the 2023-24 season at PCPGR, sowing was carried out on 8 November 2023 (TS1), 8 December 2023 (LS1), and 10 January 2024 (VLS1). During the 2024-25 season at N.E.B. CRC, sowing was conducted on 23 November 2024 (TS2), 24 December 2024 (LS2), and 12 January 2025 (VLS2). The staggered sowing dates were used to expose the crop to progressively increasing temperatures during the reproductive and grain-filling stages. Daily weather data, including maximum and minimum temperature, rainfall, relative humidity, and sunshine hours, were obtained from the University Meteorological Observatory located adjacent to the experimental site. Weather conditions during the experimental period are presented in the Results section.

2.3. Phenotypic evaluation and statistical analysis:

The DH wheat lines and their parents were evaluated for heat stress tolerance based on four phenological traits: days to heading (DTH), days to anthesis (DA), days to maturity (DM), and grain filling duration (GFD); four morphological traits: plant height (PH), peduncle length (PL), exposed peduncle length (E_PL), and flag leaf area (FLA); and nine yield and yield-related traits: spike length (SL), spikelets per spike (SPS), spike compactness (S_COMP), awn length (AL), productive tillers per meter (TPM), thousand-grain weight (TGW), grain yield (GY), biological yield (BY), and harvest index (HI). The traits were recorded under timely-sown (TS; non-stress), late-sown (LS; heat stress), and very late-sown (VLS; extended heat stress) conditions during two consecutive cropping seasons (2023-24 and 2024-25). Detailed descriptions of the traits are

provided in **Table S1**. Phenotypic data were analysed using META-R v6.04 (Alvarado et al. 2020), which uses the lmer function of the lme4 package in R and restricted maximum likelihood (REML) for estimation of variance components and genotype effects. For each environment, best linear unbiased estimates (BLUEs) were calculated using a linear mixed model with genotype treated as a fixed effect. BLUEs provide adjusted estimates of genotype performance and are commonly used as genotype-level inputs in two-stage analyses of multi-environment breeding trials when genotype effects are considered fixed (Schmidt et al. 2019; González-Barrios et al. 2019). The model used for individual environments under the alpha-lattice design was:

$$Y_{ijk} = \mu + \text{Rep}_i + \text{Block}_j(\text{Rep}_i) + \text{Gen}_k + \varepsilon_{ijk}$$

where, Y_{ijk} is the trait of interest, μ is the overall mean effect, Rep_i is the effect of the i^{th} replicate, $\text{Block}_j(\text{Rep}_i)$ is the effect of the j^{th} incomplete block within the i^{th} replicate, Gen_k is the effect of the k^{th} genotype, ε_{ijk} is the effect of the error associated with the i^{th} replication, j^{th} incomplete block, and k^{th} genotype, which is assumed to be independently and identically distributed normal with mean zero and variance $\sigma^2\varepsilon$. BLUEs were obtained for all 17 traits for each year and sowing environment separately. Pooled BLUEs were subsequently estimated for TS, LS, and VLS across years and locations and used for Pearson's correlation coefficients among traits. It was calculated based on using the agricolae package in R and visualized using the corrplot package (R Core Team, 2025).

Descriptive statistics and broad-sense heritability were estimated for each trait using the variability and METAN packages in R. Broad-sense heritability was calculated as:

$$h_{\text{bs}}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/nR}$$

where R is the number of replicates, n is the number of environments and σ^2g and σ^2e are the genotype and error variance components, respectively.

The percentage reduction in trait performance under heat stress was calculated separately for the parents and DH population using Microsoft Excel (Microsoft Corporation, 2024). Reduction under LS was calculated relative to TS, representing heat stress; reduction under VLS was calculated relative to TS, representing extended heat stress; and the difference between the two stress intensities was assessed as the reduction under VLS relative to LS. Percentage reduction was calculated according to Bennani et al. (2017):

$$\text{Reduction \%} = \frac{\text{Value under reference condition} - \text{Value under stress condition}}{\text{Value under reference condition}} \times 100$$

2.4. DNA extraction and Genotype-By-Sequencing:

Leaf samples were collected from one-month-old seedlings of the 189 DH lines along with the two parental genotypes for genomic DNA extraction. DNA was isolated using the cetyltrimethylammonium bromide

(CTAB) method described by Doyle and Doyle (1990) with minor modifications. DNA quality and integrity were assessed by electrophoresis on 0.8% agarose gel, while DNA concentration was measured using an Eppendorf™ UV Biophotometer and normalized to 200 ng μL^{-1} . High-quality DNA samples were lyophilized and submitted to NGB Diagnostics Pvt. Ltd., Noida, India, for genotyping-by-sequencing (GBS). GBS libraries were prepared following the protocol of Elshire et al. (2011) with minor modifications. Briefly, genomic DNA was digested using the restriction enzyme ApeKI, ligated to unique barcoded adapters, pooled, PCR-amplified, purified, and sequenced on the Illumina NovaSeq 6000 platform. Raw sequence data were processed using CASAVA software, and sequence reads were exported in FASTQ format for downstream analyses. Sequence quality was evaluated using FASTQC, followed by adapter trimming and quality filtering with Trim Galore (minimum Phred score of 20 and minimum read length of 20 bp). High-quality reads were aligned to the *Triticum aestivum* reference genome (IWGSC RefSeq v2.1) using BWA-MEM. SNP discovery was performed using the UGBS-GATK pipeline, and variant filtering was carried out using VCFtools. Only chromosome-mapped, bi-allelic SNPs were retained. Additional filtering removed monomorphic loci and heterozygous SNPs in the parental lines. The final SNP dataset was filtered using a minor allele frequency (MAF) $\geq 5\%$ and marker presence in at least 20% of the DH population before linkage map construction.

2.5. Linkage and QTL mapping:

The filtered SNP dataset was used for linkage map construction and QTL analysis. Markers showing significant segregation distortion (χ^2 test, $P < 0.01$) were excluded using IciMapping (<http://www.isbreeding.net/>). Marker grouping was performed at a logarithm of odds (LOD) threshold of 3.0. Marker ordering was carried out using the regression mapping algorithm implemented in JoinMap version 4.1, followed by rippling with a window size of one. Recombination frequencies were converted into genetic distances using the Kosambi mapping function to generate linkage groups corresponding to the 21 wheat chromosomes. The genetic linkage map was visualized using MapChart version 2.3.2 (Voorrips, 2002). Chromosome-wise marker density plots were generated using the LinkageMapView package (version 2.1.2) in R (Ouellette et al. 2018), enabling graphical representation of marker distribution across the 21 wheat chromosomes. QTL analysis was performed using IciMapping version 4.2.53 (Wang et al. 2015). Single-environment QTLs were detected using the Inclusive Composite Interval Mapping for additive effects (ICIM-ADD) module with a walking speed of 1 cM. Genome-wide significance thresholds were determined by 1,000 permutation tests at $P < 0.05$, and QTL positions were assigned according to the peak LOD score within each interval. Multi-environment QTL analysis was performed using the Multi-Environment Trials (MET) module of IciMapping to estimate additive effects, additive $\times$ environment interactions, and QTL $\times$ environment interactions. Mapping was conducted using the ICIM-ADD method with a scanning interval of 1 cM, a stepwise regression probability of 0.001, and a LOD threshold of 2.5. Significance thresholds were determined using 1,000 permutation tests ($P < 0.05$). QTLs explaining more than 10% of the phenotypic variance (PVE) in at least one environment and consistently detected in three or more environments were considered major and stable QTLs. QTLs were retained for further analysis if they satisfied at least one of the following criteria: (i) detected in two or more individual environments, (ii) identified in the multi-environment trial (MET) analysis, (iii) exhibited a LOD score ≥ 5.0 , (iv) explained at least 5% of the phenotypic variance (PVE), or (v) displayed overlapping confidence intervals across

environments, indicating consistent genomic positions. They were classified as Consistently detected QTLs (supported by both single-environment and MET analyses); QTLs with predominant additive effects (relatively stable; $\text{LOD}(A) \geq$ or close to $\text{LOD}(A \times E)$) and QTLs with strong QTL $\times$ environment interactions ($\text{LOD}(A \times E) > \text{LOD}(A)$, $\text{PVE}(A \times E) > \text{PVE}(A)$, and/or markedly different AbyE values). QTLs were designated using the prefix Q, followed by the trait abbreviation, *gbpu* (G.B. Pant University of Agriculture and Technology), and the chromosome identifier (Boden et al. 2023).

2.6. Candidate Gene Identification and in silico expression analysis:

Candidate genes associated with each significant QTL were identified using the physical positions of the flanking markers aligned to the IWGSC RefSeq v2.1 wheat reference genome. All annotated genes located within the physical interval of each QTL were retrieved, and overlapping QTL intervals were treated as common candidate regions. Gene annotations, genomic coordinates, and predicted protein functions were extracted for downstream analyses. Candidate-associated genes (CAGs) were functionally annotated using BLASTX against the NR database. Functional annotation of the candidate genes was performed using the UniProt database (<https://www.uniprot.org/>) to obtain gene descriptions, molecular functions and protein information. Gene Ontology (GO) classification, including Biological Process, Molecular Function and Cellular Component, was retrieved through UniProt, while pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database through the KEGG Automatic Annotation Server (KAAS) (<https://www.genome.jp/tools/kaas/>). Transcription factor families were identified using PlantTFDB v5.0 with an E -value threshold of 1×10^{-5} .

To prioritize genes associated with terminal heat stress tolerance, the annotated candidate genes were further subjected to in silico expression analysis using the WheatOmics platform (<https://wheatomics.sdau.edu.cn/>), and genes showing differential expression under heat stress conditions were retained. The candidate genes were investigated using four publicly available transcriptome datasets available through the WheatExpression Browser powered by WheatOmics (Ma et al. 2021). Only heat stress datasets were considered for expression analysis. These datasets included: (i) heat-treated seedlings of cv. TAM107 (Liu et al. 2015); (ii) leaf, root, and grain tissues of the heat-tolerant cultivar Zubkov and the heat-susceptible cultivar Atay85 under grain-filling heat stress; (iii) flag leaf, stem, and root tissues of HD2985 (tolerant) and HD2329 (susceptible) exposed to heat stress during pollination (Kumar et al. 2015); and (iv) flag leaf and grain tissues of Chinese Spring subjected to short-term heat treatments (37°C) at multiple time points. Gene expression values were retrieved as transcripts per million (TPM) and used to calculate fold change (FC) and absolute $\log_2$ fold change ($\log_2\text{FC}$) according to the following equations:

$$\text{Fold Change (FC)} = \frac{\text{Heat Stress}}{\text{Control}} \log_2\text{FC} = \log_2 \left(\frac{\text{HeatStress}}{\text{Control}} \right)$$

Genes with positive $\log_2\text{FC}$ values were considered upregulated, whereas those with negative values were considered downregulated under heat stress. Expression heatmaps were generated using R studio, and the shortlisted candidate genes were explored using gene regulatory network analysis through KnetMiner

database (https://app.knetminer.com/plants-lite/Triticum_aestivum) (Hassani-Pak et al. 2021) to identify their reported associations with heat stress and other abiotic stress responses.

3. Results:

3.1. Temperature Regimes Under Contrasting Sowing Dates

The staggered sowing schedule successfully exposed the late- and very late-sown crops to progressively higher temperatures during the post-heading and grain-filling period (Fig. 1). During the 2023-24 season, the average minimum and maximum temperatures from heading to maturity were 9.8 and 25.7°C under TS1, 12.0 and 29.0°C under LS1, and 16.2 and 34.9°C under VLS1, respectively. Compared with the timely sown crop, the late-sown crop experienced increases of 2.2°C in minimum temperature and 3.3°C in maximum temperature, whereas the very late-sown crop was exposed to substantially higher temperatures, with increases of 6.4°C and 9.2°C, respectively. Likewise, the VLS1 crop experienced 4.2°C higher minimum and 5.9°C higher maximum temperatures than the LS1 crop during the post-heading period. A similar trend was observed during the 2024-25 season. The average minimum and maximum temperatures during the post-heading period were 11.1 and 27.5°C under TS2, 13.5 and 31.8°C under LS2, and 15.8 and 33.5°C under VLS2, respectively. Compared with TS2, the LS2 crop experienced increases of 2.4°C in minimum temperature and 4.3°C in maximum temperature, while the VLS2 crop experienced increases of 4.7°C and 6.0°C, respectively. Relative to LS2, the VLS2 crop was exposed to an additional increase of 2.3°C in minimum temperature and 1.7°C in maximum temperature during the reproductive and grain-filling stages. Compared with 2023-24, the 2024-25 season experienced generally warmer conditions under timely and late sowing, whereas the 2023-24 VLS environment recorded the highest average maximum temperature (35.0°C) during the post-heading period.

3.2. Phenotypic evaluation of the DH population:

The DH population exhibited wide phenotypic variation for all measured traits across the six experimental environments. The BLUE values estimated for each environment covered broad ranges, while boxplots and violin plots demonstrated continuous variation with substantial dispersion among DH lines (Fig. 2a and 2b). Except for plant height, which exhibited a trimodal distribution, all traits showed approximately normal distributions. Transgressive segregation beyond both parents was observed for all agronomic traits, indicating the presence of novel allele combinations within the DH population. Wide variation in developmental timing was evident within the DH population, as reflected by the broad ranges observed for days to heading and days to maturity across all environments (**Table S2**). Analysis of variance revealed highly significant ($P < 0.01$) genotypic differences for all traits across environments, confirming the existence of substantial genetic variation suitable for subsequent QTL analysis. The parental lines exhibited contrasting responses across the six environments. KSG1203 consistently maintained greater peduncle length, exposed peduncle length, flag leaf size, spikelets per spike, thousand-grain weight, and grain yield under heat stress conditions than KSG1222. Under very late sowing, KSG1203 produced higher grain yield than KSG1222 in both VLS1 (422 vs. 384 g m⁻²) and VLS2 (347 vs. 329 g m⁻²). Under timely sowing, however, KSG1222 showed slightly higher grain yield, indicating superior yield potential under

optimum conditions. The contrasting phenotypic responses of the two parents, together with the extensive transgressive segregation observed in the DH population, demonstrate the suitability of this population for the identification of genomic regions associated with terminal heat stress tolerance.

Progressive delay in sowing resulted in a consistent reduction in the mean values of most phenological, morphological, and yield-related traits (**Table S2**). Percent decline analysis further quantified the impact of delayed sowing on trait performance (Table 1). Reductions were generally greater under VLS than LS environments for most traits. Phenological traits exhibited moderate reductions (approximately 10–13% under LS and 19–21% under VLS during 2023-24), whereas morphological traits were more severely affected. Among the morphological traits, flag leaf area showed the greatest reduction (48.8–50.2%), followed by peduncle length (41.2–45.9%) and exposed peduncle length (26.6–32.2%). Among the yield-related traits, biological yield exhibited the greatest reduction, reaching 47.5% under VLS2, followed by grain yield (29.7%) and thousand-grain weight (20.5%). In contrast, spike compactness and harvest index exhibited slight increases in certain stress environments, as indicated by negative percentage reduction values. Most phenological and morphological traits exhibited low to moderate coefficients of variation (CV < 10%), indicating good experimental precision and reliable phenotypic measurements. In contrast, yield-related traits showed comparatively higher CV values. Grain yield exhibited CV values ranging from 14.05 to 22.72%, harvest index from 11.50 to 21.09%, and flag leaf area from 11.37 to 20.79% across environments (**Table S2**). The highest CV values were generally observed under the very late-sown environments, indicating greater phenotypic variability under severe terminal heat stress. Broad-sense heritability was moderate to high for the majority of the evaluated traits across environments (**Table S2**). Peduncle length, exposed peduncle length, flag leaf traits, productive tillers per metre, and biological yield consistently exhibited high heritability (> 0.80), whereas grain yield showed moderate heritability (0.48–0.76). In contrast, plant height, harvest index, spike compactness, and spikelets per spike exhibited lower heritability under severe heat stress environments, reflecting a greater influence of environmental variation on their expression.

Table 1

Percent (%) decline in the mean values of DH lines for all traits in late sown (LS) and very late sown (VLS) environments in comparison to the timely sown (TS) environment in year 2023-24 and 2024-25 respectively

TRAIT	PERCENT DECLINE in year 2023-24			PERCENT DECLINE in year 2024-25		
	LS1 relative to TS1	VLS1 relative to TS1	VLS1 relative to LS1	LS2 relative to TS2	VLS2 relative to TS2	VLS2 relative to LS2
DH	10.66	20.95	11.51	11.99	16.04	8.53
DA	10.44	20.77	11.53	12.39	15.78	8.49
DM	11.46	20.34	10.03	12.31	9.79	9.32
GFD	13.46	19.51	6.99	12.13	-3.85	11.20
PH	8.69	15.06	6.97	10.63	18.60	8.63
PL	23.85	41.20	22.79	12.17	45.86	7.58
E_PL	22.05	32.16	12.97	19.30	26.56	7.98
FLA	34.26	50.23	24.28	13.91	48.76	15.32
SL	6.23	16.80	11.27	11.11	33.42	5.97
SPS	7.92	15.25	7.96	8.68	27.23	8.30
S_COMP	-2.98	0.47	3.36	1.98	9.40	-2.44
AL	2.79	18.07	15.71	0.84	10.87	11.89
TPM	20.29	28.22	9.95	1.49	3.57	57.92
TGW	9.99	15.75	6.40	19.15	20.52	2.27
GY	12.75	25.11	14.17	25.99	29.74	35.29
BY	21.09	30.43	11.84	25.66	47.50	11.48
HI	-14.65	-14.95	-0.27	-0.09	-30.02	19.27

Figure 2a Phenotypic variation for traits observed in Rabi 2023-24, under non-heat stress (blue), heat stress (green) and extended heat stress (orange) conditions. In each treatment, Violin plot represents trait distribution; box plot represents the minimum, first quartile, mean/median, third quartile, and maximum values in the dataset; and dots represent the individual genotypes. The gray lines depict the performance of same genotype across treatments with connecting dots. Diamond: Mean; Red: KSG1203; Black: KSG1222
***Significant at $p < 0.001$

Correlations among traits

Across all environments, biological yield showed the strongest positive association with grain yield ($r = 0.52-0.62$), followed by harvest index ($r = 0.41-0.56$), indicating their consistent contribution to grain yield

under both optimum and heat stress conditions (Fig. 3). Thousand-grain weight ($r = 0.36 - 0.19$) and spikelets per spike ($r = 0.19 - 0.16$) also maintained significant positive correlations with grain yield across environments, although the strength of these associations varied with stress intensity. Under timely and late sowing conditions, spike length was positively associated ($r = 0.31$) with grain yield; however, this relationship weakened ($r = 0.13$) under very late sowing. In contrast, productive tillers per metre showed a stronger association ($r = 0.18$) with grain yield under extended terminal heat stress, suggesting a greater contribution to yield maintenance under severe stress. Strong positive correlations among phenological traits (DTH, DA and DM) were observed across all environments, reflecting synchronized crop development. Peduncle length and exposed peduncle length consistently exhibited strong positive associations, while spike length and spikelets per spike remained positively correlated irrespective of sowing environment. The correlation patterns indicate that although heat stress altered the relative importance of several morphological traits, biomass production, partitioning efficiency, grain weight and spike fertility remained the principal determinants of grain yield.

3.3. Genome-wide SNP discovery and high-density linkage map construction:

Genotyping-by-sequencing of the DH wheat population generated 34,099,469 sequence variants, including 33,722,826 SNPs and 376,643 InDels. Of the identified SNPs, 33,676,232 were bi-allelic, and 33,233,605 were successfully aligned to the wheat reference genome. After removal of heterozygous loci and filtering for minor allele frequency ($MAF \geq 5\%$), marker presence ($\geq 20\%$ of the DH population), and parental polymorphism, 122,112 high-quality polymorphic SNPs were retained for downstream genetic analysis. The parental lines showed high alignment efficiencies with the wheat reference genome, with mapping rates of 92.16% for KSG1222 and 85.88% for KSG1203. The retained polymorphic SNPs were distributed across all 21 wheat chromosomes, although their distribution was uneven. Chromosome 3B harboured the highest number of SNPs (16,282), followed by 6B (13,216) and 5B (8,571), whereas chromosomes 4A (2,421), 2A (2,451), and 3A (2,895) contained the fewest markers. Among the three wheat sub-genomes, the B genome contained the largest proportion of polymorphic SNPs, followed by the D and A genomes. The high-density genetic linkage map was constructed using 12297 SNP markers spanning a cumulative genetic distance of 2737.84 cM across the 21 wheat chromosomes with an average inter-marker distance of 0.223 cM (**Table S3**). The marker distribution and density across the 21 linkage groups are shown in Fig. 4, illustrating variation in marker coverage along individual chromosomes.

3.4. QTL Analysis:

QTL mapping was performed using ICIM-ADD for individual environments (BIP) (**Table 2**; **Table S4**) and across environments (MET) (**Table S5**). Across the 17 traits, 179 QTLs were detected in at least one environment and supported by either MET analysis or detection in multiple environments. The number of QTLs detected per trait ranged from 6 for awn length to 17 for days to anthesis, with LOD scores and phenotypic variance explained (PVE) varying substantially among traits. Favourable alleles were contributed by both parents, although KSG1203 was the predominant donor for several phenological and

yield-related traits, whereas KSG1222 contributed more favourable alleles for plant height, grain-filling duration, and spikelet number. QTLs were detected on all linkage groups, although their number and distribution varied among chromosomes (Fig. 5).

Phenological traits:

For phenological traits, 15 QTLs for days to heading spanning across 11 chromosomes, 17 for days to anthesis across 12 chromosomes, 7 for days to maturity across 7 chromosomes, and 10 for grain filling duration across 8 chromosomes, were identified. For DTH, *qDTH.gbpu-7D* showed the highest LOD (31.0), whereas *qDTH.gbpu-2D.2* and *qDTH.gbpu-6A.1* accounted for the largest PVE (13.9 and 13.6%, respectively). Twelve DTH QTLs were supported by MET analysis, with *qDTH.gbpu-5D* showing the highest MET LOD (89.39). Among DTA QTLs, *qDA.gbpu-7D* was the major QTL explaining the largest proportion of phenotypic variance (15.10%). Nine of the 17 BIP QTLs were confirmed by MET, with *qDA.gbpu-2B* showing the highest MET LOD (44.79) and PVE (8.82%). The confirmed DTA loci differed in their additive and QTL × environment effects, with *qDA.gbpu-5D.1*, *qDA.gbpu-2A.1*, *qDA.gbpu-6D*, *qDA.gbpu-1B*, and *qDA.gbpu-7B* showing relatively greater additive effects, whereas *qDA.gbpu-2B*, *qDA.gbpu-6A*, *qDA.gbpu-5B.1*, and *qDA.gbpu-2D* showed stronger interaction effects. For DTM, four of seven QTLs were confirmed by MET, with *qDM.gbpu-3A* showing the highest BIP LOD (47.58) and *qDM.gbpu-5A* and *qDM.gbpu-1A* being detected across multiple environments. Five of the ten GFD QTLs were confirmed by MET, with *qGFD.gbpu-1B* and *qGFD.gbpu-5B* showing the highest MET LOD and PVE. No major QTL was detected for GFD.

Morphological and spike related traits:

For morphological and spike-related traits, 7 QTLs for plant height identified across 6 chromosomes, 11 for peduncle length across 8 chromosomes, 12 for exposed peduncle length across 10 chromosomes, 11 for flag leaf area across 8 chromosomes, 7 for spike length across 6 chromosomes, 11 for spikelets per spike across 7 chromosomes, 11 for spike compactness across 11 chromosomes, and 6 for awn length across 6 chromosomes were detected. Several loci were repeatedly detected across sowing environments and confirmed by MET analysis. For plant height, *qPH.gbpu-6D* showed the highest BIP LOD (49.46), whereas *qPH.gbpu-3D* explained the largest PVE (11.27%); four QTLs were confirmed by MET, including *qPH.gbpu-2D.1* and *qPH.gbpu-2D.2*. For peduncle length, *qPL.gbpu-2B* had the highest LOD (40.00) and PVE (13.00%), while *qPL.gbpu-5D* was repeatedly detected across three environments and confirmed by MET. For exposed peduncle length, eight of 12 BIP QTLs were confirmed by MET, with an additional locus on chromosome 1D (*qE_PL.gbpu-1D.2*) showing a high MET LOD (39.40) and PVE (20.33%). For flag leaf area, seven loci were detected in both BIP and MET analyses, with *qFLA.gbpu-7D.2* showing the highest MET LOD (15.93) and PVE (15.67%). For spike length, two QTLs were supported by both BIP and MET analyses, with *qSL.gbpu-2A.1* showing a predominantly additive effect and *qSL.gbpu-5A* a stronger interaction component. For spikelets per spike, four loci were supported by both analyses, with *qSPS.gbpu-2D.1* showing the highest MET LOD (10.33) and PVE (19.13%). Seven of the 11 spike compactness QTLs were detected in both analyses, although most confirmed loci showed substantial QTL × environment interaction. Only one awn length QTL, *qAL.gbpu-4A*, was confirmed by MET.

Yield related traits:

For yield and yield-related traits, 12 QTLs for effective tillers per meter identified across 9 chromosomes, 14 for thousand grain weight across 10 chromosomes, 11 for grain yield across 7 chromosomes, 9 for biological yield across 8 chromosomes, and 8 for harvest index across 7 chromosomes, were identified. For effective tillers per meter, four QTLs were confirmed by MET, with *qTPM.gbpu-1B.2* showing the highest MET LOD (39.16), although its effect was strongly influenced by QTL × environment interaction. Six TGW QTLs were confirmed by MET, with *qTGW.gbpu-7D* showing the highest MET LOD (25.60) and PVE (12.12%); in contrast, *qTGW.gbpu-2D.1* and *qTGW.gbpu-7B.1* showed relatively greater additive effects. *qGY.gbpu-1B.1* was identified as major QTL, explaining 11.69% phenotypic variance. Nine of the 11 grain yield QTLs were confirmed by MET, with *qGY.gbpu-6D* showing the highest MET LOD (16.12) and PVE (9.17%), although its effect was strongly influenced by QTL × environment interaction. Biological yield displayed three major QTLs, with *qBY.gbpu-7B* exhibiting the highest LOD score (38.48) and explaining 20.41% of the phenotypic variance. *qBY.gbpu-1B* with 25.36 LOD, 11.58% PVE and *qBY.gbpu-4D* with 13.90% PVE identified under stress environments were major QTLs. Four biological yield QTLs were confirmed by MET, and all showed greater interaction than additive effects. Four harvest index QTLs were similarly confirmed, with *qHI.gbpu-6D* showing the highest PVE (10.57%) but substantial QTL × environment interaction.

QTL co-localization:

Co-localization analysis revealed several genomic regions harbouring QTLs for multiple traits. On chromosome 4A, a co-localized region at approximately 72 cM harboured QTLs for thousand-grain weight (*qTGW.gbpu-4A.2*), grain yield (*qGY.gbpu-4A*), and harvest index (*qHI.gbpu-4A*). The *qTGW.gbpu-4A.2* QTL was located at 72.0 cM (within the physical interval Chr4A:187,250,785 – 187,250,845 bp), whereas *qGY.gbpu-4A* and *qHI.gbpu-4A* were detected at 72.7 cM (within Chr4A:187,250,845 – 193,659,124 bp), with overlapping genetic confidence intervals. A second prominent co-localized region was detected on chromosome 7D at approximately 69 cM, where QTLs for days to heading (*qDTH.gbpu-7D*), peduncle length (*qPL.gbpu-7D.2*), and flag-leaf area (*qFLA.gbpu-7D.2*) were mapped. All three QTLs occurred within the physical interval Chr7D:106,248,308–106,475,036 bp and showed overlapping confidence intervals spanning approximately 68.4–69.2 cM.

Table 2

Prominent QTLs identified for the traits evaluated under contrasting environments, showing QTL number and name, chromosome, peak position, flanking markers and their physical positions, LOD score, percentage of phenotypic variation explained (PVE), additive effect, confidence interval, contributing parent, and environments in which the QTL was detected.

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
1	<i>qDTH.gbpu-2A.1</i>	2	64.8	9.1	6.4	1.47	LS1 and MET
2	<i>qDTH.gbpu-2A.2</i>	2	72.8	12.8	3.7	1.49	TS2 and MET
3	<i>qDTH.gbpu-3A</i>	3	90.59	5.9	3.5	1.43	TS1 and MET
4	<i>qDTH.gbpu-4A</i>	4	68.8	4.3	8.7	-1.55	LS2 and MET
5	<i>qDTH.gbpu-6A.1</i>	6	0	17.7, 6.3	13.6, 8.6	2.18, 1.53	LS1 and VLS1
6	<i>qDTH.gbpu-6A.2</i>	6	60	10.9	2.5	-1.22	TS2 and MET
7	<i>qDTH.gbpu-7A.1</i>	7	20.8	4.6	5.1	1.31	LS1 and MET
8	<i>qDTH.gbpu-7A.2</i>	7	146.40	17.7	5.1	1.55	VLS2
9	<i>qDTH.gbpu-5B</i>	12	36.5	7.3, 7.5	4.2, 1.8	1.59, 0.95	TS1, VLS2 and MET
10	<i>qDTH.gbpu-7B</i>	14	43.2	2.7	1.9	-0.81	LS1 and MET
11	<i>qDTH.gbpu-1D</i>	15	129.60	4.3	7.0	1.37	VLS1 and MET
12	<i>qDTH.gbpu-2D.1</i>	16	68.8	3.7	3.3	1.07	LS1 and MET
13	<i>qDTH.gbpu-2D.2</i>	16	111.09	20.1	13.9	-2.83	TS1 and MET
14	<i>qDTH.gbpu-5D</i>	19	73.29	5.9	3.6	-1.44	TS1 and MET
15	<i>qDTH.gbpu-7D</i>	21	68.8	31.0	8.8	-2.29	TS2
Days to Anthesis (DA)-17							
1	<i>qDA.gbpu-2A.1</i>	2	73.09	71.86, 3.68	1.58, 3.68	0.90, 1.51	TS1, TS2 and MET
2	<i>qDA.gbpu-2A.2</i>	2	16	131.34	7.74	2.02	TS1

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
3	<i>qDA.gbpu-6A</i>	6	0	14.40, 13.75	11.03, 5.99	1.55, 1.57	LS1, VLS1 and MET
4	<i>qDA.gbpu-7A.1</i>	7	34.4	6.45	2.61	-1.03	VLS1
5	<i>qDA.gbpu-7A.2</i>	7	35.2	5.88	3.51	-1.27	VLS2
6	<i>qDA.gbpu-1B</i>	8	82.4	3.44	5.13	1.79	TS2 and MET
7	<i>qDA.gbpu-2B</i>	9	16.8	12.16, 9.69	5.94, 5.10	1.65, 1.42	LS2, VLS2 and MET
8	<i>qDA.gbpu-5B.1</i>	12	36.5	118.39, 9.80	5.30, 3.78	1.67, 1.34	TS1, VLS2 and MET
9	<i>qDA.gbpu-5B.2</i>	12	95.20	10.94, 7.88	4.50, 3.53	1.45, 1.19	LS2, VLS2
10	<i>qDA.gbpu-7B</i>	14	43.2	5.64	4.24	-0.94	LS1 and MET
11	<i>qDA.gbpu-2D</i>	16	68.8	4.55, 3.08	4.57, 1.52	0.99, 0.85	LS1, VLS2 and MET
12	<i>qDA.gbpu-3D</i>	17	68.8	3.63, 56.10	2.91, 0.90	0.78, 0.68	LS1, TS1
13	<i>qDA.gbpu-5D.1</i>	19	25.6	5.67, 5.73	7.72, 4.95	-2.19, 1.40	TS2, VLS1 and MET
15	<i>qDA.gbpu-5D.3</i>	19	59.2	9.72, 5.34	4.17, 2.74	-1.30, -1.14	LS2, VLS2
16	<i>qDA.gbpu-6D</i>	20	83.2	6.52	7.30	-2.14	TS2 and MET
17	<i>qDA.gbpu-7D</i>	21	92.00	24.46	15.10	-2.48	LS2
Days to Maturity (DM)-7							
1	<i>qDM.gbpu-1A</i>	1	85.6	10.69, 21.56	5.41, 6.08	1.55, 2.02	TS2, LS2
2	<i>qDM.gbpu-3A</i>	3	76	47.58	7.25	-1.91	VLS2
3	<i>qDM.gbpu-5A</i>	5	13.6	7.44, 10.75, 39.26	4.16, 6.36, 5.28	-1.45, -2.01, -1.89	TS1, LS1, LS2 and MET
4	<i>qDM.gbpu-7A</i>	7	15.4	10.29, 6.22, 21.87	5.94, 3.15, 2.25	1.75, 1.73, 1.07	TS1, VLS1, VLS2 and MET
5	<i>qDM.gbpu-3B</i>	10	44.8	7.14	4.27	-1.64	LS1 and MET

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
6	<i>qDM.gbpu-4B</i>	11	38.4	5.47	1.98	0.94	TS2 and MET
7	<i>qDM.gbpu-7B</i>	14	82.69	9.02	6.16	1.76	TS1
Grain Filling Duration (GFD)-10							
1	<i>qGFD.gbpu-1A.1</i>	1	30.4	3.76	6.37	-1.08	VLS2
2	<i>qGFD.gbpu-1A.2</i>	1	68.8	12.82	2.78	1.00	TS2 and MET
3	<i>qGFD.gbpu-4A</i>	4	81.09	10.98	6.90	-1.44	TS1 and MET
4	<i>qGFD.gbpu-5A.1</i>	5	54.4	11.76	7.73	1.67	TS2
5	<i>qGFD.gbpu-5A.2</i>	5	67.2	9.72, 9.34	2.33, 3.23	1.20, 0.69	LS1, LS2
6	<i>qGFD.gbpu-6A</i>	6	0	20.39	4.84	-1.75	LS1
7	<i>qGFD.gbpu-1B</i>	8	70.4	3.78	4.83	-0.94	VLS2 and MET
8	<i>qGFD.gbpu-5B</i>	12	1.5	7.64, 28.19, 17.43	4.47, 9.43, 8.57	-1.16, -2.42, -2.18	TS1, LS1, VLS1 and MET
9	<i>qGFD.gbpu-1D</i>	15	103.99	3.97	2.43	0.85	TS1 and MET
10	<i>qGFD.gbpu-5D</i>	19	46.4	11.75	5.19	-0.89	LS2
Plant Height (PH)-6							
1	<i>qPH.gbpu-7A</i>	7	99.99	5.01, 16.83	9.41, 5.50	-3.15, -3.10	TS1, LS1
2	<i>qPH.gbpu-4B</i>	11	42.4	6.99	1.43	-1.58	LS1 and MET
3	<i>qPH.gbpu-2D.1</i>	16	61.99	4.14, 10.52, 40.47	5.18, 3.25, 6.50	2.33, 2.38, 2.58	TS1, LS1, LS2 and MET
4	<i>qPH.gbpu-2D.2</i>	16	26.90	3.59	5.93	-2.49	TS1 and MET
5	<i>qPH.gbpu-3D</i>	17	67.2	3.34	11.27	-2.47	VLS1 and MET
6	<i>qPH.gbpu-6D</i>	20	69.6	6.26, 49.46	1.27, 7.81	1.49, -2.82	LS1, LS2

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
Peduncle Length (PL)-10							
1	<i>qPL.gbpu-2A</i>	2	80.8	3.29	5.16	-1.22	LS2 and MET
2	<i>qPL.gbpu-3A</i>	3	39.7	13.10	9.94	-1.55	TS1
3	<i>qPL.gbpu-7A</i>	7	36.3	12.68, 6.14	8.10, 4.79	1.40, 1.55	TS1, LS1
4	<i>qPL.gbpu-2B</i>	9	100.80	40.00	13.00	-2.62	VLS2
5	<i>qPL.gbpu-5D</i>	19	55.39	9.47, 5.36, 5.26	5.88, 3.24, 8.22	1.19, 1.27, 1.54	TS1, LS1, LS2 and MET
6	<i>qPL.gbpu-6D.1</i>	20	22.90	10.64	6.69	1.26	TS1 and MET
7	<i>qPL.gbpu-6D.2</i>	20	74.4	10.95	7.67	-1.25	TS2 and MET
8	<i>qPL.gbpu-7D.1</i>	21	66.4	5.10	4.26	0.93	TS2
9	<i>qPL.gbpu-7D.2</i>	21	68.8	39.14	10.68	2.38	VLS2
10	<i>qPL.gbpu-7D.3</i>	21	4.8	11.14	5.66	-1.69	LS1
Exposed Peduncle Length (E_PL)-11							
1	<i>qE_PL.gbpu-2A.1</i>	2	68.8	2.84	1.45	-1.12	VLS1 and MET
2	<i>qE_PL.gbpu-2A.2</i>	2	94.49	2.83	4.09	1.40	TS1 and MET
3	<i>qE_PL.gbpu-3A</i>	3	72.39	4.72	8.95	-2.07	TS1
4	<i>qE_PL.gbpu-5A</i>	5	84	2.63	3.48	0.81	TS2 and MET
5	<i>qE_PL.gbpu-6A</i>	6	58.4	4.66	6.21	1.08	TS2 and MET
6	<i>qE_PL.gbpu-6B</i>	13	29.6	9.70	8.91	1.62	LS2 and MET
7	<i>qE_PL.gbpu-1D.1</i>	15	17.6	8.89	10.09	1.38	TS2
8	<i>qE_PL.gbpu-2D</i>	16	149.6	15.38, 32.07	7.14, 6.56	-2.51, -2.38	VLS1, LS1 and MET
9	<i>qE_PL.gbpu-3D</i>	17	68.8	30.78	6.62	-2.37	LS1
10	<i>qE_PL.gbpu-4D.1</i>	18	70.4	4.21	7.31	-1.13	VLS2 and MET

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
11	<i>qE_PL.gbpu-4D.2</i>	18	116.0	5.14	0.81	0.83	LS1 and MET
Flag Leaf Area (FLA)- 11							
1	<i>qFLA.gbpu-1A</i>	1	74.4	11.92	11.70	-2.15	LS2 and MET
2	<i>qFLA.gbpu-4A</i>	4	86.4	5.35	4.20	-1.28	TS2 and MET
3	<i>qFLA.gbpu-1B</i>	8	56	89.55	8.05	-3.58	VLS1
4	<i>qFLA.gbpu-2B</i>	9	97.60	14.39	2.49	1.99	LS1 and MET
5	<i>qFLA.gbpu-7B</i>	14	86.59	4.97	6.31	-3.26	TS1 and MET
6	<i>qFLA.gbpu-2D.1</i>	16	28	3.77	9.13	2.03	VLS1
7	<i>qFLA.gbpu-2D.2</i>	16	54.4	2.88, 17.42	2.60, 0.67	-1.01, -1.02	TS2, LS1
8	<i>qFLA.gbpu-5D.1</i>	19	6.4	7.52	8.42	2.40	VLS2
9	<i>qFLA.gbpu-5D.2</i>	19	16	3.08	6.54	1.74	VLS2 and MET
10	<i>qFLA.gbpu-7D.1</i>	21	58.79	5.31	5.32	3.01	TS1 and MET
11	<i>qFLA.gbpu-7D.2</i>	21	69.09	8.65	8.93	3.87	TS1 and MET
Spike Length (SL)-7							
1	<i>qSL.gbpu-1A</i>	1	100.0	20.62	6.14	0.52	TS2
2	<i>qSL.gbpu-2A.1</i>	2	30.4	3.28	7.58	-0.34	LS1 and MET
3	<i>qSL.gbpu-2A.2</i>	2	44.8	13.47	6.99	0.52	VLS1
4	<i>qSL.gbpu-5A</i>	5	81.6	3.81	2.43	0.30	VLS1 and MET
5	<i>qSL.gbpu-7A</i>	7	99.9	7.45	7.19	0.41	TS1
6	<i>qSL.gbpu-5B</i>	12	95.20	6.28	8.42	-0.41	LS2
7	<i>qSL.gbpu-5D</i>	19	81.6	11.03	3.87	-0.33	VLS2
Spikelet per Spike (SPS)-10							

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
1	<i>qSPS.gbpu-3A.1</i>	3	108.0	9.47	1.06	-0.37	TS2
2	<i>qSPS.gbpu-3A.2</i>	3	107.20	2.56	5.81	-0.46	VLS2
3	<i>qSPS.gbpu-7A</i>	7	113.60	24.90	1.78	0.49	TS2 and MET
4	<i>qSPS.gbpu-2B.1</i>	9	33.6	3.85	8.64	0.56	VLS2 and MET
5	<i>qSPS.gbpu-2B.2</i>	9	16	32.11	8.88	-0.99	VLS1
6	<i>qSPS.gbpu-3B</i>	10	97.89	2.77	6.98	0.47	TS1
7	<i>qSPS.gbpu-5B</i>	12	64.8	7.00	5.12	0.70	LS2
8	<i>qSPS.gbpu-2D.1</i>	16	50.4	6.40, 7.17	3.43, 3.86	-0.64, -0.61	LS1, LS2 and MET
9	<i>qSPS.gbpu-2D.2</i>	16	90.40	66.20	9.81	-1.14	TS2
10	<i>qSPS.gbpu-5D</i>	19	25.6	47.48	6.72	-0.94	TS2 and MET
Spike Compactness (S_COMP)-10							
1	<i>qS_COMP.gbpu-5A.1</i>	5	86.69	9.71	3.28	-0.02	TS1 and MET
2	<i>qS_COMP.gbpu-5A.2</i>	5	81.6	6.51	0.35	-0.01	LS2 and MET
3	<i>qS_COMP.gbpu-6A</i>	6	54.4	65.11	4.21	-0.03	LS2
4	<i>qS_COMP.gbpu-1B</i>	8	99.09	18.15	6.49	0.02	TS1 and MET
5	<i>qS_COMP.gbpu-3B</i>	10	127.20	3.23	4.15	0.02	TS2 and MET
6	<i>qS_COMP.gbpu-4B</i>	11	24.60	18.57	7.73	0.03	TS1
7	<i>qS_COMP.gbpu-7B</i>	14	76.8	4.72	6.14	-0.03	VLS2 and MET
8	<i>qS_COMP.gbpu-7D.1</i>	21	18.4	54.21	2.97	-0.02	LS2 and MET

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
9	<i>qS_COMP.gbpu-7D.2</i>	21	50.4	33.71	1.27	0.02	LS2 and MET
10	<i>qS_COMP.gbpu-7D.3</i>	21	76	5.08	8.81	-0.03	TS2
Awn Length (AL)-6							
1	<i>qAL.gbpu-4A</i>	4	34.4	7.30	4.66	-0.52	VLS1 and MET
2	<i>qAL.gbpu-1B</i>	8	19.3	4.13, 7.04	2.68, 0.70	0.31, 0.15	TS1, TS2
3	<i>qAL.gbpu-2B</i>	9	18.4	6.72	4.07	0.37	LS2
4	<i>qAL.gbpu-4B</i>	11	71.2	4.91	7.34	-0.49	LS1
5	<i>qAL.gbpu-4D</i>	18	101.39	2.66, 6.72	2.64, 0.66	0.31, 0.14	TS1, TS2
6	<i>qAL.gbpu-7D</i>	21	120.80	7.41	5.27	0.33	VLS2
Tiller per Meter (TPM)-12							
1	<i>qTPM.gbpu-1A</i>	1	47.89	19.35	7.40	-14.28	TS1
2	<i>qTPM.gbpu-3A</i>	3	4	31.78	9.79	6.40	VLS2
3	<i>qTPM.gbpu-6A</i>	6	48.8	8.62	4.79	8.02	LS1 and MET
4	<i>qTPM.gbpu-1B.1</i>	8	14.4	5.62	5.73	-9.53	VLS1
5	<i>qTPM.gbpu-1B.2</i>	8	45.6	3.41	1.65	4.69	LS1 and MET
6	<i>qTPM.gbpu-2B</i>	9	22.4	25.56	7.22	5.45	VLS2 and MET
7	<i>qTPM.gbpu-3B</i>	10	92.09	21.25	5.83	-12.61	TS1 and MET
8	<i>qTPM.gbpu-4B.1</i>	11	64.49	21.36	5.02	11.75	LS1
9	<i>qTPM.gbpu-4B.2</i>	11	38.4	10.08	3.11	3.57	VLS2
10	<i>qTPM.gbpu-4D</i>	18	57.6	7.93, 13.52	4.20, 4.27	-7.51, -9.06	LS1, LS2

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
11	<i>qTPM.gbpu-5D.1</i>	19	16.3	5.34, 8.18	1.03, 5.01	5.38, 8.28	TS1, LS1
12	<i>qTPM.gbpu-5D.2</i>	19	30.4	4.96	6.27	8.51	TS2
Thousand Grain Weight (TGW)-13							
1	<i>qTGW.gbpu-1A</i>	1	24.8	10.54, 3.75	5.59, 0.34	-1.40, -0.39	LS1, VLS1
2	<i>qTGW.gbpu-3A</i>	3	60.8	9.56	1.15	0.50	LS2 and MET
3	<i>qTGW.gbpu-4A.1</i>	4	116.99	31.07, 52.22	5.84, 7.44	1.33, 1.82	TS1, VLS1
4	<i>qTGW.gbpu-4A.2</i>	4	72	53.95	6.06	2.23	TS2 and MET
5	<i>qTGW.gbpu-4A.3</i>	4	95.20	10.50	2.35, 1.93	1.39, 0.65	TS2, LS2
6	<i>qTGW.gbpu-1B</i>	8	91.20	30.68	6.54	1.19	LS2
7	<i>qTGW.gbpu-7B.1</i>	14	68.79	19.08	3.97	-1.10	TS1 and MET
8	<i>qTGW.gbpu-7B.2</i>	14	87.19	4.84, 14.87	0.73, 1.48	-0.47, -1.12	TS1, TS2
9	<i>qTGW.gbpu-2D.1</i>	16	9.6	7.98, 43.18	3.04, 4.85	-1.05, -1.49	LS1, VLS1 and MET
10	<i>qTGW.gbpu-3D</i>	17	62.4	26.95, 9.61	3.51, 1.54	1.70, 0.58	TS2, LS2
11	<i>qTGW.gbpu-6D.1</i>	20	46.39	18.44, 14.96, 33.16	3.22, 1.95, 3.52	-1.00, -0.65, -1.25	TS1, LS2, VLS1 and MET
12	<i>qTGW.gbpu-6D.2</i>	20	72.8	18.83	4.95	1.49	VLS1
13	<i>qTGW.gbpu-7D</i>	21	89.60	59.56	7.44	-2.48	TS2 and MET
Grain Yield (GY)-11							
1	<i>qGY.gbpu-1A.1</i>	1	37.6	8.24	6.89	41.41	TS2 and MET
2	<i>qGY.gbpu-1A.2</i>	1	83.2	32.01	5.00	25.09	VLS2
3	<i>qGY.gbpu-2A</i>	2	52.8	4.07	0.66	-8.78	LS2 and MET

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
4	<i>qGY.gbpu-4A</i>	4	72.69	8.17, 9.39	8.21, 8.31	27.36, 27.05	TS1, LS1 and MET
5	<i>qGY.gbpu-1B.1</i>	8	32.8	2.59, 26.01	3.60, 11.69	-17.77, -36.93	LS1, LS2 and MET
6	<i>qGY.gbpu-1B.2</i>	8	52.8	2.90	1.94	-21.91	TS2 and MET
7	<i>qGY.gbpu-4B.1</i>	11	43.2	4.93, 5.22	4.13, 6.57	19.19, 22.93	LS1, VLS1 and MET
8	<i>qGY.gbpu-4B.2</i>	11	42.4	18.79	1.94	15.58	VLS2
9	<i>qGY.gbpu-4B.3</i>	11	17.6	8.13	6.82	41.03	TS2 and MET
10	<i>qGY.gbpu-4D</i>	18	8	5.04, 3.58	8.68, 9.30	27.98, 27.36	LS1, VLS1 and MET
11	<i>qGY.gbpu-6D</i>	20	84	27.92	3.22	20.30	VLS2 and MET
Biological Yield (BY)-9							
1	<i>qBY.gbpu-3A</i>	3	44	5.09	5.61	-95.59	LS2 and MET
2	<i>qBY.gbpu-4A</i>	4	92.79	13.16	4.79	-57.80	TS1 and MET
3	<i>qBY.gbpu-6A</i>	6	16	6.18	5.34	81.97	VLS1 and MET
4	<i>qBY.gbpu-1B</i>	8	109.09	25.36	11.58	90.44	TS1
5	<i>qBY.gbpu-3B</i>	10	88.8	6.52, 6.47	7.88, 6.30	-86.99, -88.33	LS1, VLS1
6	<i>qBY.gbpu-7B</i>	14	107.20	38.48	20.41	-223.91	TS2
7	<i>qBY.gbpu-4D</i>	18	8	3.92, 10.95	5.95, 13.90	76.05, 133.32	LS1, VLS1
8	<i>qBY.gbpu-6D.1</i>	20	11.2	5.40, 3.09	4.76, 2.20	67.50, 52.27	LS1, VLS1 and MET
9	<i>qBY.gbpu-6D.2</i>	20	112.80	6.23	5.57	-131.06	VLS2
Harvest Index (HI)-6							
1	<i>qHI.gbpu-4A</i>	4	72.69	5.75	8.49	0.03	TS1 and MET
2	<i>qHI.gbpu-3B</i>	10	125.60	7.76	3.68	-0.03	LS1 and MET
3	<i>qHI.gbpu-5B</i>	12	29.6	3.31	7.65	-0.02	LS2

S.No.	QTL Name	Chromosome	QTL Peak Position (cM)	LOD	PVE (%)	Add	Environments
Days to Heading (DTH)-15							
4	<i>qHl.gbpu-1D.1</i>	15	52.8	30.27	4.14	0.04	VLS1 and MET
5	<i>qHl.gbpu-1D.2</i>	15	67.2	8.35	7.46	0.05	VLS2
6	<i>qHl.gbpu-6D</i>	20	4.8	19.26	10.57	0.05	LS1 and MET

3.5. Candidate genes associated with heat stress tolerance and in silico expression analysis

A total of approximately 2,727 putative candidate genes were identified within the confidence intervals of the detected QTLs and functionally annotated using UniProt, GO, and KEGG databases. GO annotation classified the candidate genes into cellular component, molecular function, and biological process categories, with predominant representation in cellular and metabolic processes, biological regulation, binding and catalytic activities (**Fig. S1**). KEGG analysis assigned the genes to multiple pathways, with major representation in metabolic pathways, biosynthesis of secondary metabolites, protein processing in the endoplasmic reticulum, ribosome, phenylpropanoid biosynthesis, and carbon metabolism (**Fig. S2**). The annotated candidate genes represented diverse functional groups, including transcriptional regulation, stress perception and signal transduction, protein homeostasis, oxidative stress protection, hormonal regulation, osmotic adjustment, ion transport, and photosynthetic processes (**Table S6**). The distribution of functionally relevant candidate genes varied among QTLs, with GFD, SPS, TGW, TPM, and HI QTLs showing comparatively high representation. The *qGFD.gbpu-5D* interval contained NCED2, WRKY17, Hsp70-binding protein, NF-YA10, OAT, RuBisCO large subunit-binding protein, and WAK2, whereas *qSPS.gbpu-5D* harboured WRKY62, PP2C68, bZIP44, a two-pore potassium channel, and an LRR receptor-like kinase. The *qTGW.gbpu-6D* interval contained BADH, GSTU6, thioredoxin, CIPK26, and a lectin receptor kinase, while *qTPM.gbpu-5D* included CDPK28, diacylglycerol kinase, thioredoxin, and ubiquitin ligases. The *qHl.gbpu-6D* interval contained WAK2, receptor-like protein kinase, protein disulfide isomerase, and anthocyanidin synthase. In-silico expression profiling using WheatOmics identified 602 genes showing heat-responsive expression ($|\log_2FC| > 1$), including both upregulated and downregulated genes. Of these, 286 genes were retained as consistently heat-responsive, based on their differential expression in at least two datasets. Further prioritization using KnetMiner based on gene-trait associations and biological relevance shortlisted 170 heat responsive genes for downstream analysis (**Table S7**). The prioritized genes represented several functional categories, including transcriptional regulation, ABA and receptor-mediated signaling, protein folding and endoplasmic-reticulum quality control, reactive oxygen species detoxification, chloroplast maintenance, cell-cycle and growth regulation, and carbohydrate metabolism. An HSP70-centered KnetMiner network further showed associations with heat shock factors, DREB2, heat response, heat acclimation, ER stress response, and abiotic stress tolerance (**Fig S3-a**).

4. Discussion:

4.1. Phenotypic variation and trait responses to terminal heat stress

Delayed sowing is one of the most widely adopted field-based approaches for evaluating terminal heat stress tolerance in wheat because it exposes plants to elevated temperatures during the reproductive and grain-filling stages, which are the most heat-sensitive phases of crop development. Previous studies have hailed this approach (Grolí et al. 2024). In the present study, the staggered sowing strategy successfully generated three distinct thermal environments, with progressively increasing minimum and maximum temperatures from timely to very late sowing across both cropping seasons. The observed rise in post-heading temperatures under late- and very late-sown environments confirms that the experimental design effectively simulated natural terminal heat stress conditions commonly encountered in wheat-growing regions of North-West Plain Zone.

The wide continuous variation observed for the evaluated traits, together with highly significant genotypic differences across environments, demonstrated substantial genetic variation within the DH population for terminal heat stress-related traits. The approximately normal distribution of most traits was consistent with their quantitative inheritance and suitability for QTL analysis. Similar continuous variation for heat tolerance-related traits has been reported in wheat mapping populations, reflecting the polygenic nature of adaptation to terminal heat stress (Relan et al. 2025). Extensive transgressive segregation indicated that the two parents contributed complementary alleles, with recombination generating DH lines that exceeded the parental range. Such segregation provides an opportunity to identify favourable allele combinations associated with heat adaptation. The contrasting parental responses and variation in line performance across environments further suggest that heat adaptive traits are influenced by both stable genetic effects and environment dependent responses, emphasizing the value of evaluation across contrasting environments for QTL detection (Touzy et al. 2022; Telfer et al. 2022; Kumar et al. 2026). Delayed sowing progressively reduced most traits, with generally greater reductions under very late sowing, confirming the intensification of terminal heat stress with delayed planting. The marked reductions in GFD, FLA, PL, and E_PL indicate accelerated crop development and reduced source capacity under heat stress, which can ultimately constrain grain production. The importance of peduncle traits under heat stress is supported by their association with stem water-soluble carbohydrate accumulation, suggesting their potential utility as indicators of stress resilience (Ntawuguranayo et al. 2024). Moreover, the exposed peduncle may contribute to assimilate supply during late grain filling through its photosynthetic activity, supported by relatively high stomatal density and heat-tolerant PEPCase activity (Kong et al. 2010; Paul and Duhan 2021). Heat stress also substantially reduced biological and grain yield, consistent with shortened crop duration, impaired photosynthetic capacity, and disrupted assimilate partitioning. The comparatively smaller reduction in thousand-grain weight suggests that some genotypes were able to partially sustain grain filling despite reductions in vegetative biomass, potentially through maintenance of photosynthetic activity and remobilization of stored assimilates (Bailey-Serres et al. 2019; Yu et al. 2022). In contrast, the slight increase in spike compactness observed in some stress environments likely resulted from a

proportionally greater reduction in spike length than spikelet number rather than representing a specific adaptive response (Liu et al. 2022). Similarly, increases in harvest index under some stress environments should be interpreted as changes in biomass partitioning rather than enhanced absolute productivity.

The generally low coefficients of variation observed for most traits indicate good experimental precision and reliable phenotypic evaluation across environments, whereas the relatively higher variation for grain yield and other complex productivity traits reflects their greater sensitivity to environmental fluctuations. Similar increases in CV under heat stress have been reported in wheat, suggesting that environmental stress amplifies differences in trait expression among genotypes (Kumar et al. 2023a; Groli et al. 2024). Broad-sense heritability was relatively high for several architectural traits, indicating stronger genetic control and greater potential for selection, consistent with recent findings in wheat under optimum and heat-stress conditions (Gaddam et al. 2026). In contrast, the lower heritability of GY, HI, S_COMP and SPS under severe heat stress suggests greater environmental influence on these complex traits, consistent with the complex genetic architecture and strong genotype $\times$ environment effects reported for grain yield under heat stress (Zheng et al. 2025). Thus, selection based on highly heritable component traits, together with stable QTLs, may be more effective for improving terminal heat-stress tolerance than direct selection for grain yield alone.

4.2. Correlation Analysis

Understanding the relationships among agronomic traits is essential for identifying key selection criteria for improving wheat performance. In the present study, biological yield exhibited the strongest and most consistent positive association with grain yield across all environments, followed by harvest index, indicating that biomass production and efficient assimilate partitioning are the primary physiological determinants of grain yield irrespective of stress intensity. Similar relationships have been consistently reported in wheat under both optimum and terminal heat-stress environments, where BY and HI were identified as the strongest contributors to grain yield (Jaenisch et al. 2022; Xi et al. 2024). The positive associations of thousand-grain weight and spikelets per spike with grain yield across all sowing environments further highlight the importance of sink strength in determining yield under heat stress, consistent with reports that sink capacity (grain number and grain-filling potential) is a key yield-limiting factor in wheat under high-temperature conditions (Jaenisch et al. 2022; Fang et al. 2024). The observed changes in correlation patterns with increasing stress intensity also provide insight into the differential contribution of component traits under adverse environments. The weakening relationship between spike length and grain yield under severe heat stress suggests that spike elongation becomes less important when elevated temperatures constrain grain development, consistent with reports that correlations between spike length and grain yield are strong under favourable conditions but weaker or altered under high heat stress (Kumar et al. 2023b; Ghazy et al. 2025). Conversely, the relatively stronger association between productive tillers per metre and grain yield under very late sowing indicates that maintaining a higher number of productive tillers may partially compensate for heat-induced reductions in grain size and spike productivity, consistent with reports that productive tillers per metre remain positively associated with grain yield under heat-stressed conditions and that genotypes sustaining higher tillering capacity perform better under terminal heat stress (Khairnar et al. 2018).

4.3. QTL mapping:

Among the 179 QTLs identified, 19 were major loci explaining > 10% of phenotypic variance across phenological, morphological, and yield-related traits. These loci represent promising targets for marker-assisted selection, although their breeding value should be assessed not only by effect size but also by stability across environments (Yadav et al. 2022; Telfer et al. 2022). Indeed, several major QTLs showed substantial QTL $\times$ environment interaction, indicating that large effect loci may be broadly useful when their additive effects are stable, whereas interaction driven loci may be more relevant to specific heat stress environments. The greater number of QTLs detected on the B genome was consistent with its higher marker density and greater level of polymorphism in the present population and previous wheat studies (Manjunath et al. 2024). This trend can be attributed to the greater diversity in A and B genomes than in the D genome. Genetic differences between parental genomes could also impact marker distribution. Two multi-trait regions were particularly noteworthy. The chromosome 4A region at 72 cM contained overlapping QTLs for TGW, GY, and HI, while the chromosome 7D region at 69 cM harboured QTLs for DTH, PL, and FLA. The 4A region is consistent with previously reported QTL hotspots for TGW, GY, and HI under heat stress (Guan et al. 2018) while QTL clustering on chromosome 7D has also been reported in wheat (Cerit et al. 2023). These co-localized regions may facilitate simultaneous improvement of multiple traits, although the overlap could reflect pleiotropy or tight linkage and therefore requires fine mapping and functional validation.

Both BIP and MET analyses revealed a complex genetic architecture underlying terminal heat stress adaptation, involving both relatively consistent additive effects and substantial QTL $\times$ environment interactions (QEI). Comparison of LOD(A), LOD(A $\times$ E), PVE(A), and PVE(A $\times$ E) indicated that the detected loci could broadly be characterized as predominantly additive, predominantly environment-responsive, or intermediate in their genetic behaviour. Such partitioning is important because the overall QTL signal and total PVE do not, by themselves, reveal whether the phenotypic contribution of a locus is primarily attributable to a consistent additive effect or to QTL-specific variation across environments. Multi-environment studies in wheat have similarly demonstrated that adaptation to heat stress can involve both QTL with relatively stable effects and loci showing responsiveness to contrasting environmental conditions, highlighting the importance of considering QEI when interpreting the genetic basis of stress adaptation (Telfer et al. 2022; Jia et al. 2024).

Relatively strong additive effects were observed for QTLs controlling anthesis, maturity, grain filling duration, plant height, peduncle traits, flag leaf area, spike characteristics, thousand-grain weight, grain yield, and biological yield. Particularly noteworthy was the occurrence of additive-dominated loci for economically important traits such as TGW and GY, suggesting that part of the genetic variation governing productivity under stress remained comparatively consistent across environments. Such loci represent promising candidates for marker-assisted breeding because selection based on additive effects is generally more predictable than selection for strongly environment-dependent effects. Nevertheless, their stability and transferability should be confirmed in independent genetic backgrounds and target environments before deployment in breeding programmes. Recent studies demonstrating the validation of stable wheat QTL across environments and genetic backgrounds, followed by marker development,

support this validation-oriented approach (Li et al. 2023; He et al. 2025). In contrast, substantial QEI was evident for several traits, particularly phenological development, effective tillering, spike characteristics, TGW, GY, BY, and HI. The pronounced environmental dependence of BY and HI is especially relevant because these traits integrate whole-plant biomass accumulation and assimilate partitioning, processes that are highly sensitive to the timing and intensity of heat stress (Shahi et al. 2024). Similarly, the strong interaction component associated with some GY and TGW QTLs indicates that the contribution of these loci to productivity depended on the prevailing thermal environment. These environmentally responsive QTLs should not necessarily be considered less valuable; rather, they may represent important sources of specific adaptation and could be exploited in breeding programmes targeting defined heat-stress environments. Several loci exhibited appreciable contributions from both additive and interaction components, further demonstrating that QTL stability cannot always be considered a binary property. A locus may retain a detectable additive effect across environments while the magnitude of its phenotypic expression changes substantially with environmental conditions. Such QTLs are particularly relevant to terminal heat stress adaptation because field heat stress is inherently variable across years, locations, and sowing dates (Sabir et al. 2023). Genetic effects that combine a relatively consistent component with environmentally responsive modulation may therefore contribute to phenotypic plasticity, although their breeding value will depend on the range of environments targeted by selection.

The contribution of favourable alleles from both parents highlights the complementary genetic architecture of the DH population, with neither parent possessing all favourable alleles for heat adaptation and yield-related traits. KSG1203 (P2) was the predominant donor for several phenological and productivity-related traits, including DTH, DA, TPM, TGW, GY, BY, and HI, whereas KSG1222 (P1) contributed more favourable alleles for GFD, PH, and SPS. For several architectural traits, including PI, E_PL, FLA, and SL, favourable alleles were distributed relatively evenly between the two parents. This trait-dependent distribution indicates that neither parent was uniformly superior, but rather that each contributed distinct alleles for different components of heat adaptation. The recombination of these parental alleles likely contributed to the extensive transgressive segregation observed in the population consistent with previous wheat studies reporting segregants exceeding parental values for yield and yield-related traits (Bhusal et al. 2017). From a breeding perspective, the predominance of KSG1203-derived favourable alleles for yield and associated traits highlights its potential as an important donor for improving productivity under stress, while KSG1222 provides complementary alleles for plant architecture and grain-development traits. Thus, pyramiding favourable alleles from both parents could facilitate the development of genotypes combining desirable phenology, plant architecture, biomass production, grain development, and yield under terminal heat stress (Liu et al. 2024). Importantly, the direction as well as the magnitude of QEI effects varied among environments for several traits. Positive interaction effects in one environment and negative effects in another indicate crossover QEI, demonstrating that the phenotypic consequence of an allele can depend strongly on the thermal regime in which it is expressed (Kuchel et al. 2007; Telfer et al. 2022). Such reversals were evident across maximum traits and emphasize that an allele favourable under one sowing condition may not necessarily confer the same advantage under another. This finding highlights the importance of evaluating QTLs across contrasting years, locations, and heat-stress intensities before their incorporation into marker-assisted breeding. For broad adaptation, loci with relatively consistent additive

effects would generally be preferable, whereas strongly environment-responsive loci may be useful when breeding for specific target environments.

4.5. Candidate genes and putative molecular mechanisms underlying terminal heat-stress adaptation:

Integration of QTL positions with functional annotation, heat-responsive expression data, and network information provided insights into the biological processes potentially underlying terminal heat-stress adaptation. The prioritized candidates represented diverse functional classes, suggesting that heat adaptation involves coordinated regulation of developmental timing, stress signalling, cellular protection, photosynthesis, and resource allocation rather than a single pathway **Table S6**.

Transcriptional regulation and heat responsive signalling

Transcription factors and signalling proteins were frequently identified within QTL regions. The co-localized *qDTH.gbpu-2D.1-qDA.gbpu-2D* region contained heat-stress transcription factor B-4b, MYB3, a bZIP-domain protein, and a calcium-binding protein kinase, whereas *qDTH.gbpu-1D* harboured WRKY31. Similarly, *qDA.gbpu-5D.1-qSPS.gbpu-5D* contained MYB APL, WRKY62, WRKY5, and the protein phosphatase TaPP2C68. These candidates provide plausible links between heat-responsive signalling, transcriptional reprogramming, and reproductive development. Calcium-dependent and PP2C-mediated signalling integrates stress and hormonal cues with developmental and metabolic responses, while kinase signalling coordinates heat responses with growth and reproduction (Ghanizadeh et al. 2025). Additional kinase-associated candidates occurred within *qDA.gbpu-3D*, *qGFD.gbpu-5D*, *qHL.gbpu-6D*, *qTGW.gbpu-6D.2*, and *qS_COMP.gbpu-7D.3*, including a PERK9 receptor-like kinase in *qDA.gbpu-3D*. A notable example was the major *qE_PL.gbpu-1D*, which harboured *TraesCS1D03G0035600*, encoding a serine/threonine protein kinase with ATP-binding activity. Its large-effect QTL association, kinase function, and heat responsiveness support its potential role in peduncle elongation under heat stress, although functional validation is required. Receptor-like kinases can integrate environmental signals with growth and cell-wall responses, providing a potential basis for phenotypic plasticity under stress (Whyte and Sipahi 2024). The *qDA.gbpu-5D.1-qSPS.gbpu-5D* region provided further evidence for a link between signalling and reproductive development. KnetMiner connected TaPP2C68 with TaOHN and transcriptional regulators, while the associated reproductive-development module was linked to heat response, acclimation, and ER-stress response (**Fig S3-b**). These associations support TaPP2C68 as a candidate within this co-localized region, although they do not establish causality.

Hormonal regulation and developmental plasticity

Hormone-associated candidates suggest that developmental plasticity under terminal heat stress involves interactions between stress signalling and phytohormone pathways. The *qGFD.gbpu-5D* region contained *TraesCS5D03G0859900*, encoding NCED2, a key enzyme in ABA biosynthesis, providing a plausible link between ABA-mediated stress responses and grain filling (Cui and Cheng 2025). The *qPL.gbpu-2A* region also contained a bHLH-domain protein associated with hormone signalling, while *TraesCS2A03G0612500* from the same region showed a network-level association with TaPIF4 (**Fig S3-c**). The TaPIF4-associated

network included heat response, HSP70-related functions, and regulators of plant architecture and reproductive traits, supporting a potential role for hormone-responsive transcriptional regulation in maintaining plant architecture under heat stress. Similarly, *TraesCS2D03G0306500* within the *qDTH.gbpu-2D.1-qDA.gbpu-2D* region was connected through KnetMiner to a TaBZIP17-associated network involving heat-responsive transcription factors, heat acclimation, ER-stress response, and reproductive development. Collectively, these observations suggest that hormonal and transcriptional networks may interact to modulate developmental timing and growth under variable thermal conditions (Nguyen et al. 2021; Wang et al. 2023).

Protein homeostasis and post-transcriptional regulation

Protein homeostasis and post-transcriptional regulation represented another important component of the candidate-gene set. QTL regions contained genes involved in protein folding, chaperone activity, RNA processing, and protein turnover, including ATPases, HSP70-interacting proteins, tetratricopeptide repeat proteins, protein disulfide isomerases, and RNA-binding proteins. Such functions are particularly important under heat stress, which can disrupt protein folding and RNA metabolism (Assimon et al. 2015; Gao et al. 2024; Chen et al. 2025). The *qDTH.gbpu-2D.1-qDA.gbpu-2D* region contained an ATPase associated with protein folding and a serine/arginine-rich splicing factor, while *qHI.gbpu-6D* harboured protein disulfide isomerase-like candidates. In addition, *qPH.gbpuat-3D* contained *TraesCS3D03G0508300*, encoding a 21.9-kDa small heat-shock protein domain-containing protein. Its heat responsiveness and localization within a plant-height QTL provide a plausible link between chaperone-mediated protein protection and maintenance of plant architecture under heat stress.

The *qE_PL.gbpu-4D.1* region provided further support for this mechanism. KnetMiner linked *TraesCS4D03G0504900.1* with HSP70-associated components and heat-response and acclimation processes, including TaHSFA6 and TaHSFB2B (**Fig S3-a**). This is consistent with evidence that TaHSFA6e and TaHSP70 contribute to wheat thermotolerance and stress-granule dynamics (Wen et al. 2023), while the heat responsiveness of the wheat HSP70 family (particularly within the D genome) further supports their role in heat adaptation (Lu et al. 2022).

Photosynthesis, energy metabolism and maintenance of assimilate supply

Photosynthesis- and chloroplast-associated candidates were identified within phenological and yield-related QTLs. Chlorophyll *a/b*-binding proteins occurred within *qDTH.gbpu-1D* and *qDTH.gbpu-5D*, while a cytochrome *b6/f* complex subunit (*petD*) was identified within *qDM.gbpu-7B*. Their roles in light harvesting and electron transport suggest that maintenance of chloroplast function may contribute to developmental stability and assimilate supply under terminal heat stress, particularly given the reductions in grain filling duration and yield observed under delayed sowing (Lee and Jeon 2020; Malone et al. 2021). This interpretation was further supported by KnetMiner associations of *TraesCS5D03G1027800*, *TraesCS5D03G1027900*, and *TraesCS1D03G0954900* with CAB1 and networks related to non-photochemical quenching, heat response, and heat acclimation (**Fig S3-d**). The two CAB1-associated candidates within *qDTH.gbpu-5D* therefore highlight this region as a potential heat-responsive locus affecting heading, although the causal genes remain to be established. In addition, SWEET, ERD6-like, and MST/MFS

transporters and other transport-related candidates occurred within yield-associated QTLs, suggesting that maintenance of source–sink coordination and assimilate allocation may contribute to grain filling and yield stability under heat stress (Yang et al. 2022).

Redox regulation and cellular protection

Redox-related candidates were also recurrent across heat-responsive QTLs. A glutathione metabolism associated gene occurred within *qDA.gbpu-5D.2*, while a TPR-repeat-containing thioredoxin and cinnamyl alcohol dehydrogenase related oxidoreductases were identified within QTLs for tillering and thousand grain weight. These candidates suggest a role for redox homeostasis in maintaining cellular integrity under heat stress, when excessive ROS accumulation can damage membranes, chloroplasts, and proteins and accelerate senescence (Fortunato et al. 2023). The occurrence of thioredoxin, glutathione, and oxidoreductase-related candidates across QTLs affecting tillering and grain weight is consistent with the importance of antioxidant and redox regulation for heat adaptation and yield stability (Cao et al. 2024).

Cell-wall organization and structural development

Cell-wall-related candidates were particularly associated with morphological QTLs. A pectinesterase occurred within *qE_PL.gbpu-2D*, while an XTH and a cinnamyl alcohol dehydrogenase associated with lignin metabolism were detected within *qTPM.gbpu-5D.2*. Pectin-modifying enzymes and XTHs regulate cell-wall flexibility and expansion, whereas lignin-associated enzymes contribute to tissue strengthening (Ding et al. 2025). Their occurrence within QTLs affecting peduncle and tillering traits suggests that cell-wall remodelling may contribute to maintaining structural growth under heat stress.

Integrated interpretation of candidate-gene evidence

The candidate gene evidence supports a coordinated model of terminal heat-stress adaptation involving signalling and transcriptional regulation, hormonal responses, proteostasis, photosynthetic maintenance, redox homeostasis, and cell-wall remodelling. The presence of candidates from multiple functional categories within overlapping QTL regions further suggests that some phenotypic effects may reflect interactions among interconnected biological processes. For example, the *qDTH.gbpu-2D.1-qDA.gbpu-2D* region combined candidates associated with heat-responsive transcription, calcium-dependent signalling, protein folding, and MYB regulation, whereas *qDA.gbpu-5D.1-qSPS.gbpu-5D* combined transcription factors with PP2C-mediated signalling. These regions therefore represent promising targets for further functional investigation. Nevertheless, QTL colocalization, expression evidence, and network associations provide candidate prioritization rather than proof of causality; functional validation will be required to identify the genes directly underlying the observed QTL effects.

Declarations

Author Contribution Statement

PP conducted the study, performed the field experiments, collected and curated the data, carried out phenotyping and statistical analyses, and wrote the first draft of the manuscript. JPJ conceptualized and formulated the study and provided supervision and guidance. KSG developed the DH population. SK performed the data analysis and edited the manuscript. NKS and DP contributed to the interpretation of results and revision. MT critically revised and edited the manuscript. All authors have read, revised and approved the final version of the manuscript.

Competing Interests

The authors of this manuscript declare no competing interest.

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Author Contribution

PP conducted the study, performed the field experiments, collected and curated the data, carried out phenotyping and statistical analyses, and wrote the first draft of the manuscript. JPJ conceptualized and formulated the study and provided supervision and guidance. KSG developed the DH population. SK performed the data analysis and edited the manuscript. NKS and DP contributed to the interpretation of results and revision. MT critically revised and edited the manuscript. All authors have read, revised and approved the final version of the manuscript.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Files.

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Figures

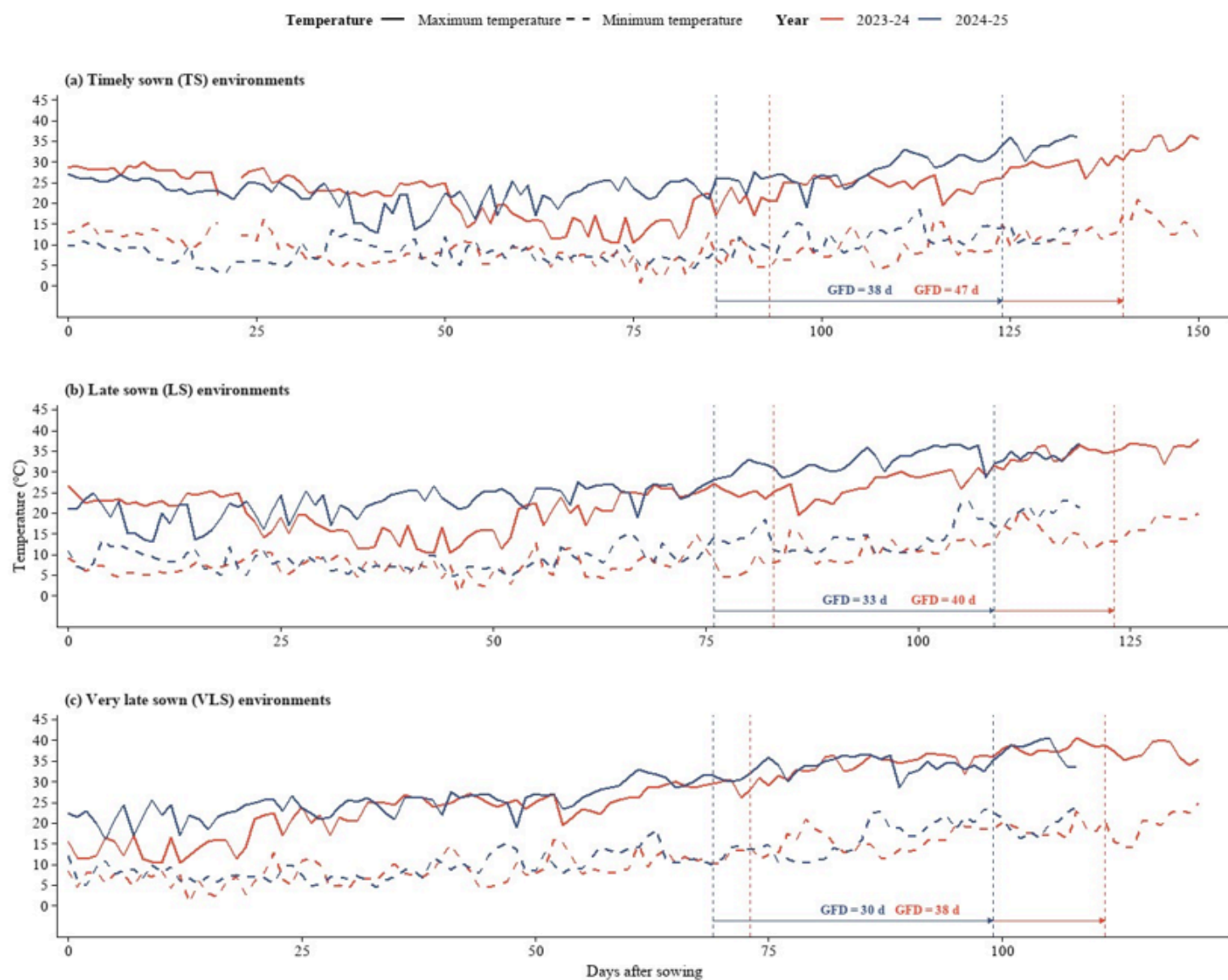


Figure 1

Fig. 1 Temperature regimes and grain-filling duration of wheat under timely sown (TS), late sown (LS), and very late sown (VLS) environments during Rabi 2023-24 and 2024-25. Solid and dashed lines represent daily maximum and minimum temperatures, respectively. Vertical dashed lines indicate anthesis and maturity, and horizontal arrows indicate the corresponding grain-filling duration (GFD) for each season.

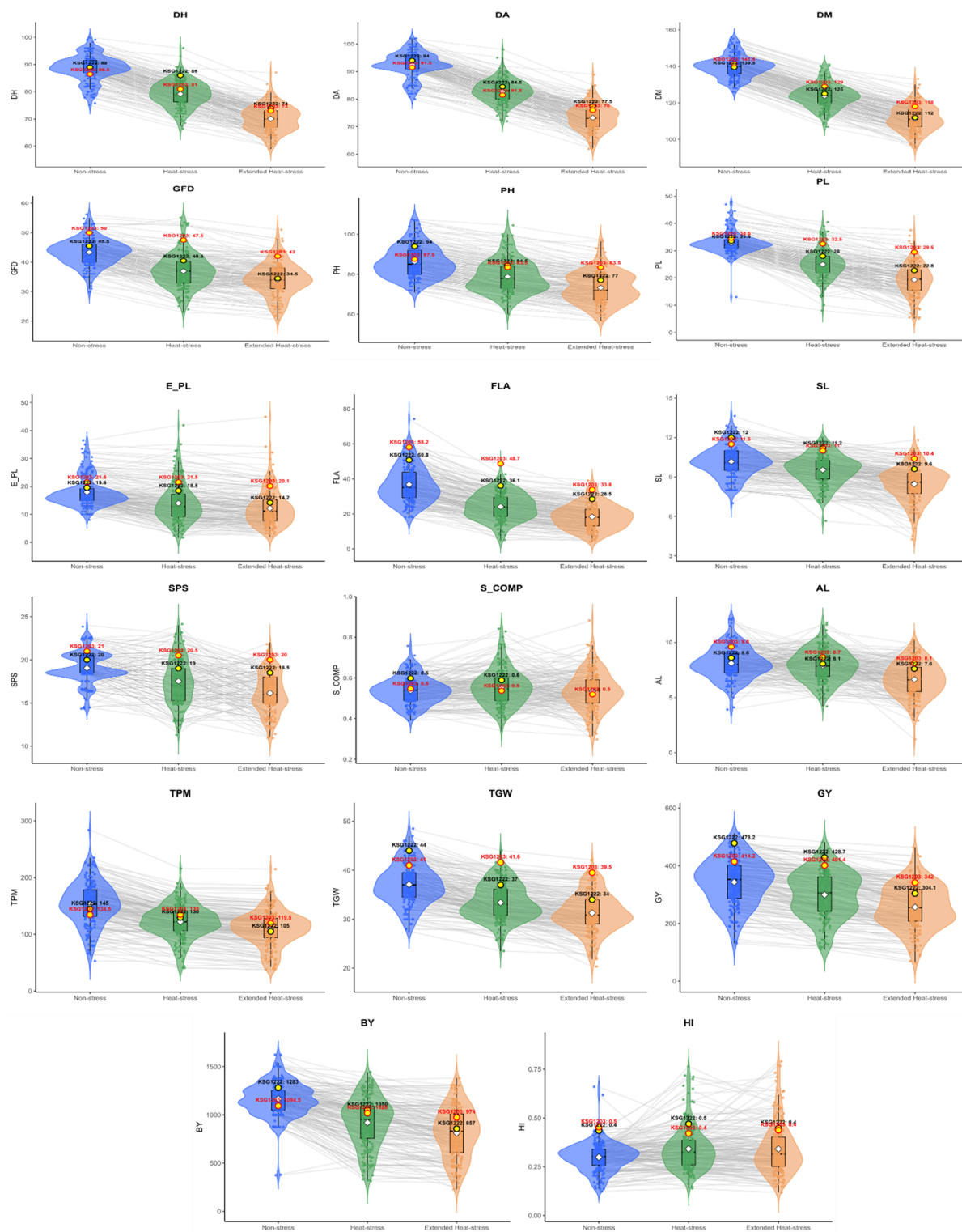


Figure 2

Fig. 2a Phenotypic variation for traits observed in Rabi 2023-24, under non-heat stress (blue), heat stress (green) and extended heat stress (orange) conditions. In each treatment, Violin plot represents trait distribution; box plot represents the minimum, first quartile, mean/median, third quartile, and maximum values in the dataset; and dots represent the individual genotypes. The gray lines depict the performance of

same genotype across treatments with connecting dots. Diamond: Mean; Red: KSG1203; Black: KSG1222
***Significant at $p < 0.001$

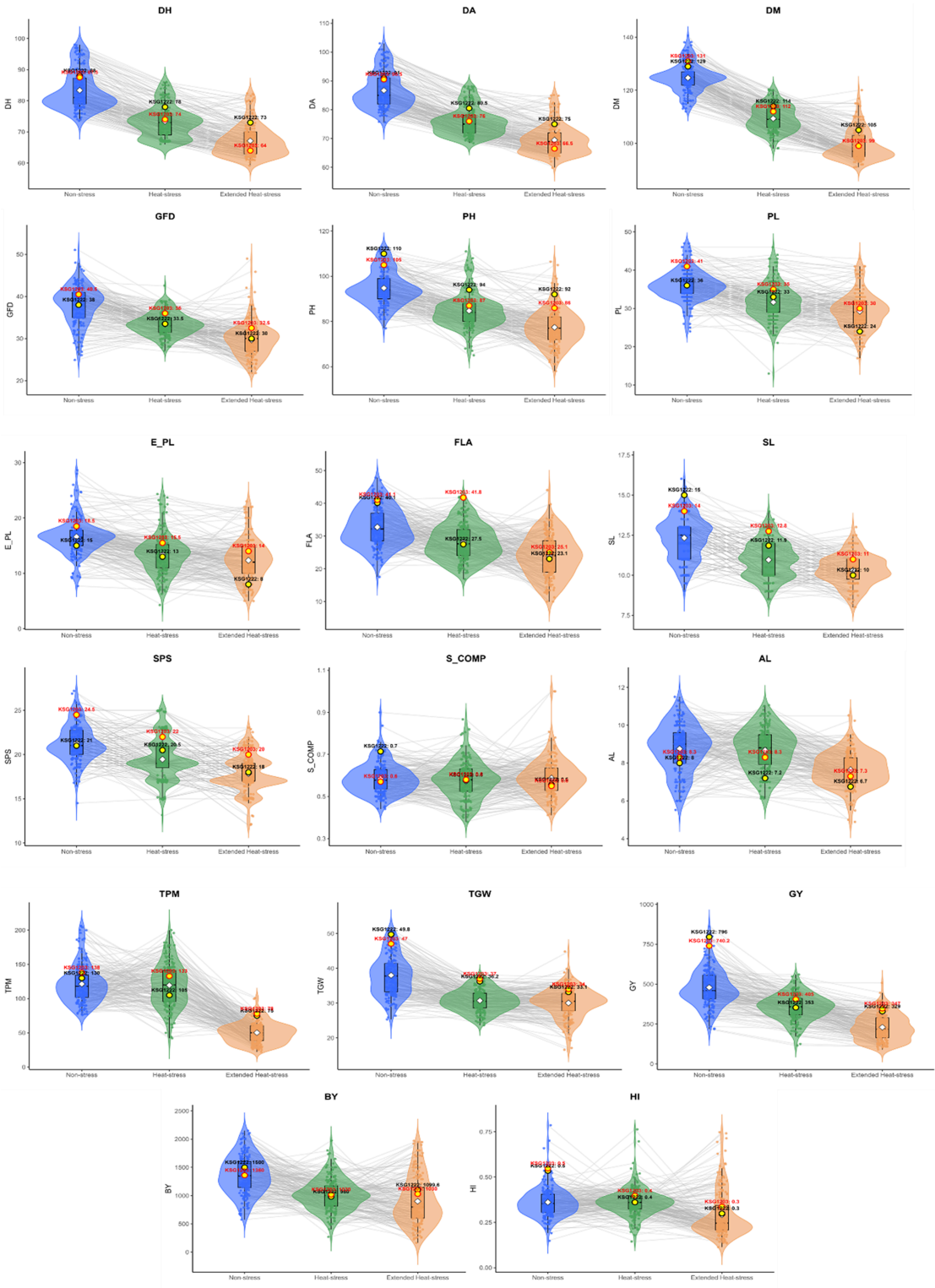


Figure 3

Fig. 2b Phenotypic variation for traits observed in Rabi 2024-25, under non-heat stress (blue), heat stress (green) and extended heat stress (orange) conditions. In each treatment, Violin plot represents trait

distribution; box plot represents the minimum, first quartile, mean/median, third quartile, and maximum values in the dataset; and dots represent the individual genotypes. The gray lines depict the performance of same genotype across treatments with connecting dots. Diamond: Mean; Red: KSG1203; Black: KSG1222

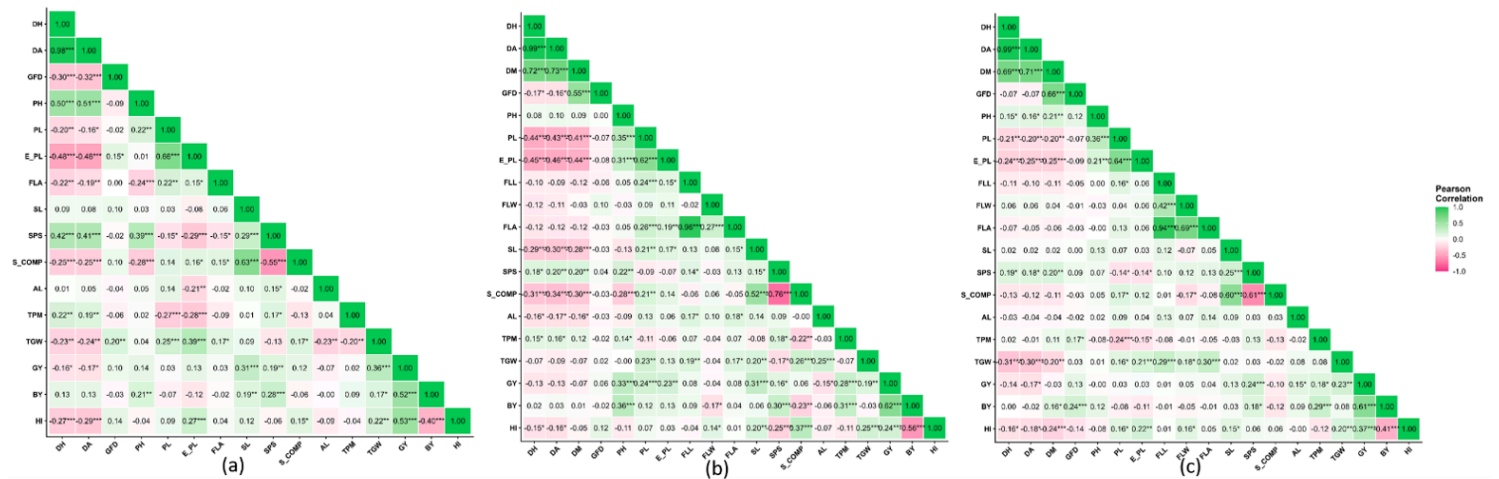


Figure 4

Fig. 3 Pearson's correlation matrix among morpho-phenological, and yield-related traits under (a) Timely Sown (Non-Heat Stress); (b) Late Sown (Heat Stress) and (c) Very-Late Sown (Extended Heat Stress). Circle size and colour indicate the magnitude and direction of correlation coefficients. Asterisks (*, **, ***) denote significant correlations at $p < 0.05$, 0.01 , and 0.001 levels, respectively.

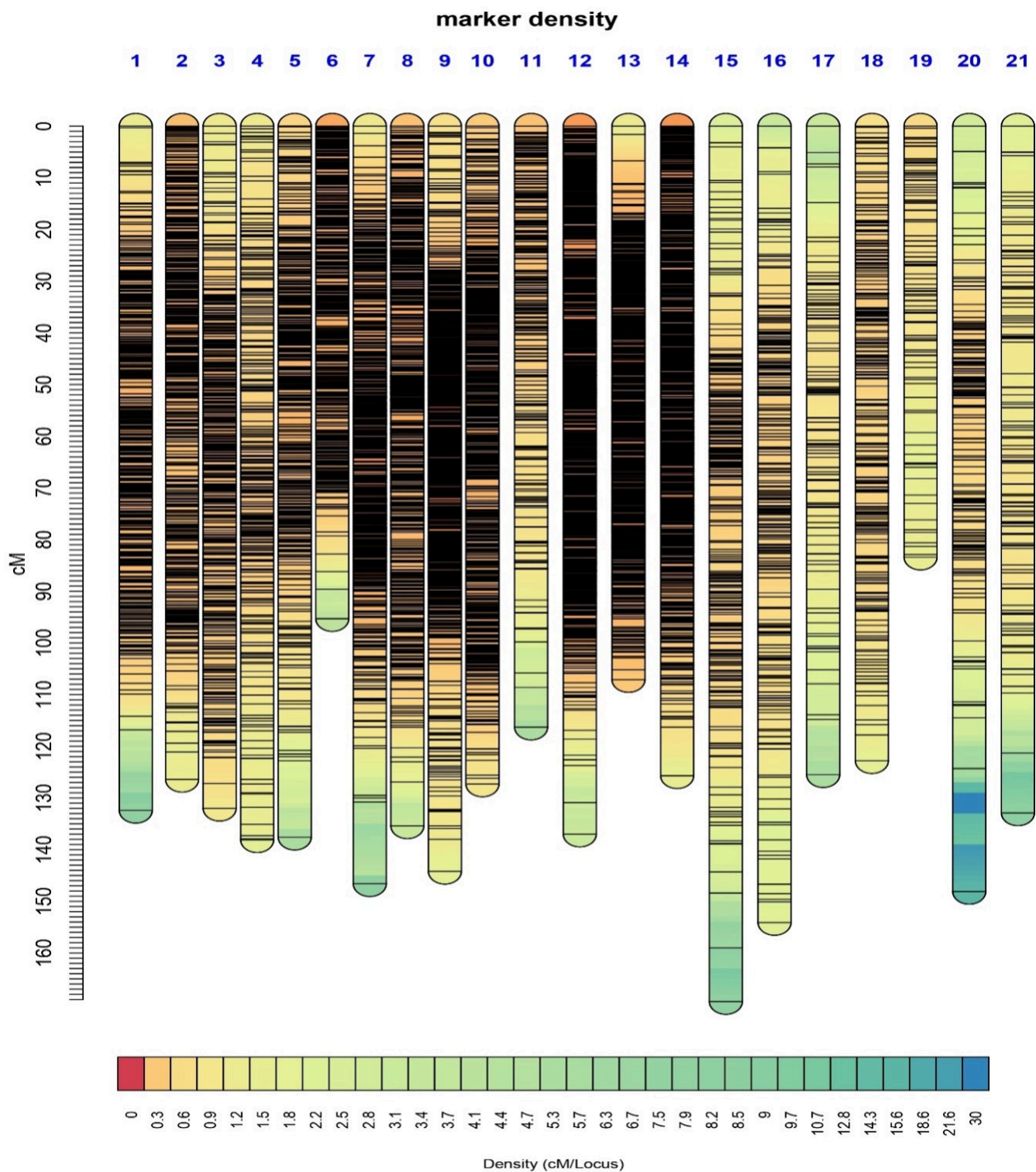


Figure 5

Fig. 4 High-density genetic linkage map and marker density across the 21 wheat chromosomes constructed using 12,297 SNP markers. The colour gradient represents marker density (cM per locus) along each linkage group.

Supplementary Files

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