JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2025 | Volume 13 | Issue 1

SNP markers associated with agronomic traits under drought and heat stress in wheat

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Original paper

Abstract: Drought and heat stresses present significant challenges in agriculture production. This study evaluated CIMMYT-Mexico Core Germplasm (CIMCOG) under drought, heat, and their combined stress over two growing seasons. The germplasm was genotyped with a 35K Axiom® SNP Array to identify associations with the studied traits under these environmental conditions. The Best Linear Unbiased Predictors (BLUPs) of each line were used to calculate marker-trait associations using 16063 SNP markers with a mixed linear model (MLM). For genome-wide association mapping (GWAM), the highest number of associations was obtained for test weight (32), followed by grain hardness (17). In the case of grain yield, six SNPs were detected in association with the trait only under heat stress conditions. When applying a P-value threshold of <0.001, GWAM identified several significant associations: 11 under normal conditions, 31 under drought stress, 10 under heat stress, and 10 under combined drought-heat stress. These associations were distributed across most wheat chromosomes. The identified SNPs explained between 18% and 35.2% of the phenotypic variance. Notably, six SNPs were shared between heat and combined drought-heat stress conditions. These findings provide valuable insights for breeding programs aimed at enhancing wheat tolerance to abiotic stresses.

Keywords: Abiotic stress tolerance, GWAS, single nucleotide polymorphism, *Triticum aestivum*.

Edited by

Prof. Junhua Peng,
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Date

Received: 10 January 2025
Accepted: 26 February 2025
Published: 01 April 2025

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Citation

Vaezi, B., Arzani, A., and Haghpanah, M.(2025). SNP markers associated with agronomic traits under drought, heat, and combined stress conditions in wheat. *J Plant Mol Breed*. *13* (1): 19-38.
doi: 10.22058/jpmb.2025.2050354.1327.

Introduction

Wheat is one of the most important staple crops in the world significantly contributing to global food security (Arzani and Ashraf, 2017). Grain yield in wheat is limited by abiotic stress with heat and drought, often coinciding as the most significant factors (Vaezi et al., 2024). The combined effects of heat and drought waves, resulting from rising temperatures, make crop production challenging. (Sato et al., 2024). While droughts naturally occur, climate change has accelerated hydrological processes, causing them to onset more rapidly and intensify (Mukherjee et al., 2018; Haghpanah et al., 2024). These effects have become significant direct consequences, severely impacting ecosystems and agriculture. Enhancing heat and drought tolerance in wheat has been approached through various strategies, including genetic analysis to identify stress-responsive genes, targeting key physiological traits, and employing indirect selection under different stress conditions (Langridge and Reynolds, 2021)

Heat stress is a crucial constraint to wheat productivity, affecting different growth stages, especially anthesis and grain filling (Mahdavi et al., 2021). The growth rate is accelerated due to increased plant temperature, which reduces the opportunity for photosynthesis as the life cycle is shortened. Heat and drought stress may also inhibit growth directly at the metabolic level. Physiological traits are often identified as contributors to improved yield potential under stress conditions (Ramya et al., 2017). Therefore, it is necessary to improve ergonomically superior, heat-tolerant wheat varieties.

Consequently, early-duration wheat varieties with traits such as early vigor and leaf rolling under stress were bred for terminal heat stress tolerance, considering the improvement of yield performance under heat stress conditions (Ramya et al., 2017).

The detrimental effects of drought and heat stress include abnormal respiration, closed stomata, inhibition of photosynthesis, and osmotic stress (Sareen et al., 2023; Haghpanah et al., 2024). These effects can be synergistic, antagonistic, or hypo-additive on plant performance. Concurrent abiotic stress events lead to interacting signaling pathways and complex responses that may promote each other. Plants theoretically maintain leaf cooling under heat stress with adequate moisture by keeping their stomata open. However, in practice, Mahdavi et al. (2021) found that most wheat genotypes closed their stomata under terminal heat stress to reduce carbon fixation and transpiration. Conversely, when plants are exposed to water scarcity, they close their stomata to avoid excessive water loss, resulting in increased leaf temperature (Sareen et al., 2023). Therefore, under heat and drought stress conditions, understanding the effects of combined stress and the plant's adaptive responses would be highly intricate.

Directly selecting for high yield potential under heat and drought stress conditions presents a significant challenge for wheat breeders. The complexity arising from combined stresses complicates the discernment of genotype × environment interactions. Hence, marker-assisted indirect selection of tolerant genotypes using markers linked to yield or associated traits represents an effective breeding approach for developing improved cultivars to combine heat and drought stress. While there are some reports on the effects of combined heat and drought stress on agro-physiological traits (Mahdavi et al., 2021; Sareen et al., 2023; Vaezi et al., 2024),lmitted research has been directed toward genome-wide association studies (GWAS) with these traits under these conditions in wheat. In a study by (Sinha et al., 2018), agro-physiological traits in 52 wheat lines were evaluated across timely sown, rainfed, and late sown environments.

The phenotypic data validated marker-trait associations of several meta-QTLs previously identified for these traits. Specifically, six meta-QTL genomic regions consistently expressed their effects under rainfed or restricted irrigation conditions, while seven meta-QTL regions showed consistent expression under late-sown environments (Sinha et al., 2018).

The objective of this research was to identify genomic regions associated with drought stress, heat stress, and combined drought-heat stress using a genome-wide association mapping (GWAM) strategy in a wheat CIMCOG panel genotyped at approximately 35000 SNP positions.

Materials and Methods

Plant materials

A set of 60 spring wheat (*Triticum aestivum* L.) lines from the CIMMYT-Mexico Core Germplasm (CIMCOG) were used in this study. CIMCOG comprises spring wheat lines with a diverse agronomic background and proven value. The pedigrees of the wheat lines used in this study are listed in Supplementary Table S1.

Plant cultivation and phenotyping assessment

Field trials were conducted during the 2012–13 and 2013–14 growing seasons at the experimental farm of Gachsaran Agricultural Research Station (located at 50°59′N, 30°18′E, and 668 m altitude) in southwestern Iran, characterized by heat and semi-heat environmental conditions. Each growing season involved planting 64 wheat genotypes in a lattice design with two replicates, using silty-clay-loam soil. Experimental plots consisted of six rows, each 4.37 m long with 20 cm spacing between rows. Four experimental conditions were applied each season: non-stress (N1 and N2), terminal drought (D1 and D2), heat stress (H1 and H2), and combined terminal drought and heat stress (DH1 and DH2). The non-stress and drought trials were planted on the normal (timely) planting dates, December 5 in the first season and December 4 in the second season. Plants were irrigated based on 50% management-allowed depletion (MAD) until the end of each experiment. For terminal drought treatment, irrigation was stopped when plants in 50% of plots reached the late booting stage.

Two trials involved late sowing on January 26 and January 25 in the first and second seasons, respectively, to induce terminal heat and combined terminal drought and heat stresses. In the first trial, genotypes received normal irrigation throughout the experiment, while in the second trial, irrigation ceased from the booting stage onward. Henceforth, the late-sown environment will be referred to as the heat stress conditions, due to the primary difference between timely-sown and late-sown plants, and 100 kg of ammonium phosphate per hectare was applied at sowing. Weed control was managed using Pomasuper and Granstar herbicides at a rate of 1 liter per hectare, following their respective instructions during the tillering and jointing stages. Plant height (cm) and spike length (cm) were measured at the same stage. Rolling flag-leaves (RFL) were measured according to a method described by (Saneoka et al., 1995). Fifty spikes were randomly selected and assembled from each plot to estimate the thousand-grain weight (TGW) (g). Single-grain hardness was measured by crushing the grains of 100 seeds (Osborne and Anderssen, 2003). Spike length, spikelet number per spike, and grain number per spike were recorded as the average of five spikes per plot. Thousand-grain weight was determined by extrapolation after counting grains of five spikes with a seed counter and obtaining the weight of the grains. Number of spikes m^{-2} was calculated by dividing the number of grains m^{-2} by grain number per spike. Final dry biomass was determined by weighing samples after 48 hours in a 60˚C oven. These samples were threshed to obtain grain weight, and the harvest index was recorded as the ratio of grain weight to oven-dried biomass of the 1-m strip. Test weight (kg hL^{-1}) was determined using standard procedures from a small sample of the grain collected at harvest. Grain yield was the total weight of seed yield in each plot divided by the plot area and expressed as kg ha^{-1}.

Physiological traits such as Chlorophyll content, flag leaf area (cm^{2}), and Fv/Fm were determined (Haghpanah et al., 2023). The SPAD 502 chlorophyll meter was used to take chlorophyll measurements. Total SPAD (chlorophyll content) was recorded before flowering (TSPDB), anthesis, and post-anthesis stages. Flag leaf area was measured using a leaf area meter. The Fv/Fm ratio was determined using a portable chlorophyll fluorometer (PAM2500, Walz, Germany). Days to heading and days to flag leaf senescence were also recorded, using the sowing date as the reference point. Water absorption of wheat flour was measured using a farinograph (Brabender OHG, Duisberg, Germany) (Okuda et al., 2016). Three hundred grams of flour (14% moisture) were mixed with water via burette titration to achieve 500 Brabender Units. The water absorption of wheat flour was evaluated using a graduated burette. A mixograph was also used to measure water absorption. Wheat flour (10 g, 14% moisture) was mixed with water in a 10-g mixograph bowl at 21°C and a speed of 86 rpm. Water absorption from a wheat flour mixogram was determined by measuring the maximum peak height (mm) using calipers.

SNP genotyping

SNP genotyping for the CIMCOG germplasm was conducted using the 35K Axiom® Array. The raw genotyping data, comprising 35143 SNP loci, was obtained from cerealsdb.uk.net. These SNPs were filtered using Tassel software, excluding loci with a minor allele frequency (MAF) of ≤ 5%. After filtering, the final genotypic dataset consisted of 16063 SNPs. The SNP data included physical positions according to RefSeqv1.0 and genetic map information.

Statistical analysis

The morpho-physiological data were analyzed by analysis of variance (ANOVA) and the means among groups (Table S1) were further tested by the Tukey HSD test. SPSS Statistics for Windows, version 13 (SPSS Inc., Chicago, Ill., USA) was used for statistical analyses. To have a better insight into the genetic structure of bread wheat, different methods were exploited. First, we applied the Bayesian model-based clustering algorithm implemented in STRUCTURE 2.2 (Pritchard et al., 2000). Admixture and correlated allele frequency models were employed with some clusters (K) ranging from 1 to 9. For each *K*, five runs were carried out. Burn-in time and replication number were both set to 100,000 for each run. Ln(PD) was derived for each K and then plotted to find the plateau of the ΔK values (Evanno et al., 2005) aimed to determine the real number of sub-populations.
For this, Structure Harvester online utility (taylor0.biology.ucla.edu/structureHarvester) was used. The proportion of the genome of an individual that belongs to each inferred sub-population (Q value) was also estimated. The population differentiation was assessed with the AMOVA implemented in the GenAlex version 6.5 software. Significance levels for variance components were estimated using 9999 permutations. Association between marker alleles and agro-physiological traits was performed using a mixed linear model (MLM) function based on population structure (Q) + relative kinship (K) in TASSEL 3.0. The relative kinship matrix was calculated by TASSEL 3.0. Significant marker-trait associations were declared by $P < 0.0001$ with relative magnitudes represented by the R2 value as the portion of variation explained by the marker. The R^2 value, representing the proportion of phenotypic variance explained by each SNP, was directly obtained from the MLM analysis in TASSEL 3.0. In TASSEL, the R^2 value is calculated as the proportional reduction in the residual sum of squares when the SNP is included in the model, compared to a model without the SNP. This value represents the proportion of the total phenotypic variance that is attributable to the effect of the individual SNP (Yao et al., 2009).

Results

Most of the studied traits, including grain yield and its components, were significantly influenced by environmental conditions in two growing seasons (means provided in Table S2).

Population structure

The analysis of population structure distinguished 5 subpopulations within the entire population, with the highest ΔK value of 231.3 (Figure 1a). Among the subpopulations, POP4 had the highest membership proportion (32.9%) and consisted of 25 genotypes. The bar plot (Figure 1b) shows the distribution of genotypes within and between subpopulations based on the inferred ancestry coefficients, with more details in Table S3. Expected heterozygosity values of subpopulations ranged from 0.123 (POP4) to 0.408 (POP1) and the fixation index (Fst) values of subpopulations ranged from 0.248 (POP1) to 0.721 (POP4). In contrast, the pairwise allele frequency divergence values were maximum between POP1 and POP4 subpopulations (details in Table S4).

Genetic differentiation of populations

Population differentiation was evaluated using an analysis of molecular variance (AMOVA). The results of the AMOVA showed that individuals within five subpopulations are highly genetically distinct compared to individuals among subpopulations. This was evident by a greater proportion of genetic variance within subpopulations than among. Specifically, 84% of the genetic variation was found among accessions within populations, while a smaller (16%) but still significant ($P<0.001$) portion of the variation was observed between populations (Table 2).

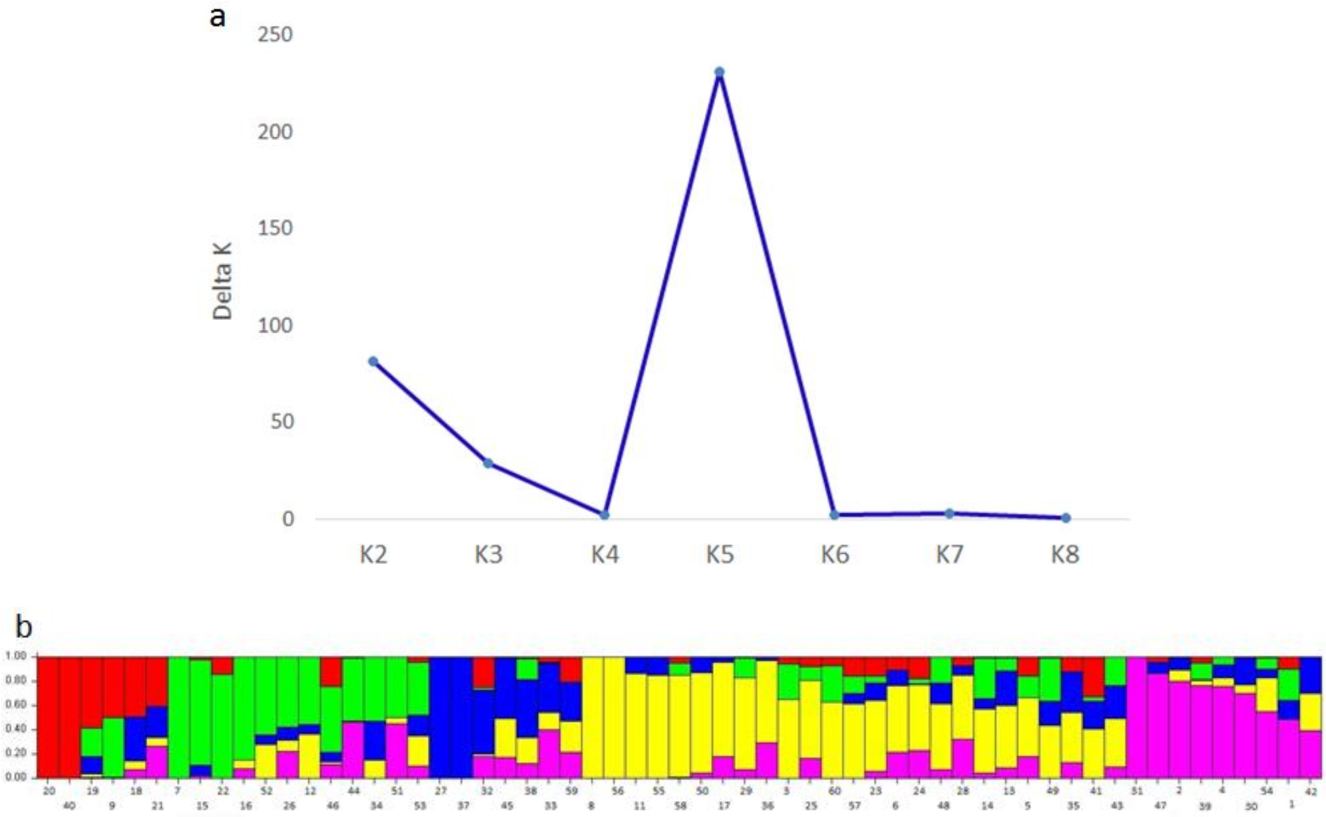

Figure 1. (a) Real number of groups inferred by the Evanno method, and (b) the bar plot showing the distribution of genotypes within and between subpopulations based on the inferred ancestry coefficients by Structure.

Table 1. Average distances (expected heterozygosity) and fixation index (Fst) between individuals in the same cluster.

Subpopulations	Proportion of membership	Average distances	Fst
POP1	0.112	0.4076	0.2481
POP2	0.203	0.222	0.5321
POP3	0.161	0.2558	0.4888
POP4	0.329	0.1228	0.7206
POP5	0.195	0.1938	0.5831

Table 2. Variability within and among five clusters of the spring wheat marker-trait associations panel based on 16063 SNP markers.

Source	df	MS	Estimated Variance	Percentage of variation
Among populations	4	8099.768	505.972	16%
Within populations	54	2632.697	2632.697	84%
Total	58		3138.669	100%

These findings suggest that there has been true differentiation between populations.

Associations in different environments

All wheat genotypes in our association panel were phenotyped for numerous morpho-physiological traits, with the overall means provided in Supplementary Table S2. Using 16063 SNPs, Tassel produced association signals for various traits under normal and stressed environments. SNPs were considered significant only after adjusting for multiple testing at a threshold level of 0.0001. The associated loci for different traits were spread across all the chromosomes.

In total, 121 marker-trait associations (MTA) for a total of 18 traits were detected using a stringent significance criterion (*P* value <0.0001) in the MLM(Q+K) method (Figure 2). All 121 SNP markers were assigned to 21 wheat chromosomes, with 45 of them (37.2%) lacking known positions on the chromosomal maps. The highest number of MTA was recorded for test weight (32) followed by grain hardness (17) and post-anthesis SPAD chlorophyll (12), while the fewest MTA were obtained for days to maturity, days to flowering, plant height, spike length, harvest index, and anthesis SPAD chlorophyll. Environment-wise, the highest number of marker-trait associations were detected in drought-heat stress conditions (37), followed by a heat environment (36). This suggests that the combined stress of drought and heat exerts a strong selective pressure on multiple traits, leading to a greater number of detectable genetic associations. The MTAs detected under drought-heat stress were primarily associated with traits such as grain yield, test weight, and chlorophyll content, suggesting that these traits are particularly sensitive to the combined stress. The late-sown conditions introduced alongside heat stress might have influenced plant performance, thus increasing the number of identified MTAs. The fewest MTA was recorded for drought stress conditions (22), while 26 MTAs were detected in normal conditions. Chromosome-wise, the highest number of marker-trait associations was detected on chromosomes 5B, 7A, and 4A, while chromosomes 2A and 4D harbored the fewest MTA in this study. No MTA was detected for chromosomes 2A and 1B (Figure 2).

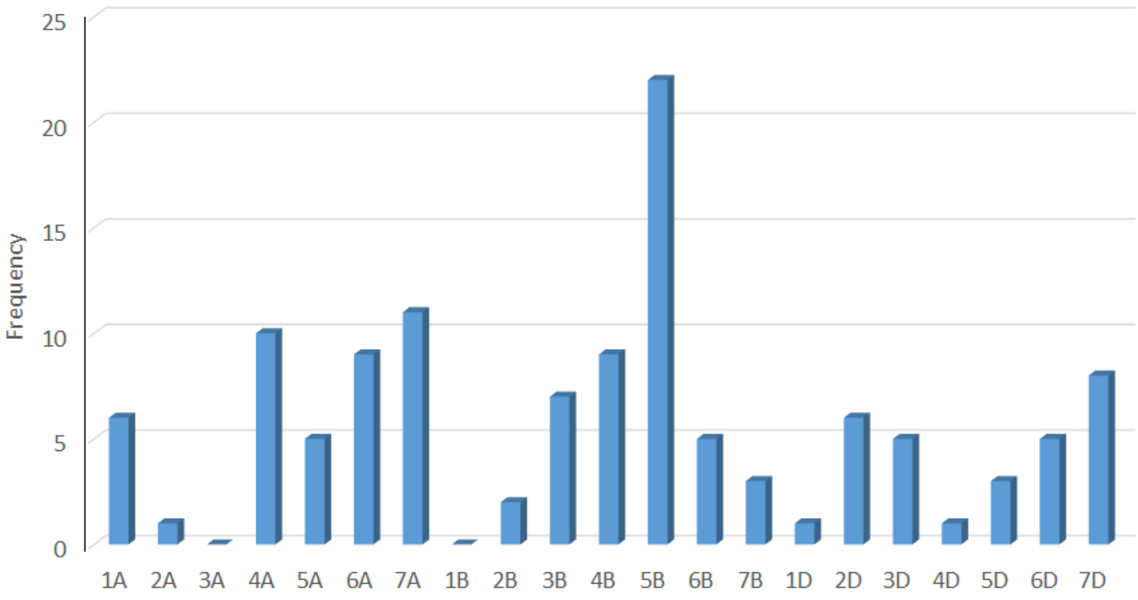

Figure 2. Chromosome-wise distribution of marker-trait associations for 18 traits significant at P<0.0001 obtained by MLM (Q+K) method.

Normal environment

Using the MLM(Q+K) approach, we identified 26 marker-trait associations under normal environmental conditions (Table 3). One SNP on chromosome 6BL showed a significant association with plant height (PLH), while, 1, 3, 5, and 1 SNPs, on chromosome 4AL, 5BL, 7AS and, 7DS respectively, showed significant associations with growth vigor. Additionally, 1 SNP on 3B showed significant associations with days to maturity, and 1, 1, 2, and 1 SNPs on 1AL, 2DL, 2DS, and 6BS respectively, showed significant associations with post-anthesis Fv/Fm. Furthermore, 1, 1, 1, 2, and 1 SNPs on 3B, 3DL, 5AS, 5BS, and 7BS respectively, showed significant associations with grain hardness. Finally, 1 and 2 SNPs on 5AS and 5BS showed significant associations with seed water absorption. The detected associations explained 30.2% to 45.9% of the phenotypic variation of the mentioned traits. The allele T of the associated SNP with days to maturity has an earliness effect of -7.83 days on wheat maturity.

Table 3. Marker-trait associations in the normal environment.

Traits	Associated SNPs	Chromosome	Position (cM)	P_value	R2
Days to maturity	SNP2818	3B	346	6.67E-05	0.318
Fv/Fm	SNP24541	1AL	21	1.84E-05	0.459
	SNP16273	2DL	40	1.20E-05	0.399
	SNP3965	2DS	547	7.88E-05	0.312
	SNP4475	2DS	373	7.88E-05	0.312
	SNP9109	6BS	16	7.88E-05	0.312
Grain hardness	SNP1624	3B	283	4.74E-05	0.334
	SNP28859	3DL	NA	4.74E-05	0.334
	SNP3892	5AS	133	2.82E-05	0.358
	SNP29253	5BS	133	4.39E-06	0.459
	SNP2934	5BS	133	2.82E-05	0.358
	SNP29622	7BS	678	7.86E-05	0.324
Plant height	SNP21106	6BL	2	8.54E-05	0.326
Growth vigor	SNP31970	4AL	327	7.45E-05	0.311
	SNP16303	5BL	579	4.28E-05	0.335
	SNP16930	5BL	714	9.66E-05	0.302
	SNP5294	5BL	579	4.28E-05	0.335
	SNP26919	7AS	NA	7.27E-05	0.312
	SNP27243	7AS	NA	7.27E-05	0.312
	SNP31160	7AS	NA	7.47E-05	0.316
	SNP5954	7AS	NA	8.20E-05	0.314
	SNP977	7AS	624	9.21E-05	0.311
	SNP17720	7DS	NA	7.27E-05	0.312
Seed water absorption	SNP3892	5AS	133	4.64E-05	0.335
	SNP29253	5BS	133	5.87E-06	0.438
	SNP2934	5BS	133	4.64E-05	0.335

NA: unknown position

Similarly, the allele G of the associated SNP with plant height has a positive effect of 12.93 on the trait (Table S5).

Drought stress conditions

Under drought stress conditions, 22 marker-trait associations were detected using the MLM(Q+K) approach (Table 4). One SNP on chromosome 3DL showed a significant association with the harvest index. Another SNP on 3DL was significantly associated with SPAD chlorophyll (anthesis). While 12 SNPs distributed on different chromosomes showed significant associations with SPAD chlorophyll (post-anthesis). Additionally, 2, 1, 1, and 1 SNPs on 5BS, 5AS, 3B, and 3DL showed significant associations with grain hardness. Finally, 2 and 1 SNPs on 5BS and 5AS showed significant associations with seed water absorption. The detected associations explained between 29% to 50.3% of the phenotypic variation of the mentioned traits.

Allele G of the associated SNP with SPAD (2 days after flowering) has a positive effect (3.2 scores) on this physiological trait, while all alleles of the associated SNPs with SPAD (3 days after flowering) have a negative effect (<-10 scores) on the trait (more details in Table S5).

Table 4. Marker-trait associations in drought stress conditions.

Trait	Associated SNPs	Chromosome	Position (cM)	P_value	R2
Grain hardness	SNP29253	5BS	133	1.5E-06	0.503
	SNP2934	5BS	133	2.1E-05	0.370
	SNP3892	5AS	133	2.1E-05	0.370
	SNP1624	3B	283	5.5E-05	0.327
	SNP28859	3DL	734	5.5E-05	0.327
Harvest index	SNP33736	3DL	20	7.2E-05	0.323
SPAD chlorophyll (anthesis)	SNP13312	2DL	39	2.8E-05	0.373
SPAD chlorophyll (post anthesis)	SNP19804	2BS	706	7E-05	0.303
	SNP24940	3B	NA	9.1E-05	0.304
	SNP25290	3B	NA	6.8E-05	0.310
	SNP12230	3DS	NA	5.6E-05	0.310
	SNP1159	4AL	118	6.5E-05	0.303
	SNP11856	4BL	228	8.9E-05	0.290
	SNP8486	4BL	NA	8.9E-05	0.290
	SNP18956	4BS	365	9.2E-05	0.293
	SNP4844	4BS	NA	6.5E-05	0.303
	SNP8797	5DS	43	6.6E-05	0.326
	SNP34288	6AS	632	9.1E-05	0.290
	SNP5500	6BS	259	4.6E-05	0.337
Seed water absorption	SNP29253	5BS	133	1E-05	0.409
	SNP2934	5BS	133	9.2E-05	0.306
	SNP3892	5AS	133	9.2E-05	0.306

NA: unknown position

Table 5. Marker-trait associations in the heat environment. NA: unknown position.

Traits	Associated SNPs	Chromosome	Position (cM)	P_value	R2
Days to flag leaf senescence	SNP17436	2AS	698	9.31E-05	0.299
	SNP23230	2DS	698	9.54E-05	0.316
Days to heading	SNP33122	1AL	139	3.39E-05	0.343
Rolling flag-leaf	SNP19927	5BL	NA	7.94E-05	0.302
	SNP24824	5BL	742	7.94E-05	0.302
	SNP33830	5BL	742	7.94E-05	0.302
	SNP7077	5BL	731	5.89E-05	0.314
	SNP22891	5DL	754	8.79E-05	0.301
	SNP3912	5DL	731	5.89E-05	0.314
Fv/Fm at anthesis	SNP31598	2DL	NA	9.71E-05	0.328
	SNP25311	4BS	NA	9.51E-05	0.304
	SNP34716	4BS	NA	9.87E-05	0.303
	SNP23862	4DS	336	4.21E-05	0.341
Fv/Fm at post anthesis	SNP2168	6AS	668	3.78E-05	0.342
	SNP24118	6AS	113	8.06E-05	0.307
	SNP3325	6DS	668	9.82E-05	0.298
Grain yield	SNP12379	6DL	NA	2.68E-05	0.276
	SNP14824	7AS	249	2.91E-05	0.270
	SNP34736	7AS	627	3.65E-05	0.260
	SNP7009	7AS	627	3.65E-05	0.260
	SNP35083	7BS	627	3.65E-05	0.260
	SNP8252	7DS	249	3.12E-05	0.280
Grain hardness	SNP29253	5BS	133	7.16E-06	0.423
Spike length	SNP22901	7BL	NA	7.21E-05	0.339
	SNP7656	7DL	1588	7.99E-05	0.333
Test weight	SNP178	6AL	NA	2.36E-05	0.394
	SNP18225	6AL	2	3.33E-05	0.349
	SNP2416	6AL	NA	3.30E-05	0.351
	SNP29469	6AL	NA	3.33E-05	0.349
	SNP33378	6AL	2	3.42E-05	0.349
	SNP9071	6AL	2	8.91E-05	0.357
	SNP2575	6BL	NA	3.27E-05	0.361
	SNP18035	6DL	2	4.86E-05	0.343
	SNP28861	6DL	NA	6.46E-05	0.350
	SNP34546	6DL	NA	3.30E-05	0.351
Seed water absorption	SNP29253	5BS	133	7.37E-06	0.421

Heat environment

In the heat stress environment, 36 marker-trait associations were detected using the MLM(Q+K) approach (Table 5). One SNP on chromosome 5BS showed a significant association with grain hardness, two SNPs on 2AS and 2DS showed significant associations with days to flag leaf senescence, and one SNP on 1AL showed a significant association with days to heading. Additionally, 4 and 2 SNPs on 5BL and 5DL showed significant associations with rolling flag-leaf, 1, 2, and 1 SNPs on 2DL, 4BS, and 4DS showed significant associations with Fv/Fm at anthesis, 2

Table 6. Marker-trait associations in the drought-heat environment.

Traits	Associated SNPs	Chromosome	Position (cM)	*P*_value	R²
Days to flag leaf senescence	SNP15000	4AS	NA	5.57E-05	0.313
	SNP24928	4AS	NA	8.30E-05	0.293
	SNP12772	4BL	NA	4.64E-05	0.362
	SNP20611	4BL	NA	4.80E-05	0.316
	SNP33386	4BS	NA	8.30E-05	0.293
	SNP32603	7DS	NA	9.65E-05	0.291
Rolling flag-leaf	SNP15121	3B	727	9.54E-05	0.291
Grain hardness	SNP1624	3B	283	8.42E-06	0.413
	SNP28859	3DL	NA	8.42E-06	0.413
	SNP3892	5AS	133	2.45E-05	0.363
	SNP29253	5BS	133	4.50E-05	0.342
	SNP2934	5BS	133	2.45E-05	0.363
SPAD chlorophyll at anthesis	SNP21259	5BL	163	7.30E-05	0.310
	SNP27662	5BL	165	9.27E-05	0.300
Total SPAD before flowering (TSPDB)SNP212595BL1635.57E-050.325Test weight	SNP13609	1AL	NA	6.08E-06	0.426
	SNP8303	1AL	105	6.08E-06	0.426
	SNP5148	1AS	157	6.08E-06	0.426
	SNP7126	1AS	157	7.08E-06	0.431
	SNP19280	1DL	105	6.08E-06	0.426
	SNP27223	1DL	105	6.73E-06	0.426
	SNP33587	1DL	106	1.08E-05	0.412
	SNP14779	2BS	473	6.08E-06	0.426
	SNP20330	4AL	NA	5.77E-06	0.445
	SNP22016	4AL	NA	1.75E-05	0.477
	SNP24923	4AL	NA	6.09E-06	0.428
	SNP26062	4AL	NA	6.94E-06	0.426
	SNP32088	4AL	NA	8.13E-06	0.422
	SNP550	4AL	NA	6.35E-06	0.429
	SNP555	6BS	NA	7.02E-05	0.535
	SNP29202	7AS	NA	6.08E-06	0.426
	SNP8061	7AS	NA	5.77E-06	0.443
	SNP8863	7AS	NA	6.08E-06	0.426
	SNP11882	7DS	NA	1.39E-05	0.424
	SNP15678	7DS	NA	6.08E-06	0.426
	SNP20749	7DS	NA	6.08E-06	0.426
	SNP8033	7DS	NA	6.08E-06	0.426

NA: unknown position

and 1 SNPs on 6AS and 6DS showed significant associations with Fv/Fm at post-anthesis. Similarly, 1, 3, 1, and 1 SNPs on 6DL, 7AS, 7BS, and 7DS showed significant associations with grain yield. Also, 1 and 1 SNP on 7BL and 7DL showed significant associations with spike length. Furthermore, 6, 1, and 3 SNPs on 6AL, 6BL, and 6DL showed significant associations with test weight. Finally, 1 SNP on 5BS showed a significant association with seed water absorption. The detected associations explained between 29.8% to 42.1% of the phenotypic variation of the mentioned traits. Allele A of the associated SNP with grain hardness has a positive effect (8.5 percent) on this trait, while all alleles of the associated SNPs with anthesis Fv/Fm and spike length have negative effects on these traits (more details in Table S5).

Heat and drought stress conditions

Under a drought and heat environment, 37 marker-trait associations were detected using the MLM(Q+K) approach (Table 6). One SNP on chromosome 3B showed a significant association with the rolling flag leaf. Two SNPs on 5BL showed significant associations with SPAD chlorophyll at anthesis. One SNP located on 5BL showed a significant association with total SPAD before flowering. While, 2, 2, 1, and 1 SNPs on 4AS, 4BL, 4BS, and 7DS showed significant associations with days to flag leaf senescence, respectively. Additionally, 1, 1, 1, and 2 SNPs on 3B, 3DL, 5AS, and 5BS showed significant associations with grain hardness, respectively. Finally, 2, 2, 3, 1, 6, 1, 3, and 4 SNPs on 1AL, 1AS, 1DL, 2BS, 4AL, 6BS, 7AS, and 7DS showed significant associations with test weight, respectively. The detected associations explained ranged from 29.1 to 53.5 percent of phenotypic variation of the above traits. Allele G of the associated SNP with rolling flag leaves has a negative effect (-0.67) on this trait, while all alleles of the associated SNPs with grain hardness exert positive effects on these traits (more details in Table S5).

Associations for grain yield

With a *P* value of <0.0001, MLM(Q+K) could only detect a few associations for grain yield (GY) under heat stress conditions. Therefore, we used the *P* value <0.001 to reduce the risk of losing probable real associations. With this relaxed criterion, Association mapping analysis detected 56 newly identified MTAs for grain yield in all environmental conditions. Specifically, 11, 31, 10, and 10 marker-trait associations were detected under normal, drought, heat, and drought-heat stress conditions, respectively (Table 7). Under normal conditions, 1, 2, 4, 2, 1, and 1 SNPs on 1DL, 2AS, 2BS, 2DS, 3AS, and 5AL, respectively, showed associations with grain yield. Under drought stress conditions, 3, 1, 2, 6, 1, 4, 1, 1, 5, 1, 2, 1, 2, and 1 SNPs were detected in association with the grain yield trait on 2AS, 2BL, 2BS, 2DS, 3AL, 3B, 3DS, 5AL, 5BS, 5DS, 6BL, 6DS, 7AS, and 7BS, respectively. Under heat stress conditions, as shown in Table 5, six marker-trait associations were observed at a low *P*-value. Additionally, four more associations (one, two, and one SNPs on chromosomes 3B, 4BS, and 4DS, respectively) were identified at a higher *P*-value (Table 7). Under drought-heat stress conditions, 1, 2, 1, 1, 3, 1, and 1 SNPs on 3AL, 3B, 3DL, 6DL, 7AS, 7BS, and 7DS were associated with grain yield, respectively (Table 7). Environment-wise, the highest explained variation of grain yield was recorded for SNP8295 on 2BS (22.5%) under normal conditions, for SNP30215 on 7AS (35.2%) under drought stress conditions, for SNP8252 on 7DS (28.0%) under heat stress conditions, and SNP23906 on 3DL (23.6%) under drought-heat stress conditions.

Discussion

This study initially describes the genetic structure and marker-trait associations in 60 CIMCOG wheat lines using the 35K Axiom® Array. Five main clusters of accessions were identified; however, a significant proportion of plants (64.4%) appeared to have ancestry in more than one cluster (more details in Table S3). This may reflect true admixture between neighboring clusters or be an artifact of attempting to model discrete populations despite continuous gradation of allele frequencies (i.e., isolation by distance, with no clear boundaries defining adjacent populations or groupings of races) (Pritchard et al., 2000; Rosenberg et al., 2002; Barbujani and Belle, 2006). Additionally, the same isolation by distance pattern would have been observed if extensive human-mediated germplasm exchange occurred, particularly in the transitional

Table 7. Significant associations (P value <0.001) of the SNPs with grain yield under normal, drought, and combined stress conditions.

Environment	Associated SNP	Chromosome	Location (cM)	*P*_value	R^2
Normal					
	SNP29838	1DL	26	3.65E-04	0.218
	SNP3766	2AS	658	7.60E-04	0.194
	SNP17950	2AS	NA	6.69E-04	0.205
	SNP14209	2BS	319	8.71E-04	0.204
	SNP29752	2BS	319	8.22E-04	0.186
	SNP431	2BS	NA	3.04E-04	0.206
	SNP8295	2BS	NA	2.51E-04	0.225
	SNP15740	2DS	303	7.10E-04	0.187
	SNP1346	2DS	319	7.10E-04	0.187
	SNP18943	3AS	NA	7.96E-04	0.180
	SNP23639	5AL	261	3.23E-04	0.211
Drought					
	SNP14404	2AS	319	4.50E-04	0.246
	SNP10223	2AS	726	1.88E-04	0.226
	SNP3176	2AS	726	5.43E-04	0.197
	SNP5575	2BL	NA	9.86E-04	0.178
	SNP24298	2BS	667	1.12E-04	0.239
	SNP7775	2BS	706	8.62E-04	0.268
	SNP33111	2DS	309	3.66E-04	0.215
	SNP19908	2DS	319	8.88E-04	0.183
	SNP32483	2DS	319	5.42E-04	0.194
	SNP33985	2DS	319	7.16E-04	0.193
	SNP4160	2DS	319	4.89E-04	0.197
	SNP4966	2DS	726	3.29E-04	0.211
	SNP6105	3AL	448	9.84E-04	0.336
	SNP31304	3B	76	1.16E-04	0.325
	SNP13756	3B	448	9.52E-04	0.246
	SNP4540	3B	784	7.21E-04	0.189
	SNP9321	3B	NA	1.81E-04	0.299
	SNP24069	3DS	NA	5.50E-04	0.284
	SNP31186	5AL	99	7.69E-04	0.184
	SNP14201	5BS	32	4.91E-04	0.196
	SNP14562	5BS	32	7.69E-04	0.184
	SNP12377	5BS	99	6.47E-04	0.186
	SNP28245	5BS	99	5.01E-04	0.199
	SNP95	5BS	99	6.47E-04	0.186
	SNP5439	5DS	99	6.47E-04	0.186
	SNP1209	6BL	NA	8.28E-04	0.182
	SNP653	6BL	NA	6.49E-04	0.202
	SNP10936	6DS	NA	3.10E-04	0.219
	SNP33259	7AS	188	8.18E-04	0.186
	SNP30215	7AS	NA	3.88E-04	0.352

	SNP19661	7BS	661	5.75E-04	0.209
Heat					
	SNP2433	3B	NA	9.24E-04	0.182
	SNP28894	4BS	NA	4.71E-04	0.198
	SNP8940	4BS	NA	4.71E-04	0.198
	SNP30521	4DS	381	4.71E-04	0.198
	SNP12379	6DL	NA	2.68E-05	0.276
	SNP14824	7AS	249	2.91E-05	0.270
	SNP34736	7AS	627	3.65E-05	0.260
	SNP7009	7AS	627	3.65E-05	0.260
	SNP35083	7BS	627	3.65E-05	0.260
	SNP8252	7DS	249	3.12E-05	0.280
Drought-heat					
	SNP10524	3AL	NA	5.29E-04	0.198
	SNP10860	3B	297	2.58E-04	0.211
	SNP2614	3B	NA	7.47E-04	0.188
	SNP23906	3DL	1082	1.09E-04	0.236
	SNP12379	6DL	NA	7.31E-04	0.189
	SNP14824	7AS	249	1.41E-04	0.229
	SNP34736	7AS	627	3.26E-04	0.204
	SNP7009	7AS	627	3.26E-04	0.204
	SNP35083	7BS	627	3.26E-04	0.204
	SNP8252	7DS	249	7.27E-04	0.195

NA: unknown position

zones between neighboring clusters (Vigouroux et al., 2008). These findings confirm that the germplasm consists of genetically diverse, advanced spring wheat lines selected for their yield potential under stressful conditions.

Association analysis using the MLM (Q+K) method with a stringent criterion ($P < 0.0001$) identified the most reliable marker-trait associations in each environment. Due to the high significance threshold, only a few studied traits have trait-marker associations that include at least one SNP marker. For example, in the normal environment, between 1 (for plant height and days to maturity) and 10 SNPs (for growth vigor) were identified. In the drought conditions, between 1 (for harvest index) and 12 SNPs (for post-anthesis SPAD chlorophyll) showed associations. In the heat environment, between 1 (for days to heading) and 10 SNPs (for test weight) were associated. In the drought-heat environment, between 1 (for RFL) and 22 SNPs (for test weight) showed associations (Tables 3-6). The more stringent the criterion for reporting a significant association, the fewer marker-trait associations are detected (Vanniarajan et al., 2012; Kumar et al., 2015).

Phenological adjustment, which involves optimizing the duration of different developmental phases, has proven to be one of the most effective strategies for helping wheat adapt to harsh and/or highly variable environmental conditions (Hyles et al., 2020). Our association analysis identified one SNP on 1AL (SNP33122) for days to heading under normal conditions and one SNP on 3B (SNP2818) for days to maturity under heat stress conditions. Time-related traits, like days to heading and days to maturity, are crucial in crop plants as time to anthesis is a key trait determining wheat adaptation to specific growing conditions (Snape et al., 2001; Zhao et al., 2023). Variability in time to anthesis can be utilized to adjust growth and development patterns to the prevailing environmental conditions in any given environment (Touzy et al., 2022).

Plant height (PLH) is a crucial agronomic trait in wheat due to various developmental and adaptability reasons. In our research, only one marker-PLH association (SNP21106 on 6BL) was detected under normal conditions. Although several genomic regions have been reported to be associated with PLH (Cadalen et al., 1998; Hu et al., 2015; Vukasovic et al., 2022), chromosome 6B has consistently emerged as an important genomic region controlling PLH in most association/QTL analyses. For instance, in the chromosome region 6BL of BF475120, the SSR markers Xfbb250-6B are significantly associated with PLH (Cadalen et al., 1998). Additionally, (Börner et al., 2002) detected two QTLs for PLH in a similar region of 6BL.

Under drought stress conditions, our research identified one associated marker (SNP33736) on chromosome arm 3DL for harvest index (HI). This is the first time that this region on 3DL has been reported in association with HI. In a study by Neumann et al. (2011) many marker-trait associations were detected for HI on chromosomes 1A, 3A, 4A, 5A, 7A, and 7B. Another analysis with 96 diverse winter wheat lines found repeatable marker-trait associations on chromosomes 1DL and 2DS (Dodig et al., 2012).

Rehman Arif et al. (2020) also reported 12 QTLs for HI on chromosomes 1B, 2B, 3B, 4B, 5B, 7A, and 7B. (El-Feki et al., 2018) reported three harvest index QTLs on chromosomes 1B, 2B, and 6B. Various reasons can be suggested to explain this discrepancy, such as chromosome rearrangements in the genetic background of our population.

Test weight, or hectoliter weight, is a key characteristic to assess and classify wheat grain quality in the marketplace. This trait is influenced by various factors such grain weight, size, endosperm hardness, and water content. Therefore, understanding the genetic basis of test weight is critical for enhancing both grain yield and end-use quality of wheat. In studied conducted under heat and drought-heat stress conditions, several associations were identified for test weight. Specifically, under heat stress, associations were found for 1, 3, and 6 SNPs on chromosomes 6BL, 6DL, and 6AL, respectively. These SNPs explained between 34% and 39.4% of the trait variation. Under drought-heat stress conditions, associations were detected for SNPs on chromosomes 2BS, 6BS, 1AL, 1AS, 1DL, 7AS, 7DS, and 4AL, explaining between 41.2% and 53.5% of the trait variation. Previous studies have also identified QTLs for test weight on chromosomes 1A, 1B, 3B, and 7A. Genome-wide association analysis, (He et al., 2023) reported the highest number of stable associations for grain hardness. Huang et al. (2006) found QTLs for test weight on chromosomes 4A, 4D, 2D, 5A, and 7A with the QTL on 4D explaining the maximum 13.1% of the phenotypic variation.McCartney et al. (2005) found 10 QTLs for test weight on chromosomes 1B, 1D, 2B, 2D, 3B, 3D, 4D, 5D, 6B, and 7D with the most significant QTLs on chromosomes 3B and 4DS. Another important QTL newly detected in this study is located on 6AL under heat stress conditions. Nonetheless, based on our findings and previous reports, the QTL located on chromosome arm 6BS seems to play a more significant role in determining test weight under water-related stress conditions.

Our analysis revealed six SNPs significantly associated with grain yield under heat stress conditions, with a *P*-value of <0.0001. However, when the threshold was relaxed to a higher *P*-value (<0.001), the mapping identified 10 SNP-grain yield associations under the same conditions, distributed across additional wheat chromosomes.

The practical application of the identified SNPs lies in their potential use as molecular markers for marker-assisted selection (MAS) in wheat breeding programs aimed at improving stress tolerance. All the detected associated SNPs explained a minimum of 18% of the variance (e.g., SNP1894 on chromosome 3AS under normal conditions) and up to 35.2% of the variance (e.g., SNP30215 on chromosome 7AS under drought stress conditions). These findings suggest that the identified SNPs are significantly associated with key traits related to stress tolerance. The six SNPs that were common between heat and combined drought-heat stress conditions (SNP2379 on 6DL, SNP14824, SNP34736, SNP7009 on 7AS, SNP35083 on 7BS, and SNP8252 on 7DS) could serve as important genetic markers for improving wheat performance under these combined stress environments. By targeting these markers in breeding programs, it is possible to develop wheat varieties that exhibit enhanced resilience to both heat and drought stress, which is essential for adapting wheat production to increasingly erratic and extreme environmental conditions.

In line with our results, many studies have demonstrated that all seven chromosome groups are involved in the genetic control of yield and yield-related traits in bread wheat (Jahani et al., 2019; Govta et al., 2022).Crossa et al. (2007) conducted an association analysis for yield and disease resistance using 170 spring wheat lines genotyping with DArT markers under normal conditions. They found marker-trait associations for yield on all chromosomes except chromosome 4D. However, our results show chromosome arm 2BS has more SNPs (4) associated with grain yield under normal conditions (Table 7). McCartney et al. (2005) detected the most significant yield QTL on chromosome 2B from QTL analysis conducted on DH lines evaluated at eight site years in Manitoba, Canada. Also, our finding aligns with previous research that identified a major quantitative trait locus (QTL) on chromosome 2B related to grain yield under drought stress (Sukumaran et al., 2018). Additionally, a study reported a significant QTL on chromosome 2B associated with heat tolerance during the booting stage, further emphasizing the importance of this genomic region in stress resilience (Erena et al., 2021). In our research, chromosome arms 2DS and 5BS as well as chromosome 3B exhibited a higher number of SNPs (6, 5, and 4, respectively) associated with grain yield under drought stress conditions. Previous studies have identified the most stable marker-trait associations for grain yield on chromosomes 2DS (Juliana et al., 2019), 5B (Zhao et al., 2021; Dan et al., 2023), and 3B (Liao, 2024).

Furthermore, our analysis revealed MTAs on chromosome 7D correlated with thermal tolerance during the grain-filling period. This observation is consistent with findings that identified a key QTL on chromosome 7D enhancing yield and grain weight under late-season heat and drought stress (Mazumder et al., 2024). While some of the MTAs identified in our study, particularly those on chromosomes 3A and 5D, were initially thought to be novel associations with heat and drought resistance, recent literature suggests that these regions may already be implicated in stress-related traits. For instance, chromosome 3A has been reported to carry productivity and adaptability-related genes, contributing to traits such as biomass, grain number, and ground cover, which are crucial for grain yield improvement under stress conditions (Bennani et al., 2022). Similarly, QTLs on chromosome 5D have been linked to days to anthesis and plant height under heat and drought stress (Schmidt et al., 2020). These findings indicate potential pleiotropic effects of these regions on grain yield and drought tolerance. As a consequence, while these MTAs show consistency across known loci, further studies need to confirm their functional implications in adaptation to stresses. These areas could contain uncharacterized processes or genes that confer wheat tolerance by adaptation; thus, further validation by means of fine-mapping as well as functional genomics studies must be conducted.

Further analysis of the detected associations reveals the co-location of some SNP loci associated with more than one trait. Examples include SNP3892 (on 5AS), associated with grain hardness and seed water absorption under normal conditions; SNP29253 (on 5BS), associated with the same two traits under normal, drought, and heat stress conditions, and SNP2934 (on 5BS), associated again with these two traits under normal and drought stress conditions.

Additionally, the analysis identified more stable marker-trait associations significant under two or more environmental conditions. For instance, aside from common SNPs for grain yield among heat and drought-heat stress conditions noted earlier, SNP29253 on 5BS showed an association with grain hardness in all four environments. Furthermore, four other SNPs (SNP3892 on 5AS, SNP2934 on 5BS, SNP1624 on 3B, and SNP28859 on 3DL) were associated with grain hardness in normal, drought, and drought-heat environments. SNP2934 (on 5BS) was associated with seed water absorption under normal, drought, and heat stress conditions, while SNP3892 (on 5AS) and SNP29253 (on 5BS) were associated with seed water absorption under normal and drought stress conditions.

Conclusion

This study has identified significant MTAs for various traits under drought, heat, and combined environmental conditions. A total of 121 MTAs were detected across 18 traits, with the strongest associations found for test weight and grain hardness. Importantly, the identified SNPs accounted for trait variances ranging from 18% to 35.2% under specific conditions. The analysis also revealed substantial genetic differentiation among populations, with 84% of genetic variation occurring within populations. This points to a robust genetic structure influenced by environmental adaptation. Furthermore, the results suggest that a deeper understanding of the genetic basis of stress tolerance could greatly benefit breeding programs focused on enhancing wheat resilience to abiotic stress. This, in turn, could lead to the development of cultivars better suited to combat the challenges brought on by climate change.

Supplementary Materials

The supplementary material for this article can be found online at: https://www.jpmb-gabit.ir/article_717860.html.

Supplementary Table S1. Combined analysis of variance for agro-physiological traits of wheat genotypes grown in four environmental (normal, Heat, drought, and combined stress) conditions, replicated (Rep) twice per two conditions and in two growing seasons, and block (BL) eight blocks per two replicated in two conditions and two growing seasons.

Author Contributions

Conceptualization, B.V. and A.A.; methodology, B.V.; software, B.V. and M.H.; validation, B.V. and A.A.; formal analysis, B.V.; investigation, B.V.; resources, B.V.; data curation, B.V.; writing—original draft preparation, B.V.; writing—review and editing, A.A. and M.H.; visualization, B.V.; supervision, A.A.; project administration, B.V.; funding acquisition, B.V. and A.A. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflict of Interest Statement

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan

2024 | Volume 11 | Issue 1

مقاله پژوهشی

نشانگرهای SNP مرتبط با صفات زراعی تحت شرایط تنش خشکی، گرما در گندم

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چکیده: تنش‌های خشکی و گرما چالش‌های عمده‌ای در تولید کشاورزی به شمار می‌آیند. در این مطالعه هسته متنوع ژرم‌پلاسم CIMMYT-Mexico (CIMCOG) تحت تنش‌های خشکی، گرما و تنش ترکیبی به مدت دو فصل زراعی مورد ارزیابی قرار گرفت. ژنوتایپینگ زرم پلاسم با استفاده از میکرو آرایه ۳۵ K ®Axiom انجام شد تا ارتباطات با صفات مورد مطالعه تحت شرایط تنش‌های محیطی مذکور شناسایی گردد. از روش آماری پیش-بینی‌های نااُریب خطی(BLUPs) برای هر لاین جهت محاسبه ارتباطات نشانگر–صفت با استفاده از ۱۶۰۶۳ نشانگر SNP و مدل خطی مختلط (MLM) استفاده شد. برای نقشه‌یابی ارتباطای گسترده ژنوم (GWAM)، بیشترین تعداد ارتباطات برای صفت وزن حجمی (۳۲) به دست آمد که پس از آن سختی دانه (۱۷) قرار داشت. برای عملکرد دانه، شش SNP تنها تحت در شرایط تنش گرمایی شناسایی شدند. با این حال، در کمتر از سطح یک دصد ($P<0.001$)، GWAM بسیاری از ارتباطات معنادار را شناسایی کرد (به ترتیب ۱۱، ۳۱، ۱۰ و ۱۰ ارتباط تحت شرایط نرمال، خشکی، گرما و ترکیبی (خشکی–گرما)). تمامی SNPهای شناسایی شده حداقل ۱۸٪ تا حداکثر ۳۵/۲٪ از واریانس را توجیه می‌کردند. از میان ارتباطات شناسایی شده، شش مورد بین تنش گرما و ترکیبی خشکی–گرما مشترک بودند. این نتایج ممکن است دریچه‌ای جدید برای برنامه‌های به‌نژادی جهت بهبود تحمل گندم به تنش‌های غیرزنده باز کند.

کلمات کلیدی: تحمل به تنش غیرزنده، GWAS، پلی‌مرفیسم نوکلئوتیدی منفرد، *Triticum aestivum*.

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مدیرتکنولوژی شرکت اگری ساینس اسپرینگ ولی، چین

تاریخ

دریافت: ۲۱ دی ۱۴۰۳
پذیرش: ۸ اسفند ۱۴۰۳
چاپ: ۱۲ فروردین۱۴۰۴

نویسنده مسئول

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ارجاع به این مقاله

Vaezi, B., Arzani, A., and Haghpanah, M.(2025). SNP markers associated with agronomic traits under drought, heat, and combined stress conditions in wheat. *J Plant Mol Breed*. *13* (1): 19-38.
doi: 10.22058/jpmb.2025.2050354.1327 .