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RESEARCH

Marker–trait associations for physiological responses to irrigation and terminal drought stress in bread wheat using SSR and iPBS markers

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Abstract

As global food demand rises, ensuring wheat (*Triticum aestivum* L.) production under intensifying climate change is critical. Late drought stress is one of the major environmental constraints limiting wheat productivity and yield stability worldwide. This study aimed to characterize drought tolerance in bread wheat by integrating physiological traits with molecular marker analysis. A panel of 64 bread wheat genotypes was evaluated in an 8 × 8 lattice design with two replications under highly controlled irrigated and rain-shelter terminal drought stress conditions over two growing seasons. Bread wheat genotypes were evaluated over two growing seasons using simple sequence repeat (SSR) and inter-primer binding site (iPBS) retrotransposon markers. Physiological assessments included canopy temperature difference (CTD), leaf senescence rate (LSR), SPAD chlorophyll content, chlorophyll fluorescence parameters (Fo, Fm, Fv, and Fv/Fm), as well as leaf and grain ash content. Significant phenotypic variation was observed among genotypes under both irrigated and drought-stressed environments. Drought stress generally increased phenotypic and genotypic variances for most investigated traits, with coefficients of variation ranging from 0.97% to 26.85%. Moreover, elevated phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) values under drought conditions indicate enhanced genetic expression in response to water deficit. Broad-sense heritability estimates were particularly high for senescence and chlorophyll fluorescence traits and were generally greater under drought stress than under irrigated conditions, suggesting stronger genetic regulation under stress environments. Association mapping identified 25 iPBS markers and 108 SSR loci significantly associated with physiological responses to drought stress. Notably, iPBS-2376 and iPBS-2390 were associated with multiple physiological traits, whereas SSR loci such as BARC 78 and CFA 2187 exhibited strong associations with chlorophyll stability and canopy temperature regulation. The findings demonstrate that integrating physiological characterization with SSR and iPBS marker systems is an effective strategy for identifying drought-tolerant wheat genotypes and detecting genomic regions valuable for marker-assisted breeding aimed at improving drought adaptation and yield stability.

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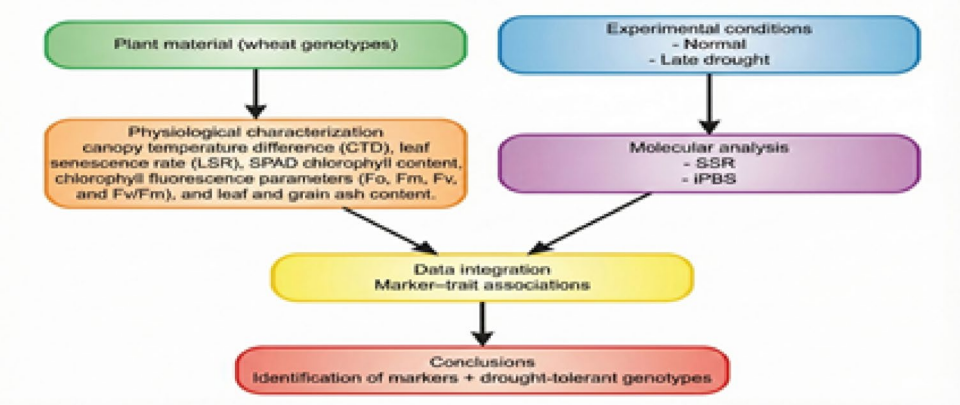
Graphical abstract



Keywords Bread wheat · Drought tolerance · SSR markers · iPBS retrotransposon · Physiological traits

Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops and a cornerstone of global food security (Bohra et al. 2024; Mutanda et al. 2025). Its grain provides a substantial share of caloric and protein intake for the human population, and its production underpins the economic stability of many regions (Aremu et al. 2025). However, the rising demand for food, combined with intensifying climate change, makes wheat particularly vulnerable to a range of stress factors, among which water deficit is of critical importance (Dave et al. 2024; He et al. 2025). Drought reduces yield more severely than any other environmental factor, and its effects are especially detrimental during the late stages of plant development, when grain formation occurs (dos Santos et al. 2025; Lohani et al. 2025; Yang et al. 2025). This so-called late season drought negatively affects key physiological processes, reduces photosynthetic potential, disrupts water balance, and accelerates leaf senescence, ultimately leading to decreases in both grain weight and grain quality (Haghpanah et al. 2024; Ingvarsson and Street 2011; Isgandarova et al. 2024; Qiao et al. 2024).

In response to these challenges, research on drought tolerance mechanisms in wheat increasingly focuses on both physiological trait assessment and the use of molecular tools that enable the identification of genetic markers associated with plant adaptation to ad-verse environmental conditions (Okselenko et al. 2025; Ramachandran et al. 2025). The development of molecular marker technologies has facilitated the investigation of polygenic inheritance and the localization of genomic regions controlling complex quantitative traits. A comprehensive understanding of the genetic architecture underlying quantitative trait variation including the number, genomic position, effect size, and identity of the contributing loci requires detailed analyses of the relationships between phenotypic variation and DNA polymorphism (Ingvarsson and Street 2011). A major goal is to accelerate breeding program's through marker assisted selection (MAS), which facilitates the rapid and precise introduction of drought resistant cultivars into production (Mapari and Mehandi 2024; Singh et al. 2025; Tharun et al. 2024).

Studies on the physiological impacts of drought stress demonstrate that reduced water availability during late growth stages leads to declines in relative water content (RWC), stomatal conductance, and photosynthetic rate, as well as increased accumulation of reactive oxygen species, which intensifies oxidative stress (Monneveux et al. 2012). These processes directly contribute to yield reductions, including decreases in thousand grain weight and grain technological quality.

In the context of phenotyping adaptive traits, widely used indicators include the Stress Susceptibility Index (SSI), Stress Tolerance Index (STI), and chlorophyll content measurements (SPAD), together with chlorophyll fluorescence parameters. These tools support the assessment of a plant's ability to maintain metabolic activity under water limited conditions (Blum 2010). Integrating physiological assessment with molecular analyses enables the identification of genotype–phenotype relationships, which is essential for understanding the complex mechanisms underlying drought tolerance.

In recent years, association mapping (AM) has emerged as a powerful approach for linking complex trait variation to underlying gene sequences by exploiting historical and evolutionary recombination events at the population level (Nordborg and Tavaré 2002). Compared with traditional quantitative trait locus (QTL) mapping, AM offers several

advantages, including reduced time and cost requirements, access to broader allelic diversity, and improved mapping resolution (Yu and Buckler 2006). Since its initial application in plant genetics (Thornsberry et al. 2001), interest in AM has increased substantially, driven by advances in statistical methodologies and high-throughput genomic technologies, as well as the growing demand for the identification of superior and novel alleles. Among molecular markers, Simple Sequence Repeats (SSR) are most frequently applied in wheat research due to their high polymorphism, reproducibility, and stability, making them valuable for genetic diversity studies, QTL mapping, and association analyses. Mohi-Ud-Din et al. (2022) used SSR markers to identify genomic regions associated with drought tolerance in diverse wheat genotypes, demonstrating their suitability for MAS. Conversely, the inter Primer Binding Site (iPBS) technique, based on conserved retrotransposon sequences, has proven effective for genetic diversity assessment and population structure analysis. Haliloğlu et al. (2023) highlighted the high utility of iPBS markers in wheat germplasm evaluation, indicating their potential for differentiating genotypes with enhanced drought tolerance. Similar conclusions were presented by Demirel et al. (2024), Dık et al. (2025a), who reported that iPBS markers generate rich polymorphism profiles and may support breeding programs targeting resistance to abiotic stresses.

The integration of physiological and molecular data is crucial for identifying genetic loci associated with drought resistance. Sukumaran et al. (2018) described the successful use of marker assisted selection in introgression QTL linked to drought tolerance into wheat breeding materials, confirming the practical value of this approach. To date, research clearly demonstrates that both SSR and iPBS markers can be effectively used to assess wheat genetic variability and identify potential markers associated with drought tolerance. However, most available studies focus on early or moderate drought stress, while late season drought critical for final yield formation remains underexplored. There is also a lack of comprehensive studies integrating physiological and molecular datasets to elucidate adaptive mechanisms during the final stages of wheat development. Moreover, despite numerous reports on the usefulness of SSR markers in QTL mapping and association studies, knowledge regarding the practical application of iPBS markers in MAS in wheat remains limited. Most iPBS based studies address germplasm diversity rather than linking these markers to physiological traits associated with drought tolerance. Therefore, there is a need for research that combines both marker systems and directly relates them to physio-logical responses of wheat grown under optimal and late season drought conditions. This gap encompasses the absence of integrative analyses combining physiological phenotyping with SSR and iPBS marker profiling during the critical grain filling stage under water deficit. Addressing this gap may contribute to the development of more precise breeding tools and improve the efficiency of selecting drought resistant wheat cultivars.

Although association mapping is increasingly used to study drought tolerance in wheat, most research has concentrated on water stress during early or mid-growth stages. Conversely, drought during the grain-filling period when yield is most vulnerable has been less examined. Moreover, while simple sequence repeat (SSR) markers are commonly employed in marker-assisted selection, little is known about the effectiveness of inter-primer binding site (iPBS) markers in linking physiological traits related to drought responses. Consequently, our understanding of how retrotransposon-based polymorphisms influence physiological adaptation under terminal drought conditions remains limited. Accordingly, the present study aimed not only to identify marker–trait associations but also to evaluate the stability, physiological relevance, and breeding potential of SSR and iPBS loci associated with drought-adaptive traits under late drought.

Materials and methods

Plant material and site description

A diverse panel of 64 bread wheat (*Triticum aestivum* L.) genotypes was evaluated, comprising 53 cultivars and 11 landraces adapted to rainfed environments in Türkiye (Table 4). Detailed information on the list of genotypes and their pedigree backgrounds has been reported previously by Öztürk and Aydin (2017). Based on prior classifications of drought tolerance, the cultivars Bezostaja-1 and Karasu-90 were included as drought-sensitive checks. Field experiments were conducted over two consecutive growing seasons (2011–2012 and 2012–2013) at the Research Farm of Atatürk University in Erzurum, Türkiye (29°55′ N, 41°16′ E; elevation 1850 m). The experimental site is characterized by a semi-arid climate, with a long-term mean annual precipitation of 398.8 mm and an average annual temperature of 5.0 °C.

Experimental design and stress treatments

As described in detail by Öztürk and Aydin (2017), the study employed an 8 × 8 lattice design with two replications under two distinct water regimes: well-watered and terminal drought stress. The experiments were established on land that had been fallowed in the previous season. Seeding rates were standardized to achieve a density of 475 viable seeds

m^{-2}. Plot dimensions varied by treatment to accommodate infrastructure; irrigated plots were machine-sown (six rows, 6 m long, 20 cm spacing), while drought plots were manually sown (two rows, 1.5 m long, 20 cm spacing). Nutrient management included 60 kg N ha^{-1} (ammonium sulfate) and 50 kg P ha^{-1} (triple superphosphate). All phosphorus and half of the nitrogen were incorporated at sowing, with the remaining nitrogen top-dressed at the onset of stem elongation (Feekes 6.0). Biotic stresses were minimized through manual weeding and prophylactic fungicide applications at the booting stage and one-week post-anthesis.

Phenotypic measurements

All agronomic and physiological traits were evaluated following standardized protocols previously described by Öztürk and Aydin (2017). Physiological assessments were performed at three key phenological stages: anthesis (A), 15 days after anthesis (15 DAA), and 25 days after anthesis (25 DAA). Canopy temperature depression (CTD) was determined as the difference between ambient air temperature and canopy temperature, the latter measured using a handheld infrared thermometer, as described by Xiao et al. (2012). Leaf senescence rate (LSR) was quantified based on the proportion of green leaf area relative to total leaf area, following the methodology proposed by Verma et al. (2004). Chlorophyll status and photosynthetic efficiency were evaluated on flag leaves using a SPAD-502 chlorophyll meter and a Handy PEA chlorophyll fluorometer, respectively, in accordance with the procedures outlined by Guo et al. (2008). At physiological maturity, ash content of both flag leaves and grains was quantified by dry ashing at 575 °C and expressed on a dry weight basis, following the approved methods of the American Association of Cereal Chemists (McCleary et al. 2013). To prevent environmental and sampling biases arising from structural differences in plot sizes between water regimes, all physiological measurements (SPAD, CTD, and chlorophyll fluorescence) were taken exclusively from standard, uniform flag leaves in the center rows of the plots. Plants on the outer margins were strictly avoided to eliminate any potential edge effects. Measurements were consistently conducted on clear days between 11:00 AM and 1:00 PM to minimize diurnal physiological fluctuations. Furthermore, prior to combined association mapping, the phenotypic data from each water regime were analyzed separately using replication and year as random effects to calculate adjusted means, effectively standardizing the trait values and accounting for micro-environmental variance.

DNA extraction

Genomic DNA isolation

Genomic DNA was isolated from young leaf material collected from 14-day-old seedlings using a modified cetyltrimethylammonium bromide (CTAB) extraction procedure, as outlined by Işık et al. (2025b). The integrity and purity of the extracted DNA were verified by electrophoretic separation on 1% agarose gels. DNA yield and purity were determined spectrophotometrically using a NanoDrop ND-1000 UV/Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Prior to polymerase chain reaction (PCR) amplification, all DNA samples were normalized to a working concentration of 25 ng μL^{-1}.

Molecular marker analysis

Two complementary marker systems, simple sequence repeats (SSR) and inter-primer binding site (iPBS) markers, were employed for molecular characterization of the wheat germplasm. Genotyping procedures followed standardized protocols previously optimized and validated in our earlier studies (Haliloğlu et al. 2023; Türkoğlu et al. 2023).

PCR amplification conditions

Polymerase chain reaction (PCR) amplifications for both marker systems were carried out using a SensoQuest Labcycler thermal cycler (Göttingen, Germany), applying primer-specific optimized conditions as described by Türkoğlu et al. (2023) and Haliloğlu et al. (2023). Each PCR reaction was performed in a total volume of 10 µL, containing 25 ng of genomic DNA, 1× reaction buffer, 2 mM $MgCl_2$, 0.25 mM of each dNTP, 1 µM (20 pmol) primer, and 0.5 U of Taq DNA polymerase. The amplification program consisted of an initial denaturation step at 95 °C for 3 min, followed by 38 cycles of denaturation at 95 °C for 60 s, primer annealing at 50–60 °C for 60 s (depending on primer-specific melting temperatures), and extension at 72 °C for 120 s, with a final elongation step at 72 °C for 10 min.

SSR genotyping

SSR genotyping was performed using the marker panel and screening strategy described by Türkoğlu et al. (2023). Initially, 425 SSR primer pairs were evaluated, of which 120 polymorphic markers displaying clear and reproducible amplification profiles were selected for genotyping the full set of wheat genotypes. Amplified fragments were resolved by polyacrylamide gel electrophoresis using a Mega-Gel dual vertical electrophoresis system (Model C-DASG-400-50).

Gels were stained with ethidium bromide (1 µg mL^{-1}), and banding patterns were visualized under ultraviolet illumination and documented using a digital imaging system (Nikon Coolpix 500, Nikon, Japan).

iPBS genotyping

Genetic diversity based on iPBS markers was assessed using 34 primers developed by Kalendar et al., previously shown to be informative in wheat germplasm analyses (Haliloğlu et al. 2023). PCR amplicons were separated on 1.5% agarose gels at a constant voltage of 100 V for 105 min. Gels were visualized under UV light and photographed using a Nikon Coolpix 500 camera for subsequent allele scoring.

Scoring of molecular data

Allelic profiles generated by SSR and iPBS markers were transformed into binary data matrices, where the presence of a band was coded as "1" and its absence as "0". Only well-resolved and consistently reproducible fragments were retained for downstream statistical analyses. Although SSR markers are inherently co-dominant, they were scored in a binary (presence/absence) format in the present study to ensure methodological consistency with the iPBS marker system and compatibility with association mapping analyses.

Statistical analyses

Estimation of genetic parameters

To quantify the extent of genetic variability among wheat genotypes and to estimate heritability for the evaluated traits, genetic parameters were calculated based on analysis of variance (ANOVA). Mean squares obtained from the ANOVA were used to partition variance components. Furthermore, to justify poolability across seasons and assess GxE interactions, a mixed-model ANOVA was performed where Genotype was treated as a fixed effect, while Year, Water Regime (Environment), and their interactions (Genotype × Year, Genotype × Environment, and Genotype × Year × Environment) were treated as random effects.

Variance components

The environmental variance (V_E), genotypic variance (V_G), and phenotypic variance (V_P) were estimated from ANOVA mean squares following the procedures described by Zaki and Radwan (2022).

$$V_E = MS_e \; V_G = \frac{MS_g - MS_e}{r} \; V_P = V_G + V_E$$

where MSg and MSe denote genotype and error mean squares, respectively, and *r* represents the number of replications.

Coefficients of variation

The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were computed to facilitate comparisons of variability among traits. Both parameters were expressed as percentages of the trait mean, following the method proposed by Burton and De Vane (1953). GCV and PCV values were classified as low (<10%), moderate (10–20%), or high (>20%) according to Sivasubramaniam and Madhava Menon (1973).

$$\%PCV = \frac{\sqrt{V_P}}{\bar{x}} \bullet 100; \; \%GCV = \frac{\sqrt{V_G}}{\bar{x}} \bullet 100.$$

Heritability

Broad-sense heritability (Hb) was estimated as the ratio of genotypic variance to phenotypic variance and expressed as a percentage (Burton 1952):

$$\%H_b = \frac{V_G}{V_P} \bullet 100$$

Heritability estimates were categorized as low (0–30%), moderate (30–60%), or high (>60%), following the classification proposed by Robinson et al. (2011)

Association mapping analysis using iPBS and SSR Markers

Population structure

Population structure was inferred using genotypic data from both SSR and iPBS markers through a Bayesian model-based clustering approach implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000). Analytical parameters were set in accordance with protocols successfully applied in our previous investigations (Haliloğlu et al. 2023; Türkoğlu et al. 2023). As the same association panel and marker dataset were used in the present study, the previously determined population structure (K = 3) was adopted for subsequent association mapping analyses. The corresponding Q-matrix was incorporated into the mixed linear model (MLM) to correct for population stratification.

Table 1 Phenotypic summary statistics (as average of 2011-12 and 2012-13 cropping seasons)

Traits	Grown condition	Min.	Max.	Mean	CV (%)	Ve	Vg	Vp	PCV (%)	GCV (%)	H (%)
Canopy temperature difference at anthesis	Irrigated	8.70	15.30	11.64	4.75	0.568	0.41	0.98	8.49	5.50	41.89
Canopy temperature difference at 15 days post anthesis		3.30	14.85	9.47	5.55	0.450	0.67	1.12	11.15	8.61	59.66
Canopy temperature difference at 25 days post anthesis		2.50	9.50	5.96	9.77	0.561	0.71	1.27	18.95	14.17	55.97
SPAD values at anthesis		40.70	59.10	48.78	3.94	4.637	13.78	18.42	8.80	7.61	74.82
SPAD value at 15 days post anthesis		34.90	61.90	48.82	4.40	7.281	16.04	23.32	9.89	8.20	68.78
SPAD value at 25 days post anthesis		6.20	58.90	36.39	10.37	26.417	60.53	86.95	25.62	21.38	69.62
Leaf senescence rate at anthesis		0.05	9.75	1.29	14.16	0.490	1.14	1.63	98.59	82.40	69.86
Leaf senescence rate at 15 days post anthesis		6.05	35.80	14.34	16.99	2.417	11.20	13.62	25.74	23.34	82.25
Leaf senescence rate at 25 days post anthesis		25.75	71.65	40.18	6.74	1.343	44.35	45.69	16.82	16.57	97.06
Initial fluorescence at anthesis		215.00	327.61	288.73	2.35	45.315	103.35	148.66	4.22	3.52	69.52
Initial fluorescence at 15 days post anthesis		267.28	339.04	308.47	2.58	70.718	56.42	127.14	3.66	2.44	44.38
Initial fluorescence at 25 days post anthesis		284.50	425.34	333.55	5.02	277.291	139.90	417.19	6.12	3.55	33.53
Maximum fluorescence at anthesis		1203.50	2180.54	1601.43	6.67	11629.247	8412.29	20041.53	8.84	5.73	41.97
Maximum fluorescence at 15 days post anthesis		1006.50	1955.97	1412.69	10.13	16467.494	10503.43	26970.92	11.63	7.25	38.94
Maximum fluorescence at 25 days post anthesis		499.50	1777.50	1196.53	8.79	11375.928	14278.08	25654.01	13.39	9.99	55.66
Variable fluorescence at anthesis		914.00	1860.37	1312.70	8.06	11467.696	7899.90	19367.60	10.60	6.77	40.79
Variable fluorescence at 15 days post anthesis		698.00	1632.70	1104.22	13.00	23245.809	7585.10	30830.91	15.90	7.89	24.60
Variable fluorescence at 25 days post anthesis		151.50	1439.68	862.98	12.21	11418.567	14361.17	25779.74	18.61	13.89	55.71
Maximum quantum efficiency of PSII at anthesis		0.76	0.86	0.82	0.97	0.000	0.00	0.00	1.22	0.00	0.00
Maximum quantum efficiency of PSII at 15 days post anthesis		0.69	0.84	0.78	2.11	0.000	0.00	0.00	3.02	2.73	81.82
Maximum quantum efficiency of PSII at 25 days post anthesis		0.30	0.81	0.70	1.91	0.000	0.00	0.00	8.46	8.34	97.18
Grain ash content		1.04	1.90	1.52	5.11	0.007	0.02	0.03	10.84	9.33	74.07
Leaf ash content		12.53	23.79	18.33	5.09	0.871	6.79	7.66	15.10	14.22	88.63

Table 1 (continued)

Traits	Grown condition	Min.	Max.	Mean	CV (%)	Ve	Vg	Vp	PCV (%)	GCV (%)	H (%)
Canopy temperature difference at anthesis	Late drought	4.00	13.60	8.93	6.45	0.800	0.20	1.00	11.20	5.02	20.12
Canopy temperature difference at 15 days post anthesis		0.20	10.00	5.98	6.51	0.175	0.92	1.10	17.52	16.06	84.05
Canopy temperature difference at 25 days post anthesis		0.50	8.00	4.12	11.35	0.220	1.94	2.16	35.67	33.80	89.79
SPAD values at anthesis		28.60	61.50	44.21	4.99	6.332	22.99	29.32	12.25	10.84	78.40
SPAD value at 15 days post anthesis		12.30	62.60	41.15	8.38	13.050	23.42	36.47	14.68	11.76	64.22
SPAD value at 25 days post anthesis		0.00	45.30	14.59	26.85	15.420	49.65	65.07	55.30	48.30	76.30
Leaf senescence rate at anthesis		0.15	19.34	2.57	20.33	0.124	6.16	6.28	97.40	96.43	98.03
Leaf senescence rate at 15 days post anthesis		11.20	65.87	25.60	14.47	1.970	68.13	70.10	32.71	32.25	97.19
Leaf senescence rate at 25 days post anthesis		71.40	100.00	86.38	3.80	0.918	39.69	40.61	7.38	7.29	97.74
Initial fluorescence at anthesis		271.83	362.90	302.95	2.88	78.625	44.86	123.48	3.67	2.21	36.33
Initial fluorescence at 15 days post anthesis		283.00	389.00	321.76	3.80	152.719	95.66	248.38	4.90	3.04	38.51
Initial fluorescence at 25 days post anthesis		11.20	515.00	273.48	5.67	236.790	14789.78	15026.57	44.82	44.47	98.42
Maximum fluorescence at anthesis		1131.50	2226.72	1542.86	5.89	8789.520	4767.27	13556.79	7.55	4.48	35.17
Maximum fluorescence at 15 days post anthesis		901.50	2092.64	1327.67	9.20	16467.490	8552.39	25019.88	11.91	6.97	34.18
Maximum fluorescence at 25 days post anthesis		16.00	1558.00	716.09	7.97	3722.887	83339.23	87062.12	41.20	40.31	95.72
Variable fluorescence at anthesis		843.50	1863.82	1239.91	7.11	8208.011	4781.61	12989.62	9.19	5.58	36.81
Variable fluorescence at 15 days post anthesis		565.00	1716.69	1005.91	11.47	15116.950	8518.17	23635.12	15.28	9.18	36.04
Variable fluorescence at 25 days post anthesis		0.50	1193.01	442.61	10.97	2744.901	36778.34	39523.24	44.92	43.33	93.05
Maximum quantum efficiency of PSII at anthesis		0.75	0.86	0.80	1.22	0.000	0.00	0.00	1.24	0.18	2.08
Maximum quantum efficiency of PSII at 15 days post anthesis		0.63	0.83	0.75	1.98	0.000	0.00	0.00	3.12	2.82	81.82
maximum quantum efficiency of PSII at 25 days post anthesis		0.01	0.77	0.49	6.43	0.001	0.02	0.02	31.34	30.68	95.83
Grain ash content		1.26	2.18	1.72	4.11	0.005	0.03	0.03	10.81	9.99	85.51
Leaf ash content		10.28	21.85	14.63	5.65	0.935	4.52	5.46	15.97	14.54	82.87

minimum (Min.), maximum (Max.), Mean, coefficient of variation (CV), genotypic variance (Vg), environment variance (Ve), phenotypic variance (Vp), phenotypic coefficient variation (PCV) genotypic coefficient variation (GCV) and heritability (H) of the measured traits for all the 64 wheat genotypes grown under drought stressed and well-watered conditions

Association mapping

Marker–trait associations (MTAs) were detected using a mixed linear model (MLM) implemented in TASSEL v5.0. To control false-positive associations arising from population stratification and kinship, both the population structure matrix (Q) and the kinship matrix (K) were included in the model. The kinship matrix was computed directly from molecular marker data within the TASSEL framework. Linkage disequilibrium (LD) between marker loci was estimated as the squared allele frequency correlation coefficient (R^2). Statistical significance of MTAs was assessed using permutation-based adjusted p-values derived from 1,000 permutations, thereby reducing the likelihood of false-positive associations due to multiple testing.(Ghomi et al. 2023). This non-parametric permutation approach rigorously accounts for the actual correlation structure among markers, preventing the over-conservatism of Bonferroni corrections while maintaining strict control over false positives (Type I errors).

Results

Phenotypic variability and genetic parameters under irrigated and late drought conditions

The descriptive statistics, including mean, range, and variance components for 64 wheat genotypes evaluation under irrigated (well-watered) and late drought stress conditions, are presented in Table 1. A wide range of phenotypic variations was observed for all measured physiological traits across both environments. Coefficient variation varied from 0.97% (for maximum quantum efficiency of PSII at anthesis in irrigated) to 26.85% (for SPAD value at 25 days post anthesis in late drought) (Table 1). CVs were characterized by higher values in irrigation than in late drought for ten observed traits. Environment variance was lower in irrigated conditions than in late drought for seven traits: canopy temperature difference at anthesis, SPAD value at anthesis, SPAD value at 15 days post an-thesis, initial fluorescence at anthesis, initial fluorescence at 15 days post anthesis, maximum quantum efficiency of PSII at 25 days post anthesis and leaf ash content (Table 1). Genotypic variance values in irrigated conditions were lower than in late drought for 13 observed traits (Table 1). In general, the Phenotypic Coefficient of Variation (PCV) values were slightly higher than the Genotypic Coefficient of Variation (GCV) for most traits, suggesting that while environmental factors influenced expression, genetic factors played a major role. Phenotypic variance was lower in irrigated conditions than in late drought for ten traits (Table 1). Phenotypic coefficient of variation was lower in irrigated conditions than in late drought for 16 observed traits (Table 1). Genotypic coefficient of variation was lower in irrigation than in late drought for 17 traits (Table 1). Broad-sense heritability estimates ranged widely but were generally high for senescence and fluorescence traits. Heritability was lower in irrigation than in late drought for 13 traits (Table 1). Of note are the very large differences in heritability values between irrigated and late drought obtained for: canopy temperature difference at 25 days post anthesis, initial fluorescence at anthesis, initial fluorescence at 25 days post anthesis, maximum fluorescence at 25 days post anthesis and variable fluorescence at 15 days post anthesis (Table 1).

Association mapping analysis using iPBS and SSR markers

iPBS marker

Twenty-five iPBS markers were associated with the observed traits (Table 2). Marker iPBS-2376 determined three observed traits: initial fluorescence at 15 days post anthesis, variable fluorescence at 25 days post anthesis, and maximum quantum efficiency of PSII at 25 days post anthesis. Marker iPBS-2298 determined initial fluorescence at 15 days post anthesis and initial fluorescence at anthesis. Marker iPBS-2390 determined leaf senescence rate at anthesis and variable fluorescence at 25 days post anthesis. Marker iPBS-2391 determined initial fluorescence at anthesis and variable fluorescence at 25 days post anthesis. Marker iPBS-2392 determined leaf senescence rate at anthesis and maximum quantum efficiency of PSII at anthesis. The remaining markers, however, determined single traits only in one environment (one year under specific conditions) (Table 1). Initial fluorescence at 15 days post anthesis was determined using seven markers (iPBS-2075, iPBS-2077, iPBS-2079, iPBS-2087, iPBS-2251, iPBS-2298, and iPBS-2375) in irrigated soils during the 2012–2013 season (Table 2). They explained from 5.4% to 14.5% of the total variance in initial fluorescence at 15 days post anthesis (Table 2).

SSR marker

120 markers were significantly associated with at least one trait in one environment (Table 3). Canopy temperature difference at anthesis was determined by 15 markers, of which only three (BARC 197, BARC 240, and CFA 152) were significant in two environments (irrigated and late drought in the 2012–2013 season) (Table 3). Canopy temperature difference at 15 days post anthesis was determined by 13 markers, of which four (CFA 152, CFD 287, WMC 320, and WMC 435) were significant in two environments (Table 3). Canopy temperature difference at 25 days post anthesis was determined by 16 markers, of which only WMC 42 was significant in two environments (irrigated in both seasons) (Table 3). SPAD value at anthesis was determined by nine markers, each in only one environment. SPAD value at 15 days post anthesis was determined by ten markers. One of them, WMC 500, was significant in two environments. In contrast, the GWM 493 marker determined the SPAD value at 15 days post anthesis in three of the four environments (Table 3). The SPAD value at 25 days post anthesis was determined by 25 markers, each in only one environment. The remaining features were determined by five (maximum quantum efficiency of PSII at 15 days post anthesis) to 28 (leaf senescence rate at 25 days post anthesis) markers (Table 3). The percentage of variation explained by individual significant markers ranged from 0.011 (marker BARC 101, irrigated, 2012–2013) to 0.73 (WMC 765, late drought, 2012–2013) (Table 3). Three markers (BARC 78, CFA 2043, and CFA 2187) were associated with 12 of the 22 observed traits (Table 3). Two markers (CFA 2040, WMS 72, as well as WMC 420 and BARC 141) were associated

Table 2 List of the iPBS markers linked to various traits and their R^2 and associated *p*-value

Traits/Markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
Canopy temperature difference at anthesis								
iPBS-2274	–	–	–	–	–	–	0.03	0.105
Canopy temperature difference at 25 days post anthesis								
iPBS-2242	–	–	–	–	–	–	0.05	0.105
Canopy temperature difference at anthesis								
iPBS-7	0.05	0.095	–	–	–	–	–	–
SPAD value at 25 days post anthesis								
iPBS-2221	–	–	–	–	–	–	0.03	0.110
Leaf senescence rate at anthesis								
iPBS-2225	–	–	0.02	0.117	–	–	–	–
iPBS-2390	–	–	–	–	–	–	0.03	0.068
iPBS-2392	–	–	–	–	–	–	0.03	0.068
Initial fluorescence at 15 days post anthesis								
iPBS-2075	–	–	–	–	0.05	0.054	–	–
iPBS-2077	–	–	–	–	0.05	0.054	–	–
iPBS-2079	–	–	–	–	0.05	0.054	–	–
iPBS-2087	–	–	–	–	0.05	0.054	–	–
iPBS-2251	–	–	–	–	0.04	0.095	–	–
iPBS-2298	–	–	–	–	0.02	0.112	–	–
iPBS-2375	–	–	–	–	0.01	0.145	–	–
Maximum quantum efficiency of PSII at 25 days post anthesis								
iPBS-2271	–	–	–	–	0.01	0.120	–	–
Leaf senescence rate at 15 days post anthesis								
iPBS-2384	–	–	0.005	0.114	–	–	–	–
Initial fluorescence at anthesis								
iPBS-2298	–	–	–	–	–	–	0.05	0.089
iPBS-2391	–	–	0.04	0.106	–	–	–	–
Initial fluorescence at 15 days post anthesis								
iPBS-2376	0.03	0.117	–	–	–	–	–	–
Variable fluorescence at 25 days post anthesis								
iPBS-2376	0.05	0.093	–	–	–	–	–	–
iPBS-2390	–	–	–	–	–	–	0.03	0.113
iPBS-2391	–	–	–	–	–	–	0.05	0.095
iPBS-34	–	–	–	–	–	–	0.03	0.113
Maximum quantum efficiency of PSII at anthesis								
iPBS-2392	–	–	0.05	0.090	–	–	–	–
Maximum quantum efficiency of PSII at 25 days post anthesis								
iPBS-2376	0.02	0.125	–	–	–	–	–	–

with 11 and ten traits, respectively (Table 3). Twenty-three markers determined single traits (Table 3).

Discussion

The present study provides a comprehensive assessment of physiological responses to late-season drought in wheat and identifies a broad set of SSR and iPBS markers associated with adaptive traits under contrasting water regimes. The combined use of chlorophyll content, fluorescence parameters, leaf senescence rate, and canopy temperature, together with two complementary marker systems, enabled the detection of loci linked to drought-responsive physiological pathways. These findings support previous reports indicating that integrating molecular markers with fine-scale phenotyping enhances the resolution of genetic dissection of drought tolerance in wheat (Cattivelli et al. 2008; Reynolds et al. 2009). A key outcome of the study is the identification of 25 iPBS markers significantly associated with the examined traits, several of which showed multi-trait associations. The detection of markers such as iPBS-2376, associated with three chlorophyll fluorescence parameters across developmental stages, suggests the presence of genomic

Table 3 List of the SSR markers linked to various traits and their R^2 and associated *p*-value

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
Canopy temperature difference at anthesis								
BARC 37	–	–	0.05	0.061	–	–	–	–
BARC 113	–	–	0.03	0.109	–	–	–	–
BARC 175	–	–	–	–	0.005	0.172	–	–
BARC 197	–	–	–	–	0.003	0.191	0.03	0.110
BARC 204	–	–	–	–	0.007	0.159	–	–
BARC 206	–	–	–	–	0.009	0.152	–	–
BARC 216	–	–	–	–	0.009	0.152	–	–
BARC 240	–	–	–	–	0.005	0.170	0.02	0.113
CFA 152	–	–	–	–	0.003	0.183	0.04	0.098
CFA 2256	–	–	0.01	0.138	–	–	–	–
CFD 190	0.04	0.070	–	–	–	–	–	–
WMC 406	–	–	–	–	0.02	0.085	–	–
WMS 52	–	–	0.02	0.088	–	–	–	–
WMS 493					–	–	0.001	0.161
WMS 566	0.05	0.101	–	–	–	–	–	–
Canopy temperature difference at 15 days post anthesis								
BARC 165	–	–	–	–	–	–	0.01	0.111
BARC 240	–	–	–	–	0.03	0.110	–	–
CFA 152	0.02	0.120	–	–	0.02	0.116	–	–
CFA 2099	–	–	0.01	0.152			–	–
CFD 287	–	–	–	–	0.03	0.071	0.005	0.137
GWM 160	–	–	–	–	0.05	0.091	–	–
GWM 340	0.02	0.079	–	–	–	–	–	–
GWM 368	–	–	–	–	–	–	0.04	0.113
GWM 493	0.02	0.079	–	–	–	–	–	–
GWM 501	0.04	0.066	–	–	–	–	–	–
WMC 320	0.004	0.135	0.01	0.107	–	–	–	–
WMC 435	0.05	0.060	–	–	–	–	0.02	0.092
WMC 500	–	–	0.03	0.072	–	–	–	–
Canopy temperature difference at 25 days post anthesis								
BARC 101	–	–	–	–	0.03	0.011	–	–
BARC 133	–	–	–	–	–	–	0.05	0.062
BARC 140	–	–	–	–	–	–	0.05	0.113
BARC 240	–	–	–	–	0.02	0.139	–	–
CFA 152	–	–	–	–	0.04	0.107	–	–
GWM 413	0.04	0.096	–	–	–	–	–	–
WMC 42	0.05	0.059	–	–	0.009	0.119	–	–
WMC 320	0.002	0.154	–	–	–	–	–	–
WMC 329	–	–	0.05	0.061	–	–	–	–
WMC 406	–	–	–	–	0.04	0.070	–	–
WMC 173	–	–	–	–	0.01	0.150	–	–
WMS 5	0.01	0.092	–	–	–	–	–	–
WMS 77	–	–	0.05	0.058	–	–	–	–
WMS 189	–	–	–	–	0.03	0.079	–	–
WMS 493	–	–	–	–	–	–	0.02	0.077
WMS 566	–	–	–	–	–	–	0.04	0.103
SPAD value at anthesis								
BARC 140	–	–	–	–	–	–	0.04	0.073
BARC 204	0.05	0.101	–	–	–	–	–	–
CFA 2190	–	–	–	–	–	–	0.05	0.100
CFD 18	–	–	–	–	0.05	0.062		
GWM 391	–	–	–	–	–	–	0.04	0.106

Table 3 (continued)

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
GWM 443	–	–	–	–	–	–	0.04	0.106
WMC 356	–	–	–	–	–	–	0.05	0.063
WMS 124	–	–	–	–	0.05	0.064	–	–
WMS 493	0.02	0.087	–	–	–	–	–	–
SPAD value at 15 days post anthesis								
CFA 2190	–	–	0.05	0.095	–	–	–	–
CFA 2	–	–	0.05	0.096	–	–	–	–
GWM 391					–	–	0.04	0.112
GWM 493	–	–	0.02	0.082	0.03	0.078	0.04	0.068
GWM 501	–	–	–	–	0.05	0.060	–	–
WMC 42	–	–	–	–	–	–	–	–
WMC 356	–	–	–	–	0.02	0.081	–	–
WMC 500	0.03	0.081	0.04	0.065	–	–	–	–
WMS 5	–	–	–	–	0.05	0.060	–	–
WMS 155	0.04	0.07	–	–	–	–	–	–
SPAD value at 25 days post anthesis								
BARC 3	–	–	0.03	0.112	–	–	–	–
BARC 78	–	–	0.03	0.071	–	–	–	–
BARC 80	–	–	0.03	0.076	–	–	–	–
BARC 88	–	–	0.03	0.076	–	–	–	–
BARC 130	0.01	0.073	–	–	–	–	–	–
CFA 2040	–	–	0.03	0.071	–	–	–	–
CFA 2049					0.05	0.059		
CFA 2187	–	–	0.03	0.071	–	–	–	–
CFA 2043	–	–	0.03	0.071	–	–	–	–
CFA 2155	0.05	0.078	–	–	–	–	–	–
CFA 2163	–	–	–	–			0.02	0.096
GWM 368	–	–	–	–	0.02	0.126	–	–
GWM 382	–	–	–	–	0.03	0.110	–	–
GWM 497	–	–	–	–	0.04	0.066	–	–
WMC 261	–	–	0.05	0.097	–	–	–	–
WMC 361	–	–	0.05	0.060	–	–	–	–
WMC 420	–	–	0.03	0.071	–	–	–	–
WMC 468	0.04	0.050	–	–	–	–	–	–
WMC 173	–	–	–	–	0.04	0.101	–	–
WMS 24	–	–	–	–	0.03	0.112	–	–
WMS 44	–	–	–	–	0.04	0.102	–	–
WMS 46	–	–	–	–	0.05	0.093	–	–
WMS 52	–	–	0.05	0.063	–	–	–	–
WMS 72	–	–	0.03	0.071	–	–	–	–
WMS 190	–	–	–	–	0.03	0.074	–	–
Leaf senescence rate at anthesis								
BARC 37	–	–	–	–	–	–	0.001	0.134
BARC 54	–	–	0.01	0.107	0.0001	0.137	–	–
BARC 73	–	–	0.01	0.107	0.0001	0.137	–	–
BARC 113	–	–	–	–	–	–	0.02	0.088
BARC 128	–	–	–	–	–	–	0.003	0.145
BARC 141	–	–	–	–	–	–	0.003	0.149
CFA 2049	–	–	–	–	–	–	0.0009	0.144
CFA 2070	–	–	–	–	0.0003	0.154	–	–
GWM 319	–	–	–	–	–	–	0.01	0.071
WMC 42	–	–	0.05	0.062	–	–	–	–

Table 3 (continued)

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
WMC 356			–	–	–	–	0.0003	0.172
WMC 524	–	–	0.01	0.104	0.001	0.096	–	–
WMC 532	–	–	–	–	0.05	0.033	–	–
WMC 553	–	–	–	–	0.05	0.033	–	–
WMC 687	–	–	0.01	0.107	0.0001	0.137	–	–
WMS 52	–	–	–	–	0.01	0.058	–	–
WMS 124	–	–	–	–	0.0002	0.127	–	–
WMS 155	–		–	–	–	–	0.05	0.045
WMS 190	–	–	0.01	0.113	–	–	–	–
WMS 493	–	–	–	–	0.0001	0.138	–	–
WMS 566	–	–	–	–	0.0001	0.143	–	–
Leaf senescence rate at 15 days post anthesis								
BARC 37	0.02	0.09	–	–	–	–	–	–
BARC 64	0.03	0.072	–	–	–	–	–	–
BARC 80	–	–	0.004	0.120	–	–	–	–
BARC 88	–	–	0.004	0.120	–	–	–	–
BARC 128	–	–	–	–	–	–	0.05	0.052
BARC 135	–	–	–	–	0.01	0.108	–	–
BARC 142	–	–	–	–	–	–	0.05	0.054
BARC 175	0.03	0.117	0.05	0.083	–	–	–	–
CFA 152	–	–	0.03	0.096	–	–	–	–
CFA 2185	–	–	–	–	–	–	0.04	0.092
GWM 314	0.03	0.116	–	–	–	–	–	–
GWM 340	0.03	0.073	–	–	–	–	0.01	0.082
GWM 350	–	–	–	–	–	–	0.04	0.057
GWM 368	–	–	–	–	0.01	0.162	–	–
GWM 382	–	–	–	–	0.009	0.168	–	–
GWM 391	–	–	0.04	0.09	–	–	–	–
GWM 413	–	–	0.02	0.110	–	–	0.008	0.145
GWM 443	–	–	0.04	0.090	–	–	–	–
GWM 501	–	–	0.04	0.057	–	–	–	–
WMC 580	0.05	0.063	–	–	–	–	–	–
WMS 46	–	–	–	–	0.04	0.112	–	–
WMS 58	–	–	–	–	–	–	0.03	0.063
WMS 63	–	–	0.01	0.084	–	–		
WMS 148	–	–	–	–	–	–	0.04	0.060
WMS 493	0.03	0.07	–	–	–	–	–	–
Leaf senescence rate at 25 days post anthesis								
BARC 24	–	–	–	–	0.02	0.112	–	–
BARC 54	0.02	0.089	–	–	0.01	0.095	–	–
BARC 59	–	–	0.002	0.168	0.03	0.062	–	–
BARC 73	0.02	0.089	–	–	0.01	0.095	–	–
BARC 78	–	–	–	–	0.03	0.062	–	–
BARC 80	–	–	0.002	0.168	–	–	–	–
BARC 141	–	–	0.008	0.170	–	–	–	–
CFA 2040	–	–	0.002	0.168	–	–	–	–
CFA 2187	–	–	0.002	0.168	0.03	0.062	–	–
CFA 2043	–	–	0.002	0.168	0.03	0.062	–	–
CFA 2185	–	–	0.05	0.099	0.02	0.111	–	–
CFA 2190	–	–	–	–	0.03	0.097	–	–
CFA 2256	–	–	–	–	0.008	0.147	–	–
CFA 2	–	–	–	–	0.03	0.103	–	–

Table 3 (continued)

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
CFD 287	–	–	0.02	0.083	–	–	–	–
GWM 319	–	–	–	–	–	–	0.03	0.054
GWM 340	–	–	–	–	0.02	0.078	–	–
WMC 361	–	–	0.01	0.095	–	–	–	–
WMC 406	–	–	0.02	0.081	–	–	–	–
WMC 420	–	–	0.002	0.168	0.03	0.062	–	–
WMC 468	–	–	0.05	0.061	–	–	–	–
WMC 524	0.003	0.151	0.007	0.123	–	–	–	–
WMC 687	0.02	0.089	–	–	0.01	0.095	–	–
WMC 807	–	–	–	–	–	–	0.03	0.095
WMS 24	–	–	–	–	0.04	0.096	–	–
WMS 44	–	–	–	–	0.05	0.084	–	–
WMS 52	0.01	0.109	–	–	–	–	–	–
WMS 72	–	–	0.002	0.168	0.03	0.062	–	–
Initial fluorescence at anthesis								
BARC 78	0.005	0.137	–	–	–	–	–	–
BARC 94	–	–	0.03	0.114	0.001	0.246	–	–
BARC 101	–	–	0.04	0.108	–	–	–	–
BARC 113	–	–	0.05	0.100	–	–	–	–
BARC 122	–	–	0.05	0.099	–	–	–	–
BARC 128	–	–	0.02	0.134	–	–	–	–
BARC 141	0.01	0.147	–	–	–	–	–	–
BARC 175	0.02	0.134	–	–	–	–	–	–
BARC 197	0.01	0.151	–	–	–	–	–	–
BARC 204	0.007	0.175	–	–	–	–	–	–
BARC 206	0.007	0.179	–	–	–	–	–	–
BARC 216	0.01	0.140	–	–	–	–	–	–
BARC 240	0.02	0.136	–	–	–	–	–	–
CFA 152	0.005	0.137	–	–	–	–	–	–
CFA 2040	0.02	0.134	–	–	–	–	–	–
CFA 2187	0.005	0.137	–	–	–	–	–	–
CFA 2043	0.005	0.137	–	–	–	–	–	–
CFD 287	0.02	0.090	–	–	–	–	–	–
WMC 336	–	–	–	–	–	–	0.03	0.076
WMC 420	0.005	0.137	–	–	–	–	–	–
WMC 765	–	–	–	–	–	–	0.03	0.73
WMS 72	0.005	0.137	–	–	–	–	–	–
WMS 493					–	–	0.05	0.097
Initial fluorescence at 15 days post anthesis								
BARC 78	0.05	0.063	–	–	–	–	–	–
BARC 130	–	–	–	–	0.009	0.094		
BARC 133	–	–	–	–	0.02	0.071	0.001	0.171
BARC 135	–	–	–	–	0.05	0.049	–	–
BARC 140	–	–	–	–	0.04	0.055	–	–
BARC 141	–	–	–	–	–	–	0.02	0.121
CFA 152	–	–	–	–	–	–	–	–
CFA 2049	–	–	–	–	–	–	0.03	0.112
GWM 368	–	–	–	–	0.05	0.080	–	–
GWM 391	–	–	–	–	0.02	0.107	–	–
GWM 443	–	–	–	–	0.02	0.107	–	–
GWM 493	–	–	0.03	0.066	–	–	0.02	0.088
WMC 320	–	–	–	–	–	–	0.0009	0.195

Table 3 (continued)

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
WMC 356	–	–	–	–	–	–	0.004	0.138
WMC 435	–	–	0.02	0.080	–	–	–	–
WMC 807	0.04	0.112	–	–	–	–	–	–
WMS 46	–	–	0.02	0.120	–	–	–	–
WMS 67	0.05	0.065	–	–	–	–	–	–
Initial fluorescence at 25 days post anthesis								
BARC 105	–	–	0.02	0.131	–	–	–	–
BARC 128	–	–	–	–	0.04	0.101	–	–
BARC 133	–	–	–	–	0.02	0.078	–	–
BARC 141	–	–	0.05	0.098	0.01	0.144	–	–
CFA 2049	–	–	–	–	0.03	0.074	–	–
CFD 190	–	–	–	–	–	–	0.0004	0.200
GWM 382	–	–	–	–	–	–	0.04	0.095
GWM 533	0.01	0.102	–	–	–	–	–	–
WMC 356	–	–	–	–	0.009	0.110	–	–
WMC 580	–	–	0.04	0.071	–	–	0.04	0.062
WMS 124	–	–	–	–	0.0002	0.232	–	–
WMS 493	–	–	–	–	0.01	0.099	–	–
Maximum fluorescence at anthesis								
BARC 48	–	–	–	–	0.05	0.53	0.04	0.66
BARC 88	–	–	–	–	–	–	0.03	0.073
BARC 141	0.002	0.218	–	–	–	–	–	–
BARC 175	0.03	0.118	–	–	–	–	–	–
BARC 204	0.05	0.105	–	–	–	–	–	–
CFA 2040	0.0007	0.211	–	–	–	–	–	–
CFA 2187	0.0007	0.211	–	–	–	–	–	–
CFA 2043	0.0007	0.211	–	–	–	–	–	–
CFD 287	0.057	0.062	–	–	–	–	–	–
WMC 356	–	–	–	–	–	–	0.03	0.068
WMC 420	0.0007	0.211	–	–	–	–	–	–
WMC 500	–	–	–	–	0.03	0.064	0.04	0.064
WMC 524	0.003	0.153	–	–	–	–	–	–
WMC 807	–	–	0.02	0.131	–	–	–	–
WMS 44	0.02	0.128	–	–	–	–	–	–
WMS 58	0.03	0.078	0.01	0.094	–	–	–	–
WMS 72	0.0007	0.211	–	–	–	–	–	–
WMS 118	–	–	0.05	0.062	–	–	–	–
Maximum fluorescence at 15 days post anthesis								
BARC 59	0.05	0.064	–	–	–	–	–	–
BARC 78	0.05	0.064	–	–	–	–	–	–
BARC 206	–	–	0.05	0.101	–	–	–	–
CFA 2040	0.05	0.064	–	–	–	–	–	–
CFA 2187	0.05	0.064	–	–	–	–	–	–
CFA 2043	0.05	0.064	–	–	–	–	–	–
CFD 287	–	–	–	–	0.04	0.065	–	–
GWM 319	–	–	–	–	0.05	0.062	–	–
WMC 42	–	–	0.05	0.061	–	–	–	–
GWM 413	0.05	0.097	–	–	–	–	–	–
WMC 114	–	–	–	–	–	–	0.05	0.103
WMC 210	–	–	–	–	–	–	0.03	0.114
WMC 333	–	–	–	–	–	–	0.03	0.078
WMC 420	0.05	0.064	–	–	–	–	–	–

Table 3 (continued)

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
WMS 46	–	–	0.02	0.134	–	–	–	–
WMS 52	–	–	–	–	–	–	0.04	0.070
WMS 58	0.05	0.060	–	–	–	–	–	–
WMS 155	0.05	0.064	–	–	–	–	–	–
WMS 297	–	–	0.03	0.075	–	–	–	–
WMS 493	–	–	–	–	0.01	0.102	–	–
Maximum fluorescence at 15 days post anthesis								
BARC 24	–	–	–	–	–	–	0.04	0.087
BARC 59	–	–	0.03	0.068	–	–	–	–
BARC 78	–	–	0.03	0.068	–	–	–	–
BARC 133	–	–	–	–	0.02	0.08	–	–
BARC 141	–	–	–	–	0.02	0.121	–	–
CFA 2040	–	–	0.03	0.068	–	–	–	–
CFA 2049	0.03	0.072	–	–	–	–	–	–
CFA 2187	–	–	0.03	0.068	–	–	–	–
CFA 2043	–	–	0.03	0.068	–	–	–	–
CFD 190	–	–	–	–	–	–	0.0002	0.215
CFD 287	–	–	–	–	0.005	0.128	–	–
GWM 340	–	–	–	–	0. 05	0.059	–	–
GWM 413	–	–	0.05	0.092	–	–	–	–
WMC 42	–	–	–	–	–	–	0.05	0.051
WMC 524	–	–	–	–	0.01	0.098	–	–
WMS 52	–	–	–	–	0.005	0.129	–	–
WMS 67	0.02	0.078	–	–	–	–	–	–
WMS 72	–	–	0.03	0.068	–	–	–	–
WMS 118	–	–	0.006	0.120	–	–	0.008	0.102
WMS 124	–	–	0.03	0.068	–	–	–	–
WMS 148	–	–	0.009	0.110	–	–	–	–
WMS 155	–	–	0.04	0.063	–	–	–	–
Variable fluorescence at anthesis								
BARC 48	–	–	–	–	0.05	0.055	–	–
BARC 59	0.0009	0.201	–	–	–	–	–	–
BARC 78	0.0009	0.201	–	–	–	–	–	–
BARC 141	0.003	0.207	–	–	–	–	–	–
CFA 2187	0.0009	0.201	–	–	–	–	–	–
CFA 2043	0.0009	0.201	–	–	–	–	–	–
CFA 2070	–	–	0.01	0.13	–	–	–	–
CFA 2155	0.05	0.102	–	–	–	–	–	–
CFD 190	–	–	–	–	–	–	0.04	0.062
GWM 493	–	–	0.02	0.08	–	–	–	–
WMC 420	0.0009	0.201	–	–	–	–	–	–
WMC 500	–	–	–	–	0.04	0.061	0.05	0.56
WMC 524	0.003	0.154	–	–	–	–	–	–
WMC 807	–	–	0.01	0.152	–	–	–	–
WMS 44	0.02	0.129	–	–	–	–	–	–
WMS 58	0.04	0.074	0.02	0.092	–	–	–	–
WMS 72	0.0009	0.201	–	–	–	–	–	–
WMS 118	–	–	0.05	0.064	–	–	–	–
Variable fluorescence at 15 days post anthesis								
BARC 59	0.05	0.064	–	–	–	–	–	–
BARC 78	0.05	0.064	–	–	–	–	–	–
BARC 133	–	–			–	–	0.03	0.077

Table 3 (continued)

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
CFA 2040	0.05	0.064	–	–	–	–	–	–
CFA 2049	–	–			0.03	0.064	–	–
CFA 2187	0.05	0.064	–	–	–	–	–	–
CFA 2043	0.05	0.064	–	–	–	–	–	–
GWM 319	–	–			0.05	0.055	–	–
GWM 413	0.05	0.102	–	–	–	–	–	–
WMC 42	–	–	0.05	0.065	–	–	–	–
WMC 420	0.05	0.064	–	–	–	–	–	–
WMS 46	–	–	0.02	0.123	–	–	–	–
WMS 52	–	–	–	–	–	–	0.04	0.067
WMS 58	0.05	0.062	–	–	–	–	–	–
WMS 72	0.05	0.064	–	–	–	–	–	–
WMS 297	–	–	0.03	0.075	–	–	–	–
WMS 403	–	–	–	–	0.01	0.088	–	–
Variable fluorescence at 25 days post anthesis								
BARC 59	–	–	0.02	0.078	–	–	–	–
BARC 78	–	–	0.02	0.078	–	–	–	–
BARC 128	0.04	0.105	V	–	–	–	–	–
BARC 133	–	–	–	–	0.01	0.106	–	–
CFA 2040	–	–	0.02	0.078	–	–	–	–
CFA 2187	–	–	0.02	0.078	–	–	–	–
CFA 2043	–	–	0.02	0.078	–	–	–	–
CFD 190	–	–	–	–	–	–	0.0004	0.207
CFD 287	–	–	–	–	–	–	0.03	0.066
GWM 319	0.05	0.059	–	–	–	–	–	–
GWM 337	0.02	0.083	–	–	–	–	–	–
GWM 340			–	–	0.05	0.062	–	–
GWM 413	–	–	0.04	0.101	–	–	–	–
WMC 361	–	–	0.05	0.056	–	–	–	–
WMC 420	–	–	0.02	0.078	–	–	–	–
WMC 524	–	–	0.05	0.056	0.03	0.074	–	–
WMS 44	–	–	0.04	0.096	–	–	–	–
WMS 52	–	–	–	–	0.006	0.124	–	–
WMS 67	0.02	0.08	–	–	–	–	–	–
WMS 72	–	–	0.02	0.078	–	–	–	–
WMS 118	–	–	0.009	0.109	–	–	–	–
WMS 124	–	–	0.05	0.058	–	–	–	–
WMS 148	–	–	0.01	0.101	–	–	–	–
WMS 155	–	–	0.04	0.064	–	–	–	–
Maximum quantum efficiency of PSII at anthesis								
BARC 59	0.002	0.163	–	–	–	–	–	–
BARC 78	0.002	0.163	–	–	–	–	–	–
BARC 94					0.02	0.128	–	–
BARC 141	0.01	0.164	–	–	–	–	–	–
CFA 2040	0.002	0.163	–	–	–	–	–	–
CFA 2187	0.002	0.163	–	–	–	–	–	–
CFA 2043	0.002	0.163	–	–	–	–	–	–
CFA 2070	–	–	0.03	0.123	–	–	–	–
CFD 18	–	–	0.01	0.110	–	–	–	–
CFD 49	–	–	0.02	0.094	–	–	–	–
CFD 190					0.02	0.080	–	–
GWM 493	–	–	0.05	0.063	–	–	–	–

Table 3 (continued)

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
WMC 356	–	–	–	–	0.04	0.063	–	–
WMC 524	0.006	0.134	–	–	–	–	–	–
WMC 807	–	–	**0.005**	**0.192**	–	–	–	–
WMS 44	0.02	0.138	–	–	–	–	–	–
WMS 58	–	–	0.03	0.074	–	–	–	–
WMS 63	0.03	0.078	–	–	–	–	–	–
WMS 72	0.002	0.163	–	–	–	–	–	–
Maximum quantum efficiency of PSII at 15 days post anthesis								
BARC 133	–	–	–	–	0.03	0.059	–	–
GWM 337	–	–	–	–	0.02	0.072	–	–
GWM 413	0.03	0.118	–	–	–	–	–	–
WMC 42	–	–	0.02	0.084	–	–	–	–
WMS 63	–	–	0.05	0.063	–	–	–	–
Maximum quantum efficiency of PSII at 25 days post anthesis								
BARC 133	–	–	–	–	0.02	0.084	–	–
BARC 141	–	–	0.02	0.127	–	–	–	–
CFA 2040	–	–	0.005	0.126	–	–	–	–
CFA 2187	–	–	0.005	0.126	–	–	–	–
CFA 2043	–	–	0.005	0.126	–	–	–	–
CFD 190	–	–	–	–	–	–	0.02	0.089
CFD 287	–	–	–	–	0.03	0.074	–	–
GWM 337	0.04	0.071	–	–	–	–	–	–
GWM 340	–	–	–	–	0.01	0.093	–	–
GWM 350	–	–	–	–	0.04	0.068	–	–
GWM 493	–	–	–	–	–	–	0.01	0.098
WMC 361	–	–	0.02	0.079	–	–	–	–
WMC 406	–	–	0.03	0.070	–	–	–	–
WMC 420	–	–	0.005	0.126	–	–	–	–
WMC 524	–	–	0.04	0.062	–	–	–	–
WMS 67	0.03	0.074	–	–	–	–	–	–
WMS 72	–	–	0.005	0.126	–	–	–	–
Grain ash content								
BARC 48	0.04	0.061	–	–	–	–	–	–
BARC 59	0.02	0.073	–	–	–	–	–	–
BARC 78	0.02	0.073	–	–	–	–	–	–
BARC 130	0.002	0.142	0.02	0.090	–	–	–	–
BARC 135	0.02	0.078	–	–	–	–	–	–
BARC 240	–	–	–	–	–	–	0.01	0.124
CFA 152	–	–	–	–	–	–	0.007	0.153
CFA 2040	0.02	0.073	–	–	–	–	–	–
CFA 2187	0.02	0.073	–	–	–	–	–	–
CFA 2043	0.02	0.073	–	–	–	–	–	–
GWM 340	–	–	–	–	–	–	0.05	0.054
WMC 320	–	–	0.02	0.086	–	–	–	–
WMC 333	–	–	–	–	0.04	0.065	–	–
WMC 420	0.02	0.073	–	–	–	–	–	–
WMC 524	–	–	–	–	0.03	0.074	–	–
WMC 580	0.02	0.074	0.05	0.064	–	–	–	–
WMS 46	–	–	–	–	–	–	0.04	0.096
WMS 72	0.02	0.073	–	–	–	–	–	–
WMS 297	–	–	–	–	–	–	0.01	0.083
WMS 566	–	–	–	–	0.01	0.054	–	–

Table 3 (continued)

Traits/markers	2011-12				2012-13			
	Irrigated		Late drought		Irrigated		Late drought	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
Leaf ash content								
CFA 2185	0.05	0.076	0.01	0.126	–	–	0.03	0.121
CFA 2190	0.04	0.081	0.01	0.122	–	–	0.01	0.152
CFA 2256	0.05	0.076	0.01	0.120	–	–	0.02	0.124
CFA 2	0.01	0.106	0.007	0.149	–	–	0.01	0.158
CFD 287	0.05	0.048	–	–	–	–	–	–
GWM 319	–	–	–	–	–	–	0.05	0.064
GWM 337	–	–	–	–	–	–	0.004	0.141
GWM 350	–	–	0.03	0.065	–	–	–	–
GWM 493	–	–	–	–	0.003	0.139	–	–
GWM 497	0.03	0.057	–	–	–	–	–	–
GWM 533	0.04	0.053	–	–	–	–	–	–
WMC 99	–	–	–	–	–	–	0.02	0.125
WMC 435	–	–	–	–	0.05	0.059	–	–
WMC 500	–	–	–	–	0.009	0.111	–	–
WMC 807	–	–	–	–	0.03	0.114	–	–
WMC 810	–	–	–	–	0.01	0.106	–	–
WMS 52	–	–	–	–	–	–	0.04	0.68
WMS 77	–	–	–	–	0.02	0.078	–	–
WMS 189	–	–	–	–	0.05	0.058	–	–

regions involved in maintaining PSII integrity under water deficit. By maintaining PSII integrity, these genotypes can effectively prevent cellular oxidative damage, thereby sustaining stable photosynthetic rates and prolonged grain filling under severe late-season water deficit.

This observation aligns with reports demonstrating that PSII efficiency is among the most drought-sensitive components of the photosynthetic apparatus and tightly linked with genetic variation in wheat (Li et al. 2015). Similarly, iPBS-2390 and iPBS-2392, which jointly explained variation in leaf senescence and fluorescence parameters, point to genetic regulation of stay-green characteristics traits previously shown to contribute to yield stability under terminal drought (Lopes and Reynolds 2012). The limited number of stable iPBS–trait associations observed across environments further indicates that these markers may preferentially reflect stress-induced genome plasticity and regulatory adjustments rather than constitutive yield-related variation. Although most iPBS markers were environment-specific, the presence of multi-environmental associations for some loci indicates that retrotransposon-based markers can identify genomic segments contributing to drought adaptation. This is consistent with earlier work demonstrating the suitability of iPBS markers for detecting environmentally responsive polymorphisms in cereals (Baránek et al. 2015; Kalendar et al. 2011).

Although the marker density in this study does not provide high-density genome-wide coverage, the methodology was designed as a targeted and cost-effective association mapping approach. An extensive initial screening of 425 SSR primer pairs and 34 iPBS primers was performed to ensure that only the most highly polymorphic and reproducible loci were utilized. To mitigate the risk of spurious associations associated with this targeted marker density, a robust Mixed Linear Model (MLM) was employed, rigorously incorporating both population structure (Q-matrix) and familial relatedness (Kinship matrix). Furthermore, the co-localization of multiple traits to single marker loci (such as BARC 78, CFA 2187, and CFA 2043) points to either genuine biological pleiotropy or tight physical linkage (linkage disequilibrium) of distinct genes. Given the functional interconnection of physiological traits—where, for instance, chlorophyll maintenance directly supports canopy cooling through sustained transpiration a pleiotropic effect of major developmental genes is highly probable, though tight linkage cannot be fully ruled out (Table 4).

SSR markers revealed extensive associations with the evaluated physiological traits, with 108 significant loci, including several markers affecting temperature regulation, chlorophyll content, and fluorescence at multiple growth stages. The strong multi-trait associations identified for markers such as BARC 78, CFA 2187, and CFA 2043, each associated with more than ten traits, indicate potential genomic "hotspots" underlying integrative drought responses. SSR markers revealed extensive associations with the evaluated physiological traits, with 108 significant loci, including several markers affecting temperature regulation, chlorophyll content, and fluorescence at multiple

Table 4 The bread wheat genotypes used in this study

Code	Genotype/variety name	Code	Genotype/variety name
G1	Aksel 2000	G33	Pehlivan
G2	Aldane	G34	Prostor
G3	Alparslan	G35	Soyer02
G4	Altay 2000	G36	Sönmez 2001
G5	Atlı 2002	G37	Sultan 95
G6	Aytın 98	G38	Süzen 97
G7	Bağcı 2002	G39	Tosunbey
G8	Bayraktar 2000	G40	Türkmen
G9	Bolal 2973	G41	Uzunyayla
G10	Bereket	G42	Yakar 99
G11	Çetinel 2000	G43	Zencirci 2002
G12	Dağdaş 94	G44	Ak-702
G13	Demir 2000	G45	Ak Buğday
G14	Doğu 88	G46	Ankara 093/44
G15	ES 26	G47	Conkesme
G16	Gerek 79	G48	Haymana 79
G17	Gün 91	G49	Hawk (Şahin)
G18	Harmankaya 99	G50	Kılçıksız Buğday
G19	İkizce 96	G51	Kırkpınar 79
G20	İzgi 2001	G52	Kırmızı Kılçık
G21	Karahan 99	G53	Kırmızı Yerli
G22	Kate A-1	G54	Koca Buğday
G23	Kıraç 66	G55	Köse 220/39
G24	Kırgız 95	G56	Özlü Buğday
G25	Kırik	G57	Polatlı Kösesi
G26	Kutluk 94	G58	Sert Buğday
G27	Lancer	G59	Sürak 1593/51
G28	Mızrak	G60	Tir
G29	Müfitbey	G61	Yayla 305
G30	Nacibey	G62	Zerin
G31	Nenehatun	G63	Bezostaja 1
G32	Palandöken 97	G64	Karasu 90

growth stages. The strong multi-trait associations identified for markers such as BARC 78, CFA 2187, and CFA 2043, each associated with more than ten traits, indicate potential genomic "hotspots" underlying integrative drought responses. The exact chromosomal locations and physical mapping positions of these associated SSR markers have been previously established and detailed in our earlier work on this association panel (Bolouri et al. 2023). Previous studies have also reported clusters of SSR loci associated with multiple drought-related traits, particularly those linked to canopy temperature and chlorophyll retention (Alsaleh 2022; Gupta et al. 2010). Furthermore, the identification of these specific multi-trait loci provides critical biological context for understanding drought adaptation. Genomic regions harboring markers such as BARC 78, CFA 2187, and CFA 2043 have frequently been co-located with major quantitative trait loci (QTLs) governing stay-green characteristics and chlorophyll stability during the critical grain-filling period. The strong phenotypic expression of these loci under terminal drought suggests their proximity to key regulatory networks, potentially including stress-responsive transcription factors like the DREB (Dehydration Responsive Element Binding) gene family or senescence-associated genes (SAGs). By modulating the plant's ability to sustain photosystem II (PSII) integrity and delay leaf senescence, these genomic hotspots enable prolonged photosynthetic activity. This alignment with known physiological pathways effectively bridges the gap between our statistical marker-trait associations and the underlying functional mechanisms of drought tolerance, highlighting these regions as prime targets for marker-assisted breeding. Previous studies have also reported clusters of SSR loci associated with multiple drought-related traits, particularly those linked to canopy temperature and chlorophyll retention (Alsaleh 2022; Gupta et al. 2010). Markers associated with canopy temperature are particularly important, as canopy cooling capacity is one of the most reliable indicators of field drought tolerance (Pinto and Reynolds 2015). The present study identified several SSR markers (e.g., SSR-30, SSR-35, SSR-51, SSR-84) that were consistently associated with canopy temperature difference across environments. Such results support previous findings suggesting that genomic regions contributing to transpiration efficiency can be identified using stable mark-er–trait associations across water regimes (Acuña-Galindo et al. 2015).

SPAD-related markers showing cross-environment significance (SSR-64 and SSR-86) further confirm the utility of SSRs in dissecting traits linked to chlorophyll stability. Maintenance of chlorophyll under drought conditions is a hallmark of stay-green genotypes, an attribute associated with prolonged photosynthetic activity and improved grain filling during terminal drought (Kumar et al. 2010). This delayed senescence (stay-green trait) is functionally interconnected with canopy temperature regulation, as sustained chlorophyll levels and active stomatal conductance allow the plant to maintain canopy cooling through continuous transpiration. Interestingly, the range of phenotypic variance explained by individual markers from nearly negligible (0.011) to very high (0.73) reflects the polygenic nature of drought adaptation and the environment-dependent expression of these traits. High R^2 values observed for some SSR markers under late drought indicate that certain loci express substantial phenotypic effects specifically under stress conditions, supporting the stress-specific QTL model proposed by Tuberosa (2012). The variance components estimated in the present study provide further insights into trait stability and genetic control. Higher genotypic and phenotypic variances observed under late drought for most traits confirm earlier findings that stress conditions enhance trait variability, thereby improving the power of marker–trait association analyses (Blum 2010; Sallam et al. 2019). The markedly

increased heritability values under late drought for traits such as canopy temperature difference and several fluorescence parameters indicate strong genetic determinism under stress conditions. These exceptionally high heritability estimates are mathematically supported by our experimental design; under the standardized rain shelter conditions, environmental background noise was strictly minimized, allowing the strong genotypic differentiation among the diverse 64 genotypes to be maximally expressed. Similar patterns have been reported for drought-induced changes in chlorophyll fluorescence and temperature traits (Abdullaev et al. 2024).

Overall, the study demonstrates that combining SSR and iPBS markers provides complementary insights into the genetic basis of physiological drought responses. SSRs, known for their high reproducibility and linkage to coding regions, proved highly useful for dissecting canonical drought traits such as chlorophyll content and canopy temperature. In contrast, iPBS markers, which target retrotransposon-rich genomic regions, were particularly effective in identifying loci associated with PSII efficiency and leaf senescence dynamics traits often influenced by regulatory and transposable-element-associated genomic regions (Kalendar and Schulman 2006). The integration of these marker systems supports the identification of genotypes with superior drought tolerance and reveals genomic regions with high potential for use in mark-er-assisted breeding programmers. These findings are consistent with recent advances emphasizing the need for multi-marker, multi-trait analyses to improve the precision of drought tolerance selection in wheat (Qaseem et al. 2018; Sukumaran et al. 2018). The identification of stable SSR and iPBS markers across environments, and particularly under late-season drought, provides valuable resources for improving breeding efficiency, especially for stress-prone regions where terminal drought is the primary yield-limiting factor. Despite the robust marker–trait associations identified, the present study is limited by the absence of functional validation of candidate loci. Future studies integrating transcriptomic or gene expression analysis will be essential to confirm the biological roles of the identified genomic regions.

Conclusions

This study provides new insights into the genetic architecture of physiological responses to late-season drought in wheat by integrating SSR and iPBS marker analyses with detailed physiological characterization. The identification of multi-trait and multi-environment marker–trait associations highlight genomic regions influencing key drought-adaptive processes, including canopy temperature regulation, chlorophyll stability, PSII efficiency, and leaf senescence dynamics. SSR markers proved particularly informative for traits traditionally linked to drought tolerance, while iPBS markers captured additional variation associated with retrotransposon-related genomic regions and regulatory elements. The strong associations observed under late drought conditions, together with high heritability's for several key traits, demonstrate that terminal drought provides a highly informative environment for dissecting genetic determinants of stress tolerance. The discovery of markers such as BARC 78, CFA 2187, CFA 2043, and iPBS-2376 as multi-trait loci underscores their potential value for marker-assisted selection. Collectively, these results demonstrate that combining SSR and iPBS marker systems enhances the resolution of genetic analyses and supports the identification of genotypes with superior drought resilience. The markers identified in this study constitute valuable resources for wheat breeding programs targeting improved performance under water-limited conditions. Their application in marker-assisted selection could accelerate the development of cultivars with enhanced physiological stability and yield potential under terminal drought a trait of increasing importance in the context of climate variability and rising global food demand.

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Declarations

Competing interest The authors declare no competing interest.

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