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RESEARCH

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Physio-biochemical and molecular characterization of wheat cultivars (*Triticum aestivum* L.) under post-anthesis heat stress

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Abstract

Background Heat stress is one of the abiotic stresses that make wheat crops vulnerable, which significantly impacts crop production around the world. An increase in temperature during the reproductive phase (anthesis) beyond the optimal range of 15–20 °C leads to decreased crop production, poor quality of the grain, and altered physiological and biochemical processes. To study the association between high temperature and physio-biochemical traits under normal and late sown, a set of fifteen genotypes was utilized.

Results Relative water content under high temperatures had an overall decrease of 8.7%. However, grain protein and malondialdehyde content were higher in the stressed conditions than in the control, with increases of 20.2% and 38.9%, respectively. Marker *Xgwm67*, located on chromosome 5B, was found to be significantly associated with malondialdehyde content ($R^2 = 21\%$) and *Xgwm570*, located on 6A, was closely linked to relative water content as well as grain protein content ($R^2 = 16\%$) revealed by regression analysis. The correlation matrix displays a positive association between the control and stressed condition by $R^2 = 0.92$, 0.82, and 0.53 in malondialdehyde, relative water content, and grain protein, respectively. However, there was a negative correlation between water content–malondialdehyde and malondialdehyde–grain protein, though there was only a 4% correlation between grain protein content (control) and relative water content (stressed). Based on the tolerance matrix, WH730 and RAJ4079 were heat tolerant, and DBW173 and HD3086 were sensitive.

Conclusions These findings indicate that to identify tolerant genotypes, physiological and biochemical traits can be utilized as an alternate criterion, and these closely associated markers can be applied for improved late-planted wheat production through MAS. The breeding scheme and genome editing by recognizing novel genes through physio-biochemical parameters, marker-assisted selection, and prospective screening of tolerant genotypes are proclaimed by the study.

Keywords Wheat, Relative water content, Malondialdehyde, Grain protein, Heat stress, SSR

Background

Wheat is the largest cultivated crop globally, comprising an estimated area of more than 218 million hectares (Giraldo et al. 2019). Wheat production in India during 2021–2022 was estimated to be 106.84 million tonnes, 2.96 million tonnes more than the last 5 years (Anonymous 2023). However, heat stress is one of the abiotic stresses that make wheat crops vulnerable, which significantly impacts crop production around the world. Under a scenario of global warming, high temperature stress is

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the major abiotic stress. The development and production of the crop are influenced by diverse climatic causes. Rise in the optimum growth temperature (15–20 °C) induces heat stress, which leads to the decreased production and quality of the grain. Due to the delayed physiological maturity of rice and cotton harvesting, 80% of wheat is planted late and 20% is planted at the normal time (Laghari et al. 2012). Heat stress during the reproductive phase leads to susceptibility and deterioration of the crop. Exposure to heat stress can alter the physiological and biochemical processes. Water intake and transpiration can improve the plant tissue temperature, as plant water status is influential under heat stress. Hence, heat stress greatly affects relative water content (RWC), stomatal conductance, and transpiration rate. Under stress, an elevated level of water loss is caused by high vapour pressure, leading to decreased RWC (Shaukat et al. 2021; Yadav et al. 2022).

Production of ROS accelerated by high temperatures causes oxidative stress in plants (Hasanuzzaman et al. 2020). Autocatalytic peroxidation of membrane lipids and pigments and modification of membrane permeability, as well as functioning, are the primary impacts of ROS. Under stress conditions, free radical damage to cell membranes has been measured by the lipid peroxidation levels. The essential and most researched polyunsaturated fatty acids (PUFAs) peroxidised by-product is MDA (malondialdehyde), and this aldehyde is more than merely a lipid peroxidation marker as it is a highly hazardous molecule. Chloroplasts are where MDA is mainly produced as a highly reactive aldehyde during peroxidation of PUFAs in biomembranes amongst different aldehydes (Mohi-Ud-Din et al. 2021).

An increase in protein concentration, grain proportion, and polymerisation is caused by a shorter grain filling stage, mainly due to the heat stress ahead of the maturity of seeds (Singh et al. 2021). Wheat genotypes were evaluated for end-use traits under normal and late sown conditions. It was discovered that wheat is susceptible to high temperatures during the reproductive phase, for instance, grain filling; as a result, protein content increases and starch production decreases. Hence, the heat stress can affect the grain's quality to produce bread (Wang et al. 2016). In agricultural farming systems, genotypes maintaining their quality under stress are an essential component. However, grain quality traits, particularly protein content, are inherited quantitatively as they are affected by genotype, environment, and gene × environment (Mahdavi et al. 2022).

Antioxidant regulation and stress-responsive genes in the wheat genotype can resist stress (Raja et al. 2020). Traits are regulated by several genes governed by heat tolerance; the better way for genetic thermotolerance analysis is by molecular markers. To produce a stress resistance crop, marker-assisted resistance strategy had been proposed due to the stress tolerance complexity and difficult phenotypic selection of heat tolerant plants. Molecular markers are undistinguished from environmental factors or developmental phases as they are reliable for identifying genotypes. Due to the significant impact of G × E interactions and complicated inheritance, enhancing stress tolerance through conventional means is challenging. Out of all the genetic markers available, microsatellite markers are a useful tool for varietal identification, genotyping, gene pyramiding, and QTL mapping. These are environment-independent and can be used in marker-assisted detection to efficiently identify genotype-to-genotype variation (Devi et al. 2023). Genetically utilized and quantitative trait loci recognized markers that alter the plant stress tolerance capacity are required for marker-assisted selection (MAS). Molecular linkage maps based QTL analysis is utilized for genetic foundation dissection to discrete components from phenotypes (Tyagi and Pandey 2022). This study covers the heat stress effect on physio-biochemical traits and their molecular marker association in fifteen wheat cultivars constituted from different agro-climatic regions of India.

Methods

Plant material

Fifteen wheat genotypes (K0307, AKAW3717, WH730, K7903, WH1142, DBW173, DBW17, WH1124, RAJ4079, PBW550, Hindi62, WCF8-W12, PBW723, HD3086, and HUW468) (Additional file 1: Table S1) procured from ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana, India; representing different agro-climatic regions were sown at Krishi Vigyan Kendra, Banasthali Vidyapith, by precise phenotyping approach during mid-Nov (timely sown) and mid-Dec (late sown) with three replications consisting 24 seeds, sown in four rows with six plants within 5 cm depth in each plot. To compare the heat stress effect on the crop, daily minimum and maximum temperatures in degrees Celsius with their calculated mean were recorded from the Automatic Weather Station at Banasthali Vidyapith. Fresh leaves for physio-biochemical tests were collected between 7–14 DAA at Z65–Z68 growth stages (Zadoks et al. 1974).

Relative water content (RWC)

In terms of the physiological effects of cellular water deficit, it was an effective indicator of plant water status. Leaves were weighed (fresh weight) and kept in water overnight; excess water was removed by blotting paper and weighed (turgid weight). Then the samples were oven dried at 80 °C for 4 h. and weighed again (dry weight).

The relative water content was calculated by the following (Suresh et al. 2013):

$$\text{Relative water content} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

Lipid peroxidation estimation

0.1 g sample was homogenized in 0.5 ml 0.1% TCA, then centrifuged at 15,000 rpm at 4 °C for 10 min. 0.5 ml of supernatant was mixed with 1.5 ml of 0.5% TBA diluted in 20% TCA, incubated at 95 °C for 25 min, and cooled on ice. Absorbance was taken at 532 and 600 nm. MDA concentration was calculated by an extinction coefficient $\varepsilon^{M} = 155\ mM^{-1}\ cm^{-1}$ (Dhindhsa et al. 1981).

Grain protein content (GPC)

1 g seeds were homogenized with 1 ml of protein extraction buffer (10 mM Tris-HCl, 10 mM EDTA, 5 mM β-mercaptoethanol) and centrifuged at 12,000 rpm for 20 min at 4 °C. 100 µl of supernatant was added with 5 ml of Bradford reagent, and absorbance was taken at 595 nm after 1 h. The graph was plotted against bovine serum albumin (BSA) as a standard curve (Bradford 1976).

Heat susceptibility index (HSI)

HSI was utilized to determine the effect of high temperature on grain yield. HSI was calculated by the formula (Paliwal et al. 2012):

$$\text{HSI of X} = \frac{1 - \text{XHeat stress}/\text{XControl}}{\text{D}}$$

where X stands for the trait, Xheat stress represents the phenotypic value of late sown genotypes, and Xcontrol stands for the phenotypic value of timely sown genotypes for the trait. D (stress intensity) is equal to (1 − Yheat stress/Ycontrol), where Yheat stress is the mean of all genotypes for Xheat stress and Ycontrol is the mean of all genotypes for Xcontrol.

DNA extraction and PCR

Genomic DNA was isolated by the *N*-cetyl-*N*, *N*, *N*-trimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al. 1984). For the molecular study, 100 SSR markers (Additional file 1: Table S2) were selected randomly from the set of 21 pairs of chromosomes from the wheat map by Röder et al. (1998), and the polymerase chain reaction was performed by the modified Röder et al. (1998) procedure. The reaction was executed in a total volume of 25 µl containing 10X Taq buffer mixed with $MgCl_2$, 200 µM dNTPs, 0.2 µM primer each, 1U Taq polymerase, and 100 ng template DNA. The PCR mixture was run in a thermocycler (Applied Biosystems, USA), and the reaction cycle involved an initial denaturation at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50, 55, and 60 °C depending on the individual primer, extension at 72 °C for 1 min, and final extension at 72 °C for 6 min. The amplified PCR product was quantified by electrophoresis on 3% agarose gel at 100 V for 45 min, visualized by ethidium bromide stain, and a photograph was taken through the GelDoc system (BioRad, USA).

Statistical analysis

Statistical analysis for experimental data was estimated by SPSS software version 20. The trait and molecular marker correlation was evaluated by simple regression analysis. The variance fraction represented by the coefficient of determination (R^2) described the physio-biochemical effects associated with markers. To find the significant differences under different conditions amongst the cultivars, Duncan's test was utilized (Table 1). Based on their response to heat stress conditions, all cultivars were categorized as S, MS, MT, and T during the study. Data on the HSI for each trait were compiled with the base value set at zero and the maximum assigned at two. 0.0–0.5 values were classified as T, 0.5–1.0 as MT, 1.0–1.5 as MS, and 1.5–2.0 as S. For each trait, a combined matrix table was created, and each of the categories received a tolerance score: S-0, MS-1, MT-2, and T-3. To acquire the final tolerance score, scores for each cultivar were added. Cultivars showing tolerant qualities in all three traits would be 9 (3 traits × 3 for T) as the highest score and zero value for sensitive characteristics as the lowest score. Score range 0–9 was distributed in four parts of 25% each. Cultivars ranging between 0–25% were considered as S, 25–50% as MS, 50–75% as MT, and above 75% as T (Table 2) (Pandey et al. 2023).

Results

Physio-biochemical factors of all the cultivars significantly changed due to heat stress, and replication means with standard deviations are concise in Table 1 after statistical analysis. All cultivars in the experiments under controlled and late-planted conditions were significant at $P \leq 0.05$ and $P \leq 0.01$.

All the traits are significantly influenced by delayed planting, leading to terminal heat stress. The average temperatures faced by the genotypes were 10.3 °C (min) and 27 °C (max). During the reproductive phase (anthesis) under normal sown conditions, the min. temperature ranged from 1.6 to 19.1 °C with an average of 9.2 °C and the max. from 17 to 35.4 °C with 26.2 °C average. However, under late sown conditions, min temperature affecting the crop ranged from 1.6 to 20.1 °C with a mean of

Table 1 Variability in physio-biochemical traits under timely and late sown in wheat cultivars

Genotypes	Relative water content (%)		Lipid peroxidation (nmoles/g FW)		Grain protein content (mg/mg FW)	
	Control	Stress	Control	Stress	Control	Stress
K0307	86.6 ± 0.38^{cd}	81.1 ± 0.49^{de}	1.7 ± 0.44^{abc}	2.7 ± 0.49^{ab}	42.4 ± 0.10^{ab}	61.3 ± 0.39^{bcd}
AKAW3717	87.3 ± 0.26^{cd}	81.1 ± 0.32^{de}	1.3 ± 0.15^{ab}	2.3 ± 0.39^{a}	61.8 ± 0.34^{g}	86.1 ± 0.57^{g}
WH730	85.1 ± 0.49^{cd}	81.6 ± 0.36^{e}	1.6 ± 0.10^{abc}	2.3 ± 0.34^{a}	57.9 ± 0.25^{efg}	61.2 ± 0.37^{bcd}
K7903	88.7 ± 0.41^{d}	81.6 ± 0.59^{e}	1.3 ± 0.46^{ab}	2.3 ± 0.36^{a}	44.6 ± 0.57^{bc}	66.7 ± 0.52^{def}
WH1142	87.5 ± 0.28^{cd}	82.3 ± 0.19^{e}	1.0 ± 0.08^{a}	2.4 ± 0.29^{a}	59.4 ± 0.24^{fg}	66.1 ± 0.46^{def}
DBW173	88.3 ± 0.63^{d}	79.8 ± 0.42^{bcde}	1.5 ± 0.15^{abc}	2.5 ± 0.29^{a}	42.6 ± 0.33^{ab}	61.3 ± 0.59^{bcd}
DBW17	88.2 ± 0.31^{cd}	77.1 ± 0.12^{abcde}	2.5 ± 0.43^{de}	3.8 ± 0.37^{bc}	36.8 ± 0.11^{a}	52.5 ± 0.33^{a}
WH1124	87.0 ± 0.62^{cd}	80.5 ± 0.24^{cde}	1.3 ± 0.12^{ab}	2.7 ± 0.16^{ab}	50.7 ± 0.21^{cde}	55.8 ± 0.42^{ab}
RAJ4079	86.1 ± 0.27^{bcd}	78.7 ± 0.39^{bcde}	1.5 ± 0.40^{abc}	2.4 ± 0.41^{a}	57.1 ± 0.30^{efg}	61.4 ± 0.47^{bcd}
PBW550	80.9 ± 0.43^{abc}	73.5 ± 0.25^{abc}	2.0 ± 0.09^{bcd}	2.9 ± 0.13^{ab}	52.7 ± 0.54^{def}	63.1 ± 0.48^{cde}
Hindi62	82.6 ± 0.44^{abcd}	72.5 ± 0.13^{ab}	1.4 ± 0.46^{ab}	2.3 ± 0.43^{a}	60.3 ± 0.46^{fg}	68.3 ± 0.51^{ef}
WCF8-W12	79.2 ± 0.38^{ab}	70.2 ± 0.24^{a}	2.2 ± 0.30^{cde}	3.3 ± 0.41^{abc}	48.4 ± 0.14^{bcd}	69.1 ± 0.34^{f}
PBW723	83.5 ± 0.55^{abcd}	75.1 ± 0.38^{abcde}	1.8 ± 0.18^{bc}	2.8 ± 0.30^{ab}	59.3 ± 0.35^{fg}	64.9 ± 0.40^{cdef}
HD3086	78.6 ± 0.30^{a}	73.7 ± 0.42^{abcd}	1.5 ± 0.25^{abc}	3.0 ± 0.42^{ab}	43.3 ± 0.29^{abc}	64.7 ± 0.53^{cdef}
HUW468	88.2 ± 0.17^{cd}	77.1 ± 0.13^{abcde}	2.7 ± 0.22^{e}	4.3 ± 0.31^{bc}	49.5 ± 0.51^{bcd}	59.5 ± 0.49^{bc}
Sig	0.020**	0.006**	0.000***	0.023**	0.000***	0.000***

**, ***Significant at $P \leq 0.05$ and $P \leq 0.001$, respectively. Data is represented as replication means with standard deviations. Mean values denoted with different symbols (a, b, c, d, e, f, and g), are significantly different as determined by Duncan's test

10.8 °C and 17–36.4 °C range of max temperature with a 27 °C mean (Fig. 1).

In Fig. 2, RWC under heat stress was lower than the control, with values ranging between 4.07–12.54% and an overall decrease of 8.7%. Cultivars with minimal decrease were WH730, WH1142, and HD3086 (4.07, 5.9, and 6.2%). RWC HSI ranged from 0.46 to 1.43, with a mean of 1.00. The maximum reduction was observed in DBW17 and HUW468 at 12.5%. Based on the HSI values, seven cultivars (DBW173, PBW550, Hindi62, WCF8-W12, and PBW723, along with DBW17 and HUW468) are heat sensitive as a value greater than 1 is contemplated as heat sensitive.

Grain protein and malondialdehyde content were higher in the stressed conditions than in the control, dissimilar to RWC. As shown in Fig. 3, in a stressed condition, WH1124 and WH1142 had the maximum lipid peroxidation of 50.9 and 56.5%, respectively, whereas WH730 and PBW550 showed a minimal increase in MDA content with 30.9 and 32.15, respectively. There was a 38.9% overall increase in MDA in delayed sowing. HSI mean with ranging values was 1.05 and 0.7–2.03, respectively. Along with WH730 and PBW550, seven more cultivars (K0307, DBW17, RAJ4079, Hindi62, WCF8-W12, PBW723, and HUW468) had HSI less than 1, constituting them as heat tolerant.

Similarly, grain protein content (Fig. 4) had a 20.2% increase in stress condition than the control. Under stress, there was a minimal increase in WH730 (5.4%) along with RAJ4079 (7.04%) and PBW723 (8.6%), whereas two cultivars had the higher protein content: HD3086 (33%) and K7903 (33.1%), and based on their HSI value higher than one, along with five more genotypes (K0307, AKAW3717, DBW173, DBW17, and WCF8-W12), these are contemplated as heat sensitive.

The correlation matrix displays a positive association between control and stressed condition by $R^2 = 0.92$, 0.82, and 0.53 in MDA, RWC, and GPC, respectively (Fig. 5). There was a negative correlation between RWC–MDA and MDA–GPC. However, there was only a 4% correlation between controlled GPC and stressed RWC. The final classification of genotypes as S, MS, MT, or T was based on the tolerance score for each characteristic acquired for each genotype (Table 2). DBW173 (score 2) and HD3086 (score 2) were classified as sensitive varieties based on the final tolerance score achieved by analysing physiological and biochemical traits. K0307 (score 4), K7903 (score 3), DBW17 (score 3), and WCF8-W12 (score 3) were classified as moderately sensitive, AKAW3717 (score 5), WH1142 (score 5), WH1124 (score 5), PBW550 (score 5), HUW468 (score 5), Hindi62 (score 6), and PBW723 (score 6) as moderately tolerant (MT), and WH730 (score 8) and RAJ4079 (score 7) were classified as tolerant to high temperature stress. The matrix table can precisely classify varieties according to their tolerance score and aid in trait-based variety selection.

Table 2 Heat tolerance matrix of wheat cultivars on the basis of physiological and biochemical traits

Genotypes	Relative water content (RWC)	Lipid peroxidation (MDA)	Grain protein content (GPC)	Tolerance score on the basis of traits
K0307	MT	MT	S	4
AKAW3717	MT	MS	S	5
WH730	T	MT	T	8
K7903	MT	MS	S	3
WH1142	MT	S	T	5
DBW173	MS	MS	S	2
DBW17	MS	MT	S	3
WH1124	MT	S	T	5
RAJ4079	MT	MT	T	7
PBW550	MS	MT	MT	5
Hindi62	MS	MT	T	6
WCF8–W12	MS	MT	S	3
PBW723	MS	MT	T	6
HD3086	MT	S	S	2
HUW468	MS	MT	MT	5

Detailed form	Abbreviation	Tolerance score
Sensitive	S	0
Moderately sensitive	MS	1
Moderately tolerant	MT	2
Tolerant	T	3

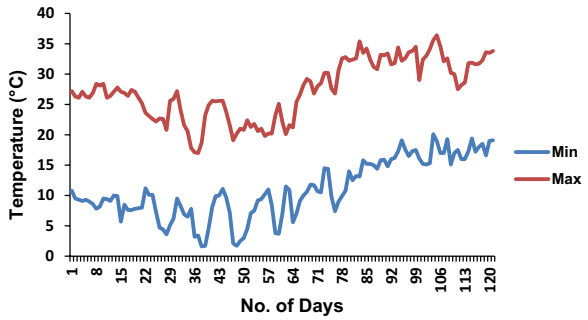

Fig. 1 Minimum and maximum temperature under TS and LS conditions

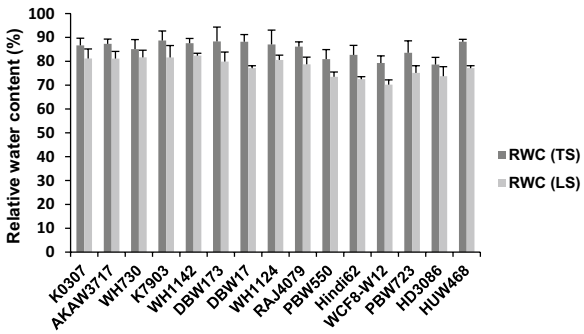

Fig. 2 Relative water content (RWC) under TS and LS conditions

Different SSR markers, such as WMC, GWM, BARC, CFD, CFA, and PSP, were utilized for the screening of all genotypes. Out of 100 markers used, 47% showed allelic variation, and marker *Xgwm67* (Fig. 6) was found to be significantly associated with malondialdehyde content with 21% phenotypic contribution located at chromosome 5B, and marker *Xgwm570*, which is located at chromosome 6A, was closely linked to relative water content as well as grain protein content with 16% phenotypic contribution, respectively, as revealed

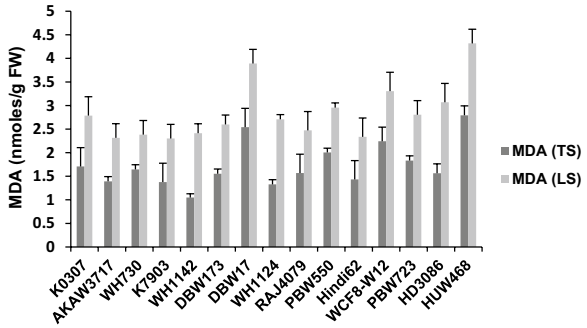

Fig. 3 Malondialdehyde content under TS and LS condition

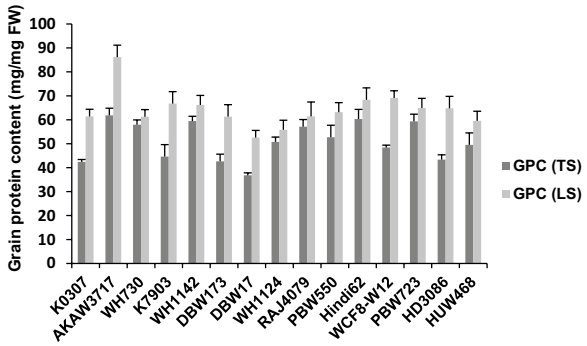

Fig. 4 Grain protein content under TS and LS condition

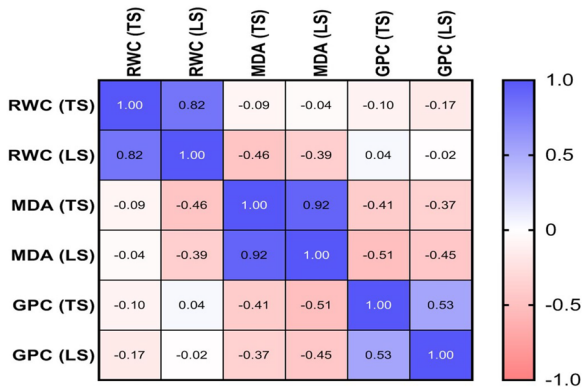

Fig. 5 Correlation matrix of traits under both conditions

by regression analysis. Marker sequences, loci, and annealing temperature are shown in Table 3.

Discussion

High temperature stress is the major abiotic stress that disrupts the crop yield mostly during the reproductive phase. Hence, the major focus of the research is on breeding for extreme temperature tolerance and verifying the wheat cultivars that are climate-adaptable. Physio-biochemical activities change under high temperature. To evaluate the high temperature adaptable genotypes, late sowing may be selected (Fleitas et al. 2020). To study the association between high temperature and physio-biochemical traits, a set of fifteen genotypes was utilized. Under heat stress, plants' water content decreases, which is directly correlated with heat tolerance. One of the heat tolerance mechanisms is the plant's ability to retain elevated relative water content implied by preceding research (Pandey et al. 2019, 2022; Nizamani et al. 2020). RWC may be related to stress tolerance and yield as it is correlated with cell water stress. Cultivars with minimal decrease were WH730, WH1142, and HD3086, whereas maximum reduction was observed in DBW17 and HUW468. However, in lipid peroxidation, WH1142 had the maximum reduction.

One of the most difficult and damaging outcomes of high temperature on all cell membranes is lipid peroxidation. Decisive factors in disclosing plant stress intensity and measurement of lipid peroxidation level are expressed in terms of MDA content, which increases with the rise in temperature (Khan et al. 2017; Kumari et al. 2020; Kaur et al. 2018). Tolerant cultivars showed less MDA content than the sensitive genotypes. MDA content was maximum in WH1124 and WH1142, implying less heat stability, high membrane fluidity, and higher leakiness compared to other cultivars, WH730, PBW550, and WCF8-W12, which are in compliance with the current discoveries regarding tolerant genotypes having lower levels of lipid peroxidation (Mohi-Ud-Din et al. 2021; Pandey et al. 2022).

Heat stress affects the grain protein content quality in wheat plants as it is susceptible to high temperature during the reproductive phase, for instance, grain filling. As a result, protein content increases and starch production decreases, hence GPC is higher under stress than in normal sown conditions, which coincides with previous studies (Wang et al. 2016; Singh et al. 2021; Osman et al. 2021). Under stress, there was a minimal protein content increase in WH730 along with RAJ4079 and PBW723, whereas two cultivars had higher protein content: HD3086 and K7903. The correlation matrix displays a positive association between control and stressed condition by $R^2 = 0.92$, 0.82, and 0.53 in MDA, RWC, and GPC, respectively. There was a negative correlation between RWC–MDA and MDA–GPC. However, there was only a 4% correlation between controlled GPC and stressed RWC (Abd El-Rady 2022). Under controlled and late-planted conditions in the experiments, genotypes significantly differed in RWC and MDA content; however, in protein content, it was similar. Based on the heat tolerance matrix, DBW173

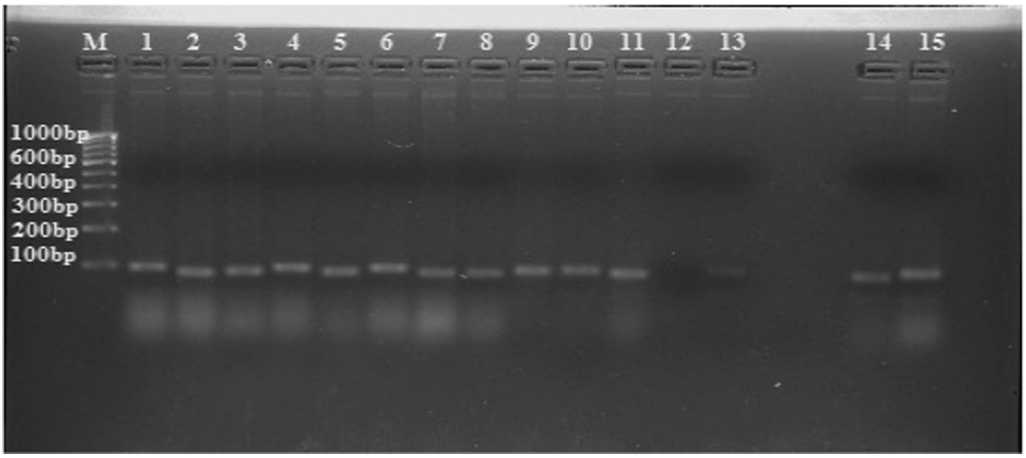

Fig. 6 *M* = 100 bp ladder, genotypes: 1 = K0307, 2 = AKAW3717, 3 = WH730, 4 = K7903, 5 = WH1142, 6 = DBW173, 7 = DBW17, 8 = WH1124, 9 = RAJ4079, 10 = PBW550, 11 = Hindi62, 12 = WCF8-W12, 13 = PBW723, 14 = HD3086, and 15 = HUW468 screened by marker *Xgwm67*

Table 3 Marker sequences, loci, and their annealing temperature

S. no.	Markers	Sequences	Chromosome no.	Annealing Temp. (°C)
1	*Xgwm67*	ACC ACA CAA ACA AGG TAA GCG CAA CCC TCT TAA TTT TGT TGG G	5B	60
2	*Xgwm570*	TCG CCT TTT ACA GTC GGC ATG GGT AGC TGA GAG CCA AA	6A	60

(score 2) and HD3086 (score 2) were classified as sensitive varieties based on the final tolerance score achieved by analysing physiological and biochemical traits, and WH730 (score 8) and RAJ4079 (score 7) were classified as tolerant to high temperature stress (Pandey et al. 2023). Identification and selection of plants with desirable and complex traits such as stress tolerance with powerful methods, which have enhanced crop improvement, owed to recent molecular genetics innovations (Hasan et al. 2021). Regression analysis signified a close association of MDA content to marker *Xgwm67* located on chromosome 5B with R^2 of 21%; similarly, RWC and GPC to marker *Xgwm570* located on chromosome 6A with R^2 of 16% phenotypic variation. These closely associated markers can be utilized for wheat improvement through MAS. For breeding programmes, it may be applied as a selection tool, and for further estimation of these markers, usage is required with high temperatures associated with diverse genotypes set. Matrix tables can also be utilized to precisely classify varieties according to their tolerance score and aid in trait-based variety selection. In order to classify tolerant or sensitive to heat stress, a physio-biochemical study of wheat genotypes was useful.

Conclusions

This study demonstrated different wheat genotypes affected by heat stress during grain filling with a detrimental impact on physiological and biochemical processes. The results of this study validated these traits that were evaluated for heat tolerant cultivars. The proposed matrix can be beneficial for trait-based breeding as well as for categorizing highly productive cultivars as tolerant and sensitive. This study showed that WH730 and RAJ4079 were heat tolerant, and DBW173 and HD3086 were sensitive. Through regression analysis, markers *Xgwm67* (MDA) and *Xgwm570* (RWC and GPC) were closely associated with the traits. These closely correlated markers can be utilized for improved late-planted wheat production through MAS. The breeding scheme and genome editing by recognizing novel genes through physio-biochemical parameters, marker-assisted selection, and prospective screening of tolerant genotypes are proclaimed by the study.

Abbreviations

TS	Timely sown
LS	Late sown
RWC	Relative water content
MDA	Malondialdehyde content
GPC	Grain protein content
SSR	Simple sequence repeat

Min Minimum temperature
Max Maximum temperature

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42269-024-01243-w.

Additional file1

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Author contributions
AV contributed in the writing, and GCP contributed in the reviewing of manuscript. All authors have read and approved the manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this article and its supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
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References

Abd El-Rady AG (2022) Evaluation of some bread wheat genotypes for heat tolerance under terminal heat stress conditions. J Cent Eur Agric 23(3):564–581
Anonymous (2023) Annual report of 2022–23. Ministry of agriculture and farmers welfare, Krishi Bhawan, New Delhi, India P1–316
Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
Devi S, Singh V, Yashveer S, Dalal MS, Paras, Chawla R, Akbarzai DK, Chaurasia H (2023) Molecular characterization of bread wheat (*Triticum aestivum* L.) genotypes using SSR markers. Indian J Agric Sci 93(9):948–953
Dhindhsa RS, Plumb-Dhidsa P, Thorne TA (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 32:93–101
Fleitas MC, Mondal S, Gerard GS, Hernández-Espinosa N, Singh RP, Crossa J, Guzmán C (2020) Identification of CIMMYT spring bread wheat germplasm maintaining superior grain yield and quality under heat stress. J Cereal Sci 93:102981
Giraldo P, Benavente E, Agugliaro FM, Gimenez E (2019) Worldwide research trend on wheat and barley: a bibliometric comparative analysis. Agronomy 9(352):1
Hasan N, Choudhary S, Naaz N, Sharma N, Laskar RA (2021) Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. J Genet Eng Biotechnol 19:1–26
Hasanuzzaman M, Borhannuddin Bhuyan MHM, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M, Fotopoulos V (2020) Reactive oxygen species and antioxidant defence in plants under abiotic stress: revisiting the crucial role of a universal defence regulator. Antioxidants 9:681
Kaur R, Bedi S, Kaur P, Asthir B (2018) Biochemical evaluation in wheat (*Triticum aestivum* L.) under heat stress conditions. Indian J Agric Biochem 31:111–115
Khan NA, Khan S, Naz N, Shah M, Irfanullah AS, Sher H, Khan A (2017) Effect of heat stress on growth, physiological and biochemical activities of wheat (*Triticum aestivum* L.). Int J Biosci 11:173–183
Kumari A, Ranjan R, Roy C, Pal A, Kumar S (2020) Effect of heat stress on inter–relationship of physiological and biochemical traits with grain yield in wheat (*Triticum aestivum* L.). Curr J Appl Sci Technol 39:19–29
Laghari KA, Sial MA, Arain MA (2012) Effect of high temperature stress on grain yield and yield components of wheat (*Triticum aestivum* L.). J Sci Technol Dev 31:83–90
Mahdavi S, Arzani A, Mirmohmmady Maibody SAM, Kadivar M (2022) Grain and flour quality of wheat genotypes grown under heat stress. Saudi J Biol Sci 29:103417
Mohi-Ud-Din M, Siddiqui N, Rohman M, Jagadish SVK, Ahmed JU, Hassan MM, Hossain A, Islam T (2021) Physiological and biochemical dissection reveals a trade-off between antioxidant capacity and heat tolerance in bread wheat (*Triticum aestivum* L.). Antioxidants 10:351
Nizamani FG, Khokhar AA, Rind RA, Nizamani MM, Mehmood A, Nizamani M, Memon S, Soothar MK, Rind MR, Nizamani AL (2020) Wheat genotypes response to heat stress tolerance normal and delay sowing dates. FUUAST J Biol 10(1):39–49
Osman R, Zhu Y, Cao W, Ding Z, Wang M, Liu L, Tang L, Liu B (2021) Modeling the effects of extreme high-temperature stress at anthesis and grain filling on grain protein in winter wheat. Crop J 9:889–900
Paliwal R, Röder MS, Kumar U, Srivastava JP, Joshi AK (2012) QTL mapping of terminal heat tolerance in hexaploid wheat (*T.aestivum* L.). Theor Appl Genet 125:565–571
Pandey GC, Mehta G, Sharma P, Sharma V (2019) Terminal heat tolerance in wheat: an overview. J Cereal Res 11:1–16
Pandey A, Mamrutha HM, Kumar R, Pandey GC, Awaji SM, Singh G, Singh GP (2022) Physio–biochemical characterization of wheat genotypes under temperature stress. Physiol Mol Biol Plants 29:131–143
Pandey A, Mamrutha HM, Kumar R, Mishra S, Khobra R, Pandey GC, Singh G, Singh GP (2023) Explicating drought tolerance of wheat (*Triticum aestivum* L.) through stress tolerance matrix. Plant Physiol Rep 28:63–77
Raja V, Qadir SU, Alyemeni MN, Ahmad P (2020) Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses and gene expression in *Solanumly copersicum*. 3Biotech 10(5):208
Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of India. Genetics 149:2009–2014
Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018
Shaukat S, Kousar I, Fatima S, Shukat R, Ali A, Ahmad J, Akhtar N, Nadeem M, Farooq J, Ramzan M (2021) Evaluation of spring wheat genotypes for terminal heat stress. SABRAO J Breed Genet 53(2):239–247
Singh N, Virdi AS, Katyal M, Kaur A, Kaur D, Ahlawat AK, Singh AM, Sharma RK (2021) Evaluation of heat stress through delayed sowing on physicochemical and functional characteristics of grains, whole meals and flours of India wheat. Food Chem 344:128725
Suresh K, Rao VP, Srinivas A, Sankar S, Govardhan V (2013) Relative water content as influenced by varied plant densities and irrigation levels in pigeon pea. Int J Appl Biol Pharm Technol 4(2):64
Tyagi M, Pandey GC (2022) Physiology of heat and drought tolerance in wheat: an overview. J Cereal Res 14(1):13–25
Wang S, Li T, Miao Y, Zhang Y, He Z, Wang S (2016) Effects of heat stress and cultivar on the functional properties of starch in Chinese wheat. Cereal Chem 49(3):443–450

Yadav MR, Choudhary M, Singh J, Lal MK, Jha PK, Udawat P, Gupta NK, Rajput VD, Garg NK, Maheshwari C, Hasan M, Gupta S, Jatwa TK, Kumar R, Yadav AK, Prasad PVV (2022) Impacts, tolerance, adaptation, and mitigation of heat stress on wheat under changing climates. Int J Mol Sci 23:2838

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14(6):415–421

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