BMC Plant Biology

RESEARCH

Open Access

Differential biochemical responses of seven Indian wheat genotypes to temperature stress

Satbhai Ravindra[1*], Bharad Swati[1] and Moharil Mangesh[1]

Abstract

Background Changes in the temperature induction response are potential tools for the empirical assessment of plant cell tolerance. This technique is used to identify thermotolerant lines in field crops. In the present investigation, ten-day-old seedlings of six wheat genotypes released by Dr. PDKV, Akola, Maharashtra, India were exposed to gradual increases in high temperature and duration (control 25 °C to 30 °C for 1 h, 34 °C for 1 h, 38 °C for 2 h and 42 °C for 3 h) to investigate their effects on some physiological and biochemical parameters to provide basic information for improving heat-tolerant cultivars.

Results Proline levels increased with increasing temperature up to 34 °C for 1 h but then decreased at higher temperatures (depending on genotype). Notably, proline levels decreased at 38 °C for 2 h in PDKV-Washim, AKAW-3722, and PDKV Sardar and at 42 °C for 3 h in all the genotypes. The relative leaf water content (RLWC) and chlorophyll 'b' content significantly decreased with increasing temperature. Hydrogen peroxide (H_2O_2) levels increased with temperature. The enzyme activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase also increased with temperature. However, these parameters, along with other biochemical indicators, generally decreased at 42 °C for 3 h.

Conclusion This study revealed positive relationships between increasing temperatures. Hydrogen peroxide levels and the activities of SOD, APX, and peroxidase enzymes across all the genotypes. The AKAW-4627 genotype presented better maintenance of physiological and biochemical parameters and lower H_2O_2 levels, indicating greater heat tolerance. Compared with PDKV-Washim and AKAW-3722, which are more susceptible to high temperatures, the WSM-109–04, AKAW-4627 and PDKV Sardar genotypes presented better adaptability to heat stress. These findings suggest that selecting wheat genotypes with higher proline accumulation and better maintenance of physiological and biochemical parameters under heat stress, such as AKAW-4627, can help in the development of heat-tolerant wheat cultivars.

Keywords Wheat, High temperature, Proline, Carotenoids, H_2O_2, SOD, APX, Peroxidase

Introduction

Wheat (*Triticum aestivum* L.) is a thermosensitive crop. The optimum temperature for wheat is 15–18 °C [1], whereas moderately high temperatures (25–32 °C) for longer durations and very high temperatures (33–44 °C) for shorter periods are very common in tropical and subtropical environments worldwide [2]. Frequent changes in climate with respect to increasing temperature have detrimental effects on all stages of wheat growth and development, which ultimately limit growth, decrease grain protein quality and reduce the number of stable food crops globally [3–5]. Heat stress at any stage of growth can alter wheat grain quality in terms of grain weight; nutrient, antinutrient, fiber, and protein contents;

*Correspondence:
Satbhai Ravindra
satbhairavindra@pdkv.ac.in
[1] Dr PDKV, Akola, Maharashtra, India

and starch granule composition [6]; thus, studies of the thermotolerance of wheat genotypes at the physiological, molecular and biochemical levels remain a major challenge for identifying heat-tolerant genotypes.

At the cellular level, high temperature increases the rate of respiration and disturbs cellular function through the generation of reactive oxygen species (ROS). The photosynthetic activity of chloroplasts is mostly disturbed [6, 7]. ROS are toxic to biological organisms, and their damage includes autocatalytic peroxidation of the cell membrane through protein degradation, enzyme inactivation, pigment bleaching and disruption of vital biomolecules such as DNA and proteins [7].

At the seedling stage, heat stress (42 °C, 24 h) inhibited root and first leaf development; increased reactive oxygen species (ROS) and lipid peroxidation (LP) products in the coleoptile and developing organs were documented in an earlier study [8]. Plants protect cell and subcellular systems by activating enzymatic and nonenzymatic antioxidant mechanisms to eliminate ROS such as superoxide dismutase (SOD) (EC: 1.15.1.1), ascorbate peroxidase (APX) (EC: 1. 11.1.11), catalase (CAT) (EC:1.11.1.6) and metabolites such as ascorbic acid, α-tocopherol, carotenoids, proline and glycine betaine. The roles of proline and glycine betaine in decreased accumulation, the detoxification of ROS and the restoration of photosynthesis under abiotic stress have been well documented [9–13]. The plant pigment carotenoids also act as antioxidants in light-harvesting processes, and protection of the photosynthetic machinery from photooxidative damage has been reported previously [14]. The associations between the apo-plastic superoxide radical level and the total activities of antioxidative enzymes such as SOD, APX and CAT were evaluated and documented in wheat previously under different light intensities [15]. In general, responses to high temperatures may involve, among other factors, alterations in transcriptional control; the synthesis of osmoprotectants and heat shock proteins [4], the modification of carbohydrate metabolism [10]; the induction of various signaling processes [16]; and the development of antioxidative systems to reduce the harmful effects of oxidative damage [9]. The exposure of plants to nonlethal, slightly elevated temperatures may protect against subsequent severe high-temperature stress; this process is called heat acclimation. The mechanisms of heat acclimation of the photosynthetic machinery have been extensively studied in various crop species, including wheat [17]. The effectiveness of heat acclimation depends on various factors. For example, it also shows season dependence, as was recently demonstrated in winter wheat [18].

One way to mitigate the adverse effects of high-temperature stress on growth and development is to develop thermotolerant varieties [19] and to develop genotypes that are early in maturity to escape terminal heat stress [20]. Physiological and some biochemical factors, such as the degradation of photosynthetic pigments and the accumulation of hydrogen peroxide ($H_2O_{2)}$ or malondialdehyde (MDA), are potential indicators of plant responses to heat stress. Previous studies on the adverse effects of high-temperature stress on wheat have been carried out at the anthesis and grain-filling stages by using different contemporary crop models [21–23]. Changes in the temperature induction response have been shown to be potential tool for empirical assessment of cell survival. By this technique, thermotolerant lines were identified from crop wheat, which performed better than the original population under high temperatures [24–27]. In this study, we used the induction response technique with five different high-temperature treatments (25–42 °C) and durations of exposure to 10-day-old wheat seedlings to evaluate their effects. To understand the mechanism for heat tolerance, the present investigation involves screening promising germplasms released by Dr. PDKV, Akola (India) for the Vidarbha region of Maharashtra for their physiological and biochemical parameters (relative leaf water content, chlorophyll, carotenoids, proline content, and profile of antioxidative enzymes) under high temperature through an acclimation process by comparison with Raj-4083, a thermotolerant cultivar used as a national check, to provide basic information to wheat breeders, which may help improve wheat improvement targeted to heat-tolerant cultivars.

Materials and methods

The seven wheat genotypes namely, PDKV Sardar, AKAW-381, PDKV-Washim, AKAW-3722, WSM-109–4, AKAW-4627 and Raj-4083 (National Check-Thermotolerant), were obtained from AICWRS, Akola. The genotypes selected for this study were developed at Wheat Research Station Dr. PDKV, Akola, based on their field trial. Among these seven genotypes, AKAW-381 (late heat tolerant) was released for the Vidarbha region of Maharashtra, India, and AKAW-3722 was released for the timely sowing of thermosusceptible genotypes. AKAW-4627, a nationally released cultivar, and PDKV Sardar State, a released cultivar, are both suitable for late sowing and are categorized as thermotolerant cultivars. WSM 109–4 is a prerelease genotype (stay green & perfuse tillering), and PDKV-Washim is suitable for rainfed and restricted irrigation (Suppl. file 1 Appendix Table S4). All genotypes are recommended for the Vidarbha region of Maharashtra by Dr PDKV. Akola, India. The seeds were cleaned and sown in earthen pots containing soil medium containing FYM (5 kg of total material) and were equilibrated with water as needed. The

seedlings were allowed to grow for 10 days under natural daylight conditions in earthen pots (diameter, 24 cm; height, 25 cm). A total of twenty-five seedlings were maintained in each pot. After ten days, these seedlings were transferred to a BOD incubator with a 15/9 h light (1500-lx) cycle with 34.5 µmol/s/m2 PPFD light intensity at 25 ± 1 °C for 24 h. A set of seedlings was subsequently exposed to temperature stress via acclimation at 30 °C for 1 h, 34 °C for hr, 38 °C for 2 h and 42 °C for 3 h. Pots of the same genotype were grown to expose the next heat temperature through the acclimation process, and the temperature was increased by one degree after five minutes to reach each heat treatment. A total of three pots from each treatment were maintained. The leaves of three plants of each genotype and treatment from the top 5 to 7 cm were separately harvested at 11 days, weighed and analyzed for selected physiological and biochemical parameters. The experiment was carried out at Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, India in November 2021.

Physiological traits

The percent RWC was measured as per the method suggested by Weatherly (1950) [28] via leaf samples (0.5 g) with the following formula: RLWC (%) = (Fresh weight—Dry weight)/(Turgid weight—Dry weight) × 100. The leaf chlorophyll content was determined by using 80% acetone per the method described by Arnon (1949) [29]. The absorbance was then read on a Spectra-20 instrument at wavelengths of 663 and 645 nm, and the chlorophyll content was calculated via the equation given by this method and expressed as mg g^{-1} Fr. Wt. ($n = 3$).

Biochemical traits

Determination of proline, leaf carotenoid, and H_2O_2 contents

The proline content was determined according to the modified method of Bates et al. (1973) [30]. A leaf sample (0.5 g) was used for proline estimation by grinding it in 5 ml of 3% (w/v) sulfo-salicylic acid. The absorbance of the toluene phase was read at 520 nm. The concentration of L-proline was calculated from the standard curve and expressed as µmoles g^{-1} Fr. Wt.

The total carotenoids were extracted and portioned in the organic solvent acetone. The carotenoid content was estimated calorimetrically at 450 nm using β-carotene as a standard, as suggested by Mahadevan and R. Sridhar (1986) [31]. A leaf sample (2 g) was ground in distilled acetone and filtered through Whatman No. 42 filter paper. The extraction was repeated three times until the tissue was free from pigments. The filtrate was pooled and partitioned thrice with equal volumes of peroxide-free ether via a separating funnel. The excess ether was evaporated, and the residue was dissolved in 10 ml of ethanol. The carotenoids that are bound as esters are hydrolyzed via aqueous 60% KOH. The mixture was then boiled for 5 to 10 min. An equal quantity of water was added, and the mixture was partitioned twice with ether. The combined layer of ether was evaporated, and the residue was dissolved in 10 ml of ethanol. The absorbance was read at 450 nm, and the quantity of total carotenoid content (mg/100 g) in the sample was calculated from a standard graph prepared against β-carotene.

The concentration of hydrogen peroxide in a wheat sample was determined as per the method described by Sinha (1972) [32]. The quantity of H_2O_2 was determined from a standard curve prepared with 100 µM H_2O_2.Fresh leaves (0.2 g) from the control and temperature-treated wheat plants were ground in 2 ml of distilled 0.8 N perchloric acid and centrifuged at 10,000 rpm for 30 min. The supernatant was collected, and the volume was 2 ml. The leaf extract (200 µl) was removed from the test tube, and the volume was adjusted to 1 ml with 0.1 M phosphate buffer (pH 7.5). Potassium dichromate-5% (2 ml) and glacial acetic acid at a ratio of 1:3 v/v were then added. The mixture was then heated in a boiling water bath for 10 min and allowed to cool. The absorbance was then recorded at 570 nm against a reagent blank without sample extract. The quantity of H_2O_2 was determined from a standard curve prepared with 100 µM H_2O_2. The biochemical estimation of all the parameters was carried out via a double-beam UV–Vis spectrophotometer (Make-Bio-Era).

Antioxidative enzyme assay

Preparation of the crude enzyme extract

For assays of SOD, APX and peroxidase, leaf samples (0.5 g) were homogenized with 5 ml of ice-cold 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA in a prechilled motor and pestle. The homogenates were filtered and then centrifuged at 15,000 rpm for 20 min at 4 °C. The resulting supernatant was used as a crude extract for enzyme assays. For the estimation of ascorbate peroxidase, the extraction buffer was further supplemented with 1 mM ascorbic acid, and the pH was adjusted to 7.5 (Almeselmani et al. 2009)[9].

Enzyme assays

SOD activity was determined by measuring its ability to inhibit the photochemical reduction of NBT via the method described by Dhindsa and Matowe (1981) [33]. The absorbances of the irradiated and nonirradiated reaction mixtures were read at 560 nm, and control was used as a blank. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction per min at 560 nm.

Ascorbate peroxidase activity was measured as per the method described by Nakano and Asada (1981) [34]. The hydrogen peroxide-dependent oxidation of ascorbic acid was followed by a decrease in the absorbance measured at 290 nm for three min at intervals of 30 s. A complete reaction mixture without ascorbic acid was used as a blank. The enzyme activity was calculated via the extension coefficient ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) and expressed as μ moles of ascorbate oxidized $min^{-1}\ mg^{-1}$ protein.

The activity of peroxidase was determined by the rate of decomposition of hydrogen peroxide by peroxidase, with guaiacol as a hydrogen donor measured by the increase in absorbance at 470 nm min^{-1} as per the method described by Castillo et al. (1984) [35]. The increase in absorbance was recorded at 470 nm for two min at an interval of 30 s. The enzyme activity was expressed as μ moles of tetraguaiacol formed min mg^{-1} protein. To estimate the antioxidative enzyme activity of mg^{-1} soluble protein, the soluble protein from leaf samples subjected to different temperature stress treatments was estimated according to the methods of Lowry et al. (1951) [36] with bovine serum albumen used as the standard protein.

Statistical analysis

To examine the significant difference in genotype, a factorial completely randomized design (FCRD) has been implemented (Gomez and Gomez, 1984) [37]. The genotype and temperature were considered as our study variables, i.e., treatment A and treatment B each with three replications. The equation for a completely randomized factorial design can be expressed as follows:

$$Yijk = \mu + \tau i + \beta j + (\tau\beta)ij + \varepsilon ijk$$

where *Yijk* represents the k^{th} observations of the i^{th} level of factor A and the j^{th} level of factor B and where μ represents the overall mean of the response variable. τi is the i^{th} level of treatment A; βj is the j^{th} level of treatment B; $(\tau\beta)ij$ represents the interaction effect between the i^{th} level of treatment A and the j^{th} level of treatment B; and ε_{ijk} is the random error. After ANOVA, Tukey's HSD test was applied as a post hoc multiple comparison test. Multiple comparison tests have been conducted at the 5% level of significance (Abdi and Williams, 2010) [38].

In this study, cluster analysis, the predominant unsupervised pattern recognition technique, was employed. The focus was on hierarchical clustering, which operates under the principle that objects nearby are more closely related than those farther apart. To measure the loss of information, the algorithm uses an increase in the criterion of the error sum of squares (ESS). During the analysis, all potential pairings of clusters were examined, and the two clusters that produced the smallest increase in ESS upon merging were combined. The resulting clusters at various distances were visualized via a dendrogram.

To reduce the dimensionality of the data, principal component analysis (PCA) was applied. The main goal of this technique is to retain a significant portion of the data's variability while reducing the number of variables to a small set of uncorrelated components. By analyzing the factor loadings, which indicate the correlations between the variables and the principal components, PCA allows for the grouping of individuals based on their top component scores. The outcomes were visualized via a biplot, which displays both the scores and loadings of the principal components (PCs). Spearman correlation analysis has also been applied to find the associations between temperature and various parameters.

Results

RLWC, chlorophyll, carotenoid and proline contents

When the wheat plants were exposed to different temperature stresses, the RLWC significantly decreased. Compared with all other genotypes, including National Check Raj-4083, the thermotolerant AKAW-4627 and PDKV Sardars maintained significantly greater RLWC values. However, PDKV-Washim and AKAW-3722 were similar ($p < 0.05$) and exhibited greater decreases (Fig-1 A, Appendix Table S1). The mean treatment RLWC ranged from 81.91 to 63.82%, i.e., a 22.08% reduction. Moreover, as the temperature increased, the percent RLWC decreased. The genotype Raj-4083 (national check) exhibited a 10% reduction. The mean varietal RLWC ranged from 81.73 to 67.26%, i.e., an 18% reduction. Compared with that in the control, the short-term temperature induction response in the RLWC was 18 to 22% lower (Appendix Table S1). The sublethal temperature of 38 °C for 2 h and the lethal temperature of 42 °C for 3 h resulted in a larger reduction in the percent relative leaf water content.

The Chl-a content recorded in seven different wheat genotypes (Fig. 1B, Appendix Table 1) slightly decreased. A significantly greater chlorophyll 'a' content was maintained in AKAW-4627 than in PDKV-Sardar, Raj-4083 and prerelease WSM-109–04, whereas PDKV-Wasim, AKAW-381, and AKAW-3722 were on par with each other ($p < 0.05$). The mean treatment values significantly decreased from 2.40 to 1.51 mg g^{-1} Fr. Wt. At 30 °C for 1 h, there was a slight increase in the chlorophyll 'a' content. A greater decline was observed in AKAW-3722 (2.59 to 0.91 mg g^{-1} Fr. Wt.) and PDKV Washim (2.24 to 0.94 mg g^{-1} Fr. Wt.), whereas a lower decrease was recorded in AKAW-4627 (2.61 to 1.94 mg g^{-1} Fr. Wt.) and the national check Raj-4083 (2.38 to 1.89 mg g^{-1} Fr. Wt.) followed by WSM-109–4 (2.63 to 1.83 mg g^{-1} Fr. Wt.) than in the other wheat genotypes (Appendix

Table 1 Spearman correlation analysis of physiological and biochemical parameters and wheat genotypes under high-temperature stress. '*', '**' and '***' denote significance at the 5%, 1% and 0.1% levels, respectively

Genotypes	Chlo. A	RLWC	APX	Peroxidase	Carotenoids	Proline	S. Protein	Chlo. B	SOD	H2O2
AKAW-381	-0.7759***	-0.8952***	-0.1911	0.4542	-0.724**	0.3161	-0.5066	-0.183	0.176	0.991***
WSM-109–4	-0.722**	-0.8515***	0.517*	-0.191	-0.7332**	0.7192**	-0.9405***	-0.1679	0.4542	0.8512***
RAJ-4083	-0.5717*	-0.9498***	0.8893***	0.8169***	-0.907***	0.6159*	-0.1176	-0.4042	0.8169***	0.8934***
PDKV Wasim	-0.8873***	-0.945***	-0.4909	-0.3466	-0.4818	-0.0198	-0.8681***	0.3656	-0.3466	0.8761***
AKAW-3722	-0.8953***	-0.9189***	-0.2077	0.3186	-0.384	0.3151	-0.9043***	0.368	0.3186	0.8015***
AKAW-4627	-0.6033*	-0.9249***	0.9157***	0.8673***	-0.6531**	0.9712***	-0.1715	-0.743**	0.8594***	0.9051***
PDKV Sardar	0.0565	-0.9642***	0.2801	-0.2619	-0.4541	0.3628	-0.2276	-0.6918**	0.8673***	0.8924***
Overall	-0.8932*	-0.991**	0.4752	0.7754	-0.9217*	0.5571	-0.814	-0.5825	0.9495*	0.9725**

Table 1). The mean heat stress treatment values significantly varied, and the Chl 'a' decreased from 2.52 to 1.70 mg g^{-1} Fr. Wt. ($p < 0.05$).

In the case of the chlorophyll 'b' content in the control and heat stress treatments at 30 °C for 1 h, the difference was not significant, but a slight increase and slight decline trend were observed (Fig. 1C). A significantly greater Chl 'b' content was recorded in Raj-4083 (1.98 mg g^{-1} Fr. Wt.) than in AKAW-4627. PDKV-Wasim, AKAW-3722 and PDKV Sardar were associated with AKAW-381 and WSM-109–04 in the control, whereas at 42 °C 3 h higher chlorophyll 'b' content was observed in AKAW-4627 (1.93 mg g^{-1} Fr. Wt.), followed by AKAW-3722 (1.95 mg g^{-1} Fr. Wt.) and Raj-4083 (1.89 mg g^{-1} Fr. Wt.). Overall, AKAW-381 showed a higher decline in the chlorophyll 'b' content. The data in Appendix Table S1 indicate that the temperature stress treatments significantly affected the chlorophyll 'b' content of the wheat genotypes. Raj-4083 (National Check), and AKAW-4627 are statistically significant, followed by WSM109-04 ($p < 0.05$), whereas PDKV Washim, AKAW-3722 and PDKV Sardar are at par with each other ($p < 0.05$).

The total carotenoid content (mg/100 g) in the wheat genotypes (Fig. 1D) significantly varied as the temperature and duration of exposure increased. The concentration of carotenoids increased and then decreased with increasing temperature and duration of exposure. A national check revealed significantly more carotenoids ($p < 0.05$), and the lowest carotenoid content was detected in AKAW-3722. AKAW-4627 and WSM109-04 were located on par with each other and presented higher carotenoid contents than AKAW-381 and PDKV-Sardar. The mean total carotenoid content decreased when the plants were subjected to sublethal and lethal temperature stress treatments (0.537 to 0.374 mg/100 g). The total carotenoid content increased significantly at 30 °C for 1 h in WSM-109–4, AKAW-4627 and AKAW-3722 (0.690, 0.780, and 0.512 mg/100 g, respectively) compared with that in the control. Raj-4083 AKAW-4622 and WSM-109–4 maintained relatively high levels even under sublethal and lethal temperature stress, i.e., at 38 °C for 2 h and 42 °C for 3 h (Appendix Table S1). The overall trend was a decrease from the control temperature for all the wheat varieties.

Under acclimation to relatively high temperatures, significant variation in proline accumulation was observed (Fig. 1E, Appendix Table 2). The highest proline content was recorded in AKAW-4627 and WSM 109–04, followed by Raj-4083. AKAW-4627 and PDKV-Sardar are at par with each other and showed a lower decline ($p < 0.05$). The mean proline content under the different temperature stress treatments increased from the control (25 °C) to 30 °C for 1 h, 34 °C for 1 h and 38 °C for 2 h and then further decreased at a lethal temperature of 42 °C for 3 h. (0.454 to 1.265 µmoles g^{-1} Fr. Wt. and 0.853 µmoles g^{-1} Fr. Wt.). The mean varietal values significantly ($p < 0.05$) greater in proline content were recorded in AKAW-3722, followed by WSM109-04 and Raj-4083 (1.186, 1.172 and 1.135 µmoles g^{-1} Fr. Wt., respectively) (Appendix Table S2). However, their indigenous levels were also greater in the control than in the other wheat genotypes. A steady increase was observed in AKAW-4627 (0.287 to 1.218 µmoles g^{-1} Fr. Wt.) and from the control (25 °C) to the lethal temperature (42°Cfor 3 h). The proline content first increased but then decreased under relatively high-temperature stress, especially for PDKV Wasim.

The soluble protein content showed mixed trends from the control (25 °C) to the lethal temperature (42 °C ^{for} 3 h) (Fig. 1F, Appendix Table S2). The data from the national check revealed that Raj-4083, AKAW-3722, and PDKV-Sardar were paired with each other, whereas AKAW-381, WSM-109–04, and AKAW-4627 were paired with each other, while PDKV-Wasim presented the lowest soluble protein among all the other wheat genotypes (Fig. 1F). The mean treatment values decreased at 30 °C for 1 h and 34 °C for 1 h (4.43 to 3.99 mg g^{-1} Fr. Wt.) increased at

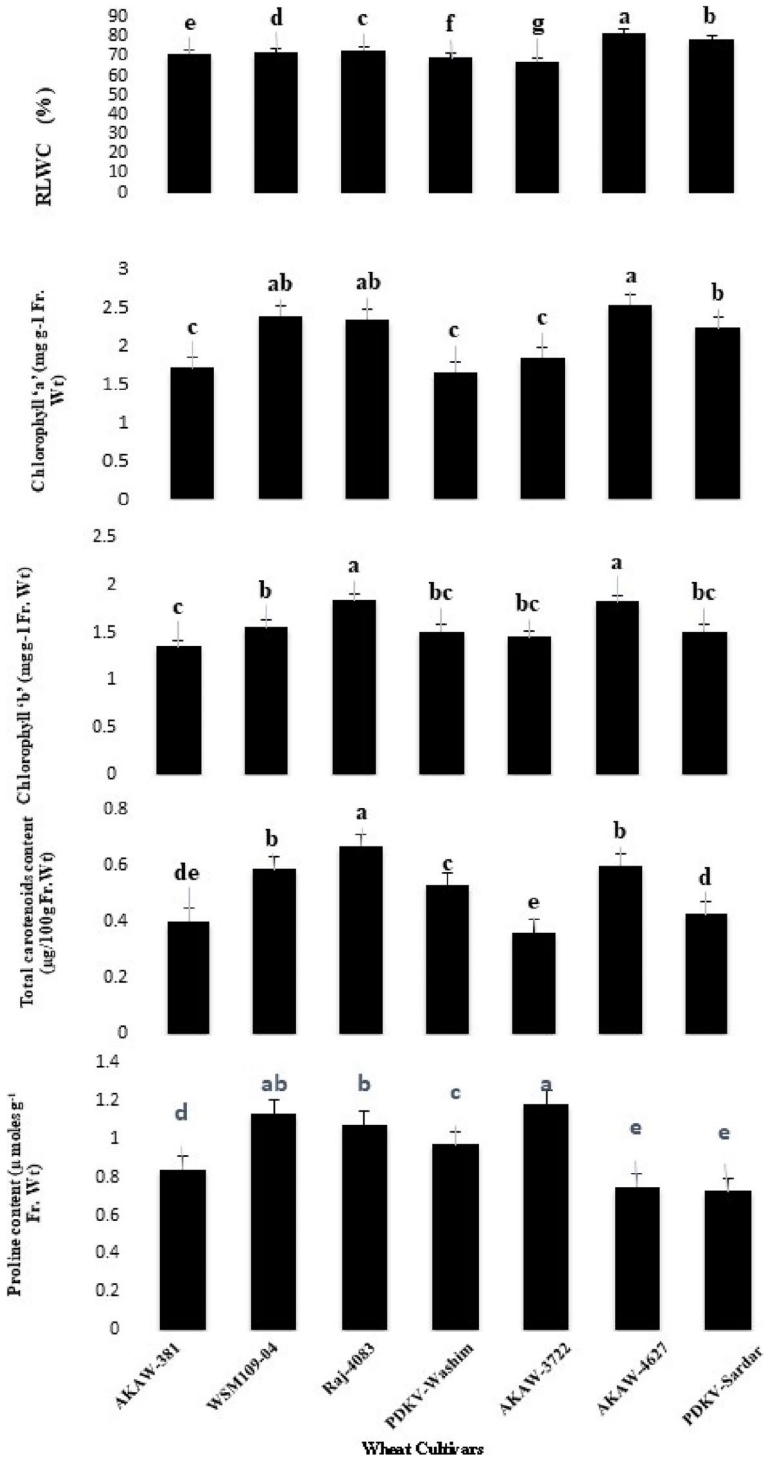

Fig. 1 (**A**, **B**, **C**, **D**, **E**) Effects of high-temperature stress on A-Relative leaf water content (%), **B**- and **C**-Chlorophyll 'a' and 'b' contents, **D**-total carotenoid content, and **E-**proline content. The vertical bar in the figures indicates the levels of the respective parameters from seven wheat cultivars after exposure to temperature stress from the control to the challenge temperature through acclimation (25°C→ 30°C for 1 hr →34°C for 1 hr→38°C for 2 hr→42°C for 3 hr). The bar indicates the SE ± the mean ($n = 3$) at $p < 0.05$ probability. For details, see the appendix of metadata files 1 and 2

38 °C 2 h (4.11 mg g^{-1} Fr. Wt.) and further declined in all wheat genotypes (Appendix Table S2). The temperature was subsequently increased (4.678 mg g^{-1} Fr. Wt.) at 34 °C for 1 h, after which it decreased to 42 °C for 3 h (2.93 mg g^{-1} Fr. Wt.). The mean varietal values revealed the highest soluble protein content (4.18 mg g^{-1} Fr. Wt.) in Raj-4083, followed by WSM-109–4 (4.08 mg g^{-1} Fr. Wt.), but overall, no significant differences in protein content were detected among all the varieties at 42 °C for 3 h in AKAW-3722. The soluble protein content decreased at 30 °C for 1 h in the wheat genotypes Raj-4083, AKAW-3722, and PDKV Washim, whereas it slightly increased in AKAW-4627 at 30 °C for 1 h, 34 °C for 1 h, 38 °C for 1 h and 42 °C for 3 h compared with the control. The overall range of soluble protein recorded from the control to 42°Cfor 3 h was from 4.580 to 3.920 mg g^{-1} Fr. Wt. A significant decline was observed in the wheat genotype PDKV Washim (5.31 to 3.25 mg g^{-1} Fr. Wt.), and the levels were higher in WSM109-04 than in the control at 42 °C for 3 h.

H_2O_2 and antioxidative enzymes

The H_2O_2 concentration increased with increasing exposure temperature and duration of exposure to wheat genotypes. A significantly lower accumulation was detected in AKAW-4627 than in Raj-4083, whereas the highest accumulation was observed in AKAW-381, PDKV-Wasim, and AKAW-3722 (Fig. 1G, Appendix Table 2). The wheat genotype AKAW-4627 (0.16 to 0.57 nMoles mg^{-1} Fr. Wt.) followed by Raj-4083 (0.45 to 0.66 nMoles mg^{-1} Fr. Wt.) presented relatively low levels, whereas AKAW-381 (0.45 to 2.14 nMoles mg^{-1} Fr. Wt.) and PDKV-Washim (0.56 to 2.23 nMoles mg^{-1} Fr. Wt.) presented relatively high levels across all the temperature stress treatments (Appendix Table S2). H_2 O_2 concentrations increased in all seven wheat genotypes from the control (25 °C) to 30 °C for 1 h, 34 °C for 1 h, 38 °C for 2 h and 42 °C for 3 h. This trend revealed a positive correlation between theH_2O_2 concentration and the increase in temperature.

The activity of superoxide dismutase increased with increasing temperature (Fig. 2H Appendix Table S2). The activity was significantly greater in PDKV-Sardar, Raj-4083 and AKAW-3722 ($p < 0.05$), followed by AKAW-4627, WSM109-04 and AKAW-381, and was lower in PDKV-Wasim (Fig-H) under all the temperature stress treatments and durations of exposure (Appendix Table S2). The activity decreased at 42 °C for 3 h. in all the genotypes except the national check strains Raj-4083, PDKV Sardar and AKAW-4627. The mean varietal SOD activity ranged from 0.74 to 1.09 U/mg protein, whereas the treatment means ranged from 0.71 to 1.09 U/mg protein.

The activity of ascorbate peroxidase in different wheat genotypes is depicted in Fig-2I and Appendix Table S3. The mean APX activity ranged from 101.26 to 168.29 µmol of ascorbate oxidized min^{-} mg^{-1} soluble protein. The activity increased in all the wheat genotypes as the temperature and duration of exposure increased, whereas at 42°Cfor 3 h, the activity decreased in almost all the wheat genotypes except Raj-4083. The highest mean varietal activity was recorded in WSM-109–4 (169.29 µmol of ascorbate oxidized min^{-1} mg^{-1} soluble protein), and the lowest was recorded in AKAW-3722 (129.60 µmol of ascorbate oxidized min^{-1} mg^{-1} soluble protein), but it was greater than the control values. The activity was higher in all the genotypes except Raj-4083, AKAW-4627 and WSM-109–4. The increase in APX activity at 30 °C for 1 h and 46 °C for 2 h may be the result of the oxidative stress induced by the high temperature.

The peroxidase activity data are depicted in Fig. 2(J) and Appendix Table S3. Higher activity was observed in AKAW-4627, Raj-4083 and AKAW-3722, followed by WSM109-04 and AKAW-381, and lower activity was observed in PDKV-Sardar, and PDKV-Washim at $p < 0.05$ (Fig. 2 J). The mean varietal peroxidase activity ranged from 0.74 to 0.1.02 µmoles of guaiacol consumed per milligram of protein. The peroxidase activity increased significantly from 30 °C for 1 h to 42 °C for 3 h in the wheat genotype AKAW-4627 and the national check Raj-4083, whereas it decreased in PDKV Sardar, WSM-109–4, and PDKV Washim at 34 °C for 1 h to 42 °C for 3 h. AKAW-4627 and Raj-4083 significantly increased the activity, i.e., 0.60 to 1.89 and 0.79 to 1.48 µmoles, respectively of tetra guaiacol consumed mg^{-1} protein (Appendix Table S3) compared with all the other genotypes at the 5% and 1% levels.

To analyze a dataset consisting of 10 physiological and biochemical parameters across five temperatures, each with three replications, a dimensionality reduction technique called principal component analysis (PCA) was utilized. The objective was to cluster the variables based on the principal components (PCs). The outcomes of this analysis can be observed in Fig. 3. The results revealed that the first three PCs accounted for more than 80% of the total variability. By examining the higher loadings among these selected PCs, the significant variables were identified. Specifically, in PC1, Chl-a and RLWC presented relatively high loadings, whereas in PC2, carotenoid and proline presented relatively high loadings. In PC3, APX and soluble protein presented relatively high loadings. These findings indicate that these parameters are important for characterizing genotypes.

Hierarchical cluster analysis revealed three distinct homogenous groups of genotypes based on different

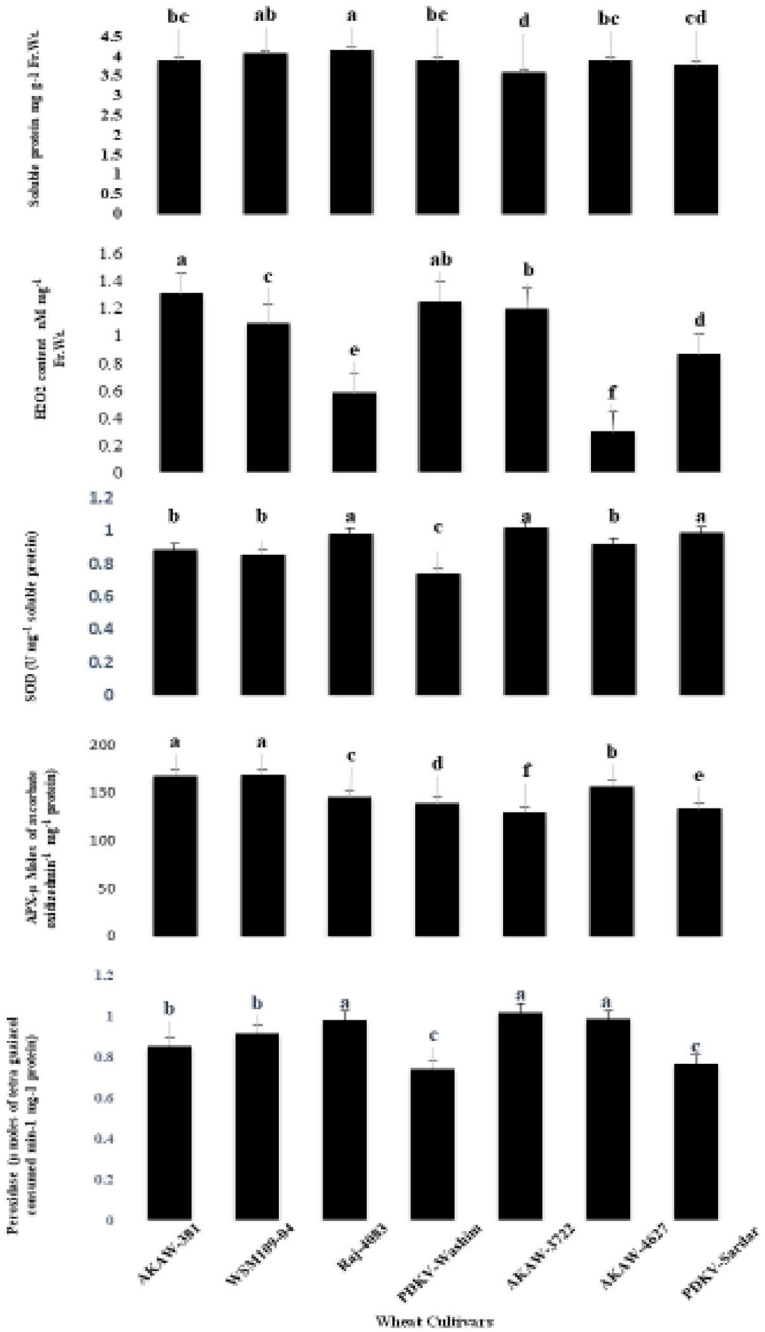

Fig. 2 (**F**, **G**, **H**, **I**, **J**) Effects of high-temperature stress on the soluble protein content (**F**) and hydrogen peroxide concentration (**G**). Superoxide dismutase (SOD) (**H**). Ascorbate peroxidase (**I**) and guaiacol peroxidase (**J**). The vertical bar in the figures indicates the levels of the respective parameters from seven wheat cultivars after exposure to temperature stress from the control to the challenge temperature through acclimation (25°C⟶ 30°C for 1 hr ⟶34°C for 1 hr⟶38°C for 2 hr⟶42°C for 3 hr). The bar indicates the SE ± the mean ($n = 3$) at $p < 0.05$ probability. For details, see the appendix of metadata files 1 and 2

physiological and biochemical properties. The heatmap with the dendrogram is presented in Fig. 4. AKAW-4627 formed a separate group; WSM-109–4 and Raj-4083 formed the second group, and the other genotypes formed the third group. Each group was treated as homogeneous for the study of physical and biological characteristics.

Table 1 clearly shows that, concerning chlorophyll 'a', all the genotypes except PDKV Sardar presented a significant negative correlation with temperature. In the case of

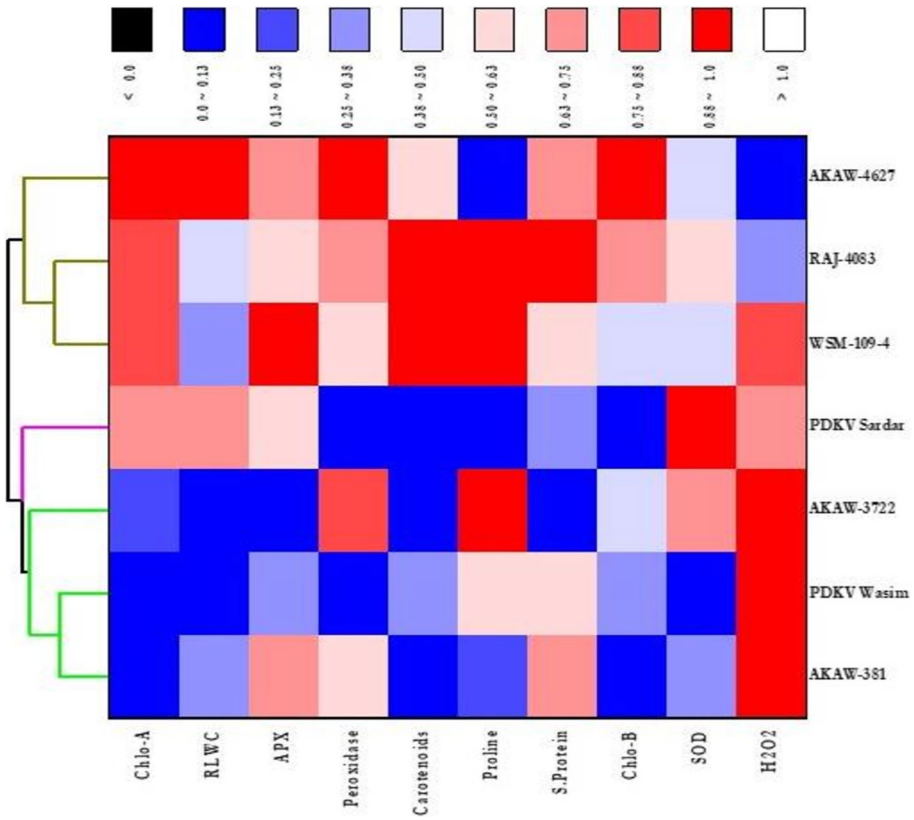

Fig. 3 PCA biplots of PC1, PC2 and PC3 with loading factors

RLWC, all the genotypes presented high and significant correlations, with PDKV Sardar having the highest value. For APX and proline, only WSM-109–4, RAJ-4083, and AKAW-4627 had significant positive correlations with temperature, and AKAW-4627 demonstrated the highest correlations among them. Peroxidase activity was significantly positively correlated with temperature only for RAJ-4083 and AKAW-4627. AKAW-381, WSM-109–4, RAJ-4083, and AKAW-4627 display significant correlations with temperature in the case of carotenoids. For soluble protein, WSM-109–4, RAJ-4083, and AKAW-4627 had significant negative correlations. In the case of chlorophyll 'b', AKAW-4627 and PDKV Sardar presented significant positive correlations, whereas for SOD, RAJ-4083, AKAW-4627 and PDKV Sardar presented significant correlations. Finally, for hydrogen peroxide H_2O_2, all the genotypes presented significant correlations, with AKAW-381 showing the highest correlation. Figure 5 illustrates the correlations among these traits. Overall, for all the genotypes, Chl-a, RLWC, and carotenoids were significantly negatively correlated, whereas H_2O_2 and SOD were significantly positively correlated. The strongest positive and negative correlations were observed between H_2O_2 and RLWC, respectively.

Discussion

Abiotic stress, such as high-temperature stress, is differentially regulated in wheat at the physiological, biochemical and molecular levels (*Triticum aestivum* L.). Every 1 °C increase in temperature at any stage of crop growth above a mean temperature of 23 °C adversely affects the wheat yield by ~ 10%. [3, 39, 40]. In the present investigation, the impacts of gradually increasing high-temperature stress treatment through the acclimation process on the physiological and biochemical parameters of six wheat genotypes released by Dr. PDKV, Akola, India, are compared with those of thermotolerant Raj-4083 (National Check). Previously, such studies were not conducted on wheat cultivars released by Dr. PDKV, Akola, which needs to provide information insight to wheat breeders.

The decline in relative leaf water potential under high-temperature stress is closely related to a reduction in water absorption capacity and to a disruption in the stomatal control of plant transpiration. [41, 42]. In this study, the relative leaf water content was relatively conserved/maintained significantly higher in the thermotolerant wheat genotype AKAW-4627, and PDKV Sardar (Fig-1 A, Appendix Table 1) might be able to tolerate

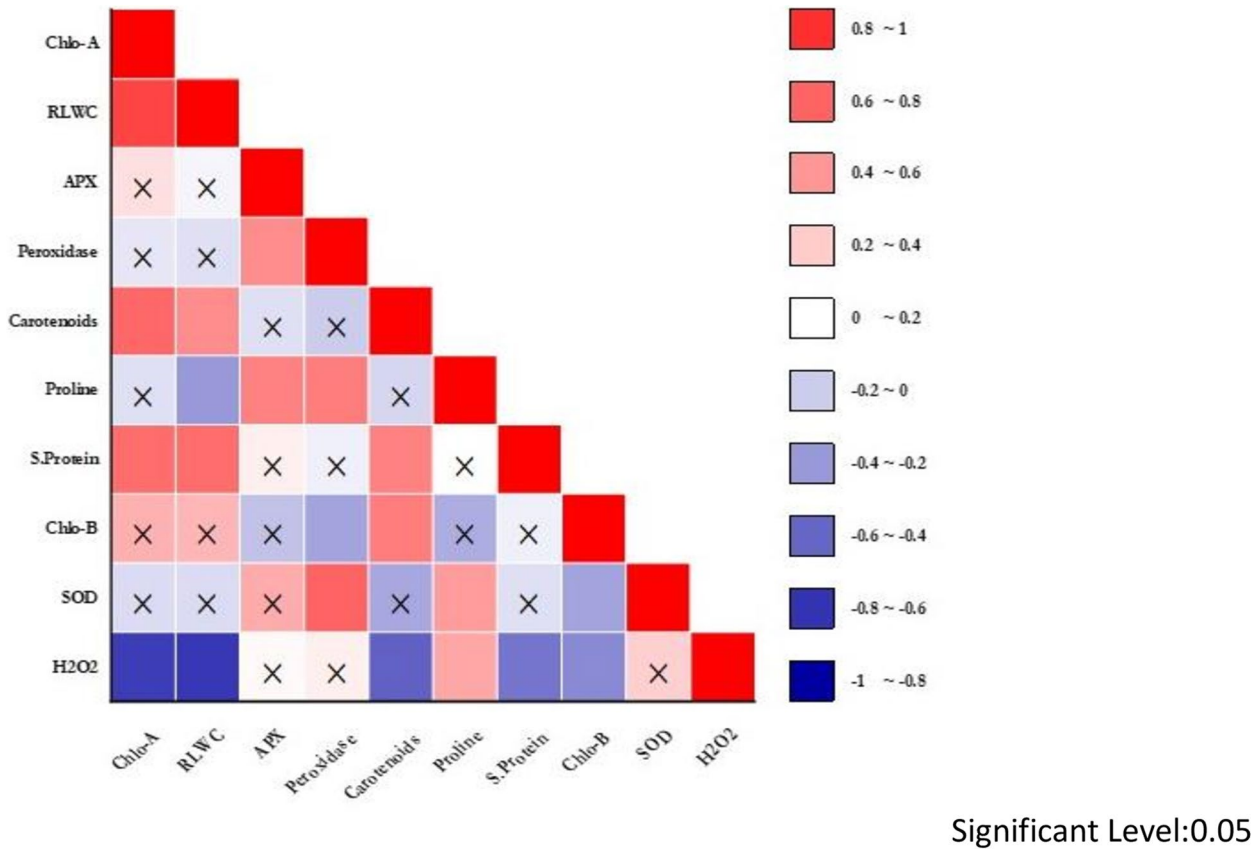

Fig. 4 Dendrogram of hierarchical cluster analysis along with heatmap of wheat genotypes

high temperatures by maintaining a lower osmotic potential than the other genotypes. A decrease in the RLWC and a reduction in membrane stability are common phenomena in hot environments and are associated with leaf water deficit and increased membrane damage [9]. Raj-4083 AKAW-381, WSM-109–4 and AKAW-381 presented similar trends and were similar ($p < 0.05$) to each other but exhibited intensive decreases in the PDKV-Washim (early heat tolerant) and AKAW3722 (timely sowing) genotypes, which showed susceptible to high temperatures. Similar observations were recorded previously in wheat when seedlings were exposed to heat stress sublethal to lethal temperature stress at 42 °C and 45 °C [21, 42]. Our results of the Chl 'a' and b' contents (Fig. 1 B & C) revealed that the level was maintained at a threshold temperature of 34 °C for 1 h and then decreased in all the wheat genotypes. However, the magnitude of decline was greater for AKAW-381, PDKV Washim, and AKAW-3722. Interestingly, the levels were maintained higher level in thermotolerant AKAW-4627, PDKV Sardar, and prerelease WSM-109–4 (Stay green), and the national check Raj-4083 showed greater stability, which might be due to the accumulation of carotenoids (Fig-1D, Appendix Table 1) to stabilize chloroplasts [7]. The results of our study confirm earlier reports that a greater reduction in chlorophyll 'b' content than chlorophyll 'a' content occurs under heat stress in wheat [19, 37]. In AKAW-3722 (timely sown) and early heat tolerant PDKV-Washim, the photosynthetic activity is more affected and might be due to damage to or disturbances in the activity of PSII or its sensitivity [43, 44], whereas acclimation at high temperature has a weaker effect on the donor side of PSII intolerant other wheat genotypes [40, 45].

In many abiotic stresses, including temperature stress, either low or high proline accumulation in cells plays a role as an osmoprotectant [46–48]. An increase in temperature induced proline accumulation in all the genotypes until a temperature threshold of 34 °C was reached at 1 h (which was dependent on genotype), but above this threshold, the proline content decreased. However, the level decreased at 38 °C for 2 h in PDKV-Washim, AKAW-3722 and PDKV Sardar and at 42 °C for 3 h in all seven wheat genotypes. In the present study, proline increased at 30 °C for 1 h and 34 °C for 1 h, whereas it decreased at 38 °C for 2 h and 42 °C for 3 h (Fig-1(E)

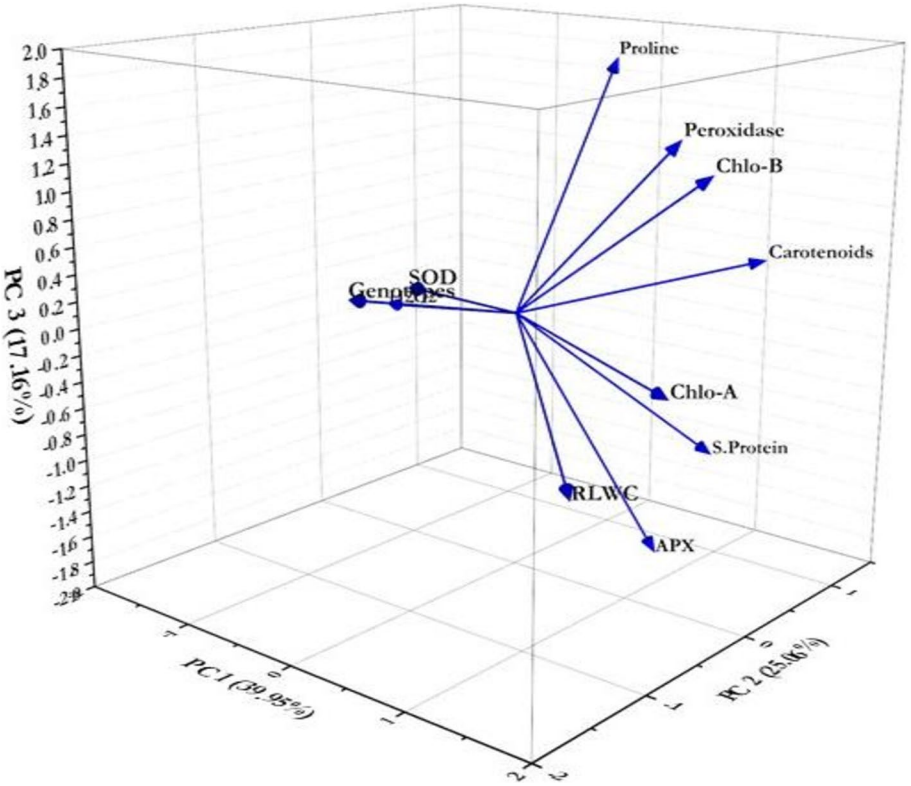

Fig. 5 Correlations among the physiological and biochemical traits. The crosses indicate non-significance at the 5% level

Appendix Table S2), which was probably due to increased ROS production via the Pro/P5C cycle, as suggested previously [49], or their utilization for the activation of antioxidative enzymes to avoid membrane damage, as reported in wheat [27, 50, 51]. The proline (Fig. 1E) and protein contents (Fig. 1F) increased in wheat seedlings at temperatures ranging from 15 to 25 °C but decreased again above 30 °C due to the decreased activities of P5CS, ornithine aminotransferase and proline dehydrogenase, as reported previously [13, 52, 53]. Similar observations were recorded earlier in the case of an increase in proline content and then a decrease in proline content in both wheat [52] and Arabidopsis [53].

Carotenoids act as light harvesters, quenchers, scavengers, and dissipators of excess energy under adverse conditions. Carotenoids stabilize membranes by reacting with lipid peroxidation products and scavenging ROS, especially singlet oxygen [54, 55]. In our study, higher levels of carotenoid pigments under high temperature might help induce the antioxidative cascade in the wheat genotypes AKAW-4627, AKAW-381, WSM-109–4 (0.588) and the national check Raj-4083 (0.671) than in the other wheat genotypes (Fig. 1(D) Appendix Table S1). This suggests their use for stability at relatively high temperatures, as reported previously [54, 56]. Improvements in photochemical efficiency due to the use of carotenoids have been documented in UV-B-exposed desert plants [57], heat stress-exposed maize [14] and sugarcane [26].

An increase in the concentration of hydrogen peroxide (H_2O_2) in cells is considered toxic to plant cells [9], whereas H_2O_2 plays a role as a signaling molecule to various stimuli in plant cells [58]. In the present investigation, the increase in H_2O_2 concentration observed in all seven wheat genotypes (Fig. 2(G), Appendix Table S2) may be involved in cellular signal transduction pathways to activate the defense mechanism [12]. The relatively high concentrations of the stay green character wheat genotypes WSM-109–04 and AKAW-3722 (timely sowing) were susceptible, whereas the relatively low accumulation in the national check variety Raj-4083 and AKAW-4627, followed by PDKV-Sardar, might be due to their strong antioxidative capacity and ability to maintain relatively high RLWC and chlorophyll a and b contents (Appendix Table S 1) and other studied biochemical parameters. A positive correlation between the increase in H_2O_2 levels and SOD levels and temperature was observed in all the wheat genotypes (Table 1). Our observations revealed that wheat responded to different heat temperature stress treatments, i.e., 22, 30, 35 and 40 °C, for 2 h periods [50, 52]. Previously, the induction of the intracellular

accumulation of H_2O_2 and ascorbate antioxidants was reported in Arabidopsis under 37 °C heat stress [58].

Plants are endowed with H_2O_2-metabolizing enzymes such as ascorbate peroxidase (APX), catalase (CAT), peroxidase, and glutathione reductase for catalyzing a dismutation reaction localized in chloroplasts and mitochondrial cells [59, 60]. APX has a relatively high affinity for H_2O_2, which reduces it to H_2O in chloroplasts, the cytosol, mitochondria and peroxisomes, and in the apo-plastic space, where it utilizes ascorbate as a specific electron donor [12, 61, 62]. A positive correlation was observed in our study, with increases in superoxide dismutase and APX activity and peroxidase activity with H_2O_2 and a gradual increase in high temperature (Fig-2 H, I & J; Appendix Tables S2 and S3), which revealed the participation of APX in the detoxification of ROS in all seven genotypes with different characteristics (Appendix S4) and that APX may play a defensive role under short term increases in temperature, as reported in wheat previously [7, 63].

Peroxidase activity is used as an indicator of free radical damage to cell membranes under stress conditions [64]. In our study, the gradual increase in temperature from the control (25 °C) to 30, 34, and 38 °C and finally to 42 °C for 3 h resulted in increased peroxidase activity, except for the PDKV Sardar, WSM-109–4, and PDKV Washim wheat genotypes (Fig-J). Conversely, the soluble protein content decreased in response to heat stress. Our results for physiological and biochemical parameters agree with the results of Sattar et al. (2020) [65], who reported that individual and combined imposition of drought and heat stresses significantly ($p \geq 0.05$) altered water relationships, osmolyte contents, soluble proteins and sugars along with an activated antioxidant defensive system in terms of superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) in wheat. In this study, the soluble protein content was highest in Raj-4083, followed by WSM-109–04, whose soluble protein content was on par with that of the other wheat genotypes. The levels of protein changed as the temperature and time changed but did not significantly change, possibly because of the synthesis of new proteins during the acclimation process to combat the situation.

Heat stress and protein denaturation cause a marked and irreversible reduction in protein solubility. Cytoplasmic proteins are more sensitive to increasing temperature, and the total protein content decreases under both heat acclimation and sudden heat stress [66]. Significant variation in biochemical parameters such as protein content, antioxidant activity, proline content and total reducing sugar content in leaves, stems, and spikes under normal (26 ± 2 °C) and terminal HS (36 ± 2 °C) conditions has been documented earlier in wheat, with the induction of unique proteins related to the photosynthesis apparatus and the upregulation of genes associated with the photosynthesis and starch biosynthesis pathways [10, 27, 67]. Similar results for protein content were documented in another study in wheat during heat stress (HS) for 1 h and 24 h at 40 °C in darkness or light, as well as after recovery from heat stress [67, 68]. In hard fescue, heat stress was applied at 38/33 °C (day/night), and the optimal temperature was 21/18 °C [69].

Compared with those in the national check Raj-4083, higher levels of carotenoids were detected in the WSM-109–4(stay green) and thermotolerant AKAW-4627samples, whereas lower levels were detected in AKAW-3722, which was released for timely sowing. The hydrogen peroxide content and relative leaf water content were found to have the strongest positive and negative correlations. As the hydrogen peroxide (H_2O_2) level increased steadily, the relative leaf water content (RLWC) and chlorophyll 'b' content both decreased. High temperature exposure increased and decreased the carotenoid content, and the increased activity of antioxidants likely ameliorated the adverse effects of high temperature for a short duration in WSM-109–4, AKAW-4627 and PDKV-Sardar.

The overall correlations among the studied biochemical parameters, temperature treatments and genotypes (Table 1, Fig. 5) revealed that Chl-a, RLWC, and carotenoids were significantly negatively correlated, whereas H_2O_2 and SOD were significantly positively correlated. The strongest positive and negative correlations were observed between H_2O_2 and RLWC, respectively. Similar observations were recorded in wheat under combined heat and drought stress [70, 71].

Conclusion and future need

The data from this investigation provide several key insights into the response of wheat genotypes to high-temperature stress: proline levels increased with increasing temperature and exposure time, indicating its role as a stress marker. However, at extreme temperatures (38 °C for 2 h and 42 °C for 3 h), proline levels decreased across all the genotypes, suggesting a possible limit to its effectiveness as a protective agent under severe stress conditions. Both the RLWC and chlorophyll 'b' content decreased with increasing hydrogen peroxide (H_2O_2) content, highlighting the negative impact of oxidative stress on these parameters. H_2O_2 levels increased with temperature, and a positive correlation was observed between H_2O_2 and superoxide dismutase (SOD) levels across genotypes. These findings indicate that increased ROS levels are associated with increased SOD activity. The activities of all four ROS scavenging enzymes increased with temperature, particularly from 34 °C for 1 h to 42 °C for 3 h. These

findings suggest an adaptive response to manage oxidative stress. Genotypes such as prerelease WSM-109–04, AKAW-4627 and PDKV Sardar followed by AKAW-381 showed better adaptability to higher temperature stress than did the national check Raj-4083. These genotypes maintained better physiological and biochemical parameters, indicating a greater level of heat tolerance. There was a notable increase in the antioxidative profile, including that of superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase and carotenoids, under high temperatures. This enhanced antioxidative response likely contributes to the observed heat tolerance. With the exceptions of PDKV Sardar, WSM-109–4, and PDKV Washim, which presented strong peroxidase activity, the other genotypes presented various responses. This variability underscores the need for further studies to confirm and better understand heat tolerance mechanisms across different genotypes. The diverse responses observed in thermotolerant genotypes warrant additional studies under controlled environmental conditions or the use of different contemporary crop models to elucidate the exact mechanisms of heat tolerance. These observations are valuable for improving wheat programs, particularly in the development and selection of heat-tolerant genotypes for better resilience to high-temperature stress.

Abbreviations

RLWC	Relative leaf water content
H_2O_2	Hydrogen peroxide
SOD	Superoxide dismutase
APX	Ascorbate peroxidase
Chl-a	Chlorophyll 'a'
Chl-b	Chlorophyll 'b

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05842-7.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
We acknowledge Associate Dean, Vasantrao Naik Govt. College of Agricultural Biotechnology, Yavatmal (MS) for providing infrastructure facilities for the current research activity and Wheat Research Unit, Dr. PDKV, Akola (MS) for providing authenticated seed material for the experimentation.

Clinical trial number
Not applicable.

Authors' contributions
1. Dr. Satbhai Ravindra Contributed to finalizing treatments, conducting experiments, recording observations and script writing, and preparing graphs, figures and interpretation of data. 2. Dr. Bharad Swati Contributed in the designing of experiments, timely guidance and procurements of wheat genotypes. 3. Dr. Moharil Mangesh. Contribute to interpretation of results and script writing, data analysis and preparation of graphs, figures and interpretation of data and timely guidance.

Funding
This work is carried out as an academic research program, and no funding has been received.

Data availability
The author declared that data supporting to the finding of this study are available within the paper and its supplementary formation files given in the submission system. Clinical trial number: not applicable.

Declarations

Ethics approval and consent to participate
All authors of this manuscript confirm that all methods were carried out following relevant guidelines and regulations. The authors also declare that the content of the manuscript has not been published, or submitted for publication elsewhere.

Consent for publication
Not applicable

Competing interests
The authors declare no competing interests.

Received: 10 April 2024 Accepted: 18 November 2024
Published online: 04 January 2025

References

1. Choudhary SI, Wardlaw IF. The effect of temperature on kernel development in cereals. Aust J Agri Res. 1978;29:205–23.
2. Narayanan S. Effects of high temperature stress and traits associated with tolerance in wheat. Open Access J Sci. 2018;2(3):177–86. https://doi.org/10.15406/oajs.2018.02.00067.
3. Tiwari V, Mamrutha HM, Sareen S, Sheoran S, Tiwari R, Sharma P, Singh C, Singh G and Rane J. (2019). Managing abiotic stress in wheat. In: Abiotic Stress Management for Resilient Agriculture(pp.313–337). Springer Nature Singapore Pte Ltd. 2017 P.S. Minhas et al., (eds.), Abiotic Stress Management for Resilient Agriculture, https://doi.org/10.1007/978-981-10-5744-1-14.
4. Hasanuzzaman M, Nahar K, Alam MM, Roy Chowdhury R, Fujita M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int J Mol Sci. 2013,3;14(5):9643–84. https://doi.org/10.3390/ijms14059643. PMID: 23644891; PMCID: PMC3676804.
5. Senthold A, Pierre M, Andrea M, et al. Climate change impact and adaptation for wheat protein. Glob Change Biol. 2019;25(1):155–73. https://doi.org/10.1111/gcb.14481.
6. Nurunnaher Akter, M. Rafiqul Islam. Heat stress effects and management in wheat. A review. Agron. Sustain. Dev. (2017) 37: 37 https://doi.org/10.1007/s13593-017-0443-9
7. Mohi-Ud-Din, M., Siddiqui, N., Rahman, M., Jagadish, S.V.K., Ahmed, J.U., Hassan, M.M., Hossain, A., Islam, T. Physiological and Biochemical Dissection Reveals a Trade-Off between Antioxidant Capacity and Heat Tolerance in Bread Wheat (*Triticum aestivum* L). Antioxidants. 2021;10(3)351. https://doi.org/10.3390/antiox10030351.
8. Savicka M, Skute N (2010) Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.) EKOLOGIJA. 2010. Vol. 56. No. 1–2. P. 26–33 https://doi.org/10.2478/v10055-010-0004-x
9. Almeselmani M, Deshmukh PS, Sairam RK. High-temperature stress tolerance in wheat genotypes: Role of antioxidative defense enzymes. Acta Agro Hung. 2009;57(1):1–14.
10. Kumar RR, Goswami S, Shamim M, Mishra U, Jain M, Singh K, Singh JP, Dubey K, Singh S, Rai GK, Singh GP, Pathak H. Chinnusamy V & Praveen Biochemical Défense Response: Characterizing the Plasticity of Source and Sink in Spring Wheat under Terminal Heat Stress. Front Plant Sci. 2017;8:1603. https://doi.org/10.3389/fpls.2017.01603.
11. Kumar RR, Goswami S, Kumar N, Pandey SK, Pandey VC, Sharma SK, Pathak H and Rai RD. Expression of novel ascorbate peroxidase

isoenzymes of wheat (*Triticum aestivum* L.) in response to heat stress. International Journal of Plant Physiology and Biochemistry. 2011; 3(11)188–194, http://www.academicjournals.org/IJPPB.
12. Pandey S, Fartyal D, Agarwal A, Shukla T, James D, Kaul T, Negi YK, Arora S, Reddy MK. Abiotic Stress Tolerance in Plants: Myriad Roles of Ascorbate Peroxidase. Front Plant Sci. 2017;8:581. https://doi.org/10.3389/fpls.2017.0058.
13. Dikilitas M, Simsek E, & Roychoudhury A. (2020). Role of Proline and glycine betaine in overcoming abiotic stresses. In A. Roychoudhury & D. K. Tripathi (Eds.), Protective chemical agents in the amelioration of plant abiotic stress (first ed., pp. 1–23). John Wiley and Sons. https://doi.org/10.1002/9781119552154.ch1.
14. Li F, Vallabhaneni R, Yu J, Rochford T, Wurtzel ET. The Maize Phytoene Synthase Gene Family: Overlapping Roles for Carotenogenesis in Endosperm, Photomorphogenesis, and Thermal Stress Tolerance. Plant Physiol. 2008;147:1334–46.
15. Robert G, Melchior M, Racca R, Trippi V, Lascano HR. Apo plastic superoxide level in wheat protoplast under photooxidative stress is regulated by chloroplast redox signals: effects on the antioxidant system. Plant Sci. 2009;177(3):168–74.
16. Proveniers MC, van Zanten M. High temperature acclimation through PIF4 signaling. Trends Plant Sci. 2013;18:59–64.
17. Janda T, Tajti J, Hamow K, Marček T, Ivanovska B, Szalai G, Pál M, Zalewska ED, Darkó É. Acclimation of photosynthetic processes and metabolic responses to elevated temperatures in cereals. Physiol Plant. 2021;171:217–31.
18. Zhou H, Xu M, Hou R, Zheng Y, Chi Y, Ouyang Z. Thermal acclimation of photosynthesis to experimental warming is season-dependent for winter wheat (*Triticum aestivum* L). Env Exp Bot. 2018;150:249–59.
19. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: An overview. Environ Expt Bot. 2007;61:199–223.
20. Joshi AK, Chand R, Arun B, Singh RP, Ortiz R. Breeding crops for reduced tillage management in the intensive rice wheat system of South Asia. Euphytica. 2007;153:135–51.
21. Nuttall, James G, and Barlow, Kirsten M, et al. "Acute High Temperature Response in Wheat". Agronomy Journal. 2018;110(4)1296–1308. https://doi.org/10.2134/agronj2017.07.0392.
22. Dwivedi SK, G. Kumar, Basu S, Kumar S, Rao KK and Choudhary AK. Physiological and molecular aspects of heat tolerance in wheat SABRAO Journal of Breeding and Genetics. 2018;50(2)192–216.
23. Yashavanthakumar KJ, Baviskar VS, Navathe S, Patil RM, et al. Impact of heat and drought stress on phenological development and yield in bread wheat. Plant Physiol Rep. 2021. https://doi.org/10.1007/s40502-021-00586-0.
24. Senthil Kumar M, Kumar G, Srikanthbabu V, Uday KM. Assessment of variability in acquired tolerance: Potential option to study genotypic responses and the relevance of stress genes. J Plant Physiol. 2006;164:111–25.
25. Almeselmani M, Deshmukh PS, Sairam RK, Kushwaha SR, Singh TP. Protective role of antioxidant enzymes under high temperature stress. Plant Sci. 2006;171:382–8.
26. Wahid A. Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. J Plant Res. 2007;120:219–28.
27. Satbhai RD, Kale AA, Naik RM. Protective Role of Osmolytes and Antioxidants during High Temperature Stress in Wheat. International Journal of Bioresource and Stress Management. 2015;6(3):220–9.
28. Weatherly PE. Studies in the water relations of the cotton plant I The field measurement of water deficits in leaves. New Phytologist. 1950;49:81–7.
29. Arnon DI. Copper enzymes in isolated chloroplast polyphenol oxidase in Beta vulgaris. Plant Physiol. 1949;24:1–25.
30. Bates LS, Waldren RP, Tears ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39:205–7. https://doi.org/10.1007/BF00018060.
31. Mahadevan A and R. Sridhar (1986). In: Methods in Physiological Plant Pathology (3rd edn.), Sivakami Publications, Chennai, pp.9–11
32. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47:389. https://doi.org/10.1016/0003-2697(72)90132-7.
33. Dhindsa RS, Matowe W. Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. J Exp Bot. 1981;32:79–91.
34. Nakano Y, Asada K. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. Plant Cell Physiol. 1981;22:867–80.
35. Castillo FJ, Penel C, Greppin H. Peroxidase release induced by ozone in Sedum album leaves: involvement of Ca^{2+}. Plant Physiol. 1981;74(4):846–51.
36. Lowry OH, Rosebraugh NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 2015;193(1):265–75 (**PMID: 14907713**).
37. Gomez, KA and Gomez, AA,1984. *Statistical procedures for agricultural research*. John Wiley & sons.
38. Abdi H, Williams LJ. Tukey's honestly significant difference (HSD) test. Encyclopaedia of research design. 2010;3(1):1–5.
39. Yang J, RG Sears, BS Gill, et al. Genotypic differences in utilization of assimilate sources during maturation of wheat under chronic heat and heat shock stresses. Euphytica. 2002;125(2):179–188.
40. Zahra N, Hafeez MB, Wahid A, Al Masruri MH, Ullah A, Siddique KHM, Farooq M. Impact of climate change on wheat grain composition and quality. J Sci Food Agric. 2022;103(6):2745–51. https://doi.org/10.1002/jsfa.12289.
41. Shah NH, Paulsen GM. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant Soil. 2003;257(1):219–26.
42. Lin KH, Huang HC, Lin CY. Cloning, expression and physiological analysis of broccoli catalase gene and Chinese cabbage ascorbate peroxidase gene under heat stress. Plant Cell Rep. 2010;29:575–93.
43. Savicka M, Skute N. Some morphological, physiological and biochemical characteristics of wheat seedlings *Triticum aestivum L*: organs after high temperature treatment. Ekologi JA. 2012;58(1):9–21.
44. Langjun CUI, Jian long LI, Yamin FAN, Sheng XU, Zhang Z. High temperature effects on photosynthesis, PS II functionality and antioxidant activity of two *Festuca arundinaceous* cultivars with different heat susceptibility. Botanical Studies. 2006;47:61–69.
45. Allakhverdiev SI, Kreslavski VD, Klimov VV, Los DA, Carpentrier R, Mohanty O. Heat stress: an overview of molecular responses in photosynthesis. Photosynthetic Res. 2008;98:541–50.
46. Ozden M, Demirel U, Kahraman A. Effects of proline on antioxidant system in leaves of grapevine (Vitis vinifera L) exposed to oxidative stress by H2O2. Sci Hortic. 2009;119(2):163–8.
47. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments. Plant Signal Behav. 2012;7:1456–66.
48. Majláth I, Szalai G, Soós V, Sebestyén E, Balázs E, Vanková R, Dobrev PI, Tari I, Tandori J, Janda T. Effect of light on the gene expression and hormonal status of winter and spring wheat plants during cold hardening. Physiol Plant. 2012;145:296–314.
49. Fuji H, Verslues PE, Zhu JK. Arabidopsis decuple mutant reveals the importance of SnRK2 kinases in osmotic stress responses in vivo. Proceeding of the National Academic of Sciences, USA. 2011;108:1717–22.
50. Kumar RR, Sharma SK, Gadpayle KA, Singh K, Sivaranjani R, Goswami S, Rai RD. Mechanism of action of hydrogen peroxide in wheat thermotolerance - interaction between antioxidant isoenzymes, proline and cell membrane. Afr J Biotech. 2012;11(78):14368–79.
51. Kumar RR, Goswami S, Sharma SK, Singh K, Gadpayle KA, Singh SD, Pathak H, Rai RD, Differential expression of heat shock protein and alteration in osmolyte accumulation under heat stress in wheat. J. Plant Biochem. Biotechnology. Springer. DOI. 2012. https://doi.org/10.1007/s13562-012-0106-5.
52. Song SQ, Lei YB, Tian XR. Proline Metabolism and Cross-Tolerance to Salinity and Heat Stress in Germinating Wheat Seeds. Russ J Plant Physiol. 2005;52(6):793–800. https://doi.org/10.1007/s11183-005-0117-3.
53. Wei-Tao Lv, Lin B, Zhang M& Hua XJ. Proline Accumulation Is Inhibitory to Arabidopsis Seedlings. Plant Physiol. 2011;156(4):1921–1933.
54. Uarrota VG, Stefen D, Leolato L, Nerling D. (2018) Revisiting Carotenoids and Their Role in Plant Stress Responses: From Biosynthesis to Plant Signaling Mechanisms During Stress. https://doi.org/10.1007/978-3-319-75088-0_10 In book: Antioxidants and Antioxidant Enzymes in Higher Plants.
55. Strzałka K, Kostecka-Gugała A, Latowski D. Carotenoids and Environmental Stress in Plants: Significance of Carotenoid-Mediated Modulation of Membrane Physical Properties. Russ J Plant Physiol. 2003;50:168–73.

56. Park S, Kim HS, Jung YJ, Kim SH, Ji CY, Wang Z, Jeong JC, Lee HS, Lee SY, Kwak SS. Orange protein has a role in phytoene synthase stabilization in sweet potato. Sci Rep. 2016;6:33563.
57. Salama HM, Al Watban AA, Al-Fughom AT. Effect of ultraviolet radiation on chlorophyll, carotenoid, protein and proline contents of some annual desert plants. Saudi J Biol Sci. 2011;18(1):79–86. https://doi.org/10.1016/j.sjbs.2010.10.002. Epub 2010 Oct 11. PMID: 23961107; PMCID: PMC3730951.
58. Lijuan Niu, Weibiao Liao. Hydrogen Peroxide Signaling in Plant Development and Abiotic Responses: Crosstalk with Nitric Oxide and Calcium. Front. Plant Sci., 04 March 2016 Sec. Plant Physiology https://doi.org/10.3389/fpls.2016.00230.
59. Volkov AA, Panchuk I, Mullineaux PM, Schöffl F. Heat stress-induced H_2O_2 is required for effective expression of heat shock genes in Arabidopsis. Plant Mol Biol. 2006;61(4–5):733–46.
60. Noctor G, Mhamdi A, Foyer CH. The roles of reactive oxygen metabolism in drought: Not so cut and dried. Plant Physiol. 2014;164:1636–48.
61. Hasanuzzaman M, Nahar K, Anee TI, Fujita M. Exogenous silicon attenuates cadmium-induced oxidative stress in Brassica napus L by modulating AsA-GSH pathway and glyoxalase system. Front Plant Sci. 2017;8:1061.
62. Sofo A, Scopa A, Nuzzaci M, Vitti A. Ascorbate Peroxidase and Catalase Activities and Their Genetic Regulation in Plants Subjected to Drought and Salinity Stresses. Int J Mol Sci. 2015;16(6):13561–78.
63. Mahla R, Madan S, Rana MK, Munjal R. Antioxidant Defense System in Wheat (Triticum aestivum L) Seedlings under heat Stress and revival Conditions. Journal of Agricultural Science and Technology. 2011;5(1):27–34.
64. Wang GP, Zhang XY, Li F, Luo Y, Wang W. Over accumulation of glycine betaine enhances tolerance to drought and heat stress in wheat leaves in the protection of photosynthesis. Photosynthetica. 2010;48:117–26.
65. Sattar A, Sher A, Ijaz M, Ul-Allah S, Rizwan MS, Hussain M, et al. Terminal drought and heat stress alter physiological and biochemical attributes in flag leaf of bread wheat. PLoS ONE. 2020;15(5):e0232974. https://doi.org/10.1371/journal.pone.0232974.
66. He Y, Liu X, Huang B. Protein Changes in Response to Heat Stress in Acclimated and Nonacclimated Creeping Bent grass. J Amer Soc Hort Sci. 2005;130(4):521–6.
67. Alafari HA, Abd-Elgawad ME. Heat-Induced Protein and Superoxide Dismutase Changes in Wild Tetraena propinqua ssp Migahidii Seedlings. Pakistan Journal of Biological Sciences. 2021;24:310–8.
68. Demirevska-Kepova K, Holzer R, Simova-Stoilova L, et al. High temperature stress effects on ribulose- 1,5-bisphosphate carboxylase/oxygenase, Rubisco binding protein and Rubisco activase in wheat leaves. Biol Plant. 2005;49:521–5.
69. Wang J, Yuan B, Xu Y, Huang B. Differential Responses of Amino Acids and Soluble Proteins to Heat Stress Associated with Genetic Variations in Heat Tolerance for Hard Fescue. J Amer Soc Hort Sci. 2018;143(1):45–55. https://doi.org/10.21273/JASHS04246-17.
70. Qaseem MF, Qureshi R, Shaheen H. Effects of Pre-Anthesis drought, heat and their combination on the growth, yield and physiology of diverse Wheat (Triticum aestivum L) genotypes varying in sensitivity to heat and drought stress. Scientific Reports. 2019;9:69–55. https://doi.org/10.1038/s41598-019-43477-z.
71. Brestic M, Zivcak M, Kalaji HM, Kalaji HM, Carpentier R, Allakhverdiev SI. Photosystem II thermostability in situ: Environmentally induced acclimation and genotype-specific reactions in *Triticum aestivum* L. Plant Physiol Biochem. 2012;57:93–105.

Publisher's Note