



Physio-biochemical characterization of wheat genotypes under temperature stress

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Abstract Thermal stress is a major abiotic stress in wheat and is highly complex in mechanism. A large area in north-western plain zones (NWPZ), which is the wheat bowl of India is affected by heat stress. Climate change also causes an abrupt increase in temperature at different growth stages of wheat. Thus, wiser selection of stress tolerant varieties is an important strategy to combat the climate change effect. The present study aims for physiological and biochemical screening of timely sown NWPZ wheat varieties (WB2, HD3086, DBW88, DPW621-50, DBW17, HD2967 and PBW550) of India for their thermal stress tolerance along with heat tolerant (RAJ3765) and susceptible checks

(RAJ4014) at seedling stage. The experiment was conducted in completely randomized design under controlled laboratory condition and heat stress was induced at 37 °C at seedling stage. Later different physio-biochemical traits were studied in both control and stress seedlings. All traits exhibited significant variations among genotypes under heat stress condition. Root and shoot weight, relative water content, chlorophyll content index and chlorophyll fluorescence reduced significantly, whereas membrane leakage, osmotic potential, catalase, ascorbate peroxidase, guaiacol peroxidase, malondialdehyde content and proline content were increased in stress plants. A tolerance matrix was prepared based on stress response of the genotypes for each trait and a final tolerance score was given to each genotype. Based on this tolerance matrix, DBW88 and PBW550 were identified as tolerant, DPW621-50, DBW17 and HD2967 as moderately susceptible and HD3086 and WB2 as susceptible to heat stress. Earlier studies parade that seedling level stress tolerance has high correlation with adult level stress tolerance under field condition in wheat. Hence, this study helps in wiser selection of varieties for sowing in NWPZ based on weather forecast of the location for creating varietal mosaic in context of climate change.

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Abbreviations

HS	Heat stress
CCI	Chlorophyll content index
CFL	Chlorophyll fluorescence
RWC	Relative water content
OP	Osmotic potential
CAT	Catalase
POX	Guaiacol peroxidase

APX	Ascorbate peroxidase
MDA	Malondialdehyde content
ROS	Reactive oxygen species

Introduction

Wheat is a primary source of nutrition and an important staple grain for humans. In the year 2021–2022, global wheat production was 778.64 million metric tons. India is third largest wheat producer in the world after European Union (EU) and China. In recent years, varying climate has significantly affected wheat yield. The average temperature and CO₂ concentration in atmosphere are on increasing trend which may result in more frequent heat waves and severe terminal drought for many cropping regions. With an increase rate of 0.3 °C per decade, by 2025 global temperature is expected to be 1 °C above present value and by 2100 it will be 3 °C above the present value (Pachauri and Meyer 2014; Chauhan et al. 2014; Gupta et al. 2016; Mamrutha et al. 2020a). Wheat, being a winter crop is highly sensitive to elevated temperatures especially during reproductive stage (Dillard 2019; Sonkar et al. 2019; Malhi et al. 2021; Daloza et al. 2021).

India has five major wheat growing zones namely Northern Hill Zone (NHZ), North Western Plains Zone (NWPZ), North Eastern Plains Zone (NEPZ), Central Zone (CZ) and Peninsular Zone (PZ). Wheat is grown on 31.76 million ha. area with a production of 109.5 mt (ICAR-IIWBR 2021) and NWPZ is the largest wheat producing zone in India. In NWPZ, thermal stress is moderate but has significant importance as it contributes for the major wheat production of the country (Mohan et al. 2011; Mamrutha et al. 2020b; Daloza et al. 2021). An increase of 6 °C in mean temperature by 2080 has been projected in Indo Gangetic Plains (IGP), as compared to present. The precipitation is also expected to increase by 20% by year 2080. Increased CO₂ and precipitation will increase the yield but enhanced CO₂ effect will get nullified by a temperature increase of above 3 °C. Kumar et al. (2014) predicted wheat yield reduction up to 25% by 2080 in India and other models also suggested a drop of 15% in wheat yield by 2100 in India. Simulated models predict a general wheat yield loss for Punjab, Haryana, Uttar Pradesh and Bihar between 1 and 8%. Increasing production in the future requires the adaptation of wheat through breeding and other suitable agronomic strategies which reduces the effect of high temperature. Under continuous climate change, the wise selection of the wheat variety based on weather forecast of the locality plays a significant role (Mukherjee et al. 2019; Ramadas et al. 2020).

Elevated temperature raises the level of water stress in plant cells, crop water requirement, respiration and also affect plant photosynthesis. In early growth stages (seed

germination), the ideal temperature range for wheat is 12–25 °C while the critical temperature at later growth stage (grain-filling) should be between 25 and 28 °C. Increased ambient temperature reduces the total crop duration by causing early flowering and shortening the grain filling period thus reducing the yield (Mohan et al. 2017; Lamaoui et al. 2018). Studies suggest that for 1 °C rise in air temperature in north India, the flowering date is reduced by 5 days and maturity date by 4 days (Zampieri et al. 2017) with wheat yield reduction upto 3–5% (Akter and Islam 2017).

Exposure to intermittent or constant high temperatures result in an array of morpho-anatomical, physiological and biochemical changes in plants affecting their growth and development and consequently causing drastic reduction in crop yield. Although supply of assimilates is boosted due to increased temperature and photosynthesis but this doesn't fully compensate for reduced duration of starch deposition. Thus, resulting in smaller grains and low yield (Dhyani et al. 2013; Hossain et al. 2013; Asthir et al. 2014; Ding et al. 2021). Plants survive in high temperature by two inherent mechanisms, mainly by basal tolerance (inherent ability to survive) followed by acclimation (adaptability to lethal temperature). Both these mechanisms are found in wheat with activation of different genetic systems (Akter and Islam 2017; Islam et al. 2017; Ding et al. 2021). Protein denaturation, aggregation and enhanced fluidity of membrane lipids are direct injuries to the plants resulting from high temperatures, whereas protein degradation, inhibition of protein synthesis, inactivation of enzyme in chloroplast and mitochondria and loss of membrane integrity are low heat injuries or indirect injuries to the plants (Khan et al. 2017; Sharma et al. 2019). Heat stress is extremely complex and the physiological and biochemical changes occurring inside the plant under heat stress condition have not been fully explored (Wang et al. 2014; Pandey et al. 2019; Sharma et al. 2019).

Timely sowing (TS) is done in majority of the wheat growing area in NWPZ and there is a need to study the response of timely sown wheat varieties on temperature fluctuations for wiser selection of varieties pertaining to the area to sustain higher yield (ICAR-IIWBR 2021). Previous studies have shown high correlation between seedling level heat stress tolerance with adult level heat stress tolerance under field conditions in wheat (Rinki et al. 2016). Hence, the present study aims at precise screening of TS wheat varieties of NWPZ based on their heat stress tolerance and segregating them into stress tolerant and susceptible groups considering different physio-biochemical traits. Such classification can serve varieties as signature in breeding program for heat stress tolerance, it helps in wise selection of the variety for sowing based on weather forecast of the location and also as

a good source for transgenic and genome editing to improve abiotic stress tolerance in wheat.

Material and methods

Plant materials and experimental conditions

Nine wheat genotypes, including seven NWPZ-TS released varieties (WB2, PBW550, DBW17, DPW621-50, HD3086, HD2976 and DBW88) and two checks RAJ4014 (heat-susceptible), and RAJ3765 (heat-tolerant), were used in the present study (Table 1). Seeds were obtained from the germplasm unit, ICAR-Indian Institute of Wheat and Barley Research, Karnal, India. Seeds were surface sterilized with 0.1% mercuric chloride solution for 5 min and then rinsed with 70% ethanol for 2 min and subsequently with distilled water as described by Kumar et al. (2017). The experiment was conducted in plastic 4" pots having potting mixture of soil, sand and peat in the ratio 2:1:1 (v/v) and grown at 22 °C, 65 ± 10% RH, 16/8 h photoperiod in laboratory condition. A completely randomized design (CRD) with three replications per genotype per treatment was used. Seven seeds were sown in each of the pots, after successful germination, two least grown plants were removed from each pot leaving only five plants in each pot. Forty-five days old seedlings were subjected to heat stress in growth chamber at 37 °C for 12 h and another set was maintained as control. Leaf samples were collected from treated and non-treated plants for studying different physio-biochemical traits immediately after stress treatment.

Morpho-physiological traits

Shoot, root length and root weight

Shoot and root were harvested immediately after stress treatment and their length were measured in cm using a standard scale and root weight was measured using a precision weighing scale (Afcoset, ER-182A).

Relative water content (RWC)

After stress treatment, RWC was measured in three randomly chosen plants. About 100 mg Fresh leaf samples (FW) from treated and control plants were immersed in distilled water in 50 ml centrifuge tubes overnight at room temperature. Next morning, the leaf samples were weighed for recording turgid weight (TW). Subsequently, the samples were placed in a paper bag and dried in a hot air oven at 80 °C until they obtained a constant weight and dry weight (DW) was recorded (Barrs and Weatherley 1962). A precision analytical balance (Afcoset, ER-182A) was used for all weight measurements.

The RWC of a leaf was calculated as:

$$\text{RWC}(\%) = \left[\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \right] \times 100$$

where FW = fresh weight, TW = turgid weight, DW = dry weight.

Membrane leakage

Membrane leakage of leaves after treatment was measured using electrical conductivity meter (Eutech PC700, Thermo Scientific, USA) in EC mode. Equal weighing (100 mg) leaf samples were excised and were immersed in 50 ml distilled water in 50 ml tubes. The initial leakage was measured immediately after immersion in water. Then, all the samples were autoclaved at 121 °C for 15 min. The tubes were cooled at room temperature and final leakage was recorded. The

Table 1 Pedigree of the wheat genotypes used in the study

Genotype name	Pedigree	Year of release
WB2	T.DICOCCON CI9309/AE.SQUARROSA(409)// MUTUS/3/2*MUTUS	2017
PBW550	WH 594/RAJ 3858//W 485	2007
DBW17	CMH79A.95/3*CNO-79//RAJ-3777	2006
DPW621-50	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	2010
HD3086	DBW14/HD2733//HUW468	2014
HD2967	ALD/COC//URES/HD2160M/HD2278	2011
DBW88	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	2014
RAJ4014	DL 8025/K 9011	2014
RAJ3765	HD2402/VL639	1995

membrane leakage of each sample was calculated using the formula: Membrane leakage% = (Initial leakage/Final Leakage) × 100 (Maia et al. 2010).

Chlorophyll content index (CCI)

Leaf chlorophyll content is an indirect measure of the photosynthetic capacity of the plants. A chlorophyll content meter (SPAD-502 Plus, Konica Minolta) was used to estimate CCI of the leaves. The measurements were done immediately after stress treatment in seedling leaves, by placing the instrument in such a way that its optical portion is covered by the leaf and three biological readings were taken from each treatment of the varieties as replications. The CCI values of the instrument ranges from 1 to 100.

Chlorophyll fluorescence (CFL)

CFL (F_v/F_m) measures the photosynthetic efficiency of the plants and is severely affected under abiotic stress. CFL was estimated using a chlorophyll fluorescence meter (Model OS30P+, Opti-Sciences, Inc., USA). It was recorded after 20 min of dark adaptation using the leaf clips (for normalizing the electron flow of the photosystems) and later placing the instrument at the same position with a flash of light to measure the efficiency of photosystem II in terms of F_v/F_m in varieties after the stress treatment. The CFL values ranges from 0 to 0.84.

Leaf osmotic potential

Leaf osmotic potential measurements were done as described by Blum et al. (1999). About 100 mg of fresh leaf sample was taken and immediately stored in liquid nitrogen. Then the leaf sample was put in spin tube and crushed via glass rod to extract the sap. The sap received from pressed leaves was analyzed for osmolarity (mmol/kg^{-1}) using a Vapor Pressure Osmometer (model 5520, Wescor Inc., Logan, UT). Osmolarity of the cell sap was converted from mmol/kg to osmotic potential (MPa) using the formula $\text{MPa} = -C \times 2.58 \times 10^{-3}$

Extraction and determination of antioxidant enzyme activities

Leaf samples were collected from seedlings of treated and control plants. Enzyme extract for each sample was prepared by grinding 100 mg leaf in an ice-cold pestle and mortar with cold extraction buffer (100 mM potassium phosphate buffer, pH 7.5) containing 0.5 mM EDTA and then spinning the extract at 15,000 rpm in a centrifuge at 4 °C for 20 min. The supernatant obtained was used for the spectrophotometric assay of different antioxidant enzymes.

Catalase (CAT)

CAT activity was determined by observing the decrease in concentration of H_2O_2 in reaction mixture at 240 nm for 5 min (Aebi 1984; Jebara et al. 2005). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0) and 25 μl plant extract in 3.0 ml reaction volume. The reaction was initiated by adding 10 μl of 6 mM H_2O_2 to the reaction mixture. The enzyme activity was calculated using the extinction coefficient of H_2O_2 ($39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as units/gram fresh weight.

Ascorbate peroxidase (APX)

Separate extraction was carried out for the estimation of APX activity with the extraction buffer solution containing 100 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 1.0 mM ascorbate and 1 mM DTT. APX activity was calculated by monitoring the rate of oxidation of ascorbic acid in assay buffer that contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate and enzyme extract, in a total volume of 1 ml (Jebara et al. 2005). The oxidation of ascorbic acid was initiated by adding 10 μl of 10% (v/v) H_2O_2 and the decrease in absorbance was monitored at 290 nm (extinction coefficient $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$) for 2 min. One unit of enzyme activity was defined as amount of enzyme required to oxidize 1 μmol of ascorbate per minute.

Guaiacol peroxidase (POX)

POX activity was determined using the method described by Jebara et al. (2005). The final assay volume of 3.0 ml containing 50 mM potassium phosphate buffer (pH 7.0), 9 mM guaiacol, 19 mM H_2O_2 and 0.1 ml of enzyme extract was prepared. Five absorbance readings were recorded for each sample at one-minute interval at 470 nm. Peroxidase activity was calculated using the extinction coefficient of $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$.

Malondialdehyde content (MDA)

The method by Dhindsa et al. (1981) and Zhang and Kirkham (1994) were used to estimate the level of lipid peroxidation in the leaf tissue in terms of MDA, a product of lipid peroxidation, content determined by thiobarbituric acid (TBA) reaction. About 0.25 g leaf sample was homogenized in 5.0 ml of 0.1% TCA. The homogenate was then centrifuged at 10,000 \times g for 5 min. 4.0 ml of 20% TCA containing 0.5% TBA was added in 1 ml of enzyme extract. This mixture was then heated at 95 °C for 30 min and followed by immediate quenching in an ice bath. After centrifugation of this mixture at 10,000 \times g for 10 min, the absorbance of the supernatant was recorded at 532 nm and the

value for non-specific absorption was subtracted at 600 nm. The MDA was calculated using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Proline content

About 500 mg fresh tissue of the shoots was homogenized in 10 ml of 3% sulphosalicylic acid (w/v) with pestle and mortar in ice cold bath. Homogenate was centrifuged at $10,000 \times g$ for 15 min. Reaction mixture was prepared by taking 2 ml of the supernatant and mixing it with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid. The mixture was then incubated at 100°C for 1 h until a colored complex developed and the reaction was terminated by instant cooling in ice. Then 4.0 ml toluene was added to the colored complex and the mixture was vortexed for 15–20 s. Optical density of chromophore layer was taken at 520 nm (Bates et al. 1973; Carillo et al. 2008). Proline content was estimated by using standard curve of L-proline (Bates et al. 1973).

Data analysis

All the experimental data were analyzed using the SAS statistical software, PROC GLM, SAS version 9.3 (SAS Institute Inc. Cary, NC, USA) at different statistical significance level. Least significant difference (LSD@5%) and Tukey–Kramer’s test were used to find the significant differences among varieties under different treatments. The mean values with different alphabets are found to be statistically significant. During the study of each physio-biochemical trait, all the genotypes were classified as sensitive (S), moderately sensitive (MS), moderately tolerant (MT) and tolerant (T) based on their response under heat stress condition. For each trait, percent increase/decrease data was compiled and the highest percent increase/decrease value was taken as 100% and base value was taken as Zero. The genotypes falling in 0–25% were categorized as T, 25–50% as MT, 50–75% as MS and 75–100% as S. A consolidated matrix table was prepared categorizing all nine varieties in aforementioned groups for each of the thirteen traits studied. A tolerance score was assigned to each category where S-0, MS-1, MT-2 and T-3. The scores were added for each variety to obtain a final tolerance score. Thus, the total highest score for a variety exhibiting tolerant characteristic for each trait would be “39” (13 traits \times 3 for “T”) and the lowest score would be zero if a variety exhibits sensitive behavior for all the traits. Again, the highest score of 39 was considered at 100% and the range 0–39 was divided in four segments of 25% each. The genotypes having scores from 0 to 25% of the range were categorized as S, 25–50% as MS, 50–75% as MT and above 75% as T.

Results

All the morpho-physiological and biochemical parameters changed significantly in response to stress condition for all the genotypes (Fig. 1). After statistical analysis, the LS means of replications for control and stress conditions are summarized in Tables 2 and 3 along with their standard deviations. The traits to stress interactions for different genotypes were significant at $*P \leq 0.05$ and $**P \leq 0.01$ (Figs. 2, 3).

Effect of heat stress on morpho-physiological traits

The heat stress (37°C for 12 h) was imposed on forty-five days old seedlings of wheat (Fig. 1) and the significant variation on morpho—physiological traits was observed in response to temperature stress among studied genotypes as observed by LSD and Tukey–Kramer’s test (Table 2 and Fig. 2).

Under control condition, shoot length varied from 22.5 to 29 cm but under stress condition a significant reduction was observed in shoot length and it ranged from 20.5 to 26.2 cm. Higher reduction was observed in WB2 (15.9%), RAJ4014 (15.5%) and DBW17 (15.2%) genotypes whereas less reduction was seen in DBW88 (2.7%). Root length in control condition varied from 9.2 to 18.8 cm but under thermal stress it varied from 8.1 to 13.1 cm. Less reduction in root length was observed in RAJ3765 (2.7%) and higher reduction was observed in WB2 (39.4%) under heat stress condition. Root weight varied from 198 to 321 mg in control condition and 171–241 mg in stress condition with DPW621-50 (32.0%), WB2 (30.3%) and HD3086 (29.6%) genotypes exhibiting highest reduction whereas DBW88 (5.6%) displayed least reduction in root weight (Fig. 2a–c).

Leaf RWC is the best growth and biochemical index revealing the stress intensity. RWC indicates the water stress of the cells and has significant association with yield and stress tolerance. During heat stress, the water balance of plant gets disrupted and the RWC of leaves is reduced. The RWC in control plants varied from 86% (RAJ3765) to 96.4% (HD2967), while in stress conditions it varied from 47.1% (HD3086) to 83.3% (DBW88) (Table 2, Fig. 2). Reduction was more significant in HD3086 (45.6%) and RAJ4014 (47.7%) compared to DBW88 (4.2%) and RAJ3765 (11.1%). Membrane Leakage explains the percentage relative injury to cell membrane and results revealed that membrane leakage in control condition varied from 20.3% to 41.3% and in stress condition it varied from 26.4 to 47.0%. Percent increase in membrane leakage was more in HD3086 (30.3%) and RAJ4014 (28.9%) under stress condition compared to control condition (Fig. 2d, e). Similar pattern was observed for CCI

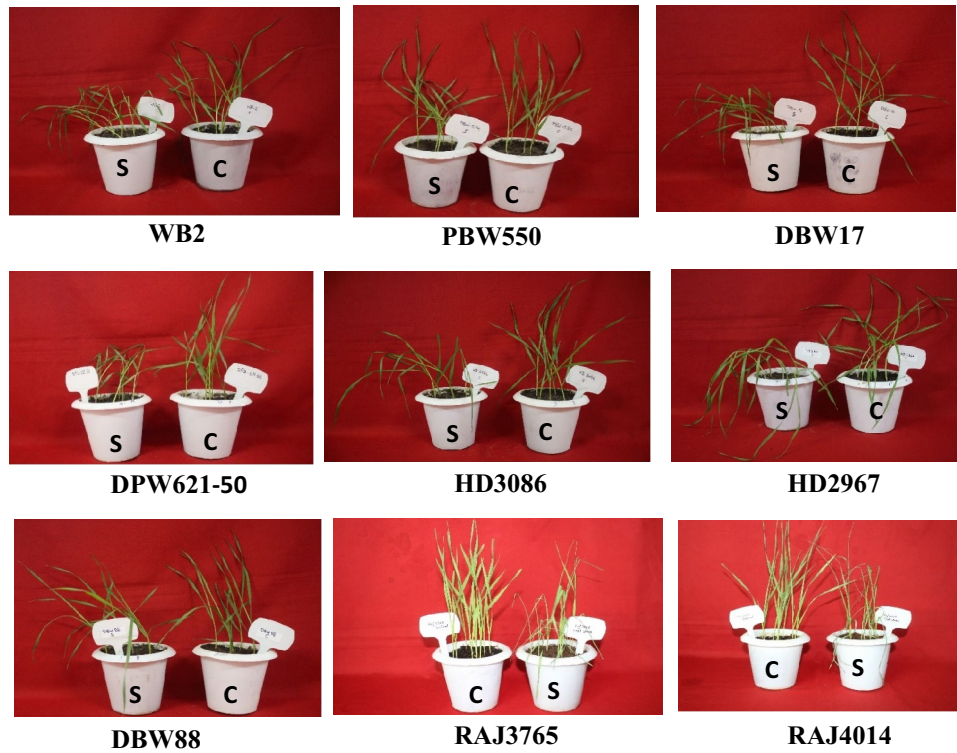


Fig. 1 Variation in phenotype of wheat genotypes under control (C) and heat stress (S) (37 °C for 12 h) condition

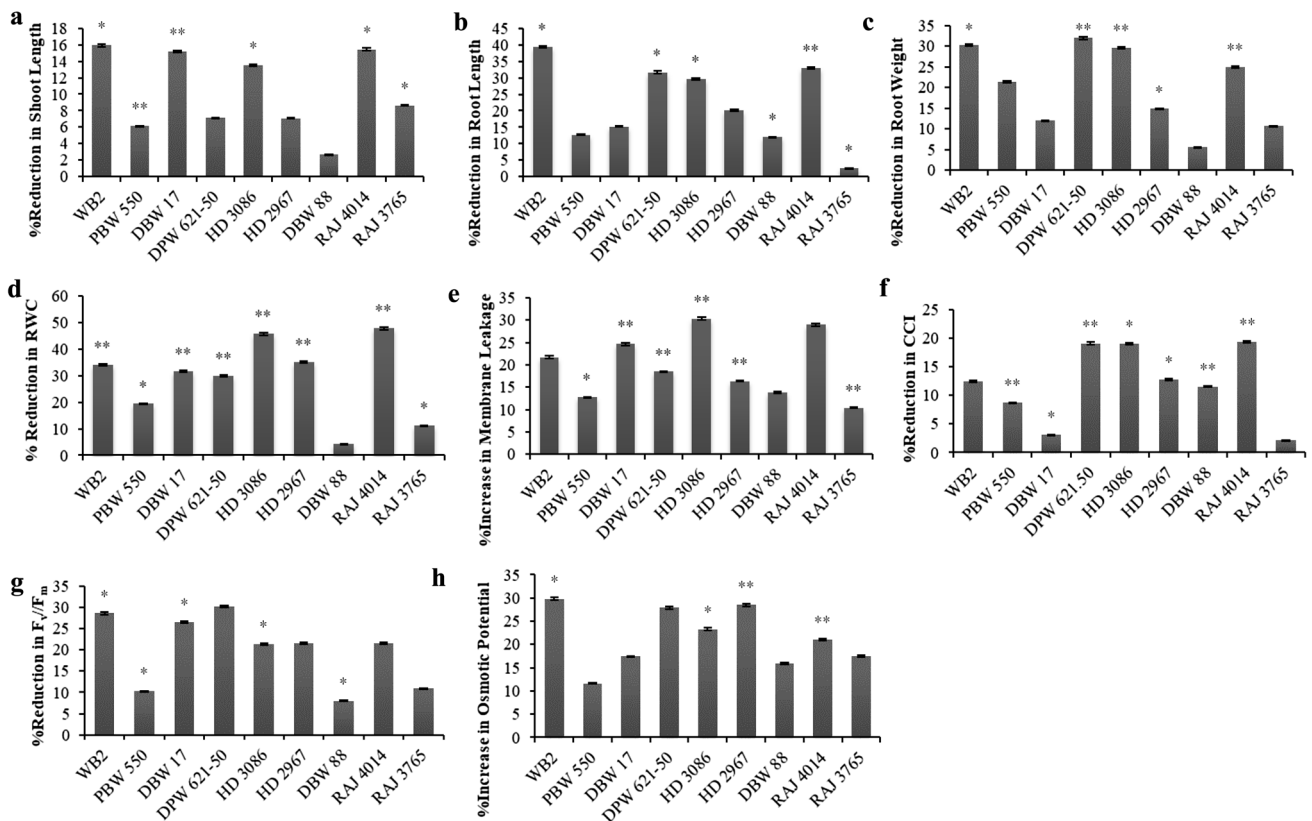


Fig. 2 Percent increase or decrease in physiological traits under heat stress compared to control condition in wheat genotypes. **a** Shoot length, **b** root length, **c** root weight, **d** relative water content (RWC),

e membrane leakage, **f** Chlorophyll content index (CCI), **g** variable fluorescence/maximal fluorescence (F_v/F_m), **h** osmotic potential (OP). * $P \leq 0.05$; ** $P \leq 0.01$

Table 2 Variation in morpho-physiological traits under control and heat stress conditions in wheat genotypes

Genotypes	Shoot length (cm)		Root length (cm)		Root weight (mg)		Relative water content %		Membrane leakage %		Chlorophyll content index (CCI)		Chlorophyll fluorescence (Fv/Fm)		Osmotic potential (-MPa)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
WB2	25.7 ± 0.70 ^a	21.6 ± 0.66 ^a	18.8 ± 1.65 ^a	11.4 ± 0.26 ^a	300 ± 19 ^a	209 ± 20 ^a	94.4 ± 2.32 ^a	62.2 ± 1.34 ^a	37.1 ± 2.5 ^a	43.4 ± 3.9 ^a	28.4 ± 0.61 ^a	24.9 ± 1.29 ^a	0.78 ± 0.01 ^a	0.29 ± 0.06 ^a	1.0 ± 0.11 ^a	1.3 ± 0.05 ^a
PBW550	24.5 ± 0.72 ^a	23.0 ± 0.56 ^b	15.0 ± 0.41 ^a	13.1 ± 0.64 ^{ab}	271 ± 18 ^{ab}	213 ± 12 ^b	90.5 ± 1.61 ^a	73.0 ± 1.59 ^b	39.9 ± 3.8 ^a	57.4 ± 2.6 ^{ab}	32.9 ± 0.61 ^b	30.0 ± 0.25 ^a	0.79 ± 0.05 ^a	0.65 ± 0.05 ^{ab}	0.9 ± 0.12 ^a	1.0 ± 0.12 ^b
DBW17	25.0 ± 0.53 ^b	21.2 ± 0.87 ^b	14.5 ± 0.56 ^b	12.3 ± 0.73 ^{ab}	225 ± 16 ^{bc}	198 ± 22 ^{abc}	87.9 ± 0.49 ^b	60.1 ± 0.96 ^c	36.4 ± 2.9 ^a	46.9 ± 1.6 ^{ab}	30.9 ± 0.26 ^c	29.9 ± 0.31 ^a	0.76 ± 0.04 ^a	0.25 ± 0.04 ^{ab}	0.8 ± 0.07 ^a	0.9 ± 0.09 ^b
DPW621-50	22.5 ± 1.56 ^{bc}	20.9 ± 0.62 ^c	18.0 ± 0.71 ^{bc}	12.3 ± 0.82 ^{bc}	278 ± 8 ^d	189 ± 16 ^{bc}	93.9 ± 0.45 ^b	65.8 ± 1.50 ^d	30.3 ± 2.6 ^a	58.0 ± 2.4 ^b	36.5 ± 0.8 ^{abc}	29.5 ± 0.74 ^a	0.78 ± 0.06 ^a	0.15 ± 0.11 ^{abc}	0.9 ± 0.10 ^a	1.1 ± 0.04 ^b
HD3086	23.7 ± 0.62 ^{cd}	20.5 ± 0.35 ^{cd}	13.5 ± 1.27 ^{bc}	9.5 ± 0.65 ^{bcd}	243 ± 5 ^{de}	171 ± 7 ^{cd}	86.5 ± 0.90 ^b	47.1 ± 2.57 ^e	20.3 ± 1.0 ^b	49.5 ± 2.3 ^b	29.7 ± 1.36 ^c	24.0 ± 0.44 ^b	0.78 ± 0.03 ^a	0.32 ± 0.03 ^{abc}	0.9 ± 0.06 ^a	1.1 ± 0.09 ^b
HD2967	28.2 ± 1.32 ^{cd}	26.2 ± 0.70 ^{de}	11.9 ± 0.78 ^{cd}	9.5 ± 0.62 ^{cd}	236 ± 7 ^{cd}	201 ± 5 ^{cd}	96.4 ± 2.23 ^{bc}	62.5 ± 2.40 ^e	27.8 ± 3.7 ^b	55.2 ± 1.8 ^c	23.8 ± 0.42 ^c	20.8 ± 0.27 ^c	0.76 ± 0.06 ^a	0.43 ± 0.06 ^{bc}	0.9 ± 0.08 ^a	1.2 ± 0.01 ^b
DBW88	22.5 ± 0.17 ^{de}	21.9 ± 0.95 ^{de}	9.2 ± 0.96 ^{de}	8.1 ± 1.17 ^{de}	251 ± 18 ^{ef}	237 ± 12 ^{cd}	86.9 ± 1.32 ^c	83.3 ± 2.81 ^e	41.3 ± 2.0 ^{bc}	54.5 ± 3.6 ^{cd}	30.7 ± 0.71 ^c	27.1 ± 1.03 ^c	0.78 ± 0.05 ^a	0.68 ± 0.07 ^c	1.0 ± 0.23 ^a	1.2 ± 0.06 ^{bc}
RAJ4014	29.1 ± 0.36 ^e	24.6 ± 0.72 ^{de}	15.5 ± 1.09 ^f	10.4 ± 1.42 ^{de}	321 ± 10 ^f	241 ± 16 ^d	94.7 ± 2.43 ^c	49.5 ± 0.62 ^f	24.3 ± 3.5 ^{cd}	40.7 ± 3.4 ^{de}	31.0 ± 0.66 ^d	25.0 ± 0.33 ^c	0.79 ± 0.05 ^a	0.33 ± 0.06 ^c	0.9 ± 0.06 ^a	1.1 ± 0.03 ^{cd}
RAJ3765	26.6 ± 0.36 ^e	24.3 ± 0.79 ^e	11.3 ± 0.77 ^f	11.0 ± 0.83 ^e	198 ± 13 ^g	177 ± 18 ^d	86.0 ± 1.61 ^c	76.4 ± 1.09 ^f	31.1 ± 2.5 ^d	62.1 ± 0.9 ^e	29.9 ± 0.81 ^e	29.3 ± 0.35 ^d	0.78 ± 0.07 ^a	0.70 ± 0.06 ^c	0.9 ± 0.18 ^a	1.1 ± 0.06 ^d
LSD@5%	1.48	1.27	1.76	1.44	23.99	27.45	2.94	2.65	4.66	4.56	1.22	1.00	0.089	0.10	0.22	0.12

*Data is represented as means of replications along with their standard deviations
 Values within a group in a column bearing different letters are significantly different as determined by Tukey's test

where in, higher percent reduction in CCI was observed in RAJ4014 (19.4%), HD3086 (19.1%), and DPW621-50 (19.1%) genotypes compared to RAJ3765 (2.1%), DBW17 (3.1%) and PBW550 (8.7%) under stress compared to control condition. Chlorophyll is one of the major chloroplast components for photosynthesis and relative chlorophyll content has a positive relationship with photosynthetic rate. Maximum reduction in CFL (Fv/Fm) was observed in three genotypes DBW621-50 (30.2%), WB2 (28.5%) and HD3086 (26.6%) whereas minimum reduction was found in DBW88 (8%), PBW550 (10.3%) and RAJ3765 (10.9%) under stress compared to control condition. Osmotic potential increased with stress exposure. The highest Osmotic potential (1.33 MPa) was observed under stress as compared to control (0.77 MPa) and percent increase under stress was higher in WB2 (29.8%), HD2967 (28.5%) and DPW621-50 (27.9%) as compared to control condition (Fig. 2f–h).

Quantification of antioxidant enzymes and proline concentration

Heat stress is associated with an increase in ROS production in different cell compartments and elevated activities of antioxidant enzymes is an adaptation by plants to ameliorate the heat stress-induced oxidative stress. Under stress conditions, ROS homeostasis plays an important role in cellular ionic balance. Activities of APX, CAT, MDA and POX showed a significant increase in all the tested varieties under stress (Table 3 and Fig. 3).

The percent increase in CAT activity under stress was higher in four genotypes DBW88 (27.7%), DPW621-50 (25.9%), RAJ3765 (23.9%) and PBW550 (23.4%) compared to others. APX activity under stress was found to be maximum in four genotypes HD2967 (30.1%), RAJ3765 (27.7%), DBW88 (27.1%) and PBW550 (26.1%) and the activity of POX continued to increase in RAJ3765 (29.8%) and DBW88 (26.2%) but less increase was seen in case of WB2 (9.6%) and RAJ4014 (10.1%) under stress condition. It was noted that the activity of MDA enhanced greatly with stress in DPW621-50 (47.8%) compared to PBW550 (10.2%), RAJ3765 (15.6%) and DBW88 (17.0%). The proline content was increased in RAJ4014 (26.7%), WB2 (23.5%) and HD3086 (20.9%) compared to DBW88 (9.5%) and RAJ3765 (9.8%) under stress condition (Fig. 3a–e).

Classification of varieties as heat tolerant and susceptible

The overall results revealed that various physio-biochemical plant traits i.e., osmotic potential, proline, APX, GPX, CAT, MDA and membrane leakage increased significantly under heat stress compared to control conditions. Whereas,

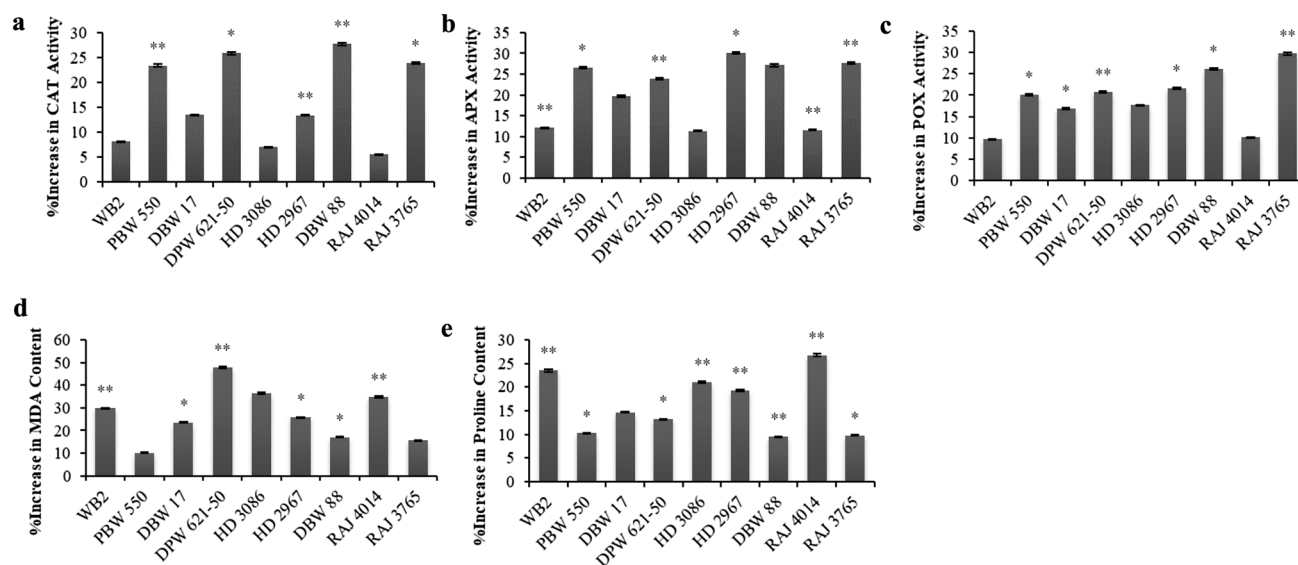


Fig. 3 Percent increase in antioxidant enzyme activities and proline content under heat stress compared to control condition in wheat genotypes. **a** Catalase (CAT), **b** ascorbate peroxidase (APX), **c** guai-

acol peroxidase (POX), **d** lipid peroxidase (MDA), **e** proline content. * $P \leq 0.05$; ** $P \leq 0.01$

RWC, CCI, CFL, shoot length, root length and root weight decreased under heat stress conditions. By considering all these physiological and biochemical traits expression scores, RAJ4014 (score 3), HD3086 (score 6) and WB2 (score 6) were grouped as heat sensitive genotypes. PBW550 (score 31), DBW88 (score 35) and RAJ3765 (score 36) as heat tolerant genotypes and DPW621-50 (score 14), DBW17 (score 17) and HD2967 (score 19) as moderately heat sensitive genotypes (Table 4). Finally, this matrix table explains the cause for tolerance and susceptibility of genotypes in precision based on traits.

Discussion

Among the different abiotic stresses in NWPZ, heat stress causes huge damage to wheat production at all growth stages. Thus, breeding for heat stress tolerance and identifying the climate resilient wheat varieties is the main thrust area of research in NWPZ. Heat stress changes different physiological, morphological and biochemical processes in wheat. The present study is to evaluate the timely sown NWPZ wheat varieties (WB2, DPW621-50, PBW550, DBW17, HD3086, HD2967, DBW88) along with two check varieties (RAJ4014 and RAJ3765) under heat stress condition at seedling stage. All studied physiological and biochemical traits (RWC, OP, CCI, CFL, Membrane leakage, APX, POX, CAT, MDA, proline) showed significant variations in traits between tolerant and susceptible groups among the tested varieties as indicated by tukey's test and it also confirmed the proper induction of heat stress for classifying the varieties.

Heat response in plants is the cumulative outcome of various morpho-physiological and biochemical variations triggered at seedling stage. In present study, heat stress hindered the seedling growth corroborating the results reported in early- stage heat induced reduction in shoot length, root length, root weight, CCI, CFL, Membrane leakage, RWC and increase in OP. Growth depends upon the maintenance of turgor pressure inside the cell. During HS, plant is not able to maintain its water potential and thus growth is hindered by affecting various morphological traits. Shoot length, root weight and root length were found to be correlated with heat tolerance and are documented in different studies (Hasanuzzaman et al. 2013; Xin et al 2019; Poudel and Poudel 2020). Thus, the decrease in wheat seedling biomass could be associated with the reduction in shoot length, root length and root weight caused by thermal stress. Many researchers reported that germination time as well as seedling growth gradually decrease under short duration heat stress which shows involvement of multiple factors at the time of germination and at seedling stage (Sharma et al. 2021; Kumar et al. 2020; Gupta et al. 2013). Our results were consistent with earlier studies in maize and rice (Jagadish et al. 2021).

RWC of plants decreases under heat stress conditions (Hasanuzzaman et al. 2013; Sharma et al. 2015; Sattar et al. 2020) and it is closely related with heat tolerance. Previous studies have also suggested that the ability of plants to maintain higher RWC is one of the mechanisms of heat tolerance (Hasanuzzaman et al. 2013; Iqbal et al. 2017; Pandey et al. 2019). The decrease in RWC could be attributed to the decrease in the biomass and growth of the roots under heat stress, which eventually restricts the supply of water and

Table 3 Variation in biochemical traits and proline concentration under control and heat stress conditions in wheat genotypes

Genotypes	Catalase (U/mg protein/min)		Ascorbate Peroxidase (U/mg protein/min)		Guaiacol Peroxidase (U/mg protein/min)		Lipid Peroxidation (U/min/gm FW)		Proline (µmol/gm FW)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
WB2	2.34 ± 0.22 ^a	2.53 ± 0.43 ^a	7.15 ± 0.40 ^a	8.01 ± 0.41 ^a	3.00 ± 0.34 ^a	3.29 ± 0.39 ^a	5.48 ± 0.22 ^a	7.11 ± 0.07 ^a	15.75 ± 0.42 ^a	19.45 ± 0.63 ^a
PBW550	5.79 ± 0.13 ^b	7.14 ± 0.13 ^b	6.93 ± 0.24 ^{ab}	8.77 ± 0.57 ^{ab}	3.52 ± 0.39 ^a	4.23 ± 0.47 ^a	7.68 ± 0.34 ^b	8.46 ± 0.30 ^b	16.84 ± 0.21 ^a	18.57 ± 0.26 ^{ab}
DBW17	4.31 ± 0.35 ^b	4.89 ± 0.14 ^b	6.32 ± 0.82 ^{abc}	7.56 ± 0.25 ^{bc}	5.18 ± 0.24 ^a	6.05 ± 0.28 ^a	6.19 ± 0.48 ^{bc}	7.65 ± 0.23 ^{bc}	16.37 ± 0.48 ^{ab}	18.77 ± 0.69 ^{bc}
DPW621-50	7.46 ± 0.20 ^c	9.39 ± 0.24 ^c	7.62 ± 0.44 ^{bcd}	9.44 ± 0.52 ^c	7.35 ± 0.19 ^b	8.87 ± 0.41 ^b	6.86 ± 0.17 ^{bc}	10.14 ± 0.51 ^{bc}	16.65 ± 0.42 ^{abc}	18.85 ± 0.28 ^{cd}
HD3086	4.31 ± 0.42 ^c	4.61 ± 0.37 ^c	5.13 ± 0.09 ^{cd}	5.71 ± 0.20 ^c	3.95 ± 0.24 ^c	4.65 ± 0.20 ^c	5.40 ± 0.46 ^c	7.36 ± 0.40 ^{cd}	15.46 ± 0.56 ^{abc}	18.70 ± 0.28 ^d
HD2967	6.34 ± 0.16 ^d	7.19 ± 0.25 ^c	4.88 ± 0.55 ^{de}	6.34 ± 0.74 ^c	7.76 ± 0.07 ^d	9.43 ± 0.60 ^d	6.67 ± 0.20 ^c	8.39 ± 0.24 ^{de}	16.77 ± 0.59 ^{abc}	20.00 ± 0.26 ^{de}
DBW88	8.12 ± 0.24 ^e	10.37 ± 0.30 ^d	6.16 ± 0.33 ^{ef}	7.84 ± 0.56 ^d	7.48 ± 0.12 ^d	9.44 ± 0.43 ^d	6.69 ± 0.34 ^d	7.83 ± 0.18 ^{de}	16.45 ± 0.38 ^{bcd}	18.01 ± 0.19 ^{def}
RAJ4014	6.41 ± 0.24 ^e	6.76 ± 0.27 ^d	4.91 ± 0.26 ^f	5.48 ± 0.23 ^{de}	3.64 ± 0.46 ^d	4.01 ± 0.25 ^d	6.13 ± 0.34 ^d	8.27 ± 0.46 ^e	15.90 ± 0.21 ^{cd}	20.15 ± 0.28 ^{ef}
RAJ3765	7.63 ± 0.19 ^f	9.45 ± 0.39 ^e	5.83 ± 0.66 ^f	7.44 ± 0.65 ^e	5.88 ± 0.20 ^e	7.63 ± 0.29 ^e	4.74 ± 0.27 ^e	5.48 ± 0.27 ^f	16.46 ± 0.37 ^d	18.07 ± 0.61 ^f
LSD@5%	0.45	0.49	0.81	0.79	0.44	0.64	0.53	0.57	0.77	0.62

U/mg protein/min, unit/milligram protein/minutes; U/min/gm FW, unit/minutes/gram fresh weight; µmol/gm FW, micromole/grams fresh weight

*Data is represented as means of replications along with their standard deviations

Values within a group in a column bearing different letters are significantly different as determined by Tukey's test

nutrients to the shoot of the plants. RWC is directly linked with water stress of cells and can be associated with yield and stress tolerance.

Cell membrane is sensitive to different abiotic stresses including heat stress. With increasing heat stress, electrolyte leakage increases and lipid peroxidation starts. The membrane leakage of most of the genotypes was significantly affected by thermal stress, which may reveal the heat sensitivity of genotypes. Poudel and Poudel (2020) reported that stable cell membrane system which remains functional during heat stress apparently controls the adaptation to high temperatures. Our results were in conformance with the findings of Khan et al. (2013), who reported increase in membrane leakage under heat stress. Increase in membrane leakage reflects the level of lipid peroxidation caused due to active oxygen species. Lower membrane leakage of the genotypes RAJ3765, PBW550 and DBW88 might be due to their heat tolerant nature due to stronger membrane integrity as compared to sensitive HD3086 and RAJ4014.

Exposure to heat stress results in reduction in chlorophyll synthesis and increase in its loss. The chlorophyll pigments are crucial for harvesting light. In chloroplast, thylakoid is known as the most heat labile cell structure that harbors the chlorophyll (Qaseem et al. 2020; Mamrutha et al. 2020b) and any damage to thylakoid from heat will result in chlorophyll loss. Several studies indicated the presence of genetic variability for chlorophyll retention under heat stress as the rate of decrease in chlorophyll content varied across wheat genotypes under heat stress (Kumari et al. 2013). Many studies have also reported that heat tolerant wheat genotypes show lower reduction in chlorophyll content under heat stress condition (Pandey et al. 2015; Sharma et al. 2015; Kumar et al. 2018). In present study, variation in percent reduction of chlorophyll indicates that the genotypes RAJ3765 and DBW17 were able to retain more chlorophyll under heat stress which will also contribute to stay green trait of variety in later stages.

Chlorophyll fluorescence reveals the efficacy of photosystem II (PSII) and subsequently the photosynthetic efficiency. In the previous studies, genotypes having higher CFL were also found to have higher yield which indicates that CFL can be used to screen for heat tolerant genotypes (Kumar et al. 2012; Hasanuzzaman et al. 2013; Pandey et al. 2015). Total 1274 contrasting wheat varieties of diverse origin were screened on the basis of maximum quantum efficiency of PS-II (F_v/F_m) and physiological traits (Sharma et al. 2018, 2019). In the present study, PBW550, DBW88 and RAJ3765 found having high PS-II efficacy while DPW621-50, DBW17 and WB2 were least efficient under the heat stress conditions. Kumar et al. (2020) also observed that RAJ3765 has more efficient PS-II as compared to RAJ4014 under heat stress. Osmotic potential is a decisive trait of plant physiology, by which they respond to thermal stress.

Table 4 Heat tolerance matrix of wheat genotypes based on different morphological, physiological and biochemical traits

Genotypes	Shoot length	Root Length	Root weight	Relative water content (RWC%)	Membrane Leakage %	Chlorophyll Content Index (CCI)	Chlorophyll fluorescence (CFL)	Osmotic Potential	Catalase (CAT)	Ascorbate Peroxidase (APX)	Cuaiacal Peroxidase (POX)	Lipid Peroxidation (MDA)	Proline Content	Tolerance score of the genotype based on traits
WB2	S	S	S	MS	MS	MS	S	S	MS	S	S	MT	S	6
PBW550	MT	MT	MS	MT	T	MT	T	T	T	T	MT	T	MT	31
DBW17	S	MT	MT	MS	MS	T	S	MT	MS	MS	MS	MT	MS	17
DPW621-50	MT	S	S	MS	MT	S	S	S	T	MT	MT	S	MT	14
HD3086	MS	MS	S	S	S	S	MS	MS	S	S	MS	MS	S	6
HD2967	MT	MS	MT	MS	MT	MS	MS	S	MS	T	MT	MT	MS	19
DBW88	T	MT	T	T	T	MS	T	MT	T	T	T	T	T	35
RAJ4014	S	S	S	S	S	S	MS	MS	S	S	S	MS	S	3
RAJ3765	MT	T	MT	T	T	T	T	MT	T	T	T	T	T	36

Full form	Denotation	Tolerance score
Sensitive	S	0
Moderately Sensitive	MS	1
Moderately Tolerant	MT	2
Tolerant	T	3

Osmotic potential helps plants to maintain turgor pressure and cell volume in stress conditions, to maintain their metabolic functions, as well as assisting in the recovery of metabolic activities after relief from heat stress. Osmotic potential significantly increased in all genotypes with stress exposure. Osmotic potential was expressed more in drought susceptible genotypes that maintained lower shoot water potential as compared to tolerant genotypes maintaining higher shoot water potential under stress conditions. Heat-susceptible genotypes WB2, DPW621-50 and HD2967 that maintained lower shoot water potential had higher OP values, as compared to tolerant genotypes maintaining higher shoot water potential under stress conditions.

Different abiotic stresses affect plant process and results in loss of cellular homeostasis along with formation of ROS that cause oxidative damage to membranes, lipids, proteins and nucleic acids. In normal conditions, the oxidative damage to cellular components is less due to effective processing of ROS by a rapid antioxidant system which comprised of several enzymes and redox metabolites. However, under environmental stress, production of ROS exceeds the antioxidative capacity resulting in cellular damage. Heat stress tolerance in crop plants is found to be linked with increase in antioxidant enzyme activity. Different stresses cause different response on enzymatic activities. The activities of CAT, POX and APX increased under heat stress in genotypes like DBW88 and PBW550. The generation of destructive ROS such as singlet oxygen (O^-), hydroxyl radical (OH^-) and Hydrogen peroxide (H_2O_2) is the cause of increased enzyme activities (Caverzan et al. 2016; Kumar et al. 2018). Production of ROS often induces the production of abscisic acid that acts as signal molecule under stressed conditions and regulates the gene expression that control the production

of enzymatic antioxidants such as CAT. The activities of CAT, POX and APX were enhanced in variable magnitude under heat stress conditions (Kumar et al. 2013; Puthur 2016), these findings are concurrent with our study. Variable increase in the activities of these antioxidants in wheat genotypes indicates their differential ability to adapt to heat stress conditions (Sattar et al. 2020). APX activity under heat stress was more discernible in the leaves of heat tolerant genotypes than in sensitive ones. Many researchers have also reported upregulation of APX and POX activities in leaves of heat tolerant genotypes under heat stress. Heat tolerance of varieties were found to be directly linked with percent increase in the activities of CAT, APX and POX in varieties DBW88 and PBW550.

Lipid peroxidation in cell membranes is found to be one of the most challenging and detrimental effects of heat stress in the membranes of all the cells and the degree of lipid peroxidation measured in terms of MDA content is one of the determining factors in revealing the severity of stress experienced by any plant (Khan et al. 2017; Kumari et al. 2020). Kaur et al. (2018) reported increase in MDA content with increase in the degree of heat stress in wheat. Turkan et al. (2005) observed lower MDA content in the leaves of heat tolerant genotypes as compared to heat sensitive genotypes. The accumulation of MDA content was higher in DPW621-50 as compared to PBW550, DBW88 and RAJ3765 genotypes. Sairam and Srivastava (2001) reported lower lipid peroxidation levels in heat tolerant genotypes than heat sensitive genotypes which is in conformity with the present findings.

Proline serves as a membrane protectant and due to its zwitter ion character it accumulates in higher concentration in cell cytoplasm under stress condition without

interfering with the cellular structure or metabolism. Kaur et al. (2018) suggested that proline accumulation is a wide spread plant response to environmental stresses including heat. The present findings are also in agreement with Khan et al. (2015) who reported that heat stress conditions increased proline contents in wheat crop. In present study, heat tolerant genotypes DBW88 and RAJ3765 exhibited less increase in proline as compared to heat sensitive genotypes which is in accordance with Janda et al. (2019) who found higher amount of proline in heat sensitive genotypes. The overall physio-biochemical studies of NWPZ wheat varieties helped in categorizing the genotypes as tolerant and susceptible under heat stress conditions. The study also supports trait-based selection of genotypes for further studies and also unveils timely sown varieties for their best utilization in the context of climate change in wheat bowl of India.

Conclusions

In this study, a novel tolerance matrix was prepared based on the stress response of the varieties for each trait and a final tolerance score were calculated for each genotype. On the basis of relative variations in tolerance scores, DBW88, RAJ3765 and PBW550 were identified as heat tolerant, RAJ4014, HD3086 and WB2 as heat susceptible and DPW621-50, DBW17 and HD2967 as moderately sensitive to heat stress. The study also reconfirmed the use of studied physiological and biochemical traits for potential screening of heat tolerant genotypes which can be further used for trait-based breeding programs and also for identifying novel genes for transgenic and genome editing in wheat. This study assists in wise selection of wheat varieties for different anticipated heat stress areas of NWPZ by considering weather forecast, so that a varietal mosaic can be created for minimizing yield loss under climate change to meet the growing wheat demand.

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Author contributions MHM conceived and designed the research with AP. AP conducted experiments. AP, RK and SMA collected the experimental data. AP, RK, GCP and MHM wrote the manuscript. MHM, GS and GPS edited the manuscript. All authors read and approved the manuscript for publication.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval The present research study does not involve any human participants, their data, or biological material.

References

- Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105:121–126. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
- Akter N, Islam M (2017) Heat stress effects and management in wheat: a review. *Agron Sustain Dev* 37:37. <https://doi.org/10.1007/s13593-017-0443-9>
- Asthir B, Bala S, Bains NS (2014) Effect of terminal heat stress on yield and yield attributes of wheat. *Indian J Appl Res* 4:1–2. <https://doi.org/10.15373/2249555X/June2014/1>
- Barrs H, Weatherley P (1962) A Re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust J Biol Sci* 15:413–428. <https://doi.org/10.1071/BI9620413>
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39:205–207. <https://doi.org/10.1007/BF00018060>
- Blum A, Zhang JX, Nguyen HT (1999) Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. *Field Crop Res* 64:287–291. [https://doi.org/10.1016/S0378-4290\(99\)00064-7](https://doi.org/10.1016/S0378-4290(99)00064-7)
- Carillo P, Mastrolonardo G, Nacca F, Parisi D, Verlotta A, Fuggi A (2008) Nitrogen metabolism in durum wheat under salinity: accumulation of proline and glycine betaine. *Funct Plant Biol* 35:412–426. <https://doi.org/10.1071/FP08108>
- Caverzan A, Casassola A, Brammer SP (2016) Antioxidant responses of wheat plants under stress. *Genet Mol Biol* 39:1–6. <https://doi.org/10.1590/1678-4685-GMB-2015-0109>
- Chauhan BS, Singh B, Kaur P, Mahajan G, Randhawa RK, Singh H, Kang MS (2014) Global warming and its possible impact on agriculture in India. *Adv Agron* 123:65–121. <https://doi.org/10.1016/B978-0-12-420225-2.00002-9>
- Daloza AS, Rydsaa JH, Hodnebrog O, Sillmann J, van Oorta B, Mohr CW, Agrawal M, Emberson L, Stordal F, Zhang T (2021) Direct and indirect impacts of climate change on wheat yield in the Indo-Gangetic plain in India. *J Agri Food Res* 4:100132. <https://doi.org/10.1016/j.jafr.2021.100132>
- Dhindsa RS, Plumb-Dhindsa P, Thorpe 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. <https://doi.org/10.1093/jxb/32.1.93>
- Dhyani K, Ansari MW, Rao YR, Verma RS, Shukla A, Tuteja N (2013) Comparative physiological response of wheat genotypes under terminal heat stress. *Plant Signal Behav* 8:1–6. <https://doi.org/10.4161/psb.24564>
- Dillard HR (2019) Global food and nutrition security: from challenges to solutions. *Food Secur* 11:249–252. <https://doi.org/10.1007/S12571-019-00893-3>
- Ding S, Ali EF, Elmahdy AM, Ragab KE, Seleiman MF, Kheir AMS (2021) Modeling the combined impacts of deficit irrigation, rising temperature and compost application on wheat yield and water productivity. *Agric Water Manag*. <https://doi.org/10.1016/j.agwat.2020.106626>
- Gupta NK, Agarwal S, Agarwal VP, Nathawat NS, Gupta S, Singh G (2013) Effect of short-term heat stress on growth, physiology and antioxidative defence system in wheat seedlings. *Acta Physiol Plant* 35:1837–1842. <https://doi.org/10.1007/s11738-013-1221-1>
- Gupta R, Somanathan E, Dey S (2016) Global warming and local air pollution have reduced wheat yields in India. *Clim Change* 140:593–604. <https://doi.org/10.1007/s10584-016-1878-8>

- Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int J Mol Sci* 14:9643–9684. <https://doi.org/10.3390/IJMS14059643>
- Hossain A, Sarker MAZ, Saifuzzaman M, da Silva JAT, Lozovskaya MV, Akhter MM (2013) Evaluation of growth, yield, relative performance and heat susceptibility of eight wheat (*Triticum aestivum* L.) genotypes grown under heat stress. *Int J Plant Prod* 7:615–636
- ICAR-IIWBR (2021) Director's reports of AICRP on Wheat and Barley 2020–21. In: Singh GP (ed) AICRP on wheat and barley progress report 2020–21 crop improvement. ICAR—Indian Institute of Wheat and Barley Research, Karnal, Haryana, India, p 76
- Iqbal M, Raja NI, Yasmeen F, Hussain M, Ejaz M, Shah MA (2017) Impacts of heat stress on wheat: a critical review. *Adv Crop Sci Technol* 5:1–9. <https://doi.org/10.4172/2329-8863.1000251>
- Islam AU, Chhabra AK, Dhanda SS, Peerzada OH (2017) Genetic diversity, heritability and correlation studies for yield and its components in bread wheat under heat stress conditions. *IOSR J Agric Vet Sci* 5:71–77. <https://doi.org/10.9790/2380-1005017177>
- Jagadish SK, Way DA, Sharkey TD (2021) Plant heat stress: concepts directing future research. *Plant Cell Environ* 44:1992–2005. <https://doi.org/10.1111/pce.14050>
- Janda T, Khalil R, Tajti J, Pál M, Darkó E (2019) Responses of young wheat plants to moderate heat stress. *Acta Physiol Plant* 41:137. <https://doi.org/10.1007/s11738-019-2930-x>
- Jebara S, Jebara M, Limam F, Aouani ME (2005) Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. *J Plant Physiol* 162:929–936. <https://doi.org/10.1016/j.jpiph.2004.10.005>
- Kaur R, Bedi S, Kaur P, Asthir B (2018) Biochemical evaluation in wheat (*Triticum aestivum* L.) under heat stress conditions. *Ind J Agric Biochem* 31:111–115. <https://doi.org/10.5958/0974-4479.2018.00019.9>
- Khan M, Iqbal N, Masood A, Per T, Khan N (2013) Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signal Behav* 8:11. <https://doi.org/10.4161/psb.26374>
- Khan SU, Din JU, Qayyum A, Jan NE, Jenks MA (2015) Heat tolerance indicators in Pakistani wheat (*Triticum aestivum* L.) genotypes. *Acta Bot Croat* 74:109–121. <https://doi.org/10.1515/botcro-2015-0002>
- 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.). *IJB* 11:173–183. <https://doi.org/10.12692/ijb/11.4.173-183>
- Kumar R, Goswami S, Sharma S, Singh K, Gadpayle K, Kumar N (2012) Protection against heat stress in wheat involves change in cell membrane stability, antioxidant enzymes, osmolyte, H₂O₂ and transcript of heat shock protein. *Int J Plant Physiol Biochem* 4:83–91. <https://doi.org/10.5897/IJPPB12.008>
- Kumar S, Singh R, Nayyar H (2013) α -Tocopherol application modulates the response of wheat (*Triticum aestivum* L.) seedlings to elevated temperatures by mitigation of stress injury and enhancement of antioxidants. *J Plant Growth Regul* 32:307–314. <https://doi.org/10.1007/s00344-012-9299-z>
- Kumar SN, Aggarwal PK, Rani DNS, Saxena R, Chauhan N, Jain S (2014) Vulnerability of wheat production to climate change in India. *Clim Res* 59:173–187. <https://doi.org/10.3354/cr01212>
- Kumar R, Kaur A, Mamrutha HM, Grewal A (2017) Synergistic effect of cefotaxime and timentin to suppress the *Agrobacterium* overgrowth in wheat (*Triticum aestivum* L.) transformation. *Asian J Microbiol Biotechnol Environ Sci* 19(4):961–967
- Kumar A, Sharma S, Giri K, Goswami A, Chaudhary B, Sengar RS (2018) Morpho-physiological and biochemical characteristics of wheat (*Triticum aestivum* L.) Varieties under heat stress condition. *Progress Agric* 18:195–200. <https://doi.org/10.5958/0976-4615.2018.00044.3>
- Kumar R, Masthigowda MH, Kaur A, Bhusal N, Pandey A, Kumar S, Mishra C, Singh G, Singh GP (2020) Identification and characterization of multiple abiotic stress tolerance genes in wheat. *Mol Biol Rep* 47:8629–8643. <https://doi.org/10.1007/s11033-020-05906-5>
- Kumari M, Pudake RN, Singh VP, Joshi AK (2013) Association of stay green trait with canopy temperature depression and yield traits under terminal heat stress in wheat (*Triticum aestivum* L.). *Euphytica* 190:87–97. <https://doi.org/10.1007/s10681-012-0780-3>
- 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 Appl Sci Technol* 39:19–29. <https://doi.org/10.9734/cjast/2020/v39i1930786>
- Lamaoui M, Jemo M, Datla R, Bekkaoui F (2018) Heat and drought stresses in crops and approaches for their mitigation. *Front Chem* 6:26–30. <https://doi.org/10.3389/fchem.2018.00026>
- Maia J, Voigt E, Macêdo C, Ferreira-Silva S, Silveira J (2010) Salt-induced changes in antioxidative enzyme activities in root tissues do not account for the differential salt tolerance of two cowpea cultivars. *Braz J Plant Physiol* 22:113–122. <https://doi.org/10.1590/S1677-04202010000200005>
- Malhi GS, Kaur M, Kaushik P (2021) Impact of climate change on agriculture and its mitigation strategies: a review. *Sustainability* 13:1318. <https://doi.org/10.3390/su13031318>
- Mamrutha HM, Khobra R, Sendhila R, Munjal R, Sai Prasad SV, Bira-dard S, Mavi GS, Dhar T, Bahadur R, Bhagwan JH, Prakash S, Singh H, Shukla RS, Srivastava M, Singh C, Gosavi AB, Salunke VD, Dhyani VC, Singh GP (2020a) Developing stress intensity index and prioritizing hotspot locations for screening wheat genotypes under climate change scenario. *Ecol Ind* 118:106714. <https://doi.org/10.1016/j.ecolind.2020.106714>
- Mamrutha HM, Rinki K, Venkatesh K, Gopalareddy K, Khan H, Mishra CN, Kumar S, Kumar Y, Singh G, Singh GP (2020b) Impact of high night temperature stress on different growth stages of wheat. *Plant Physiol Rep* 25:707–715. <https://doi.org/10.1007/s40502-020-00558-w>
- Mohan D, Singh SS, Gupta RK (2011) Vibrancy of the Indian wheat in upholding yield and quality under global environmental change. In: Wheat: productivity enhancement under changing climate. New Delhi, pp 87–94
- Mohan D, Mamrutha HM, Tyagi BS (2017) Weather conditions favouring wheat (*Triticum aestivum* L.) productivity in hot climate of central India and congenial environment of north-western plains. *Indian J Agric Sci* 87:278–281
- Mukherjee A, Simon Wang SY, Promchote P (2019) Examination of the climate factors that reduced wheat yield in northwest India during the 2000s. *Water* 11:43. <https://doi.org/10.3390/w11020343>
- Pachauri RK, Meyer LA (2014) IPCC, 2014: climate change 2014: synthesis report. Contribution of working groups I, II and III... I Tribal Climate Change Guide. 10013/epic.45156.d001
- Pandey GC, Mamrutha HM, Tiwari R, Sareen S, Bhatia S, Siwach P, Tiwari V, Sharma I (2015) Physiological traits associated with heat tolerance in bread wheat (*Triticum aestivum* L.). *Physiol Mol Biol Plants* 21:93–99. <https://doi.org/10.1007/s12298-014-0267-x>
- Pandey GC, Mehta G, Sharma P, Sharma V (2019) Terminal heat tolerance in wheat: an overview. *J Cereal Res* 11:1–16. <https://doi.org/10.25174/2249-4065/2019/79252>
- Poudel PB, Poudel MR (2020) Heat stress effects and tolerance in wheat: a review. *J Biol Today's World* 9:217
- Puthur JT (2016) Antioxidants and cellular antioxidation mechanism in plants. *South Indian J Biol Sci* 2:14–17

- Qaseem MF, Qureshi R, Shaheen H (2020) 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. *Sci Rep* 9:6955. <https://doi.org/10.1038/s41598-019-43477-z>
- Ramadas S, Kiran Kumar TM, Pratap Singh G (2020) Wheat production in India: trends and prospects. In: Shah F, Khan Z, Iqbal A, Turan M, Olgun M (eds) Recent advances in grain crops research. IntechOpen, London. <https://doi.org/10.5772/INTECHOPEN.86341>
- Rinki MHM, Kumar R, Tiwari V (2016) Comparison of seedling and adult stage heat stress tolerance in wheat. *Wheat Barley News Lett* 10:9
- Sairam RK, Srivastava GC (2001) Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. *J Agron Crop Sci* 186:63–70. <https://doi.org/10.1046/J.1439-037X.2001.00461.X>
- Sattar A, Sher A, Ijaz M, Ul-Allah S, Rizwan MS, Hussain M et al (2020) Terminal drought and heat stress alter physiological and biochemical attributes in flag leaf of bread wheat. *PLoS ONE* 15:e0232974. <https://doi.org/10.1371/journal.pone.0232974>
- Sharma DK, Andersen SB, Ottosen CO, Rosenqvist EW (2015) Heat cultivars selected for high Fv/Fm under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. *Physiol Plant* 153:284–298. <https://doi.org/10.1111/pp1.12245>
- Sharma D, Tiwari R, Gupta VK, Rane J, Singh R (2018) Genotype and ambient temperature during growth can determine the quality of starch from wheat. *J Cereal Sci* 79:240–246. <https://doi.org/10.1016/j.jcs.2017.11.006>
- Sharma D, Singh R, Tiwari R, Kumar R, Gupta V (2019) Wheat responses and tolerance to terminal heat stress: a review. In: *Wheat production in changing environments*, pp 149–173. https://doi.org/10.1007/978-981-13-6883-7_7
- Sharma P, Singh R, Dahiya M, Kumar V, Kumar A, Sharma AK (2021) Screening of heat stress tolerant in early stage of wheat seedling using morphological parameters. *Asian J Biol Sci* 10:667. <https://doi.org/10.5530/ajbls.2021.10.89>
- Sonkar G, Mall R, Banerjee T, Singh N, Kumar T, Chand R (2019) Vulnerability of Indian wheat against rising temperature and aerosols. *Environ Pollut* 254:112946. <https://doi.org/10.1016/j.envpol.2019.07.114>
- Turkan I, Bor M, Ozdemir F, Koca H (2005) Differential response of lipid peroxidation and antioxidants in the leaves of drought tolerant *P. Acutifolius Gray* and drought-sensitive *P. vulgaris L.* subjected to polyethylene glycol mediated water stress. *Plant Sci* 168:223–231. <https://doi.org/10.1016/j.plantsci.2004.07.032>
- Wang X, Cai J, Liu F, Dai T, Cao W, Wollenweber B, Jiang D (2014) Multiple heat priming enhances thermo-tolerance to a later high temperature stress via improving subcellular antioxidant activities in wheat seedlings. *Plant Physiol Biochem* 74:185–192. <https://doi.org/10.1016/J.PLAPHY.2013.11.014>
- Xin M, Peng H, Ni Z, Yao Y, Hu Z, Sun Q (2019) Wheat responses and tolerance to high temperature. In: *Wheat production in changing environments*, pp 139–147. https://doi.org/10.1007/978-981-13-6883-7_6
- Zampieri M, Ceglari A, Dentener F, Toreti A (2017) Wheat yield loss attributable to heat waves, drought and water excess at the global, national and subnational scales. *Environ Res Lett* 12:064008. <https://doi.org/10.1088/1748-9326/aa723b>
- Zhang J, Kirkham MB (1994) Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant Cell Physiol* 35:785–791. <https://doi.org/10.1093/oxfordjournals.pcp.a078658>

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