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RESEARCH ARTICLE

Transgenerational analyses and transcript memory profiling of *SKP1* gene expression after seed priming with IAA and GA3 shows positive modulation of terminal heat stress tolerance in bread wheat (*Triticum aestivum* L.)

Praful Jaiswal, Anant Narayan Sahi[1] and Sharmistha Barthakur*

Abstract

Obligate exposure to high temperatures during the anthesis of wheat is a reality and one of the major challenges in wheat breeding today. Seed priming is a valuable technology to increase seed vigour and stress resilience. Seed priming of wheat cv. HD2967 was carried out with two plant hormones, auxin (IAA) and gibberellins (GA3) and the primed plants were analyzed in two consecutive seasons in a field at two planting times. The main aim was to determine how these hormones modulate various pre-germinative metabolic pathways, which lead to enhanced stress tolerance and finally affect crop yield after a triggered high-temperature stress at the anthesis stage. At the molecular genetic level, transcriptional memory was analyzed by expression profiling of the *SKP1*(S-phase kinase protein1)gene, an essential component of the ubiquitin-proteasome system that is specifically involved in protein homeostasis and plant hormonal signaling. The results revealed that the grain yield at all sowing times was significantly increased by IAA and GA3 seed priming (50 mg/L) under high ambient temperature. The *SKP1* transcript showed maximum induction in the P1-D, P2-D, and P2-N conditions in the flag leaf and ear head under heat stress. The *SKP1* gene expression showed a higher correlation with chlorophyll content at the post-anthesis stage (S3) by *SKP1*-FL r = .92 and *SKP1*-EH r=0.85 ($p < 0.01$) and a strong positive correlation with S3 r = 0.77and NOGPH r=0.77 ($p < 0.01$). These results document the potential of this technology as an effective approach to cope with severe high-temperature stresses during grain filling in wheat cultivation. To the best of our knowledge, this is one of the first reports of IAA, GA3, in combination with heat priming of seeds and analyses in natural field conditions in wheat.

Keywords: Wheat, seed priming, high temperature, anthesis stage, *SKP1*.

Introduction

Sustainable crop production is drastically being affected by global climate change. Millions of people get their dietary energy and protein from wheat, one of the world's most crucial cereal crops. Wheat-growing regions around the world are experiencing and are expected to withstand more severe heat waves and extreme temperature events in the future. The expected changes in temperature over the next 30 to 50 years are predicted to be in the range of 2 to 3°C as per the Intergovernmental Panel on Climate Change (IPCC, 2023). Many of the wheat-growing areas around the world today suffer from terminal or reproductive stage heat stress during flowering, which results in severely decreased yields.

Plant hormones have vital roles in the regulation of plant growth and development as well as in plant immunity and defense. Under various stress conditions, plant hormones function as stress signaling molecules, response determiners, and modulators, thereby in stress tolerance

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development by influencing/regulating them both directly and indirectly. Auxin (IAA) and Gibberellic acid (GA3) are two important plant hormones that govern processes such as seed germination, leaf expansion, stem elongation, flower and trichome initiation, source-sink interaction, flower, fruit, seed development, etc. (El Karamany et al. 2019; Salehin 2024).

Seed priming technology is increasingly becoming popular as a useful approach to enhance rapid and uniform emergence along with high seedling vigor and enhanced yields in vegetables, floriculture, and field crops. In this technique, regulated hydration followed by a rehydration procedure activates the pre-germination metabolic processes (Damalas et al. 2019; Okello et al. 2022). The fundamental principle of priming is pre-exposure to eliciting factors or specific mild stress at a seed or early growth phase in parent or grandparent generations, which can lead to enhanced adaptation in terms of stress tolerance and expression of favorable traits in the future. Priming-induced stress tolerance has been shown to persist to descendants, improving tolerance to stress reoccurring in the next generation (Rasmann et al. 2011; Staacke et al. 2025). Acquisition and maintenance of stress memory involves stress sensing, signal transduction and gene regulation. However, the mechanisms remain largely unexplored. A few of the studies report epigenetic changes, protein homeostasis and non-coding RNA inheritance, which positively modifies plant metabolism and physiology to reoccurring stress in the same generation or in the next generation (Kambona et al. 2023; Nishio et al. 2024).

The ubiquitin-proteasome system (UPS) is one of the protein turnover-regulating mechanisms in a cell which is also intricately involved with plant hormone signaling and metabolism. The UPS plays a crucial role in nearly every stage of plant growth and development. A network of three enzymes, E1, E2, and E3, work together in a three-step enzymatic cascade during UPS activity to covalently transfer ubiquitin to the target proteins, leading to degradation of the labeled protein by 26S proteasome. UPS-26 proteasome degrades damaged, misfolded, or no longer required proteins within cells through a robust signaling mechanism. The multimeric complex SCF (SKP1/Cullin/F-box) E3 ligase, an essential component of UPS, is intricately involved in response to development, stress and plant hormonal signaling (El Beji et al. 2019). The SKP1 protein has approximately 160 amino acids and is essential for cell-cycle progression, hormone and light signaling, and vegetative and flower development (Parry and Estelle. 2006). The discovery of the F-box protein TIR1, a component of SCF^{TIR1} E3 ligase, led to the first indication that E3-SCFs are involved in auxin signaling, which was later identified as an auxin receptor. Similarly, GA3 is perceived by a protein called gibberellin insensitive dwarf1 (GID1), another F-box protein, through a UPS-mediated signaling mechanism. Thus, both auxin and GA3 are perceived by components of UPS, leading to degradation of their repressors, a well-established signal transduction process today (Woodward and Bartel 2005; Isoda et al. 2021).

With documented regulatory and protective roles of various phytohormones in imparting plant robustness under stress we aimed to elucidate the role of auxin and gibberellin under terminal heat stress in wheat. We hypothesized that seed priming with IAA and GA3 prior to heat exposure at the most vulnerable anthesis stage and further evaluation of primed plants will provide an interesting opportunity to dissect the role of these two plant hormones in terminal heat stress response in wheat. Thus, this study was designed with the objectives to carry out seed priming with IAA and GA3 individually with and without additional heat priming, further exposure of the plants to high temperature and analyze phenotypically *in-situ* and to transcript profile *SKP1*gene expression to determine transcriptional memory and evaluate its role as a molecular hallmark under heat stress.

Materials and methods

Plant material, planting time, seed priming, crop establishment and in situ high-temperature exposure

A popular wheat genotype, cv. HD2967, released by ICAR-Indian Agriculture Research Institute, New Delhi, India, was used to carry out all the experiments. Initially, growth chambers were used to evaluate seedling root and shoot length, germination percentage and vigor index. Subsequently, seeds were sown in the December 2015-2016 crop season (P1-D) in a field-based net house with three biological replicates for each treatment using 10-inch pots with 8.7 kg soil per pot in a random block design. For the next season, 2016-2017, sowing was done in November (P2-N) and December (P2-D) in regular and late sowing conditions. Healthy seeds were surface sterilized with 1% sodium hypochlorite solution, rinsed with sterilized water, and air-dried on filter paper. Fungicide treatment with Bavistin 1% was done for 2 hours, rinsed with sterile distilled water and soaked in hormone solutions of 50 mg/L concentration. About 25 healthy seeds were selected for three replicates of each of the priming events. Seeds were fully immersed in the priming medium and incubated for 24 hours at 22 ± 2°C under dark conditions. All seeds were removed from the priming solution at the same time, given three surface washings with distilled water, and dried thoroughly on filter paper to a pre-primed state. One set of seeds was given heat priming at 42°C for 2 hours. Water soaking was used as a non-primed control.

Nearing the anthesis stage, the plants were divided into two groups and one group was exposed to heat stress in a heat trap chamber indigenously built and the other group was kept in ambient conditions as a heat stress control.

The heat exposure experiments were done in two levels of air temperature (ambient and high temperature) and six treatments: i) without priming (NP) treated as control, ii) no hormone only heat primed (HT), iii) IAA iv) GA3 v) IAA plus heat (IAA+HT) and vi) GA3 plus heat (GA3+HT). The heat stress treatment was given at anthesis (Zadoks growth stage 59-60; i.e., the beginning of pollination) on 8-11th March 2016 for three consecutive days, at peak heat time of the day from 11:00 am to 03:00 pm for 4 hours under heat trap chamber in *in-situ* conditions. The heat trap chamber is made up of aluminum and covered with transparent plastic sheets (PVP) with dimensions of front 64×58 and side 62×79 inches and a rooftop open air window (12×12 inches) to maintain ventilation, keeping the same concentrations of carbon dioxide and air humidity on the inside and outside and with light transmittance of 92% from outside (Fig. 1). The chamber maintained temperature 4 to 5°C above the existing ambient temperature (Khomdram and Barthakur 2015).

After the treatments, tissue samples were collected from flag leaf and ear head under both temperature conditions, frozen in liquid nitrogen and stored at -80°C for further RNA isolation and expression profiling. The P1-D seeds were collected at the end of the experiments and planted in the next year 2016-2017 season, in two staggered sowing dates. High-temperature stress was given in March for P2-N plants from 25-27th February and P2-D plants from 23-25th March. Samples were collected for transcript profiling and morpho-physiological observations, and seeds were harvested at maturity at the end of the experiments.

Estimation of leaf chlorophyll content

Fully grown and expanded flag leaves were utilized to measure the chlorophyll content. Five flag leaves were chosen to represent each of the three measuring stages, which were booting, pre-anthesis and post-anthesis at Zadok's wheat growth scale, ZGS 47, 50, 59, respectively (Zadoks et al. 1974) using a SPAD- 502 (Minolta Camera Co., Osaka, Japan) chlorophyll meter.

Estimation of H_2O_2 concentration in primed plants

The DAB technique was used to quantify H_2O_2 (Daudi and O'Brien 2012). The leaf samples were collected, the edges were removed, and the discs were cut into 3cm lengths, dipped in DAB solution, vacuum infiltrated for 20 minutes and incubated at 28°C overnight. The DAB solution was discarded the next day, and a bleaching solution was added. After boiling the solution at 95°C for 15 minutes, the bleaching solution was changed and samples were incubated at room temperature for 20 minutes before taking leaf photographs.

Measurement of grain parameters and yield estimation

At maturity, plants were harvested and separate spikes were collected. Grain yield per pot and thousand-grain

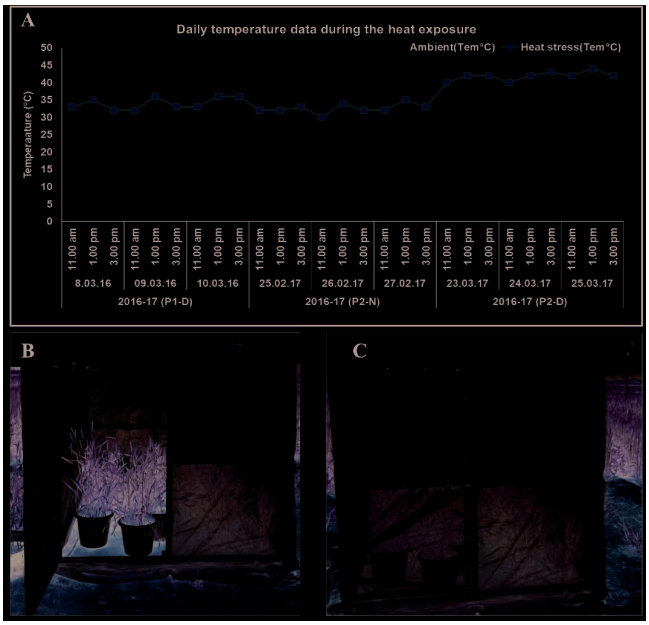


Fig. 1. (A) Daily temperature data recorded during the in situ heat exposure period from 11.00 am to 03.00 pm for 3 consecutive days during the growth period in two years 2015–16 and 2016–17, (B) Plants during exposure to heat stress in heat trap chamber, and (C) Picture of the heat trap chamber used in the experiments

weights were determined along with the number of spikelets per plant (NOS/P), length of ear head (LOEH), and number of grains per spikelet (NOG/S) was recorded after thrashing of the spikes. Grain weight was measured using a sensitive balance and the thousand-grain weight (TGW) was determined by the following formula (Luo et al. 2006).

$$1000 \text{ grain weight} = \frac{\text{Total no. of grain weight per spike}}{\text{Total no. of seed per spike}} \times 100$$

Semi-quantitative reverse transcription PCR (sqRT-PCR) and expression analyses

Total RNA was prepared from flag leaf and ear head using a one-step RNA reagent (Bio-Basic, Canada) followed by DNase I treatment (Promega). First-strand cDNA was synthesized from total RNA using AccuScript High Fidelity 1st strand cDNA synthesis kit (Agilent, USA). The internal standard gene used was 18SrDNA (Accession no.Y357916), with primer sequences (forward-5'TTTGACTCAACACGGGGAAA3' and reverse 5'CAGACAAATCGCTCCACCAA3'). For transcript expression profiling of wheat *SKP1* (Accession number KJ830759), cloned earlier in the lab and identified as *TaSKP1-6B-4* from International wheat genome sequence version 2.1 (https://www.ensemblgenomes.org) was used. The primer sequences (forward-5'TGGCTGCCAACTACCTGAACA3' and reverse 5'ACATGTTCACCGACACCACCT3'). The locus or motif-specific primers were designed in a way that all three homologs of *TaSKP1-6B-4* were amplified. The PCR program followed was preheating at 94°C for 4 minutes

followed by 35 cycles of 94°C for 45 sec-denaturation, 60°C for 35 sec-annealing, 72°C for 1-minute-extension and a final extension at 72°C for 10 min for both the primer sequences. The sqPCR was performed with the Emerald Amp Max PCR Master mix (TaKaRa-Clontech, Japan) using a T-personal Thermocycler system (Biometra, Germany). The results were confirmed using three independent biological replicates and quantification was done with gene tool software version 4.02 from (Syngene, UK).

Statistical analyses

One-way analysis of variance (ANOVA) was used to test the effect of priming treatments with all data presented as mean ±SE of three independent measurements, analyzed using the OPSTAT software package (HAU Haryana, India). Duncan's multiple range test was applied to determine significant differences amongst the treatments ($p < 0.05$) using the latest version of R studio package software (2023).

Results and discussion

Plant hormone seed priming maintained chlorophyll content under ambient and high temperature

Chlorophyll is the most important factor that determines crop yield and productivity. It is also the most sensitive physiological factor and its ratios have often been used as indicators for stress tolerance in plants (Wahid et al. 2007; Wang et al. 2011). We found that the chlorophyll index of the primed plants IAA+ HT and GA3+HT was significantly higher when observed across three crucial stages of reproductive phases exposed or triggered with heat stress at the anthesis stage. Analysis of variance (ANOVA) showed a significant effect of seed priming on chlorophyll content after different treatments. The chlorophyll content was recorded under ambient as well as heat stress conditions at three stages, viz., booting, anthesis and post-anthesis in the three consecutive growth seasons.

In the P1-D season, increase in chlorophyll content under ambient temperature was observed at booting stage for HT, IAA, IAA+HT, GA3 by 12.74, 18.96, 10.15 and 12.04%, respectively as compared to NP, while under heat stress environment, primed plants increased as HT (5.64%), IAA+HT (8.69%) and GA3 (13.94%) respectively over corresponding NP plants. At the anthesis stage, HT, IAA and IAA+HT exhibited significant increases ($p \leq 0.05$) at 11.82, 6.14 and 7.60%, respectively under ambient conditions; while GA3 (33.15%) and GA3+HT (16.65%) showed a rise over NP plants under stress exposed plants again by HT (10.95%), IAA (15.47%) and GA3 (39.09%) over corresponding NP plant. In contrast, at the post-anthesis stage high, temperature-exposed plants showed highly significant retention of green activity in all the priming events over NP plants viz., HT, IAA, IAA+HT, GA3 and GA3+HT by 23.46, 53.14, 50.71, 47.95, and 51.75%, respectively (Fig. 2A).

In the P2-N sown condition at the booting stage, IAA and GA3 treated plants reflected significantly ($p \leq 0.05$) higher chlorophyll values as observed as 10.22 and 14.11% over corresponding NP plants under ambient and also under high-temperature stress conditions. The same trend was followed by IAA (15.53%) and GA3 (13.66%). At the anthesis stage, GA3-treated plants showed higher chlorophyll over NP plants by 16.70%, while under heat stress, the plants treated with IAA (15.01%), IAA+HT (20.27%), GA3 (13.66%) and GA3+HT (12.44%) had significantly ($p \leq 0.05$) higher values over corresponding NP plants. At the post-anthesis stage, again similar trend was observed in HT, IAA, IAA+HT, GA3 and GA3+HT induced plants by 23.36, 23.85, 27.82 and 23.99%, respectively, over corresponding NP plants (Fig.2B).

Furthermore, in P2-D sown condition, the hormone-primed plants with IAA, GA3 and GA3+HT showed enhanced chlorophyll content over corresponding NP by 7.20, 8.34, and 11.84% under ambient while under heat stress, it had higher chlorophyll content than the ambient grown plants, as the treatments with IAA (17.80%), IAA+HT (15.63%), GA3(13.83%) and GA3+HT (18.80%) ($p \leq 0.05$) had an improved value over corresponding NP plants. At the anthesis stage, the plants grown under ambient conditions showed moderate chlorophyll content when compared with NP plants, while the plants exposed to heat stress showed higher chlorophyll content in IAA, IAA+HT, GA3 and GA3+HT by 23.55, 27.30, 18.81, and 18.32% respectively over NP plants. At the post-anthesis stage same pattern was found as P1-D and P2-N conditions. The plants maintained significantly high stay green traits or delayed senescence at HT (15.57%), IAA (22.27%), IAA+HT (19.65%), GA3 (24.75%), and GA3+HT (21.98%)over corresponding NP plants($p \leq 0.05$) (Fig.2C).

The results demonstrate that hormonal and heat priming significantly increased chlorophyll content across all growth stages under both ambient and heat stress conditions. In particular, the post-anthesis stage showed the highest chlorophyll retention, especially in plants treated with HT, IAA, GA3, and their combinations, indicating delayed senescence and improved stress resilience. These results suggest that seed priming with IAA, GA3, and mild heat can enhance chlorophyll content and promote stay-green traits, thus improving the plant's tolerance to high-temperature stress.

Hydrogen peroxide accumulation was reduced in primed plants.

Reactive oxygen species (ROS) produced during heat stress, such as H_2O_2, cause oxidative damage to cells and tissues. ROS can cause lipid peroxidation, protein oxidation and DNA damage, leading to cellular damage and impaired plant growth and development. In our findings, qualitative measurement with DAB staining exhibited a lower level of brown coloration with intact chlorophyll content retained

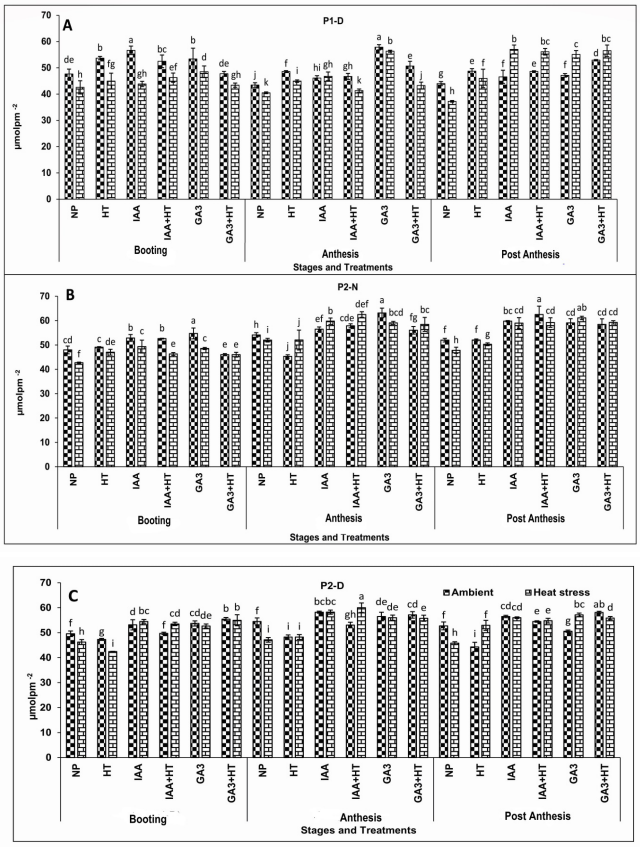


Fig. 2. Effect of hormonal seed priming (P) as well as heat stress at the anthesis stage chlorophyll content in two consecutive years, 2015–16 and 2016–17. In 15–16 (P1-D) seeds were sown in December and in 2016–17 season, regular sowing during November (P2-N) and late sowing during December (P2-D) was done and at the anthesis stage exposed to heat stress. NP- no priming; HT-priming with high temperature; priming with IAA; priming with GA3; priming with IAA+HT; priming with GA3+HT (A) Chlorophyll content 2015–16 (P1-D), (B) Chlorophyll content 2016–17 (P2-N), and (C) Chlorophyll content 2016–17 (P2-D). The data are means ± SE and the lowercase letters refer to significant differences between the treatments at the ($p \leq 0.05$) levels as determined using Duncan's multiple range test

in hormone-primed plants under ambient as well as in high ambient temperature conditions. In the DAB staining, a visible brown precipitate could be seen in the presence of H_2O_2 in NP and HT-primed treated plants under stress conditions.

In P2-N sown ambient plants, no coloration was observed when infiltration was carried out in NP, IAA, and GA3 treated plants, whereas HT, IAA+HT and GA3+HT showed higher H_2O_2 accumulation. In the stress condition, the stain was retained by NP, HT, IAA+HT, and GA3 priming, while less content of H_2O_2 was observed in IAA and GA3+HT plants (Fig. 3A). In P2-D grown plants, most of the treatments exhibited a strong level of retention of the stains, showing the high content of H_2O_2 production by all the treatments except IAA-treated plants. Similarly, this increment was detected in all the treatments under stress conditions (Fig.3B). The result indicates that excessive ROS accumulation was countered in response to heat stress in IAA and GA3-primed plants. Li et al. (2021) found that excessive H_2O_2 accumulation in wheat leaves under heat stress caused oxidative damage and reduced plant growth and yield. However, low levels of H_2O_2 can act as a signaling molecule to trigger defense responses that help the plant cope with heat stress (Wang et al. 2019).

Primed plants showed improved grain yield and yield-related components

High temperature during the reproductive stages in wheat is associated with a reduction in grain yield (Djanaguiraman et al. 2020). Various yield-related parameters were recorded and found to be considerably modulated by various treatments across all the generations. Seed priming enhanced yield and yield-related parameters, including the number of spikes per plant, spike length, number of grains per spike and thousand-grain weight. Spike length (SL) is an important component in determining grain yield and was found to be higher in IAA and GA3 primed plants at timely sown P2-N as well as in P2-D late sown condition.

The results documented showed that in the P1-D season, the number of spikes per plant under ambient conditions significantly increased vide IAA, IAA+HT and GA3+HT seed priming by 50.33, 33.33, and 100%, respectively, whereas, under stress conditions only GA3 treatment, plants showed maximum spike per plant by 83.33% over corresponding NP plants ($p \leq 0.05$) (Fig. 4A). In the P2-N shown conditions, GA3+HT (17.65%), IAA+HT (20%) and GA3 (53.33%) got improved spikes per plant over corresponding NP plants, under ambient and heat stress respectively. In the P2-D sown plants, higher spikelet numbers were observed under both ambient and high-temperature environments. In the case of plants grown under ambient environment,

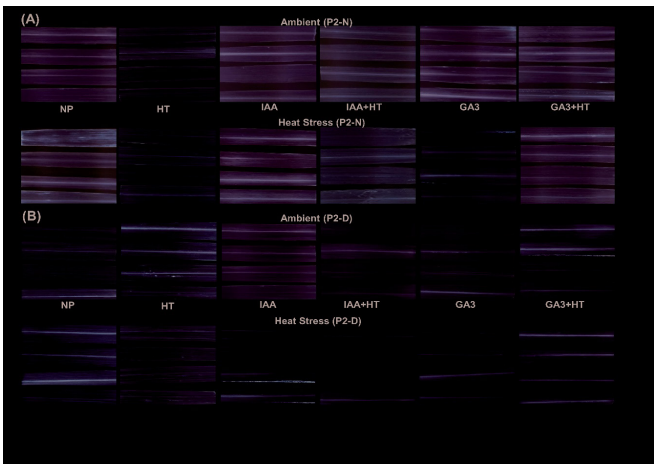


Fig. 3. *In situ* determination of hydrogen peroxide level by DAB staining of P2-N and P2-D primed wheat leaves. In 2016–17 two staggered planting at November (P2-N) and December (P2-D) was done. NP- no priming; HT-priming with high temperature; priming with IAA; priming with GA3; priming with IAA+HT; priming with GA3+HT. (A) P2-N wheat leaves stained under ambient and heat stress. (B) P2-D primed wheat leaf stained under ambient and heat stress

the plants treated with IAA, IAA+HT, GA3 and GA3+HT, showed increase by 27.27, 90.91, 63.64 and 90.91% over corresponding NP plants, whereas, under heat stress environment, treated plants showed significantly ($p \leq 0.05$) higher spikelet numbers with IAA (112.50%), IAA+HT (150%), GA3 (125%) and GA3+HT (212.50%).

Visible results of priming, with respect to the control, were observed in case of spikelet lengths. In P1-D, the maximum spikelet length was observed with IAA, IAA+HT, GA3 and GA3+HT at 24.39, 5.03, 25.72, and 28.02%, respectively over corresponding NP plants under heat stress condition. In case of P2-N plants, a notable significant increment was observed in IAA (24.29%), IAA+HT(11.70%), GA3 (22.72%) and GA3+HT (21.15%) treated plants over corresponding NP plant ($p \leq 0.05$) while under heat stress environment, IAA+HT, GA3 and GA3+HT values were 8.17, 13.19 and 20.20% higher than the corresponding NP plants. In the P2-D condition, maximum spikelet length was seen in HT, IAA, IAA+HT, GA3 and GA3+HT by 9.45, 7.86, 18.06, 21.94, and 26.49% over the corresponding NP plants, while under heat stress, significant increment was observed for IAA (6.37%), IAA+HT (19.75%), GA3 (22.72%) and GA3+HT (27.26%) over corresponding NP plants at ($p \leq 0.05$) (Fig.4B).

In the case of NOG/Sin the P1-D sown conditions, maximum grain number were counted for IAA (10.53%) over corresponding NP plants while under heat stress condition, a significant observation was observed for HT (16.11%), IAA (26.13%), IAA+HT (37.92%), GA3 (21.41%) and GA3+HT (52.06%) over corresponding NP plants ($p \leq 0.05$). Additionally, significant differences between individual treatments were noted in the P2-N sown condition. The plants treated with hormones IAA, IAA+HT, GA3 and GA3+HT possessed maximum grain numbers of 62.25, 51.15, 46.03 and 59.69% over corresponding NP plants whereas, under heat stress conditions, the maximum number was observed in IAA (32.88%), IAA+HT (17.87%) and GA3 (11.11%) over corresponding NP plants ($p \leq 0.05$). In the P2-D sown conditions, improved grain yield and sustained grain were observed in both the ambient and heat stress conditions. In the ambient condition, Plants treated with IAA, IAA+HT, GA3 and GA3+HT showed statistically significant ($p \leq 0.05$) grain no. by 12.08, 48.14, 47.35, 49.71 and 51.27% respectively, while under heat stress conditions IAA (44.98%), IAA+HT (50.19%), GA3 (50.19%) and GA3+HT (59.11%) over corresponding NP plant ($p \leq 0.05$) (Fig.4C).

As a comparative analysis to quantify yield, the thousand-grain weights (TGW) were determined. Thousand-grain weight measurements showed moderate variation in P1-D and P2N conditions as well as in all the priming samples. In the P1-D condition, maximum grain weight was observed for IAA+HT (9.31%), GA3 (13.26%) and GA3+HT (20.30%) under ambient conditions. While under heat stress, only IAA-treated plants showed significantly higher values by 13.35% over corresponding NP-treated plants($p \leq 0.05$). In the P2-N sowing conditions, only IAA+HT treated plants had significantly higher grain weight at 25.27% over corresponding NP plants. In late-sown P2-D conditions, a remarkable increase was seen for HT, IAA, IAA+HT, GA3 and GA3+HT at 46.48, 87.54, 121.08, 113.49, and 120.36%, respectively, while under heat stress, same trend was followed by HT (7.05%), IAA (44.92%), IAA+HT (46.90%), GA3 (55.02%) and GA3+HT (46.49) over corresponding NP plant($p \leq 0.05$). However, high temperature prolonged the active grain-filling period only for superior spikes; the mean number of grains rate was significantly increased by HT, IAA, IAA+HT, GA3 and GA3+HT for both conditions (Fig. 4D)

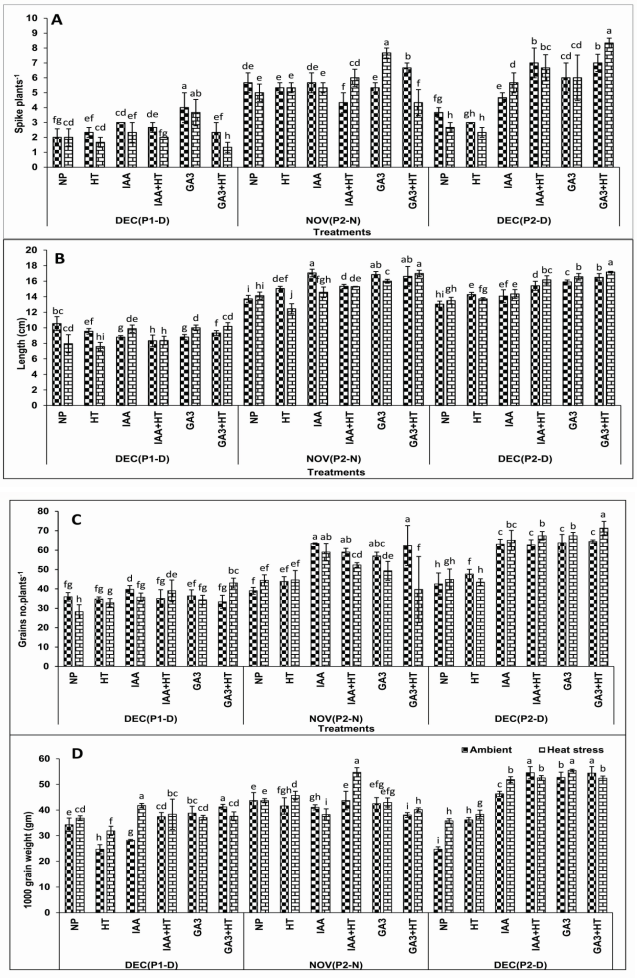


Fig. 4. Effects of seed priming and anthesis stage heat stress on yield related traits in two consecutive years, 2015–16 and 2016–17. The yearly data with various priming and anthesis heat stress were measured NP no priming; HT-priming with high temperature; priming with IAA; priming with GA3; priming with IAA+HT; priming with GA3+HT. (A) No. of spikes per plants, (B) Length of the ear head, (C) No. of grains per spike, (D) Thousand grains weight. The data are means ± SE and the lowercase letters refer to significant differences between the treatments at the ($p \leq 0.05$) levels as determined using Duncan's multiple range test

An increase in grain characteristics with kinetin seed priming has been reported earlier (Jaiswal et al., 2022). Overall, hormone-primed plants showed significantly higher grain yield under heat stress conditions over the ambient conditions. It is important to highlight here that as the experiments were carried out in field conditions, the daily variations in environmental conditions also affected the traits documented. Thus, our results provide a realistic picture of plant response to rising temperature as compared to experiments carried out under controlled conditions in the laboratory.

Transcript expression profiling of SKP1 gene showed differential and induced modulation after seed priming

Towards identifying genetic components involved in plant stress memory, effectors and their regulators need to be identified. Here, we selected an important component of UPS and investigated its transcript modulation under high ambient temperature. *SKP1*transcripts were differentially modulated when analyzed by semi-quantitative RT-PCR within a season as well as between the three different crop seasons, P1-D, P2-N and P2-D (Fig.5).

For comparison of the absolute expression of *SKP1* transcripts in the P2-N condition, the expression value of flag leaf NP was taken as 1 and all the other values were calculated accordingly. Then, each treatment expression value at ambient was compared with corresponding high-temperature values and percentage differences were calculated. Under heat stress expression of SKP1 was recorded as NP (50.91%), IAA (42.04%), IAA+HT (37.57%), GA3 (210.04%), GA3+HT (29.23%) increase showing significant enhancement ($p \leq 0.05$) while only HT priming under heat stress, the expression of SKP1 got reduced by 59.10% ($p \leq 0.05$) (Fig. 6A).

In the P2-D condition with similar calculation, IAA+HT, NP, GA3, HT, and IAA, showed highest induction of SKP1 expression by 637.99, 364.79, 247.72, 60.56 and 17.86% ($p \leq 0.05$) respectively while GA3+HT showed reduced expression (8.92%) under stress condition. In the P1-D sown condition, SKP1 was induced significantly under heat stress in NP (36.62%), HT (52.69%), IAA (255.10%), IAA+HT (174.18%), GA3+HT (180.90%) ($p \leq 0.05$). In contrast to this, GA3 showed a reduction of 24.68%.

Next, the relative fold change in RNA expression was carried out in all the three seasons for all the priming conditions. Higher transcript expression of SKP1 was observed under heat stress conditions. Here, also flag leaf ambient NP expression value was taken as 1 and relative fold changes in expression were calculated for the remaining treatments with all the sowing conditions. In the P2-N condition, IAA, IAA+HT, GA3 and GA3+HT got maximum fold changes of 31.39, 31.74, 33.20 and 26.50 fold, respectively under heat stress(Fig. 6C). In the P2-D sown condition, in NP 7.74, HT 9.67, IAA 11.95, IAA+HT 51.71 and GA3 25.0 folds were observed while in GA3+HT 10.16 fold which is less in fold change as compared to the corresponding ambient treated plant. In the case of P1-D sown conditions, maximum fold change was observed for IAA, IAA+HT, GA3+HT at 53.51, 51.52, 67.45 fold, respectively, whereas, GA3 showed a maximum 52.19 fold to the corresponding ambient tissue.

SKP1 transcript profiling in ear head tissues showed enhanced expression in the case of hormonally primed samples (Fig.6B). In P1-D season, HT primed ambient value showed the least value and was taken as 1 and all other values were calculated. The NP plants reduced the expression by 45.35%, whereas HT, IAA, IAA+HT, GA3, and GA3+HT significantly increased expression by 280.91, 154.74, 57.80, 157.05% ($p \leq 0.05$). Furthermore, in the P2-N conditions, NP and GA3+HT reduced the fold change by 47.85 and 4.06%, while HT (255.06%), IAA (98.42%), IAA+HT (60.52%) and GA3 (92.28%) were expressed significantly high ($p \leq 0.05$). In the P2-D season, a similar trend like the flag leaf of P2-D condition was observed. The NP (405.85%), HT (276.12%), IAA (88.07%), IAA+HT (339.34%) and GA3 (136.77%) showed significantly higher expression, while GA3+HT expression declined by 46.31%.

Relative fold change comparison of ear head tissues of P2-N, P1-D and P2-D SKP1 transcripts, higher expression was observed under heat stress (Fig. 6D). In the case of P2-N condition, the NP plants showed a reduction of 3.17 fold change under the heat stress. Whereas HT, IAA, IAA+HT, GA3, and GA3+HT showed higher fold change under heat stress compared to corresponding ambient temperature by 3.5, 31.80, 31.53, 21.83 and 33.37 fold, respectively. In the

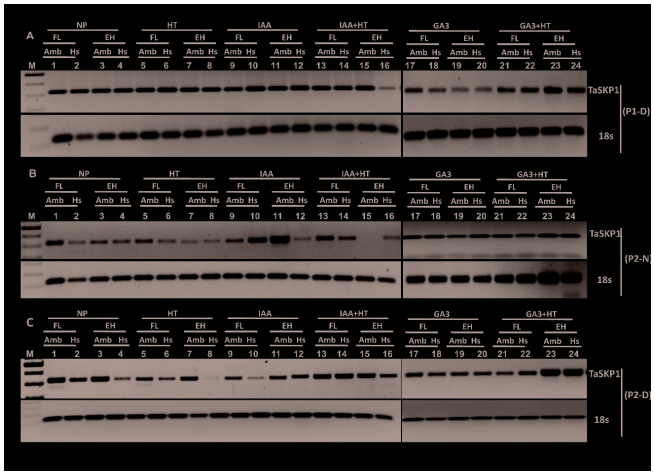


Fig. 5. Transcript expression profiling by semi quantitative RT-PCR of TaSKP1 gene in flag leaf and ear head under ambient and heat stress conditions of variously primed plants (A) P1-D, where M is 100 bp ladder ,Lane 1–4 NP (No priming), Lane 5–8 HT (Heat), Lane 9–12 IAA, Lane 13–16 IAA+HT, Lane 17–20 GA3, Lane 21–24 GA3+HT. Same sequence is followed in, (B) P2-N and P2-D, FL = Flag leaf; EH = Ear head

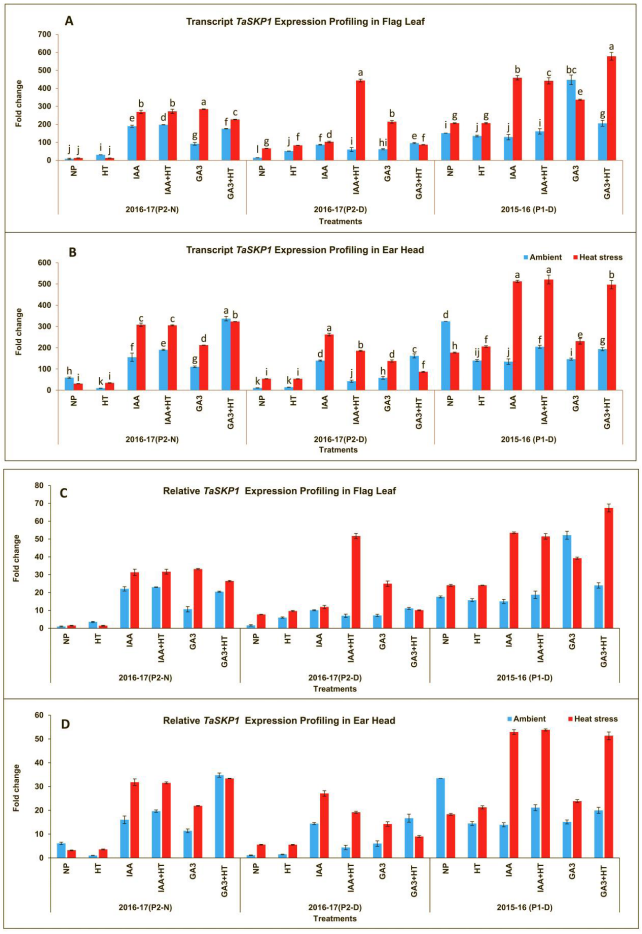


Fig. 6. Absolute and relative *TaSKP1* transcript expressions profiling in flag leaf and ear head in two growth seasons with three sowing time points. (A) Comparative absolute transcript expression profiling in flag leaf. (B) Comparative absolute transcript expression profiling in ear head (C) Relative transcript expression profiling in flag leaf where ambient NP is taken as 1 and relative fold changes in expression were calculated for the remaining treatments with all the sowing conditions. (D) Relative transcript expression profiling, ear head HT ambient was taken as 1 and relative fold changes in expression were calculated for the remaining treatments with all the sowing conditions.NP no priming; HT-priming with high temperature; priming with IAA; priming with GA3; priming with IAA+HT; priming with GA3+HT. The data are means ± SE and the lowercase letters refer to significant differences between the treatments at the ($p < 0.05$) level as determined using Duncan's multiple range test

P-2D plants, higher induction of SKP1 was observed over corresponding heat stress treatment by NP (5.56), HT (5.51), IAA (27.09), IAA+HT (19.16), GA3 (14.21) fold whereas GA3+HT reduced the fold change by 8.96. In the P1-D condition again NP plant showed reduction in SKP1 expression under heat stress by 18.29 fold while HT samples showed an increase of (21.23), IAA (52.92), IAA+HT (53.80), GA3 (23.84) and GA3+HT (51.32) fold.

Transcriptional memory is defined as a transcriptional response to recurring stress that differs from the transcriptional response to a primary stress (Ding et al. 2012, Tehrani et al., 2023) Priming strengthens the induction of genes that are up regulated by heat stress in plant defence. Thus analysing the output of stress triggering at the molecular level is a simple way to demonstrate the priming effect and the existence of memory (Charng et al. 2023). The *SKP1* gene expression induction in hormonal treated plants showed higher correlation with chlorophyll content at post anthesis stage under heat stress. Although heat tolerance was measured in both the IAA and GA3 primed plants after exposure to high temperature at anthesis, plants primed with GA3 exhibited superior attributes in terms chlorophyll and grain yield. The effects of seed priming were very much evident in both the years and three time points where plants were raised. Several fold increase in spike length, number of grains per spike and thousand grain weight was recorded in primed plants over non primed plants in case of both IAA and GA3 priming. As cvHD2967 is a timely sown variety, as expected maximum yield was observed under ambient condition in all the primed experiments and minimum or negligible differences were observed amongst the treatments. Exception to the other treatments at P2-N under anthesis heat trigger, IAA primed plants exhibited enhanced thousand grain weights.

Estimation of correlation coefficients of SKP1 expression, chlorophyll and grain parameters

Correlation analysis confirmed the beneficial effects of seed priming in two broad conditionsof ambient and high temperature. Chlorophyll content was recorded at anthesis, booting and post anthesis stages (S1, S2, S3) and grain attributes i.e., No. of spikelet per plant, (NOS/P), length of ear head (LOEH), No. of grain per spike (NOG/S), Thousand grain weight (TGW) and *SKP1* transcript expression analysis in flag leaf and ear head (SKP1-FL, SKP1-EH) tissues. In the P1-D condition a positive relationship was established between examined parameters. The chlorophyll content of S3 stage was positively and significantly interrelated with SKP1-FL expression by r=0.89 under ambient while under heat stress by SKP1-FL r =0.92 and SKP1-EH r=0.85 ($p < 0.01$) (Table 1A). Similarly, the P2-N sown condition under ambient condition also positive and significant correlation with S3, r=0.77 and NOGPH r=0.77 ($p < 0.01$) whereas under heat stress by r=0.92 and 0.85 with S3, respectively (Table 1B). Furthermore, in the P2-D condition, the *SKP1* expression of FL and EH again were significantly positively interrelated with chlorophyll S2 and S3 stage by r = 0.80, 0.92, whereas with SKP1-EH by r=0.79, 0.85 ($p < 0.01$) under ambient condition (Table 1C).

Number of Spike/plant (NOS/P), length of ear head (LOEH), No. of grains/spikelet (NOG/S), Thousand grain weight (TSW), Booting stage (S1), Anthesis stage (S2), Post anthesis stage (S3), Flag leaf (FL) and Ear head (EH).

Attuning a plant's own defense system to counter

Table 1. Pearson correlation matrix of ambient (upper diagonal) and heat stress (lower diagonal) in (A) December (P1-D) 2015–16 (B) November (P2-N) 2016–17 (C) December (P2-D) 2016–17

(A)

Traits	S1	S2	S3	NOS/P	LOEH	NOGPH	TGW	SKP1-FL	SKP1-EH
S1	1	0.37	0.24	0.07	-0.07	0.14	-0.22	-0.01	-0.05
S2	0.01	1	0.30	0.44	0.13	-0.02	0.11	-0.04	-0.24
S3	-0.11	0.30	1	-0.22	0.08	0.53	0.19	0.80	0.73
NOS/P	0.33	0.44	-0.04	1	-0.10	-0.44	-0.14	-0.57	-0.61
LOEH	-0.10	-0.26	-0.15	-0.22	1	0.19	0.05	0.28	0.02
NOG/S	0.42	-0.03	-0.18	-0.20	0.28	1	-0.19	0.64	0.55
TGW	-0.54	0.36	0.28	0.18	-0.21	-0.27	1	0.35	0.43
SKP1-FL	-0.01	0.89	0.05	0.53	-0.20	-0.06	0.48	1	0.90
SKP1-EH	-0.57	-0.51	-0.35	-0.35	0.53	-0.12	0.29	-0.25	1

DEC (P1-D)

(B)

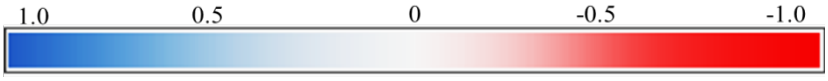
Traits	S1	S2	S3	NOS/P	LOEH	NOGPH	TGW	SKP1-FL	SKP1-EH
S1	1	0.34	0.59	0.30	0.17	0.37	-0.27	0.49	0.39
S2	0.50	1	0.78	0.08	0.79	0.46	-0.14	0.80	0.79
S3	0.32	0.55	1	0.35	0.68	0.20	-0.06	0.92	0.85
NOS/P	-0.19	-0.09	-0.23	1	-0.03	-0.01	0.25	0.35	0.03
LOEH	0.17	0.44	0.44	-0.06	1	0.13	-0.13	0.67	0.68
NOG/S	0.14	0.49	0.58	-0.12	0.89	1	0.06	0.25	0.19
TGW	0.24	0.05	-0.25	-0.01	-0.19	-0.18	1	-0.02	-0.05
SKP1-FL	0.22	0.43	0.77	-0.03	0.55	0.77	-0.20	1	1.00
SKP1-EH	-0.18	0.41	0.51	0.24	0.44	0.64	-0.34	0.78	1

NOV (P2-N)

(C)

Traits	S1	S2	S3	NOS/P	LOEH	NOGPH	TGW	SKP1-FL	SKP1-EH
S1	**1**	0.34	0.59	0.30	0.17	0.37	-0.27	0.49	0.39
S2	0.34	**1**	0.78	0.08	0.79	0.46	-0.14	0.80	0.79
S3	0.59	0.78	**1**	0.35	0.68	0.20	-0.06	0.92	0.85
NOS/P	0.30	0.08	0.35	**1**	-0.03	-0.01	0.25	0.35	0.03
LOEH	0.17	0.79	0.68	-0.03	**1**	0.13	-0.13	0.67	0.68
NOG/S	0.37	0.46	0.20	-0.01	0.13	**1**	0.06	0.25	0.19
TGW	-0.27	-0.14	-0.06	0.25	-0.13	0.06	**1**	-0.02	-0.05
SKP1-FL	0.49	0.80	0.92	0.35	0.67	0.25	-0.02	**1**	0.92
SKP1-EH	0.39	0.79	0.85	0.03	0.68	0.19	-0.05	0.92	**1**

DEC (P2-D)



(Note: In bold font, the values equal to 1 because each measure has a perfect linear correlation with itself. The matrix is essentially mirrored from bottom left to top right as the measures are correlated in both directions)

high-temperature stress can be an effective strategy for sustainable wheat production. Here, we demonstrate that seed priming with IAA and GA3 promotes reproductive stage high-temperature stress by positively modulating chlorophyll content and countering ROS production. The transgenerational effect of seed priming was most evident in late-sown plants. At the molecular level, transcriptional memory of the *SKP1* gene showed involvement of protein homeostasis in maintaining heat tolerance at the generative stage. Further molecular dissection and mechanistic analyses of UPS and *SKP1* under terminal heat stress will elucidate their exact functional role and reveal other key players involved.

Authors' contribution

Conceptualization of research (SB); Designing of the experiments (SB, PJ); Contribution of experimental materials (ANS); Execution of field/lab experiments and data collection (PJ); Analysis of data and interpretation (PJ, SB); Preparation of the manuscript (PJ, SB).

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References

Charng Y. Y., Mitra S. and Yu S. J. 2023. Maintenance of abiotic stress memory in plants: lessons learned from heat acclimation. The Plant Cell, **35**(1): 187–200. https://doi.org/10.1093/plcell/koac313.

Damalas C. A., Koutroubas S. D. and Fotiadis S. 2019. Hydro-priming effects on seed germination and field performance of fababean in spring sowing. MDPI Agriculture. https://doi.org/10.3390/9090201.

Daudi A. and O'Brien J. A. 2012. Detection of hydrogen peroxide by DAB staining in Arabidopsis leaves. Bio-protocol 2, **18**: e263. DOI: 10.21769/BioProtoc.263.

Ding Y., Fromm M. and Avramova Z. 2012. Multiple exposures to drought "train" transcriptional responses in Arabidopsis. Nature Commun., **3**(1): https://doi.org/10.1038.

Djanaguiraman M., Narayanan S., Erdayani E. and Prasad P. V. V. 2020. Effects of high temperature stress during anthesis and grain filling periods on photosynthesis, lipids and grain yield in wheat. BMC Plant Biol., **20**(1): https://doi.org/10.1186/s12870-020-02479-0.

El Beji I. H., Mouzeyar S., Bouzidi M. F., et al. 2019. Expansion and functional diversification of SKP1-like genes in wheat (*Triticum aestivum* L.). MDPI. https://doi.org/10.3390/ijms20133295.

El Karamany M. F., Sadak, M. S. and Bakry B. A. 2019. Improving quality and quantity of mungbean plant via foliar application of plant growth regulators in sandy soil conditions - Bulletin of the National Research Centre. Springer Open. https://doi.org/10.1186/s42269-019-0099-5.

IPCC 2023, Lee H., Calvin Katherine et al. 2023. Synthesis report: a report of the intergovernmental panel on climate change. http://www.kma.go.kr/kma.

Isoda Reika., Yoshinari Akira., Ishikawa Yuuma., SadoineMayuri., Simon Rüdiger., Frommer Wolf B. and Masayoshi Nakamura. 2021. Sensors for the quantification, localization and analysis of the dynamics of plant hormones. The Plant J., **105**(2): 542-557. https://doi.org/10.1111/tpj.15096.

Jaiswal P., Sahi A. N. and Barthakur S. 2022. Cytokinin seed priming mediated induction of terminal heat stress tolerance and expression profiling of SKP1 transcripts, a component of ubiquitin proteasome system in bread wheat (*Triticum aestivum* L.): a transgenerational analysis. Plant Growth Regulation, **98**(2): 259–280. https://doi.org/10.1007/s10725-022-00861-6.

Kambona C.M., Koua P.A., Léon, J. et al. 2023. Stress memory and its regulation in plants experiencing recurrent drought conditions. Theor. Appl. Genet., **136**(26): https://doi.org/10.1007/s00122-023-04313-1.

Khomdram S. and Barthakur S. 2015. In situ evaluation of high temperature stress in wheat (*Triticum aestivum* L.)-Validation of a portable heat trap chamber. Ann. Agric. Res., **36**(2): 123–131. https://epubs. icar. org. in/ index. php/ AAR/ artic le/ view/ 55463/ 23265.

Li M., Feng J., Zhou H. et al. 2021. Overcoming reproductive compromise under heat stress in wheat: Physiological and genetic regulation, and breeding strategy. Front. Plant Sci., **13**: 881813. https://doi: 10.3389/fpls.2022.881813.

Luo P., Ren Z., Wu X. et al. 2006. Structural and biochemical mechanism responsible for the stay-green phenotype in common wheat. Chinese Sci. Bull., **51**: 2595–2603. https://doi.org/10.1007/s11434-006-2175-0.

Nishio H., Kawakatsu T. and Yamaguchi N. 2024. Beyond heat waves: Unlocking epigenetic heat stress memory in Arabidopsis. Plant Physiol., **194**: 1934–1951. https://doi.org/10.1093/plphys/kiad558.

Okello D., Komakech R., Gang R. et al. Influence of various temperatures, seed priming treatments and durations on germination and growth of the medicinal plant *Aspilia africana*. Sci. Rep., **12**: 14180 2022. https://doi.org/10.1038/s41598-022-18236-2.

Parry G., Estelle M. 2006. Auxin receptors: a new role for F-box proteins. Curr. Opin. Cell Biol., **18**(2): 152-156. https://doi.org/10.1016/j.ceb.2006.02.001.

Rasmann S., De. Vos. M., Casteel C. L. et al. 2011. Herbivory in the previous generation primes plants for enhanced insect resistance. Plant Physiol., **158**(2): 854–863. https://doi.org/10.1104/pp.111.187831.

Salehin M. 2024. Emerging roles of auxin in plant abiotic stress tolerance. Physiologia Plantarum, **176**(6): e14601. 176:e14601. https://doi.org/10.1111/ppl.14601.

Staacke T., Mueller-Roeber B. and Balazadeh S. 2025. Stress resilience in plants: the complex interplay between heat stress memory and resetting. The New phytologist, **245**(6):

2402–2421. https://doi.org/10.1111/nph.20377.

Tehrani S. S. H., Kogan A., Mikulski P., et al. 2023. Remembering foods and foes: emerging principles of transcriptional memory. Cell Death and Differentiation. https://doi.org/10.1038/s41418-023-01200-6.

Wahid A., and Close T. J. 2007. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. Biologia Plantarum, **51**(1): 104–109. https://doi.org/10.1007/s10535-007-0021-0.

Wang Yaliang., Wang Lei., Zhou Jianxia., Hu Shengbo., Chen Huizhe., Xiang Jing., Zhang Yikai., et al. 2019. Research progress on heat stress of rice at flowering stage. Rice Sci., **26**(1-10). https://doi.org/10.1016/j.rsci.2018.06.009.

Wang Xiao and Cai, et al. 2011. Pre-anthesis high-temperature acclimation alleviates damage to the flag leaf caused by post-anthesis heat stress in wheat. J. Plant Physiol., **168**(6): 585-593. https://doi.org/10.1016/j.jplph.2010.09.016.

Woodward A. W. and Bartel B. 2005. Auxin: regulation, action, and interaction. J. Exp. Bot., **83**(5): 707–735. https://doi.org/10.1093/aob/mci083.

Zadoks J.C., Chang T.T. and Konzak C.F. 1974. A decimal code for the growth stages of cereals. Weed Res., **14**: 415-421. http://dx.doi.org/10.1111/j.1365-3180.1974.tb01084.x.