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Physiological and molecular response of *Brassica rapa* to moderate and extreme heat

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Abstract – One of the key factors affecting plant survival and agricultural yield production is temperature. The magnitude of temperature extremes is increasing as a result of global climate change. The present study evaluated the impact of elevated temperature treatments on *Brassica rapa* seed germination, as well as of prolonged exposure of seedlings to temperatures of 37 °C and short-term exposure to the temperature of 45 °C. Elevated temperatures reduced seed germination rate and affected germination pattern. Both applied heat stresses negatively affected seedling development and root growth, and showed a differential physiological and molecular response. Under prolonged exposure to 37 °C seedling growth and development patterns were impaired but with no sign of oxidative stress, which could be related to increased indole-3-acetic acid (IAA), abscisic acid, enhanced heat shock protein 90 (HSP90) and reduced 1-aminocyclopropane-1-carboxylate levels. The short-term exposure to a temperature of 45 °C, a treatment mimicking a heat wave event, had more negative effects on seedling growth, which correlated with the appearance of oxidative stress. The extreme temperature significantly stimulated the gene expression of heat stress transcription factors *HSFs* and dehydration-responsive element-binding protein *DREB2A*, and induced the accumulation of auxin IAA and HSP90 proteins. Our study confirms the great importance of phytohormones and HSP90 in the heat stress response of *B. rapa* and emphasizes the potential for their manipulation in phytoprotection and breeding programs for adaptation to climate change.

Key words: cabbage, germination, heat, phytohormones, root growth, seedling growth, stress markers

Introduction

Plants have the ability to adapt their phenotype in response to different environmental influences. To withstand adverse conditions, they have evolved numerous molecular strategies to adapt to environmental challenges and deal effectively with all types of stressful conditions. Environmental and endogenous signals precisely regulate plant growth and development (Peleg and Blumwald 2011) and ambient temperature is one of the most important parameters influencing all aspects of plant development, such as germination, seedling establishment, vegetative growth and reproductive development (Begcy et al. 2018, Angadi et al. 2000). Germination is a particularly strictly regulated stage in the life cycle of plants. It is the first step in the successful establishment of plant growth and influences species distribution (Yamauchi et al. 2004, Heschel et al. 2007). Although all plant growth stages can be affected by heat stress, high temperatures can negatively impact seed germination and limit subsequent plant growth, sometimes leading to irreversible damage and plant death (Yeh et al. 2012). To adapt to elevated temperatures, plants undergo a series of changes in their transcriptome, proteome, metabolome and lipidome (Mittler and Blumwald 2015, Raza et al. 2023), while vulnerability depends on temperature intensity and duration. Recently, great progress has been made in research into the response mechanisms of plants to elevated temperatures (Kan et al. 2023). However, further research is needed as the ways in which heat stress is perceived and transduced into physiological and morphological responses are still unclear.

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Important players in the adaptation to high temperatures are phytohormones, which influence growth in a dose-dependent manner and whose fine-tuning and interactions are particularly important in the regulation of a variety of developmental processes under heat stress (Peleg and Blumwald 2011, Vanstraelen and Benková 2012, Ahammed et al. 2016). Heat exposure particularly influences the metabolism and accumulation of so-called stress hormones such as auxin, ethylene, abscisic acid (ABA), salicylic acid and jasmonic acid, which is reflected in plant growth and development (Vanstraelen and Benková 2012, Qin et al. 2019). But our knowledge of how exposure to different temperatures affects the metabolism and accumulation of phytohormones and how this fine-tuning affects the development and growth of specific plant is still limited.

The frequency and magnitude of temperature extremes are increasing as a result of global climate change, which is expected to result in ever more intense and frequent extreme weather events (Seneviratne et al. 2021). The optimal growth temperature, as well as an upper threshold at which development stagnates or stops have significant effects on plant distribution, performance and yield (Ahmad et al. 2022). *Brassica rapa* has a long history of human cultivation and is a feral plant found around the world. Due to its high nutritional value, health-promoting effects and mild flavour, it is a very popular leafy vegetable. It prefers cool weather (15-20 °C), so climate change and the associated rise in temperature have a negative impact on growth and seed yield (Angadi et al. 2000). Still, little is known about how different types of heat stress, such as prolonged exposure to moderately elevated temperature or short exposure to extreme temperature affect morphological, the physiological and molecular responses of *B. rapa* seedlings. The temperatures applied in our experiments reflect the current rise in global temperature and are of ecological importance. The temperature of 37 °C is commonly measured during the summer season in areas with a continental climate, while short exposures to 45 °C reflect the extreme heat wave events encountered intermittently during summer. Therefore, we investigated the effects of elevated temperatures on the germination and emergence of *B. rapa* seedlings. Additionally, we analysed growth, proline (Pro) and malondialdehyde (MDA) content, as well as heat-inducible HSP90 protein and *HSFA7A*, *HSFB2A* and *DREB2A* genes in seedlings exposed to prolonged moderately elevated temperature or to an extreme and short heat wave. Special attention was devoted to the accumulation of the major stress related phytohormones since, as far as we know, there are currently no data available on their heat-induced changes in *B. rapa* seedlings.

Materials and methods

Plant material and sterilization

For all experiments, *B. rapa* L. subsp. *pekinensis* (purchased from ISP-International Seeds Processing GmbH, Germany) was used. Seeds were sterilized by soaking in 70% ethanol for 1 min, and then incubated in 3% Izosan G (100% sodium dichloroisocyanurate dihydrate, Pliva) for 10 min. After a 5-fold rinsing step with sterile distilled water, seeds were sown on Murashige and Skoog (1962) medium (MS) and stratified at 4 °C for 5 days. Plates with seeds were then moved to a growth chamber (PHC Corporation, Tokyo, Japan), positioned horizontally (for the germination assay) or vertically (for the seedling growth assay) and incubated for 16 h with light at 150 μmol m^{-2} s^{-1} and 8 h in darkness.

Seed heat treatment and germination

To examine temperature effects on seed germination, plates with seeds were incubated at constant temperature of 24 °C (control) and at elevated temperatures (up to 41 °C). After 3 days, the germination rate was evaluated as percentage (%) of seeds with a visible radicle tip and hypocotyl length was measured.

Seedling heat treatments

Seven-day-old *B. rapa* seedlings were exposed to a heat stress treatment at two different temperatures: 45 °C for 2 h and 15 min followed by a recovery at 24 °C, or 37 °C for 24 h. A preliminary experiment showed that 2 h and 15 min exposure to 45 °C did not result in immediate lethality to the seedlings (On-line Suppl. Fig. 1).

For the growth parameters, the plant material was analysed seven days (168 h) after the start of the heat treatment. Gene expression, HSP90 protein levels and phytohormone concentrations were assessed at a single time point, 24 h after the start of treatment. Pro and MDA content were measured at three time points: 2 h and 15 min, 24 h and 48 h after the start of treatment at 37 °C and 45 °C. Control seedlings were kept at 24 °C and analysed at the same time points as the treated seedlings.

Seedling growth assay

Seedling growth was monitored 7 days from the beginning of treatments (until the root tip of control seedlings reached the bottom of the dish). Root length of heat-treated and control seedlings was determined by ImageJ v.1.49 (Schneider et al. 2012). Lateral root number was counted manually. Total root length was measured as the sum of primary and lateral root lengths. Primary root growth rate was expressed as the difference between the final (168 h after the start of heat treatment) and initial (immediately prior to heat treatment) primary root length. Average fresh weight per seedling was calculated by weighing 10-12 whole seedlings at the end of the experiment.

Malondialdehyde and proline evaluation

MDA and Pro contents were determined as markers of oxidative damage during heat exposure and after recovery from heat treatments at 24 °C. Whole seedlings exposed to heat or control conditions were sampled, weighed and immediately frozen in liquid nitrogen before analyses.

MDA determination was done according to Hodges et al. (1999) and Sunkar et al. (2003) and Pro determination was done according to Bauer et al. (2022).

Gene expression evaluation

Whole seedlings exposed to heat-treated or control conditions were sampled, weighed and frozen in liquid nitrogen. RNA was isolated from 40-50 mg of frozen homogenized seedlings using the MagMaxTM Plant RNA Isolation Kit according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 1 µg of isolated RNA using 200 U of RevertAid H Minus Reverse Transcriptase and Oligo(dT)18 primer (Thermo Fisher Scientific) in a total volume of 20 µL. The RT-qPCR was performed in technical duplicates on the MIC platform (Bio molecular Systems) by using GoTaq® qPCR Master Mix reagent (Promega), gene specific primers (On-line Suppl. Tab. 1) and 10 ng of cDNA. Thermocycling conditions were set to 5 min at 95 °C, followed by 40 cycles at 95 °C for 10 s and 60 °C for 10 s. Specific amplification was verified by no template controls and melting curves generated by increasing the temperature from 55 °C to 95 °C at 0.5 °C s^{-1}. Quantification cycle (Cq) values and primer efficiencies were calculated with MIC qPCR Cycler software (Bio Molecular Systems). Relative gene expression of heat stress-related genes *HSFA7A*, *HSFB2A* and *DREB2A* was calculated with the ΔΔCq method (Pfaffl 2001, Vandesompele et al. 2002). The *B. rapa* genes *OGIO* (2-oxoglutarate and Fe (II)-dependent oxygenase) and *PUX* (plant UBX domain-containing protein) were used as references. Genes, accession numbers and primer sequences are listed in On-line Suppl. Tab. 1.

HSP90 determination

Total soluble proteins were extracted from 150-200 mg frozen heat-treated or control seedlings. The seedlings were homogenized in 400-800 µL Staples and Stahmann (1964) extraction buffer (92.5 mM TRIS-HCl, 500 mM sucrose, 6.48 mM dithiothreitol, pH 7.6). After sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 12% polyacrylamide gels, proteins were transferred onto a polyvinylidene difluoride (PVDF; Immobilon-P, Sigma-Aldrich) membrane. HSP90 was detected by using anti-HSP90-1 (Agrisera) and anti-Rabbit IgG-horse raddish peroxidase (Sigma Aldrich) antibodies and Immobilon® Forte Western HRP substrate (Millipore). PVDF membranes were stained with Coomassie brilliant blue (0.1% (w/v) Coomassie R-250 in 40% methanol and 10% acetic acid). Images were analysed in ImageJ v.1.49 (Schneider et al. 2012) as described in Taylor and Posch (2014). To calculate the changes in protein levels (FD), the respective control values were set to one.

Phytohormone determination

Free endogenous indole-3-acetic acid (IAA), abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylate (ACC) were measured by gas chromatography–mass spectrometry (GC–MS) according to previously adapted protocols (Villas-Bôas et al. 2003, Rawlinson et al. 2015, Ludwig-Müller et al. 2021). Heat-treated and control seedlings were sampled, weighed, frozen in liquid nitrogen and homogenised. After homogenization, at least 100 mg of frozen plant tissue was transferred to 2 mL tubes. A hundred nanograms of labelled standards $^{13}C_6$-IAA (Cambridge Isotope Laboratories, Andover, MA, USA; 10 ng μL^{-1}) and $^{2}H_4$-ACC (Eurisotop GmbH, Saarbrücken, Germany; 10 ng μL^{-1}), and 200 ng of $^{2}H_6$-ABA (Cambridge Isotope Laboratories, Andover, MA, USA; 10 ng μL^{-1}) were added to each tissue sample as internal standards. Samples were weighed, processed and measured in technical duplicates. ABA, IAA and ACC concentrations were determined using principles of isotope dilution (Cohen et al. 1986) from diagnostic ion ratios of endogenous and labelled hormones at a m/z of 190/194 for ABA, 130/136 for IAA and 141/145 for ACC, at respective retention times of 11.8-11.9 min, 10.5-10.6 min and 6.9-7.0 min, using the formula: $Y=\left(\frac{Ci}{Cf}-1\right)\times X$. Ci is the sum of peak areas of standard and endogenous hormone, Cf the peak area of the standard, X the amount of labelled standard added, and Y the hormone concentration. Values obtained by this formula were then divided by the fresh weight and expressed as such.

Statistical methods

At least three biological replicates per treatment or control were analysed. Germination rates were compared between treatments and control using a Chi-square test of independence. For other parameters, normality of data was assessed using the Shapiro-Wilk test and homogeneity of variances using the Levene test. Normality and equal variances were assumed if $P > 0.05$. Although the data were normally distributed, the variances were not always homogeneous. Therefore, depending on the homogeneity of variances, a one-way ANOVA followed by Tukey's HSD post hoc test or Welch's ANOVA followed by a Games-Howell post hoc test was performed. Statistical significance was set at $P \leq 0.05$. Statistical analysis was performed in the TIBCO Statistica 13.5.0.17 software package (TIBCO Software, USA).

Results

Heat significantly affects seed germination

Seeds of *B. rapa* were exposed to a set of rising temperatures and germination rate (% of seeds with visible radicle tips) was monitored (Fig. 1). In control conditions, *B. rapa* germination started as early as after 8 h (On-line Suppl. Fig. 2) and after 3 days all seeds (100%) had developed into green seedlings. At slightly elevated temperature (28.5 °C), hypocotyls elongated significantly (Fig. 1A, C) while a further temperature rise (e.g. 32.7 °C) significantly inhibited hypocotyl elongation. At temperatures above 38 °C, the germination rate was significantly reduced compared to the control. In addition, these temperatures blocked hypocotyl elongation, apical hook opening and cotyledon opening, ex-

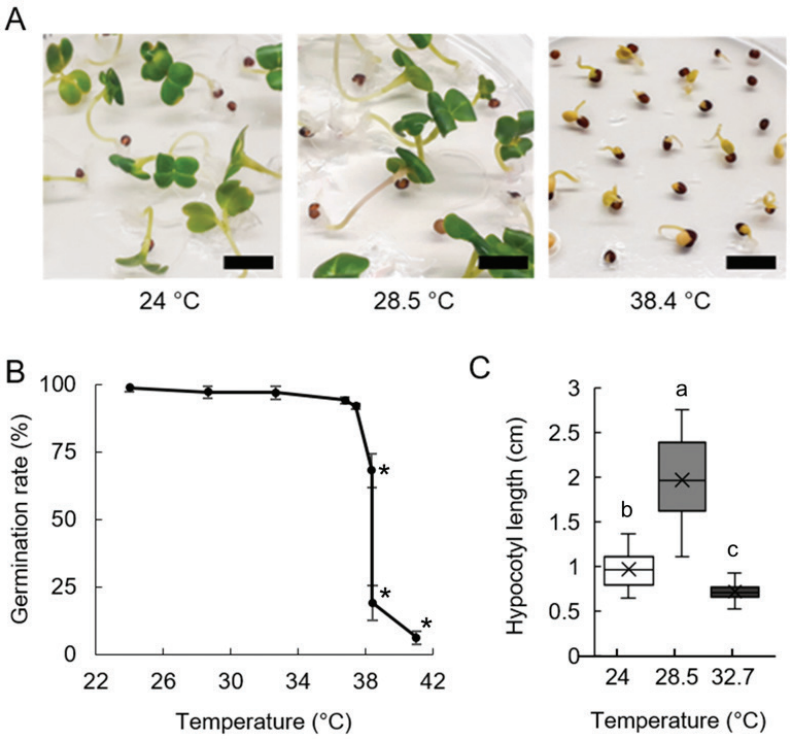

Fig. 1. Effect of elevated temperatures on *Brassica rapa* seed germination. Following stratification, seeds were exposed for three days to a set of temperatures when seedlings were documented (A), the germination rate (% of seeds with visible radicle tip) determined (B), and hypocotyl length measured (C). Data in B are the average of three biological replicates (mean ± SD) each consisting of ~100 seeds. Asterisks (*) indicate a significant difference between the control (24 °C) and the different temperatures at $P \leq 0.05$ (Chi-square test of independence). Data in C are represented as boxes that indicate the lower and upper quartiles while means and medians are denoted with a horizontal line and a cross in the box, respectively. Whiskers represent the highest and lowest scores in the data set. Different letters indicate a significant difference at $P \leq 0.05$ (Welch's ANOVA, Games-Howell test). Scale bar in (A) = 1 cm.

pansion and greening (Fig. 1A, B). The highest temperature at which a minimal number of *B. rapa* seeds still germinated was 40 °C.

Heat affects seedling growth, morphology and biomass accumulation

Although both prolonged exposure (24 h) to 37 °C and short-term (2 h and 15 min) heat treatment at 45 °C, influenced the growth of *B. rapa* seedlings, the short exposure to 45 °C had more severe effects. A heat wave at 45 °C blocked lateral root development and affected primary root growth rate, total root length and biomass accumulation significantly more than the prolonged exposure to 37 °C (Fig. 2). Exposure to 37 °C induced hypocotyl elongation (Fig. 2A) while both heat treatments induced leaf yellowing, inhibited leaf growth (On-line Suppl. Fig. 3) and significantly reduced biomass accumulation (Fig. 2E).

Heat wave affects proline and malondialdehyde accumulation

To determine the severity of the applied heat treatments, Pro and MDA levels as markers of oxidative damage were

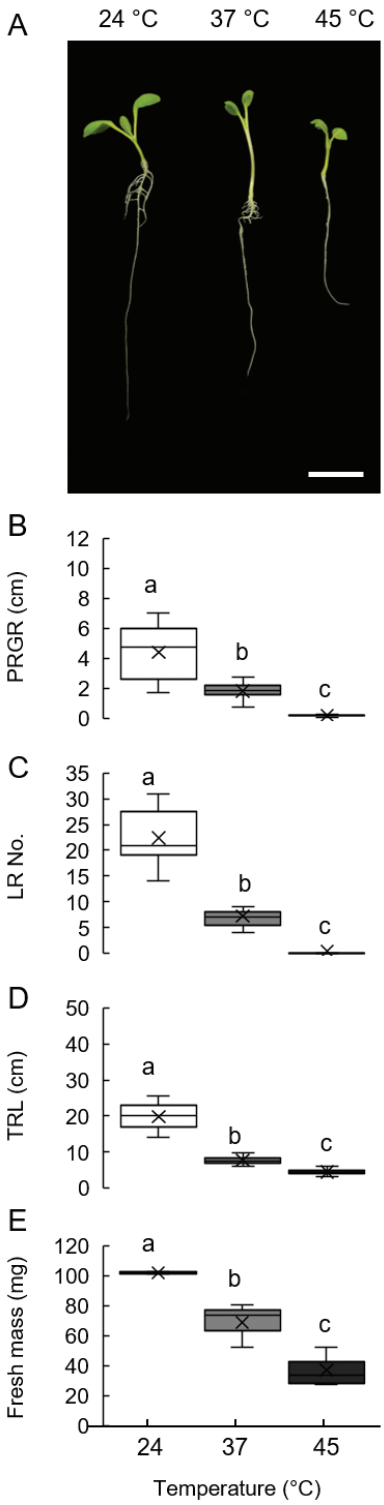

Fig. 2. The effect of heat stress on *Brassica rapa* seedling development. Seedlings were treated continuously at 37 °C for 24 h, or at 45 °C 2 h and 15 min followed by recovery at 24 °C. Control plants were kept at 24 °C. Seedlings were photographed (A) and analyzed 7 days after the start of heat stress treatments. Primary root growth rate, PRGR (B), lateral root number, LR No. (C), total root length, TRL (D) and biomass accumulation (E) were determined. Data in B-E are represented as boxes that indicate the lower and upper quartiles while means and medians are denoted with a horizontal line and a cross in the box, respectively. Whiskers represent the highest and lowest scores in the data set. Different letters indicate a significant difference at $P \leq 0.05$ (Welch's ANOVA, Games-Howell test). Scale bar in (A) = 2 cm.

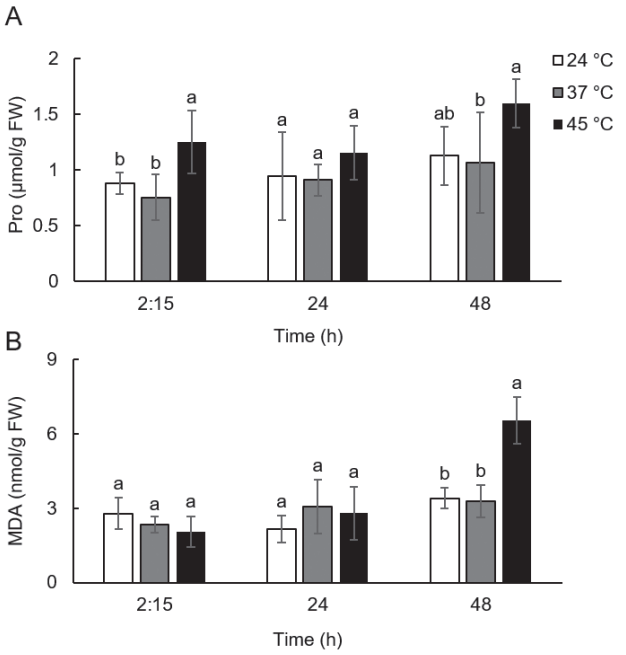

Fig. 3. Proline, Pro (A) and malondialdehyde, MDA (B) accumulation following heat stress in *Brassica rapa*. Pro and MDA levels were measured in seedlings either treated at 37 °C for 24 h or exposed to 45 °C for 2 h and 15 min (2:15) followed by recovery. Control plants were kept at 24 °C. Pro and MDA levels were measured at 2:15 h, 24 h and 48 h from the start of the treatments and expressed per fresh weight (FW). Data represent the average of six replicates with standard deviations denoted by vertical bars. Different letters indicate a significant difference at $P \leq 0.05$ (ANOVA, Tukey's test).

measured in heat treated (37 °C for 24 h, or 45 °C for 2 h and 15 min followed by recovery) *B. rapa* seedlings and compared to the corresponding control. Pro and MDA levels were measured in three time points – 2 h and 15 min, 24 h and 48 h from the start of the treatments. Exposure to 45 °C immediately induced Pro accumulation by 42% and elevated Pro levels were also detected even 48 h after heat wave treatment (Fig. 3A). In accordance, MDA content was significantly higher in seedlings 48 h after heat wave treatment (Fig. 3B). Exposure to 37 °C did not affect Pro and MDA accumulation indicating that the stress response of *B. rapa* seedlings to the heat wave-like treatment was more pronounced than that to prolonged exposure to a moderately elevated temperature.

Heat wave induces expression of heat stress-related genes and proteins

Plant stress response is mediated by transcription factors that perceive stress signals and direct downstream defence gene expression. To further investigate the effect of heat treatments, we quantified gene expression of major heat stress-responsive transcription factors *HSFA7A*, *HSFB2A* and *DREB2A* by RT-qPCR. Short-term exposure to 45 °C revealed a drastic induction of the expression of the examined genes, while a prolonged exposure to 37 °C only moderately enhanced *HSFA7A*, *HSFB2A* and *DREB2A* gene expression (Fig. 4A). HSP90 protein accumulation was evaluated after immunodetection and normalized to the RuBisCO protein (Fig. 4B, On line Suppl. Fig. 4). Both heat stress treatments induced accumulation of HSP90 proteins. Under control conditions, a low level of one HSP90 protein form (Fig. 4B, white arrowhead) was detected, while under heat stress enhanced accumulation as well as additional forms of HSP90 proteins were observed (black arrowheads).

Heat treatments affect phytohormone levels

Evident morphological changes of heat-treated *B. rapa* seedlings encouraged us to explore the fluctuations of phytohormones IAA, abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylate (ACC, the precursor of ethylene)

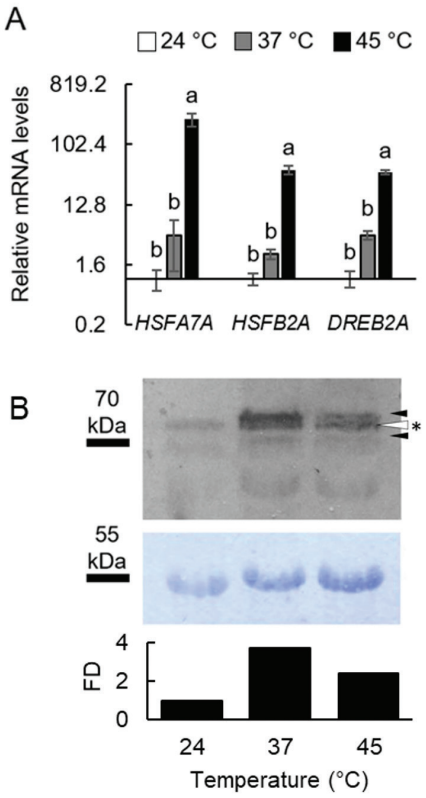

Fig. 4. Expression of heat stress-related transcription factor genes and proteins in *Brassica rapa*. Seedlings were treated at 37 °C for 24 h or exposed to 45 °C for 2 h and 15 min followed by recovery. Control plants were kept at 24 °C. Gene expression of heat stress-responsive transcription factors (A) and HSP90 proteins (B) were measured 24 h after the start of the treatments. For the relative quantification of *HSFA7A*, *HSFB2A* and *DREB2A* genes were normalized to the reference genes *OGIO* and *PUX*. Gene expressions are ΔΔCq values (with corresponding controls taken as one) on a log2 scale and presented as averages of three biological replicates with standard deviations denoted by vertical bars. Different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Tukey's test). Immunoassay signals of HSP90 were normalized to the RuBisCO protein intensities on PVDF membranes after staining with Coomassie brilliant blue and expressed as fold difference (FD), where corresponding control (24 °C) is set to one. The asterisk/white arrowhead indicates HSP90 present at control conditions and black arrowheads indicate heat-induced HSP90 forms.

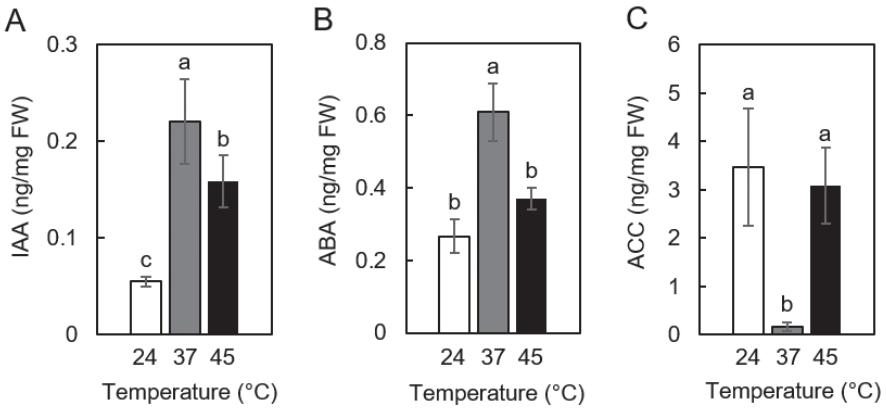

Fig. 5. Phytohormone levels following heat stress in *Brassica rapa*. Seedlings were either treated at 37 °C for 24 h or exposed to 45 °C for 2 h and 15 min followed by recovery. Control plants were kept at 24 °C. Indol-3-acetic acid, IAA (A), abscisic acid, ABA (B), and 1-aminocyclopropane-1-carboxylic acid, ACC (C) were measured by GC-MS from whole seedlings harvested 24 h from the start of the treatments and expressed per fresh weight (FW). Data represent the average of three replicates with standard deviations denoted by vertical bars. Different letters indicate a significant difference between control and heat treatments at $P \leq 0.05$ (ANOVA, Tukey's test).

(Fig. 5). Plant growth regulators were affected more severely by prolonged exposure to 37 °C than by short-term exposure to 45 °C followed by recovery. IAA content was significantly upregulated by both heat treatments (Fig. 5A) while exposure to 37 °C, but not to 45 °C, significantly induced ABA (Fig. 5B) and reduced ACC levels (Fig. 5C).

Discussion

The magnitude of temperature extremes is increasing as a result of global climate change and is accompanied by more frequent heat waves. Elevated temperatures may alter germination timing and rate, decrease biomass accumulation and reduce species' performance and yields by impairing reproduction (Anderson et al. 2020, Ahmad et al. 2022). Since temperature is one of the most important factors in plant growth and development, significant changes in plant distribution, abundance and yield are expected in the near future. Temperature increases of 1 °C cause a 17% yield reduction in *Brassica* species (Kaushal et al. 2016). Here, we studied the heat effects on *B. rapa* seed germination, and seedlings' performance under a moderately elevated temperature of 37 °C, reflecting the commonly measured temperature during the summer season in areas with a continental climate, and the effect of a short exposure to an extreme temperature of 45 °C, reflecting extreme heat wave events that occasionally occur during summer days. We analysed early stages of seedling establishment when plants are more vulnerable and temperature particularly affects growth rate and development. Successful seed germination is the major prerequisite of species' survival and distribution (Heschel et al. 2007) and may significantly impact crop yield (Boter et al. 2019). Several works have shown a negative correlation between rising temperatures and *Brassica* species seed germination rates. Wilson et al. (1992) showed that 40 °C is a critical temperature decreasing germination rates significantly in 11 different cultivars of *B. rapa*, *B. oleracea* and *B. napus*, while Motsa et al. (2015) showed that 36 °C is the maximum germination temperature for *B. rapa* ssp. *chinensis*. In accordance with these results, we showed that although *B. rapa* seed radicule protrusion occurred under heat, post-germination seedling development was strongly impaired indicating 36 °C as the temperature threshold for *B. rapa* distribution and seedling establishment in the field. Once germinated, *B. rapa* seedlings tolerated 37 °C and could survive for as long as 2 h and 15 min at 45 °C. However, the accumulation of biomass, as well as root growth and development were significantly disturbed by exposure to both temperature regimes (Fig. 2), and the effect was more pronounced when seedlings were exposed to a heat wave at 45 °C. Indeed, short exposure to 45 °C temporarily blocked root growth of *B. rapa* seedlings. In other *Brassica* species heat stress often reduces growth, especially of aboveground organs, which is an adaptation that can help plants reduce transpiration and conserve water (Gunasekera et al. 2006, Rodríguez et al. 2015, Munns and Millar 2023), but heat response and tolerance can vary greatly among species, varieties or even accessions (Pavlović et al. 2018, 2019, Bauer et al. 2022). For example, exposure to elevated temperature reveals accession-specific diversity in *B. oleracea* var. *acephala* and *B. napus* (Bauer et al. 2022, Boter et al. 2023), with some having enhanced growth and accumulation of biomass, while most remain sensitive to heat, which fits with our results. Moreover, a significant difference in the phenotypic root system architecture of *B. napus* varieties is associated with differences in the transcriptional dynamics of the heat shock and hormonal response genes (Boter et al. 2023). Furthermore, comprehensive analyses of *B. rapa* genes expressed only at one type of heat treatment, such as 45 °C (Dong et al. 2015, Quan et al. 2023), 42 °C (Yu et al. 2023) or 40 °C (Zhang et al. 2022) have been performed. We compared how different types of heat treatments affect the gene expression of heat-related *HSF* and *DREB2A* transcription factors and further determined HSP90 protein and phytohormone levels, which to our knowledge, has not previously been done in heat stress treated *B. rapa* seedlings.

At 37 °C, *HSFA7A*, *HSFB2A* and *DREB2A* were slightly enhanced, while the 45 °C-treatment significantly upregulated these genes (Fig. 4A). Both types of heat treatment enhanced HSP90 protein accumulation and induced the expression of new HSP90 forms (Fig. 4B). Additional HSP90 proteins recognised by immunodetection may be heat-induced splice variants, post-translationally modified HSP90 proteins or newly induced HSP90 proteins, as 17 genes encoding *HSP90* have been reported for *B. rapa* (Wang et al. 2022). Differential expression of HSP90s participates in the responses of *B. napus* to salt stress and *Sclerotinia sclerotiorum* infection (Wang et al. 2022), and in the response of *B. oleracea* to cold (Sajad et al. 2022), but as far as we know, no accurate expression analyses of individual HSP90 genes and proteins have been determined in *Brassica* species under heat stress.

In many plant species, Pro accumulates under heat stress and mediates tolerance by participating in signalling networks, helping to maintain redox balance as an ROS scavenger, and serving as an osmoprotectant (Raza et al. 2023). If they are not neutralised, ROS can damage proteins, lipids, carbohydrates and nucleic acids. Lipid peroxidation, characterised by the accumulation of MDA, destabilises cell membranes by increasing their fluidity. Increased Pro is confirmed for different *B. juncea* cultivars (Hayat et al. 2011) and rapeseed *B. napus* (Mohamed et al. 2020) subjected to abiotic stress. When *B. rapa* seedlings are exposed to 45 °C for 4 h, the MDA content increases significantly under heat stress (Rai et al. 2021). Here, *B. rapa* seedlings exposed to 37 °C showed no changes in Pro and MDA levels, while exposure to 45 °C immediately caused an accumulation of Pro that remained elevated for at least 2 days after the applied stress (Fig. 3). MDA accumulated with a delay of 2 days, indicating a heat-induced overproduction of ROS and consequent lipid peroxidation (Mittler et al. 2012), which led to an extreme reduction of plant growth after the short-term exposure to 45 °C (Fig. 2). Our results indicate that Pro and MDA are not suitable markers of heat stress when examining a single species or cultivar and their use as indicators of stress state depends on the type of temperature applied. However, comparing Pro and MDA accumulation among different cultivars for the purpose of screening and selecting those more resistant to a particular type of abiotic stress has been proven to be a good indicator for *Brassica* species (Pavlović et al. 2019, Bauer et al. 2022).

Although seedlings treated at 37 °C also showed significant growth deviations, they were accompanied by only small changes in Pro, MDA and heat-related gene expression levels so we further analysed heat treatment effects on phytohormone fluctuations that are known to be involved in *Brassica* species' stress tolerance mechanisms (Pavlović et al. 2018, 2019). Indeed, an exposure to either 37 °C or 45 °C had significant and differential effects on IAA, ABA and the immediate ethylene precursor ACC accumulation. These changes in phytohormone levels may be responsible for detected heat-related root growth disturbances (Figs. 2, 5). Phytohormones affect growth in a dose-dependent manner and their finetuning and interactions are especially important under stress exposure (Vanstraelen and Benková 2012). The perturbation in phytohormone levels under heat exposure mediates plant adaptive responses by impacting nutrient synthesis and allocation and guiding growth and developmental changes known as thermomorphogenesis (Casal and Balasubramanian 2019). Auxin, ABA and ethylene have a central role in determining root architecture, guiding the induction and growth of the main roots, lateral roots, adventitious roots and root hairs (Olatunji et al. 2017). They also promote hypocotyl elongation at elevated temperature (Emenecker and Strader 2020), which is in accordance with our results. Elevated IAA and ABA, and a decrease of the ethylene precursor ACC may be the main reasons for the changes induced at 37 °C in *B. rapa* seedling morphology, while significant changes of IAA seem to govern growth and developmental changes of seedlings exposed to short extreme heat stress (Figs. 1, 2). In accordance with previous studies (Gray et al. 1998, Bianchimano et al. 2023), IAA level was significantly enhanced after both heat treatments and IAA can be considered a good heat stress marker in *B. rapa* seedlings. ACC was barely detectable at 37 °C and showed no significant change upon short-term exposure to 45 °C, which could be due to the imbalanced activity of the ACC synthase and ACC oxidase enzymes as described by Antunes and Sfakiotakis (2000) for kiwifruit grown at 40 °C. ABA content increased at 37 °C but after exposure to 45 °C (followed by recovery) was close to the control value. We assumed that a strong heat-induced rise in ABA level during treatment at 45 °C would instantly suppress seedling root growth and time-dependent screening of ABA levels under heat will be considered in our future research.

Heat-induced fluctuations of different hormones are not unequivocal and uniform among different species and may vary under different heat treatments (Prerostova et al. 2020, Poór et al. 2022) which is in agreement with our results. *In planta* auxin and ethylene synergistically affect root elongation and root hair formation, but act antagonistically in lateral root initiation and hypocotyl elongation (Muday et al. 2012). ABA induces primary root growth and suppresses lateral roots at low concentrations (Tiwari et al. 2022) and is important in mediating plant adaptation to stress (Baron et al. 2012). ABA confers thermotolerance by supporting photosystem II (PSII), switching on antioxidants, producing osmolytes and inducing gene expression of HSFs and HSPs (Ahammed et al. 2016, Jha et al. 2022). We measured a significant induction of ABA levels at 37 °C, and control-like ABA levels after short-term heat treatment at 45 °C followed by a recovery period at 24 °C (Fig. 5B) indicating that ABA accumulation is possibly instantly and strongly affected by heat. Similar ABA perturbances are noticed in *S. lycopersicum* and *A. thaliana* plants treated at 40 °C. After initial heat-induced ABA accumulation, a post-treatment ABA decline was observed (Dobrá et al. 2015). ABA may also have a crucial role in heat-induced *B. rapa* seed germination arrest observed here (Fig. 1). Exposure to extreme heat blocked hypocotyl elongation, apical hook opening,

cotyledon opening, expansion and greening of *B. rapa* seedlings (Fig. 1). ABA is known to mediate inhibition of post-germination seedling establishment in the dark, while under light conditions, ABA is degraded enabling seedling greening and growth (Yadukrishnan and Datta 2021). Whether heat disturbs ABA degradation and induces seed germination and seedlings growth arrest of *B. rapa* should be further investigated in the future.

The results of our work highlight *B. rapa* vulnerability at early developmental stages. Prolonged, moderately elevated temperature changed the growth and development pattern by significant perturbation of phytohormone level and HSP90 protein accumulation. Short exposure to extreme heat significantly stimulates the expression of *HSFs* and *DREB2A* transcription factors, induces the accumulation of auxin IAA and HSP90 proteins, and almost stops seedling growth. The above confirms the great importance of phytohormones, especially IAA and ABA, in regulating *B. rapa* growth under heat stress and indicates the potential of targeting auxin and ABA metabolic and signalling pathways for phytoprotection or for use in breeding programs with aim to enhance stress tolerance and improve climate change adaptation.

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References

Ahammed, G. J., Yu, J. Q., Li, X., Zhou, J., Zhou, Y. H., Yu, J. Q., 2016: Role of hormones in plant adaptation to heat stress. In: Ahammed, G., Yu, J. (eds.), Plant hormones under challenging environmental factors, 1–21. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-7758-2

Ahmad, M., Imtiaz, M., Shoib Nawaz, M., Mubeen, F., Imran, A., 2022: What did we learn from current progress in heat stress tolerance in plants? Can microbes be a solution? Frontiers in Plant Science 13, 1–19. https://doi.org/10.3389/fpls.2022.794782

Anderson, R., Bayer, P. E., Edwards, D., 2020: Climate change and the need for agricultural adaptation. Current Opinion in Plant Biology 56, 197–202. https://doi.org/10.1016/j.pbi.2019.12.006

Angadi, S. V., Cutforth, H. W., Miller, P. R., McConkey, B. G., Entz, M. H., Brandt, S. A., Volkmar, K. M., 2000: Response of three *Brassica* species to high temperature stress during reproductive growth. Canadian Journal of Plant Science 80(4), 693–701. https://doi.org/10.4141/P99-152

Antunes, M., Sfakiotakis E. M., 2000: Effect of high temperature stress on ethylene biosynthesis, respiration and ripening of `Hayward` kiwifruit. Postharvest Biology and Technology. 20(3), 251-259. https://doi.org/10.1016/S0925-5214(00)00136-8

Baron, K. N., Schroeder, D. F., Stasolla, C., 2012: Transcriptional response of abscisic acid (ABA) metabolism and transport to cold and heat stress applied at the reproductive stage of development in *Arabidopsis thaliana*. Plant Science 188–189, 48–59. https://doi.org/10.1016/j.plantsci.2012.03.001

Bauer, N., Tkalec, M., Major, N., Talanga Vasari, A., Tokić, M., Vitko, S., Ban, D., Ban, S. G., Salopek-Sondi, B., 2022: Mechanisms of kale (*Brassica oleracea* var. *acephala*) tolerance to individual and combined stresses of drought and elevated temperature. International Journal of Molecular Science 23(19), 11494. https://doi.org/10.3390/ijms231911494

Begcy, K., Sandhu, J., Walia, H., 2018: Transient heat stress during early seed development primes germination and seedling establishment in rice. Frontiers in Plant Science 9:1768, 1–13. https://doi.org/10.3389/fpls.2018.01768

Bianchimano, L., De Luca, M. B., Borniego, M. B., Iglesias, M. J., Casal, J. J., 2023: Temperature regulation of auxin-related gene expression and its implications for plant growth. Journal of Experimental Botany 74(22), 7015–7033. https://doi.org/10.1093/jxb/erad265

Boter, M., Calleja-Cabrera, J., Carrera-Castaño, G., Wagner, G., Hatzig, S. V., Snowdon, R. J., Legoahec, L., Bianchetti, G., Bouchereau, A., Nesi, N., Pernas, M., Oñate-Sánchezm L., 2019: An integrative approach to analyze seed germination in *Brassica napus*. Frontiers in Plant Science 10:1342.. https://doi.org/10.3389/fpls.2019.01342

Boter, M., Pozas, J., Jarillo, J. A., Piñeiro, M., Pernas, M., 2023: *Brassica napus* roots use different strategies to respond to warm temperatures. International Journal of Molecular Sciences 24(2), 1143. https://doi.org/10.3390/ijms24021143

Casal, J., Balasubramanian, S., 2019: Thermomorphogenesis. Annual Review of Plant Biology 70. 321–346. https://doi.org/10.1146/annurev-arplant-050718-095919

Cohen, J. D., Baldi, B. G., Slovin, J. P., 1986: 13C6-[benzene ring]-indole-3-acetic acid. Plant Physiology 80(1), 14–19. https://doi.org/10.1104/pp.80.1.14

Dobrá, J., Černý, M., Štorchová, H., Dobrev, P., Skalák, J., Jedelský, P. L., Lukšanová, H., Gaudinová, A., Pešek, B., Malbecka, J., Vanek, T., Brzobohatý, B., Vanková, R., 2015: The impact of heat stress targeting on the hormonal and transcriptomic response in *Arabidopsis*. Plant Science 231, 52–61. https://doi.org/10.1016/j.plantsci.2014.11.005

Dong, X., Yi, H., Lee, J., Nou, I. S., Han, C. T., Hur, Y., 2015: Global gene-expression analysis to identify differentially expressed genes critical for the heat stress response in *Brassica rapa*. PLoS One 10, e0130451. https://doi.org/10.1371/journal.pone.0130451

Emenecker, R. J., Strader, L. C., 2020: Auxin-abscisic acid interactions in plant growth and development. Biomolecules 10(2), 281. https://doi.org/10.3390/biom10020281

Gray, W. M., Östin, A., Sandberg, G., Romano, C. P., Estelle, M., 1998: High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*. Proceedings of the National Academy of Sciences 95(12), 7197–7202. https://doi.org/10.1073/pnas.95.12.7197

Gunasekera, C., Martin, L., Siddique, K. H. M., Walton, G. H., 2006: Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*B. napus* L.) in Mediterranean-type environments. European Journal of Agronomy 25(1), 1–12. https://doi.org/10.1016/j.eja.2005.08.002

Hayat, S., Mir, B. A., Wani, A. S., Hasan, S. A., Irfan, M., Ahmad, A., 2011: Screening of salt-tolerant genotypes of *Brassica jun-*

cea based on photosynthetic attributes. Journal of Plant Interactions 6(1), 53–60. https://doi.org/10.1080/17429145.2010.521592

Heschel, M. S., Selby, J., Butler, C., Whitelam, G. C., Sharrock, R. A., Donohue, K., 2007: A new role for phytochromes in temperature-dependent germination. New Phytologist 174(4), 735–741. https://doi.org/10.1111/j.1469-8137.2007.02044.x

Hodges, D. M., DeLong, J. M., Forney, C. F., Prange, R. K., 1999: Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611. https://doi.org/10.1007/s004250050524

Jha, U. C., Nayyar, H., Siddique, K. H. M., 2022: Role of phytohormones in regulating heat stress acclimation in agricultural crops. Journal of Plant Growth Regulation 41, 1041–1064. https://doi.org/10.1007/s00344-021-10362-x

Kan, Y., Mu, X. R., Gao, J., Lin, H. X., Lin, Y., 2023: The molecular basis of heat stress responses in plants. Molecular Plant 16(10), 1612–1634. https://doi.org/10.1016/j.molp.2023.09.013

Kaushal, N., Bhandari, K., Kadambot, H. M., Siddique, K. H. M., Nayyar, H., 2016: Food crops face rising temperatures: An overview of responses, adaptive mechanisms, and approaches to improve heat tolerance. Cogent Food & Agriculture, 2(1). https://doi.org/10.1080/23311932.2015.1134380

Ludwig-Müller, J., Rattunde, R., Rößler, S., Liedel, K., Benade, F., Rost, A., Becker, J., 2021: Two auxinic herbicides affect *Brassica napus* plant hormone levels and induce molecular changes in transcription. Biomolecules 11(8), 1153–1175. https://doi.org/10.3390/biom11081153

Mittler, R., Blumwald, E., 2015: The roles of ROS and ABA in systemic acquired acclimation. Plant Cell 27(1), 64–70. https://doi.org/10.1105/tpc.114.133090

Mittler, R., Finka, A., Goloubinoff, P., 2012: How do plants feel the heat? Trends in Biochemical Sciences 37(3), 118–125. https://doi.org/10.1016/j.tibs.2011.11.007

Mohamed, I. A. A., Shalby, N., Bai, C., Qin, M., Agami, R. A., Jie, K., Wang, B., Zhou, G., 2020: Stomatal and photosynthetic traits are associated with investigating sodium chloride tolerance of *Brassica napus* L. cultivars. Plants 9(1), 62. https://doi.org/10.3390/plants9010062

Motsa, M. M., Slabbert, M. M., van Averbeke, W., Morey, L., 2015: Effect of light and temperature on seed germination of selected African leafy vegetables. South African Journal of Botany 99, 29–35. https://doi.org/10.1016/j.sajb.2015.03.185

Muday, G. K., Rahman, A., Binder, B. M., 2012: Auxin and ethylene: collaborators or competitors? Trends in Plant Science 17(4), 181-195. https://doi.org/10.1016/j.tplants.2012.02.001

Munns, R., Millar, A. H., 2023: Seven plant capacities to adapt to abiotic stress. Journal of Experimental Botany 74(15), 4308–4323. https://doi.org/10.1093/jxb/erad179

Murashige, T., Skoog, F., 1962: A revised medium for rapid growth and bio assays with tobacco tissue cultures. Plant Physiology 15, 474–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Olatunji, D., Geelen, D., Verstraeten, I., 2017: Control of endogenous auxin levels in plant root development. International Journal of Molecular Sciences 18(12), 2587. https://doi.org/10.3390/ijms18122587

Pavlović, I., Mlinarić, S., Tarkowská, D., Oklestková, J., Novák, O., Lepeduš, H., Vujčić Bok, V., Radić Brkanac, S., Strnad, M., Salopek-Sondi, B., 2019: Early Brassica crops responses to salinity stress: a comparative analysis between Chinese cabbage, white cabbage and kale. Frontiers in Plant Science, 10, 450. https://doi.org/10.3389/fpls.2019.00450

Pavlović, I., Petřík, I., Tarkowska, D., Lepeduš, H., Vujčić, V., Radić Brkanac, S., Novák, O., Salopek-Sondi, B. 2018: Correlations between phytohormones and drought tolerance in selected Brassica crops: Chinese cabbage, white cabbage and kale. International Journal of Molecular Sciences, 19(10), 2866; https://doi.org/10.3390/ijms19102866

Peleg, Z., Blumwald, E., 2011: Hormone balance and abiotic stress tolerance in crop plants. Current Opinion in Plant Biology 14(3), 290–295. https://doi.org/10.1016/j.pbi.2011.02.001

Pfaffl, M. W., 2001: A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Research 29(9), 45. https://doi.org/10.1093/nar/29.9.e45

Poór, P., Nawaz, K., Gupta, R., Ashfaque, F., Khan, M. I. R., 2022: Ethylene involvement in the regulation of heat stress tolerance in plants. Plant Cell Reports 41, 675–698. https://doi.org/10.1007/s00299-021-02675-8

Prerostova, S., Dobrev, P. I., Kramna, B., Gaudinova, A., Knirsch, V., Spichal, L., Zatloukal, M., Vankova, R., 2020: Heat acclimation and inhibition of cytokinin degradation positively affect heat stress tolerance of *Arabidopsis*. Frontiers in Plant Science 11, 1–14. https://doi.org/10.3389/fpls.2020.00087

Qin, H., He, L., Huang, R., 2019: The Coordination of ethylene and other hormones in primary root development. Frontiers in Plant Science 10, 874. https://doi.org/10.3389/fpls.2019.00874

Quan, J., Li, X., Li, Z., Wu, M., Zhu, B., Hong, S. B., Shi, J., Zhu, Z., Xu, L., Zang, Y., 2023: Transcriptomic analysis of heat stress response in *Brassica rapa* L. ssp. *pekinensis* with improved thermotolerance through exogenous glycine betaine. International Journal of Molecular Sciences 24(7), 6429. https://doi.org/10.3390/ijms24076429

Rai, A. N., Mandliya, T., Kulkarni, P., Rao, M., Suprasanna, P., 2021: Evolution and transcriptional modulation of lipoxygenase genes under heat, drought, and combined stress in *Brassica rapa*. Plant Molecular Biology Reporter 39, 60–71. https://doi.org/10.1007/s11105-020-01234-x

Rawlinson, C., Kamphuis, L. G., Gummer, J. P. A., Singh, K. B., Trengove, R. D., 2015: A rapid method for profiling of volatile and semi-volatile phytohormones using methyl chloroformate derivatisation and GC–MS. Metabolomics 11, 1922–1933. https://doi.org/10.1007/s11306-015-0837-0

Raza, A., Charagh, S., Abbas, S., Hassan, M. U., Saeed, F., Haider, S., Sharif, R., Anand, A., Corpas, F.J., Jin, W., Varshney, R.K., 2023: Assessment of proline function in higher plants under extreme temperatures. Plant Biology 25(3), 379–395. https://doi.org/10.1111/plb.13510

Rodríguez, V. M., Soengas, P., Alonso-Villaverde, V., Sotelo, T., Cartea, M. E., Velasco, P., 2015: Effect of temperature stress on the early vegetative development of *Brassica oleracea* L. BMC Plant Biology 15(1), 145. https://doi.org/10.1186/s12870-015-0535-0

Sajad, S., Jiang, S., Anwar, M., Dai, Q., Luo, Y., Hassan, M. A., Tetteh, C., Song, J., 2022: Genome-wide study of *Hsp90* gene family in cabbage (*Brassica oleracea* var. *capitata* L.) and their imperative roles in response to cold stress. Frontiers in Plant Science 13, 908511. https://doi.org/10.3389/fpls.2022.908511

Schneider, C. A., Rasband, W. S., Eliceiri, K. W., 2012: NIH Image to ImageJ: 25 years of image analysis HHS public access. Nature Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089

Seneviratne, S. I., Zhang, X., Adnan, M., Badi, W., Dereczynski, C., Di Luca, A., Ghosh, S., Iskandar, I., Kossin, J., Lewis, S., Otto, F., Pinto, I., Satoh, M., Vicente-Serrano, S. M., Wehner, M., Zhou, B., 2021: Weather and climate extreme events in a changing climate. doi: 10.1017/9781009157896.013. In: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M. I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J. B. R.,

Maycock, T. K., Waterfield, T., Yelekçi, O., Yu, R., Zhou B. (eds.), Climate change 2021: The physical science basis. Contribution of working group i to the sixth assessment report of the intergovernmental panel on climate change. Cambridge University Press, 1513–1766. Cambridge, United Kingdom and New York.

Staples, R. C., Stahmann, M. A., 1964: Changes in proteins and several enzymes in susceptible bean leaves after infection by the bean rust fungus. Phytopathology 54, 760–764.

Sunkar, R., Bartels, D., Kirch, H. H., 2003: Overexpression of a stress-inducible aldehyde dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance. Plant Journal 35(4), 452–464. https://doi.org/10.1046/j.1365-313X.2003.01819.x

Taylor, S. C., Posch, A., 2014: The design of a quantitative western blot experiment. BioMed Research International 2014, 361590–361598. https://doi.org/10.1155/2014/361590

Tiwari, M., Kumar, R., Min, D., Jagadish, S. V. K., 2022,: Genetic and molecular mechanisms underlying root architecture and function under heat stress—A hidden story. Plant, Cell & Environment 45(3), 771–788. https://doi.org/10.1111/pce.14266

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002: Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3, https://doi.org/10.1186/gb-2002-3-7-research0034

Vanstraelen, M., Benková, E., 2012: Hormonal interactions in the regulation of plant development. Annual Review of Cell and Developmental Biology 28, 463-487. https://doi.org/10.1146/annurev-cellbio-101011-155741

Villas-Bôas, S. G., Delicado, D. G., Åkesson, M., Nielsen, J., 2003: Simultaneous analysis of amino and nonamino organic acids as methyl chloroformate derivatives using gas chromatography-mass spectrometry. Analytical Biochemistry 322(1), 134–138. https://doi.org/10.1016/j.ab.2003.07.018

Wang, L., Liu, F., Ju, L., Xue, B, Wang, Y., Wang, D., Hou, D., 2022: Genome structures and evolution analysis of *HSP90* gene family in *Brassica napus* reveal the possible roles of members in response to salt stress and the infection of *Sclerotinia sclerotiorum*. Frontiers in Plant Science 13, 854034. https://doi.org/10.3389/fpls.2022.854034

Wilson, R. E., Jensen, E. H., Fernandez, G. C. J., 1992: Seed germination response for eleven forage cultivars of *Brassica* to temperature. Agronomy Journal 84(2), 200–202. https://doi.org/10.2134/agronj1992.00021962008400020015x

Yadukrishnan, P., Datta, S., 2021: Light and abscisic acid interplay in early seedling development. New Phytology 229, 763-769. https://doi.org/10.1111/nph.16963

Yamauchi, Y., Ogawa, M., Kuwahara, A., Hanada, A., Kamiya, Y., Yamaguchi, S., 2004: Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. Plant Cell 16(2), 367–378. https://doi.org/10.1105/tpc.018143

Yeh, C. H., Kaplinsky, J. N., Hu, C., Charng, Y., 2012: Some like it hot, some like it warm: Phenotyping to explore thermotolerance diversity. Plant Science 195, 10–23. https://doi.org/10.1016/j.plantsci.2012.06.004

Yu, J., Li, P., Tu, S., Feng, N., Chang, L., Niu, Q., 2023: Integrated analysis of the transcriptome and metabolome of *Brassica rapa* revealed regulatory mechanism under heat stress. International Journal of Molecular Sciences, 24(18), 13993. https://doi.org/10.3390/ijms241813993

Zhang, L., Dai, Y., Yue, L., Chen, G., Yuan, L., Zhang, S., Li F., Zhang, H., Li, G., Zhu, S., Hou, J., Tang, X., Zhang, S., Wang, C., 2022: Heat stress response in Chinese cabbage (*Brassica rapa* L.) revealed by transcriptome and physiological analysis. PeerJ 10:e13427. https://doi.org/10.7717/peerj.13427