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Heat-stress-induced changes in enzymatic antioxidant activities and biochemical processes in bell pepper (*Capsicum annuum* L.) seedlings

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Abstract: Heat stress (HS) is expected to become a significant abiotic stressor with the continued rise in global temperatures, severely limiting the development and production of bell pepper (*Capsicum annuum* L.), especially in arid and semiarid regions. Thus, this study looked into the impact of HS on the antioxidant activity, as well as the biochemical responses of bell pepper seedlings under varying temperatures (40, 32, and 25 °C) for 5 days. The results showed that exposure to higher temperatures led to an increase in enzymatic and antioxidant processes. At 40 and 32 °C, HS led to a decrease in chlorophyll a (4.327 mg g^{-1} FW), b (1.710 mg g^{-1} FW), total chlorophyll (5.202 mg g^{-1} FW), and total carotene (1.092 mg g^{-1} FW) compared to the control at 25 °C (chlorophyll a 5.383 mg g^{-1} FW, chlorophyll b 2.358 mg g^{-1} FW, total chlorophyll 6.692 mg g^{-1} FW, and total carotene 1.817 mg g^{-1} FW). On the other hand, seedlings exposed to 32 and 40 °C showed higher antioxidant activity and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid radical scavenging activity compared to the control group, as the activity significantly increased in response to high temperatures. Similarly, the catalase activity also significantly increased in response to 40 and 32 °C compared to the control group at varying temperatures. The proline content of the stressed seedlings increased at 32 and 40 °C compared to the control at all temperatures. These results indicate that HS damages the antioxidant activity, biochemical processes, and growth of bell pepper seedlings; thus, a scientific approach is needed to mitigate the effects in arid regions like Qatar.

Key words: Bell pepper, heat stress, antioxidant/enzymatic activity, a biochemical process, arid region

1. Introduction

In arid and semiarid regions like Qatar, high temperatures have a significant impact on crop growth and productivity. Plants can be genetically engineered through breeding to thrive and produce even in adverse conditions to address environmental stress challenges (Idrissa et al., 2022). To accomplish this, it is essential to acquire a deeper understanding of the mechanisms by which plants can adapt to heat stress (HS), maintain growth, and sustain productivity during challenging times. The activities and metabolic processes of plants are very sensitive to changes in temperature, and plants cannot survive or develop well under too much HS. To mitigate the effects of HS on crops in arid regions, it is essential to investigate and comprehend these dynamics. Generally, HS refers to a particular period of elevated temperature that leads to irreversible damage to plants, mostly happening when the rise in temperature levels exceeds the benchmark of 10–15 °C (Raza, 2020; Zahra et al., 2021; Raza et al., 2022; Saeed et al., 2023). HS alone will not directly affect plant growth and development but it does with the addition of other abiotic stresses such as drought, salinity, etc., due to their interactions. The increase in air temperature will result in a corresponding increase in the rate of transpiration, while in return causing greater water demand by plants for metabolic and biochemical reactions (Asim et al., 2021; Saeed et al., 2023). Moreover, the effect of HS often manifests in the soil temperature by increasing it, which ultimately intensifies the rate of evaporation, causing plant water availability reduction, resulting in water scarcity that might be temporary or prolonged, which is termed drought stress (Hassan et al., 2021; Hassan et al., 2021). Similarly, the occurrence of an increase in salt accumulation is imperative because of the higher rate

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of evaporation leading to salinity stress (Liu et al., 2021; Saeed et al., 2023). Moreso, nutrient stress is not inevitable as a result of the nutrient imbalances that could occur due to HS (Sakhonwasee et al., 2017; Safdar et al., 2019). No doubt, when plants are susceptible to HS, they undergo different biochemical, physiological, and molecular modifications (Demirel et al., 2017; Asim et al., 2021; Yildirim et al., 2021). Therefore, accurately evaluating the gravity of the impact of HS during critical growth and development for specific vegetable crops is very essential for good performance. Temperature fluctuations, as an environmental factor, have a detrimental impact on crop yields and quality (Rykaczewska, 2015; Lu et al., 2017; Scheelbeek et al., 2018; Kawasaki et al., 2019; Aleem et al., 2020). Plants respond to HS in many ways, including stomatal closure, leaf rolling, and osmotic adjustment, but the biochemical mechanisms behind these responses have not been fully explored. Recent evidence has shown that environmental stress often results in the formation of activated oxygen and free radicals through the disruption of electron transport systems, causing damage to membranes in plants under HS. The production of reactive oxygen species (ROS) superoxide (O2−), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH)/peroxides, singlet oxygen ($1[O_2]$ or $1O_2$)), and alpha-oxygen (α-O) are normal from the cellular metabolism of plants (Verma et al., 2019; Sharma et al., 2020; Mittler et al., 2022). However, different environmental stresses such as heat may cause excessive production of ROS, thus leading to oxidative stress in plants (Mazzeo et al., 2018). Furthermore, ROS hinders enzyme activity and causes deleterious effects on essential cellular components (Ergin et al., 2012; Krishnamurthy et al., 2013). Interestingly, the most important ROS-scavenging strategy includes superoxide dismutase, catalase (CAT), peroxidases, ascorbate peroxidase, and glutathione reductase (Demirel et al., 2017; Asim et al., 2021; Yildirim et al., 2021). In a previous study, it was shown that any alterations in the antioxidant enzymes will enhance the plant's resistance to excessive temperature and other abiotic stresses (Bello et al., 2021). Furthermore, high temperature is expected to become a hindering factor for bell pepper cultivation, and many other plant species, owing to the persistent increase in global warming, putting future farming in jeopardy. Thus, investigating the mechanisms associated with high-temperature stress in plants is inevitable to guarantee food security in the future.

Bell pepper is one of the vegetables that is cultivated year round under hydroponic systems with higher productivity during the spring in Qatar. Bell pepper is characterized by a high yield during this period when the temperature is mild and moderate. However, slightly elevated temperatures exceeding the optimum temperature during bell pepper flowering might result in blossom shedding and invariably affect the yield and quality. Nevertheless, these persistent temperature changes and harsh climatic conditions have been the regular circumstances in Qatar, which is in an arid region. Cultivation under a hydroponic system is a sustainable technology that is expected to mitigate these environmental stress issues during the summer, but the result is often contrary. Thus, it was exciting to investigate the antioxidant activities and biochemical responses in the leaves of bell pepper seedlings exposed to heat stress at different temperature levels to see the attributable effects associated with the temperature variation.

2. Materials and methods

2.1. Plant materials and growth parameters

The present study was conducted at the Department of Biological and Environmental Sciences (DBES), Qatar University within their greenhouse and growth chamber facilities. The seeds of bell pepper (*Capsicum annuum* L.), particularly the F1 hybrid, were procured from the Technical Agricultural Company, a licensed agricultural input supplier situated in Doha, Qatar. The seeds were sown in the germinating box inside the greenhouse. On day 30, when the seedlings had attained a healthy state, they were transplanted into 192-mL glass vases for cultivation in a hydroponic deep-water culture system utilizing the Hoagland solution shown in Figure 1. The seedlings were grown in a controlled, illumination, and aerated growth chamber (16:8 h day/night photoperiod, day/night temperatures: adjusted according to the treatments at 25, 32, and 40 °C, respectively), and the nutrient solution was replenished once on the third day.

2.2. HS tolerant assay

Exposure to HS was conducted using 30-day old bell pepper seedlings. For every replicate treatment, 16 seedlings were submerged into the Hoagland nutrient solution (Arnon, 1948) and 3 replicates were used (48 seedlings/per treatment). Three treatments: optimal/control (25 °C), moderate (32 °C), and extreme (40 °C) temperatures were applied, as shown in Figure 2. All of the treatments were done in replicates of 3 and subjected to 3 to 5 days of exposure during which the Hoagland nutrient solutions and the whole system were continuously monitored. The plant sampling commenced after the third day of exposure until the end of the exposure period. The collected plant samples were stored at –80 °C for further analysis.

2.3. Chlorophyll content analysis (acetone assay)

During and at the end of the exposure time after the completion of the treatments, approximately 12 to 14 fresh leaves were randomly selected from the plant shoot. The green leaves were macerated into a fractional part, in which approximately 0.5 g of the sample was homogenized in 10 mL of chilled 80% acetone in a mortar and pestle

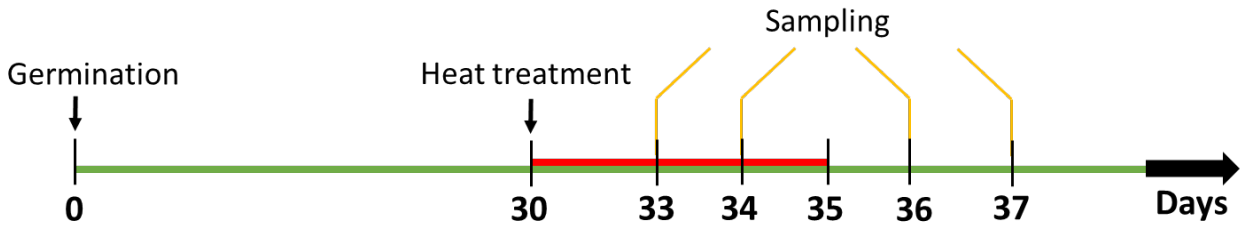

Figure 1. Experimental layout in the greenhouse and thereafter in the growth chamber.

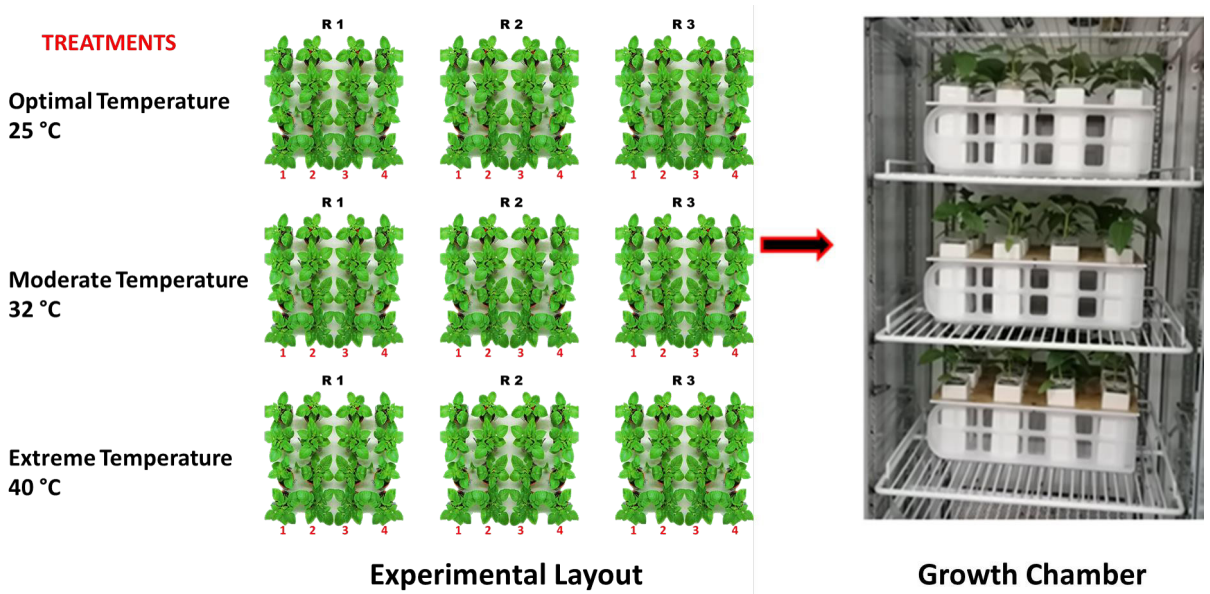

Figure 2. Scheme of the germination and heat treatment (age of the bell pepper plants)
Heat treatment was maintained for days 30–35, and during days 33–37, plant sampling was carried out.

(Azpack Mortar and Pestle; Thermo Fisher Scientific, UK). The final acetone extract was filtered (Advantec GC-50, Tokyo, Japan) and the final volume was scaled up to 20 mL with 80% acetone. Determination of the chlorophyll a, chlorophyll b, and total chlorophyll content (chlorophyll a + chlorophyll b) absorbance values was consequently obtained utilizing a Jenway 6715 ultraviolet (UV)-vis spectrophotometer (Cole-Parmer, Vernon Hills, IL, USA) at 2 wavelengths, 663 and 645 nm (Arnon, 1948; Saoussen et al., 2012), with 80% acetone as a reference (Kakade et al., 2020). The concentrations of the chlorophyll contents were finally determined using Eqs. (1) to (3) (Inskeep et al., 1985; Bello et al., 2022) below:

$$\text{Chlorophyll a (mg g}^{-1}\text{fw)} = \frac{12.7(A_{663}) - 2.79(A_{645})}{1000 \times W \times a} \times V. \quad (1)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{fw)} = \frac{20.7(A_{645}) - 4.62(A_{663})}{1000 \times W \times a} \times V. \quad (2)$$

$$\text{Chlorophyll a + b (mg g}^{-1}\text{fw)} = \frac{17.9(A_{645}) + 8.08(A_{663})}{1000 \times W \times a} \times V. \quad (3)$$

Here, A denotes the absorbance, a is the length of the light path in the cell (1 cm-constant), V is the extract volume in milliliters, and W is the fresh weight of the sample in grams.

2.4. Total carotene determination

Determination of the carotene followed the same pattern as the chlorophyll extraction explained above, except that the absorbance values were obtained at 3 different wavelengths. The total carotene was, therefore, determined spectrophotometrically at 470, 663, and 645 nm, respectively. Thereafter, the carotene content was calculated using the formula in Eq. (4) below:

$$\text{Total carotene } (C_{x+c}) = \frac{1000(A_{470}) - 1.82(C_a) - 85.02(C_b)}{198}. \quad (4)$$

Here, A is the absorbance, C_a is chlorophyll a, and C_b is chlorophyll b, respectively.

2.5. Proline content determination assay

The proline content assessments were carried out at the end of the experiment according to the methods of Bates et al. (1973) and Sapre et al. (2022). Extraction was performed using a sample of approximately 0.5 g of fresh expanded leaf material, chopped in 3% sulfosalicylic acid (w/v), and subsequently estimated by applying freshly prepared ninhydrin solution. The dissolved proline in the solution was separated by fractionation using toluene. The absorbance of the topmost liquid phase (fraction) was determined using the UV-vis spectrophotometer at 520 nm. Afterward, the concentration of the proline content was estimated from a calibration curve in the standard unit of μ mol. proline g^{-1} fresh weight (FW) after calculation as μmoles g^{-1} FW against standard proline (Sigma-Aldrich, Chemie, Germany) using Eq. (5):

$$\text{Proline content } (\mu\text{moles g}^{-1}\text{FW}) = \left\{\begin{matrix}(\mu\text{g proline ml}^{-1} \times \text{mL toluene}) \\ /115.5\end{matrix}\right\} \times \left\{\begin{matrix}5 \\ /(\text{g sample})\end{matrix}\right\} \quad (5)$$

Plant extraction

To investigate the CAT activity, fresh leaf tissue weighing 0.5 g was submerged in liquid nitrogen, and the frozen leaf tissue was ground in 5 mL of freshly prepared extraction buffer that contained 0.1 M of phosphate buffer with a pH range of 7 to 7.5, 0.0005 M of ethylenediaminetetraacetic acid, as well as 0.1% polyvinyl pyridine. Next, centrifugation of the mixture was performed at 15,000x *g* at 4 °C for 1200 s. The supernatant obtained was utilized for various analyses, as described below. The preparation as well as the activity of the enzyme were kept at a constant temperature of 4 °C throughout the process.

2.6. 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) assay

After the exposure period, determination of the antioxidant capacity was immediately carried out on the leaf tissues. First, fresh leaves were taken from the 3 replicates at random. To extract the enzyme and subsequent measurement of its activity, the instructions outlined in the assay kit were followed (Antioxidant assay CS0790, Sigma-Aldrich Co., LLC., St. Louis, MO, USA). Briefly, approximately 10^2 mg of liquid nitrogen frozen leaf tissue was ground, and then homogenized in 1X assay buffer (0.5 mL). The mixture was centrifuged at 12,000 *g* and 4 °C for 15 min. Subsequently, the assay was carried out on a 96-well plate. Wells 1 to 12 were used for the synthetic Trolox, which served as the standard to generate a calibration curve for quantifying the antioxidant activities. Trolox standard solution (10 μL) and Myoglobin working solution (20 μL) were subsequently added to each well. The experimental sample wells 1 to 12 contained 10 μL of leaf tissue sample, 20 μL of Myoglobin, and 1.5×10^2 μL of ABTS working solution to form the final mixture. After incubating the homogenates for 5 min at room temperature, the reaction was terminated with the addition of 10^2 μL of stop solution, which had been warmed to room temperature and blended to attain homogeneity before it was added to each well. The endpoint absorbance was measured using the plate reader at 405 nm. The antioxidant activity of the leaf tissue samples was determined using Eq. (6) below. The results of the antioxidant activities were expressed as the Trolox equivalents per gram of the FW of the samples (mM of Trolox/g FW) (Xia et al., 2017; Bello et al., 2022).

$$\text{X(mM)} = \frac{\text{y } (A_{405}) - \text{Intercept}}{\text{Slope}} \times \text{dilution factor} \quad (6)$$

Here, X (mM) is the concentration of antioxidant, Intercept is the interception of the Y-axis by the standard curve, y (A_{405}) is the mean average of the leaf tissue sample absorbance at 405 nm, and Slope is the slope of the standard curve, while the need to use a dilution factor only emanated if sample dilution was required before its addition to the well, which was regarded as the original sample dilution fold.

2.7. Analysis of the CAT activity

The measurement and estimation of the CAT activity were carried out according to the method of Aebi et al. (1984). The reaction mixture was prepared from the combination of 0.1 mL of enzyme extract, 9.9. ml of 0.1 M phosphate buffer (pH 7.0), and 0.5 mL of 0.03 M H_2O_2, which added up to 1.5 mL as the final volume. The H_2O_2 was added last, and absorbance was read at 240 nm. The enzymatic reaction of breaking down the enzymes and the final disappearance of the substrate (H_2O_2) were closely monitored for 30 min as the absorbance decreased. This is summarized below in Eq. (I):

$$H_2O_2 \xrightarrow{CAT} 2H_2O + O_2 \quad (I)$$

This shows the catalytic metabolism of breaking down H_2O_2 to give H_2O and O_2, respectively.

The CAT activity was computed using Eq. (II) below:

$$\text{CAT activity } (\mu\text{mol mg}^{-1} \text{ protein min}^{-1} = \Delta A_{240}(1000|\varepsilon_i * \text{PC}) \quad (II)$$

Here, the change in CAT absorbance at 240 nm is denoted as ΔA_{240}, ε_i is the coefficient of extinction, and the protein content is represented as PC.

2.8. Data analysis

The data were processed using SPSS Statistics for Windows 17.0 (SPSS Inc., Chicago, IL, USA) software. The statistical significance of the obtained data was determined by one-way analysis of variance (ANOVA) and reported as the mean ± standard deviation (SD). The mean comparison

of the treatment values was obtained using the post hoc Tukey honestly significant difference test at $p \leq 0.05$ (Tukey's test for mean comparisons).

3. Results

3.1. Effects of HS on the chlorophyll pigments and carotene

Exposure to HS had a significant effect on the chlorophyll a and b contents, and total chlorophyll content, as shown in Table 1.

The increase in temperature during the entire period had a negative impact on these contents when compared with the control, as visually observed. Under regulated conditions, the bell pepper seedlings had the highest chlorophyll a (5.383 mg g^{-1} FW), chlorophyll b (2.358 mg g^{-1} FW), and total chlorophyll (6.692 mg g^{-1} FW) values at the optimal temperature of 25 °C, respectively, compared with the moderate temperature of 32 °C; chlorophyll a (4.705 mg g^{-1} FW), chlorophyll b (1.876 mg g^{-1} FW), and total chlorophyll (5.674 mg g^{-1} FW). However, when the bell pepper seedlings were subjected to the extreme temperature of 40 °C, a noticeable reduction in chlorophyll a, chlorophyll b, and total chlorophyll content was equally recorded as 4.327, 1.710, and 5.202 mg g^{-1} FW, respectively. In terms of the HS impact, the average chlorophyll concentration decreased compared to the control at 32 and 40 °C by 12.614% for chlorophyll a, 20.44%, for chlorophyll b, and 15.213% total chlorophyll; and 19.610% for chlorophyll a, 27.481%, chlorophyll b, and 22.26%, total chlorophyll, respectively. The minimum decrease in the chlorophyll concentration was for chlorophyll a, while the maximum was chlorophyll b. Interestingly, it is also important to note that at the maximum temperature, the bell pepper seedlings did not show any signs of wilting or chlorosis despite the significant decrease in the chlorophyll a and b, and total chlorophyll content, respectively. The carotene content followed a similar trend, as it reduced as the temperature increased when compared with the control. At 25, 32, and 40 °C, the carotene content was 1.817, 1.094, and 1.092 mg g^{-1} FW, respectively, as shown in Table 2.

3.2. Effect of HS on the proline content

An assessment of the proline content showed a considerable increase (μmol g^{-1} FW) in the leaves of the bell pepper seedlings in response to HS. The highest proline content was seen with the extreme temperature of 40 °C (4.064 μmol. g^{-1} FW), followed by the moderate temperature of 32 °C (3.261 μmol. g^{-1} FW) compared with the control, at

Table 1. ANOVA showing the statistically significant effect of various treatments on the pigments (chlorophyll a and b, total chlorophyll, and carotene) composition.

Source of variation	DF	ANOVA - mean square			
		Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotene (mg g^{-1} FW)
Treatments	2	3.4350*	1.3599*	6.960*	2.0957*
Error	33	0.9540	0.1715	1.410	0.1043
Total	35				
p-value		0.039	0.02	0.013	0.000

* Significant statistical difference at 0.05 (5%), ns: not significant, ** indicates a significant difference at 5% significance ($p < 0.05$).

Table 2. Effects of the heat treatments on the chlorophyll and carotenoid contents of the bell pepper seedlings (mean ± SD).

Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotene (mg g^{-1} FW)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control 25 °C	5.383 ± 0.997 a	2.358 ± 0.412 a	6.692 ± 1.195 a	1.817 ± 0.514 a
Moderate 32 °C	4.705 ± 0.468 ab	1.876 ± 0.217 b	5.674 ± 0.591 ab	1.094 ± 0.102 b
Extreme 40 °C	4.327 ± 1.284 b	1.710 ± 0.545 b	5.202 ± 1.566 b	1.092 ± 0.196 b

Mean values that do not share a letter are significantly different according to the Tukey pairwise comparisons of the treatments.

25 °C (0.938 μmol. g^{-1} FW), respectively. Consequently, the mean fold of the proline accumulation over the control followed the trend: extreme (76.92%) > moderate (71.24%), respectively, as shown in Table 3.

Hence, HS significantly increased the production of free proline ($p < 0.05$), as higher accumulation levels of proline occurred in at moderate and extreme temperatures, but at different concentrations, as shown in Table 4.

However, the proline content greatly relies on the age of the plant, the leaves, and the position of the leaves or leaf parts (Chiang et al., 1995; Mafakheri et al., 2010). Generally, at the vegetative stage, HS increased the accumulation of proline by multiple folds to mitigate the impact of stress on the plant. Interestingly, proline accumulation has been considered a very reliable factor/parameter of consideration for stress resistance (Mafakheri et al., 2010)

3.3. ABTS assay

The effect of HS on the antioxidant activity of the leaves of the bell pepper seedlings is shown in Table 3. After 5 days of HS treatment, the antioxidant activities of the bell pepper leaves were measured using ABTS assay, indicating a tremendous increase compared to the control, optimum 25 °C (0.6339 mM of TE g^{-1} FW). The activity was highest with the extreme temperature of 40 °C (1.8118 mM of TE g^{-1} FW), followed by the moderate temperature of 32 °C (1.6333 mM of TE g^{-1} FW), respectively.

3.4. Effect on the CAT activity

It can be seen in Table 3 that the effect of HS on the bell pepper seedling leaves after 5 days of HS treatment caused a significant increase in the CAT activity compared with the control. At an extreme temperature (40 °C), the CAT activity significantly increased (8.34 units mg of $protein^{-1}$ min^{-1}) and at a moderate temperature (32 °C), there was a significant increase (5.72 units mg of $protein^{-1}$ min^{-1}) compared to the control (25 °C), where the activity was maintained (3.86 units mg of $protein^{-1}$ min^{-1}).

4. Discussion

The bell pepper plant is often exposed to abiotic stresses that negatively affect its vegetative growth, physiological development, and productivity. Of all these stresses, drought, and HS are responsible for the most destruction in plant development. Generally, plant responses to extreme temperatures are regulated by their genetic potential to withstand it and their ability to develop a tolerance to HS. In this study, when the seedlings of bell pepper (*Capsicum annuum* L.) were subjected to an extreme temperature of 40 °C, the pigment contents, such as chlorophyll a and b, total chlorophyll, and carotene, exhibited a significant decrease compared to the control. This decline in the pigment content is a result of structural damage to the chloroplasts within the seedlings. Surprisingly, even at a comparatively

Table 3. Effects of the heat treatments on the proline content, Trolox equivalent, and CAT activity of bell pepper.

Treatments	Proline (μmoles g^{-1} FW)	ABTS (mM of TE g^{-1} FW)	CAT (unit mg $protein^{-1}$ min^{-1})
	Mean ± SD	Mean ± SD	Mean ± SD
Control 25 °C	0.9379 ± 0.07 a	0.6339 ± 1.284 a	3.861 ± 0.653 b
Moderate 32 °C	3.261 ± 0.71 b	1.6333 ± 0.468 b	5.718 ± 1.289 b
Extreme 40 °C	4.064 ± 0.79 c	1.8118 ± 0.997 b	8.339 ± 1.289 a

Values are the mean of 3 replicates ± SD. Different letters within each column show statistically significant differences at 5% ($p \leq 0.05$).

Table 4. ANOVA showing the statistically significant effects of various treatments on the proline content, Trolox equivalent, and CAT activity.

Source of variation	DF	ANOVA - mean square		
		Proline (μmoles g^{-1} FW)	ABTS (mM of TE g^{-1} FW)	CAT (unit mg $protein^{-1}$ min^{-1})
Treatments	2	31.6248*	4.83623*	15.1836*
Error	33	0.3769	0.09836	0.8811
Total	35			

* Significant statistical difference at 0.05 (5%), ns: not significant, ** indicates a significant difference at 5% significance ($p < 0.05$).

moderate temperature of 32 °C, similar negative effects were observed. The decline in the chlorophyll content, as found in the HS-susceptible bell pepper seedlings, complies with the results of a study conducted by Wang et al. (2022) and other researchers, in which they reported a decline in chlorophyll a and b, and total chlorophyll content (Rossi et al., 2017; Mattila et al., 2018; Şimşek et al., 2018; Yildirim et al., 2021). Moreover, it has been reported that depending on the severity of the abiotic stress, such as HS and drought, different proline synthesis patterns have been observed (İpek, 2015; Yildirim et al., 2021). As an osmotic regulator, the increase in proline synthesis decreased the water potential of the cells and made them more tolerant of high evaporation by preventing water loss between them. Proline is an osmotic regulator in addition to its biological role as an energy source and antioxidant (Hussain et al., 2019). Several studies have also indicated that proline, the main osmolyte, increased in almost all plants under stress, offering a defense against stressful situations (Abid et al., 2018; Hussain et al., 2019). Rajametov (2021) found that proline synthesis increased in stressed pepper plant tissues, indicating that enhanced proline resistance levels can be a sign of a plant's ability to withstand stress. Additionally, proline synthesis has been found to increase under stress conditions in previous studies (Fiasconaro et al., 2019; Furlan et al., 2020; Yildirim et al., 2021). Therefore, proline accumulation was found to be positively correlated with HS severity in the current study; thus, proline accumulation increases as the HS severity increases, which supported these previous studies. Moreover, in the present study, after exposing the bell pepper seedlings to different temperatures, the HS caused a significant increase in the CAT enzyme activity, which is a probable indication that an antioxidant enzyme could be triggered to scavenge or remove ROS, such as O_2^-, H_2O_2, OH^-, etc., to countereffect their harmful effects. The significant increase in the CAT activity is attributable to the scavenging of H_2O_2. The findings in this study concerning the significant increase in antioxidant enzymatic activity were supported by several reports (Zrig et al., 2015; Yildirim et al., 2021).

5. Conclusion

Based on the findings herein, an increase in the antioxidant activity and biochemical processes as indicators of the HS response demonstrated effectiveness at scavenging ROS under high stress, which is responsible for higher membrane degradation and reductions in photosynthetic activities. Thus, it is evident that a single indicator or set of indicators can be identified as the sole factor responsible for the HS effects on the bell pepper seedlings. However, these results established that there is a deleterious effect of HS stress on bell pepper production, while these findings add to our understanding of how bell pepper adapts to HS in unfavorable environments and provide novel insights into the mechanisms involved. Future studies will focus on the investigation of how bioresource extract (plant-derivative biostimulant) can mitigate these adverse effects.

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Author contributions

Bello AS: Conceptualization, methodology, investigation, biochemical, and statistical analysis, writing original draft, preparation of the final manuscript, review, and editing; Ahmed T: supervision, experimental design, statistical analysis, review, and editing, resources; Saadaoui I: conceptualization, review, and editing; Hamdi H: methodology, review, and editing; Ben-Hamadou R: supervision, methodology, resources, review, and editing.

All of the authors have carefully gone through and agreed to the published version of the manuscript.

Conflict of interest

The authors unanimously declared that no conflicts of interest exist between them.

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