INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2023, Vol. 2, NO. 1, 59-68.
doi: 10.56612/ijaeb.v2i1.51
https://eminentscientists.com/

Exogenous applications of salicylic acid alleviate the damaging effects of heat stress in chili (*Capsicum frutescens* L.) through improved antioxidant defense system

Fozia Saeed[1], Sumaira Rasul[1], Sidra Batool[1], Zafar Ullah Zafar[2], Hamid Manzoor[1]

[1]Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan 60800, Pakistan
[2]Institute of Botany, Bahauddin Zakariya University, Multan 60800, Pakistan

Abstract

Due to global climate change, heat stress is a critical environmental factor for adversely affecting plant growth and productivity. It induces changes in cellular machinery and antioxidant enzymes' functions in plants. The role of phytohormones especially salicylic acid (SA) is well documented to alleviate the heat stress-induced adverse effects in plants. Here, we investigated the ameliorative role of SA in heat-stressed chili (*Capsicum frutescens* L.) plants. For this purpose, four-week-old chili plants of three local cultivars (Moro, Tilhari and Ren-02) pretreated with 1 mM and 2 mM SA were subjected to heat stress (42 °C) for 72 h. Our results revealed that heat stress significantly reduced plant height, shoot biomass, chlorophyll contents and relative water contents, while it increased membrane peroxidation and hydrogen peroxide in all three chili cultivars. However, foliar spray with both concentrations of SA (particularly 2 mM) showed prominent recovery effects in high temperature exposed chili cultivars (Moro and Ren-02) with significantly improved biomass production, photosynthetic pigments, relative water contents, and catalase and peroxidase activities compared to their respective controls. Moreover, the foliar spray of 2 mM SA significantly reduced hydrogen peroxide and malonaldehyde contents under the heat stress conditions. In conclusion, SA significantly improved heat tolerance of chili plants by decreasing oxidative stress via increasing antioxidant activity and membrane stability.

HANDLING EDITOR
Muhammad Ashraf

ARTICLE HISTORY
Received: 17 Jul 2021
Accepted: 20 Dec 2022
Published: 30 Dec 2022

KEYWORDS
Heat stress;
Hormones;
Plant growth regulators;
Chlorophyll contents;
Oxidative damage;
Antioxidant enzymes

Introduction

As sessile organisms, plants unavoidably encounter various abiotic stress factors that are detrimental to their growth and productivity. Among these environmental stresses, heat stress (almost 10–15 °C higher than ideal growth temperature) negatively affects cellular catabolism and anabolism, seedling growth and development (Mittler et al., 2012; Abdelrahman et al., 2020). Plant species exhibit varying responses to heat stress depending on plant type, temperature range and exposure time. Heat stress disturbs plant growth through cell division alterations, root and stem growth inhibition, plant relative water content reduction, and decreased photosynthetic pigments and plant photosynthetic activity that lead to biomass reduction (Hasanuzzaman et al., 2013). Heat stress may cause significant protein damage, disturb protein synthesis, inactivate key enzymes, and damage membranes (Fichman and Mittler, 2020). High temperature causes oxidative damage ultimately resulting in cellular and metabolic imbalance (Szymańska et al., 2017; Cohen et al. 2021). All these above-mentioned changes can adversely affect plant growth, development and, finally, the yield.

***CONTACT** Hamid Manzoor, 💻 dr.hamid@bzu.edu.pk, ☎ +92 61 9210463 Ext: 3902 🖂 Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan 60800, Pakistan

TO CITE THIS ARTICLE: Saeed, F., Rasul, S., Batool, S., Zafar, Z.U., Manzoor, H. (2023). Exogenous applications of salicylic acid alleviate the damaging effects of heat stress in chili (*Capsicum frutescens* L.) through improved antioxidant defense system. *International Journal of Applied and Experimental Biology* 2(1): 59-68.

Plants activate various morphological and phenological adaptations to acclimate heat stress. For example, plants avoid or tolerate heat stress by over-production of heat shock proteins and osmoprotectants as well as limiting water loss with reduced transpiration (Ghani et al., 2020). To deal with overloading reactive oxygen species (ROS), plants have developed many protective strategies including production of non-enzymatic and enzymatic antioxidants (Zhao et al., 2020). The role of ROS scavenging enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) are well documented to boost plant tolerance to heat stress. Whereas, carotenoids are among the non-enzymatic ROS scavengers that prevent plant pigments and fatty acids from free radical-induced damage and maintain the physical characteristics of photosynthetic membranes (Gruszecki et al., 1991). However, under extremely stressful conditions, antioxidant potential may not be adequate to mitigate the adverse effects of oxidative damage. Therefore, searching for chemical messengers that trigger stress resistance with an improved antioxidant defense system is an essential step toward understanding how plants adapt to extreme conditions.

Salicylic acid (SA) is a natural phenolic phytohormone that activates plant's defense system and promotes growth and development. The key role of SA in regulating plant responses to a variety of abiotic stresses like drought, salinity, ultraviolet light, chilling, heat, etc. is well-documented (Chavoushi et al., 2019; Abdelaal et al., 2020; Bukhat et al., 2020; Hasan et al., 2020; Miao et al., 2020). Jahan et al. (2019) reported that SA foliar spray stabilized membrane damage and alleviated heat stress effects via increased antioxidant enzymes' activity in tomatoes. Foliar spray of SA in alfalfa plants subjected to heat stress resulted in increased plant biomass by an improved height and photosynthetic efficiency, reduced electrolyte leakage (EL) and malondialdehyde (MDA) contents, and significant elevations in the activities of POD, CAT and SOD (Wassie et al., 2020). Munir and Shabbir (2018) showed that SA treatment (foliar and seed) to different heat-stressed wheat cultivars led to the activation of enzymatic antioxidants and enhancement in chlorophyll and proline contents as well as net yield increase. These findings suggest a protective role of SA in inducing high-temperature tolerance in plants.

Chili (*Capsicum frutescens* L.) is among the most widely used vegetable and spice around the world. Globally, chili production exceeded to 38 million tons in 2019 (FAOSTAT, 2019). In Pakistan, chilies are grown on a large scale as a cash crop with an estimated production of 126,943 tons during 2018-19 (http://www.mnfsr.gov.pk/). Chili has an optimum growth temperature of 22–30 °C. However, rise in temperature above this optimal level significantly affects vegetable production by disturbing the physiological and biochemical functions of chili plants. As little information on the mechanism of heat tolerance in chili is available in the literature, the present research was conducted to elucidate the SA role in regulating key physiological processes in chili plants exposed to heat stress.

Materials and Methods

Seed collection and growth conditions

Ayub Agricultural Research Institute, Faisalabad, Pakistan provided seeds of three *Capsicum frutescens* L. varieties, Tilhari, Moro, and Ren-02. A triplicate pot experiment was carried out in the following controlled conditions of the growth room: 16/8 h light/dark cycle at 30/25 °C (day/night) with 80% relative humidity. Seeds were grown in 10-cm-diameter plastic pots filled with sandy loam soil and were irrigated with Hoagland's nutrition solution. Four-week-old chili plants were treated through leaves with 0, 1, and 2 mM SA. After two days of SA spray, one group of plants was kept as control, while another group was heat stressed in the growth chamber with a day/night temperature stress of 42 °C/34 °C. After 72 h of heat treatment, the plant samples were collected to perform various biochemical and physiological assays.

Measurement of growth parameters

For shoot and root length measurement, plants were precisely rooted out from the pots and washed with water to remove the attached sand particles. Shoot and root lengths were then recorded with a measuring scale. The fresh weights of the shoot and the root were measured by a digital weighing balance. To measure dry biomass, the samples were dried in an oven (65 °C) for 7 days, and dry biomass for root and shoot were recorded separately.

Estimation of relative water content

Relative water contents (RWC) were estimated following the recommendations of Conroy et al. (1988). Chili leaf discs, each of 1.5 cm^2 diameter, were used for the estimation of fresh, dry and turgid weights. The following formula was used to calculate plant RWC percentage:

$$\text{RWC (\%)} = (\text{FW}-\text{DW})/(\text{TW}-\text{DW})\times 100$$

Estimations of chlorophyll and carotenoid contents

Arnon's method (1949) was used to estimate the amount of chlorophyll *a, b* and carotenoid contents. Briefly, chili leaf samples (each 0.2 g) were ground with 80% acetone (10 mL). The homogenate was filtered and then centrifuged (6000 x *g*) at 4 °C for 15 min. Then, the absorbance of the individual samples was recorded at 645, 663 and 480 nm using a spectrophotometer. Final calculations were made by using Arnon's equation (1949).

Estimation of lipid peroxidation

Malondialdehyde (MDA) levels in leaf tissues were quantified to estimate the lipid peroxidation using the Cakmak and Horst (1991) method with minor modification. Each (0.2 g) of the collected samples was ground in a pestle mortar using 0.1% trichloroacetic acid (TCA) solution (1 mL). The resulting homogenate was then centrifuged at 2000 x *g* for 15 minutes. Then, 1 mL of thiobarbituric acid (TBA) solution (0.5% TBA and 20% TCA) was mixed with 0.5 mL of the supernatant. The reaction mixture was given heat treatment at 95 °C for 50 min in a shaking water bath. The tubes were immediately transferred to an ice box to stop the reaction process. Final centrifugation was made at 12000 x *g* for 10 minutes. The absorbance readings of the collected supernatant were taken at 532 and 600 nm. Lipid peroxidation was quantified through the below given formula:

$$\text{Malondialdehyde (MDA)} = \Delta(\text{A 532 nm} - \text{A 600 nm})/1.56\times10^5$$

H_2O_2 measurement

Hydrogen peroxide (H_2O_2) concentration was calculated using the method of Velikova et al. (2000). Fresh leaf (0.2 g) sample was ground by adding 2 mL TCA (0.1%), and the homogenate was subjected to 6000 x *g* centrifugation for 15 minutes. Then, 100 µl of 50 mM phosphate buffer (pH 7.2) and 200 µl of 1 M potassium iodide were added in 100 µl of the obtained supernatant. The absorbance was spectrophotometrically recorded at 390 nm. Finally, H_2O_2 concentration was calculated with a standard curve.

Enzymatic antioxidants activity

Antioxidant enzymes' activities were appraised following the method of Ananieva et al. (2004). In brief, a proportion (200 mg) of fresh leaf tissue was extracted in 2 mL of chilled sodium phosphate (50 mM) buffer (pH 7.8) followed by 20 minutes centrifugation at 15000 *g* using a refrigerated centrifuge (4 ^{o}C). The resulting supernatant was used for the quantification of enzyme activities. Peroxidase (POD) enzyme activity was estimated with the protocol suggested by Chance and Maehly (1955). Enzyme extract (50 µl) was transferred into a tube containing 250 µl H_2O_2 (40 mM), 200 µl guaiacol (20 mM) and 1 mL of phosphate buffer (50 mM, pH 7.0). Finally, the changes in absorbance values due to guaiacol oxidation were read at 470 nm for 2 minutes with a 20 second interval. POD activity was measured by using the molar extinction coefficient (ε) of guaiacol (26.6 mM^{-1} cm^{-1}). Catalase activity was estimated following Chance and Maehly (1955). Briefly, 50 µl enzymatic extract was added in a tube containing 1 mL of phosphate buffer (50 mM, pH 7.0) and 450 µl of hydrogen peroxide (5.9 mM). Optical density was measured spectrophotometrically at 240 nm for 1 min with a 20 seconds interval. The activity of catalase was measured using a molar extinction coefficient (ε) of hydrogen peroxide (36 M^{-1} cm^{-1}).

Statistical analysis

To calculate the treatment differences, the data collected during this work were analyzed with an ANOVA (analysis of variance) test using the statistical tool, COSTAT version 6.3 (Cohort Software, Berkley, California). Similarly, the least significance difference test (LSD test) was conducted to compare treatment means ($P < 0.05$).

Results

Heat stress significantly decreased plant height, and shoot fresh and dry weights in all three chili cultivars. However, all these parameters showed a significant improvement by the supplementation of varying concentrations of SA (1 mM and 2 mM; **Figure 1**). Out of three investigated cultivars, Moro and Ren-02 showed a maximal increase in plant height after 2 mM SA treatment under heat stress (Table 1, **Figure 1e**). Similarly, Moro and Ren-02 showed a maximal increase in shoot fresh and dry weights with pretreatment of 2 mM of SA under heat stress, whereas Tilhari showed a similar response in all these parameters at both SA levels (**Table 1; Figure 1a and 1b**). Heat stress also suppressed the fresh and dry weights of root and their length in all chili cultivars (**Figure 1 c-d and f**). The foliar spray of salicylic acid improved these parameters, but the interaction SA × Heat × Var was non-significant for these physiological parameters.

SA had an interactive impact on the photosynthetic pigments of plants during heat stress in all three chili varieties. Chlorophyll (*a*, *b*, total) and carotenoids reduced prominently after heat stress, and a maximal decrease was observed in Tilhari (**Figure 2a, b, c and d**). In all chili cultivars, exogenous treatment of SA, specifically 2 mM, significantly increased the photosynthetic pigments. This recovery effect of SA was maximum in Moro and Ren-02; however, in Tilhari, both concentrations of SA (1 & 2 mM) were effective (**Table 1; Figure 2a-d**).

Heat stress significantly decreased RWC in all three investigated chili varieties. Heat induced relative water content reduction in different chili cultivars was alleviated by foliar-applied SA (1 and 2 mM) under both control and heat stress conditions (**Table 1; Figure 2**). Foliar spray of 2 mM SA had a maximal increasing effect on the relative water content of Moro and Ren-02 in contrast to that in Tilhari, which showed the best response at 1 mM level of SA.

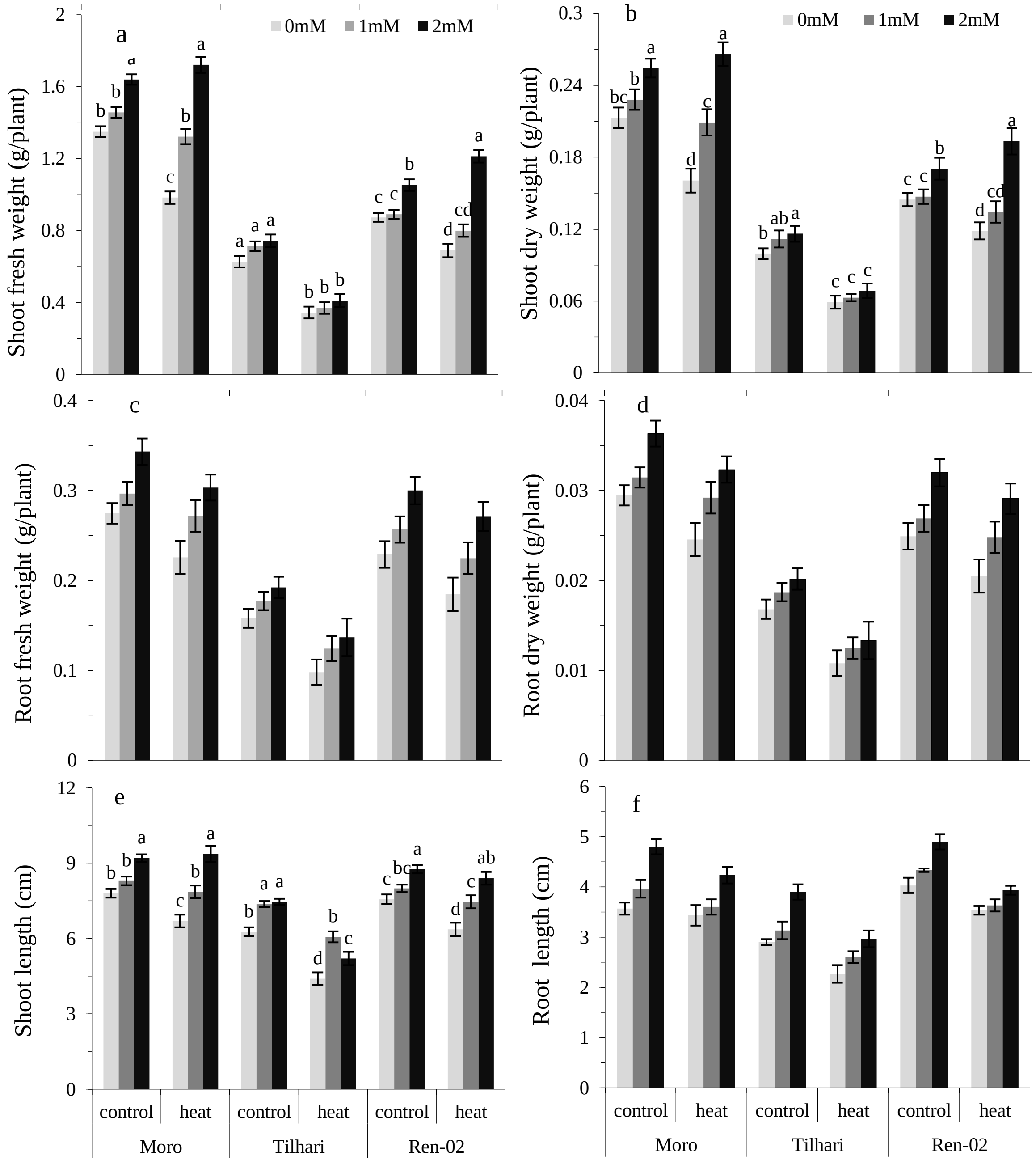

Figure 1. Effects of heat stress and foliar application of salicylic acid (0, 1 and 2 mM) on the biomass of three chili cultivars (a) Shoot fresh weight, (b) Shoot dry weight, (c) Root fresh weight, (d) Root dry weight, (e) Shoot length, and (f) Root length when chili plants were subjected to high temperature (42 °C for 72 h). Bar graphs represent mean values ± SE of three independent biological replicates (*n* = 9). Different letters indicate significant difference between the mean values at *P* < 0.05.

Table 1. Analysis of variance test for shoot fresh weight (Shoot FW), shoot dry weight (Shoot DW), root fresh weight (Root FW), root dry weight (Root DW), shoot length, root length, chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, relative water content (RWC), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and activities of catalase (CAT) and peroxidase (POD) enzymes of three chili cultivars under control and heat stress conditions.

Source of variation	df	Shoot FW	Shoot DW	Root FW	Root DW	Shoot length
Heat	1	0.37***	0.008***	0.025***	2.61***	13.0***
SA	2	0.47***	0.010***	0.017***	1.64***	11.24***
Varieties	2	3.48***	0.082***	0.090***	0.001***	21.92***
Heat * SA	2	0.07***	0.001 ***	0.0002ns	0.000002ns	0.56*
Heat * Var	2	0.09***	0.002***	0.0005ns	0.00001ns	2.47***
SA * Var	4	0.08***	0.002***	0.0008ns	0.00001ns	1.16***
Heat * SA * Var	4	0.03*	0.0005*	0.00004ns	0.00001ns	0.40*
Error	36	0.008	0.0001	0.0006	0.000006ns	0.148
Source of variation	**df**	**Root length**	**Chl *a***	**Chl *b***	**Total chl**	**Carotenoids**
Heat	1	3.89***	1.20***	0.56***	3.68***	0.492***
SA	2	2.74***	0.30***	0.19***	0.78***	0.232***
Varieties	2	6.16***	0.001ns	0.12***	0.36***	0.145***
Heat * SA	2	0.37*	0.014*	0.021***	0.18***	0.037***
Heat * Var	2	0.13ns	0.30***	0.018***	0.44***	0.016**
SA * Var	4	0.10ns	0.06***	0.013***	0.26***	0.021***
Heat * SA * Var	4	0.012ns	0.028***	0.005*	0.043***	0.007**
Error	36	0.076	0.0028	0.0019	0.005	0.0020
Source of variation	**df**	**RWC**	**ROS**	**MDA**	**CAT**	**POD**
Heat	1	1297***	0.72***	61.67***	28.88***	21.77***
SA	2	2496***	0.78***	10.13***	25.21***	36.90***
Varieties	2	3312***	0.31ns	17.95***	13.58***	25.53***
Heat * SA	2	324***	0.45***	1.21***	2.98***	13.16***
Heat * Var	2	572***	0.075*	4.01***	1.26***	1.17*
SA * Var	4	162***	0.082*	0.67**	1.32***	2.12***
Heat * SA * Var	4	87.40**	0.146***	0.358*	0.63**	1.65**
Error	36	21.41	0.021	0.118	0.138	0.35

Heat stress resulted in higher H_2O_2 and MDA contents in Moro, Ren-02 and Tilhari (**Figure 3a-b**). However, foliar treatment of SA decreased the levels of these oxidative stress related biochemicals. Our results showed that foliar spray of 2 mM SA significantly reduced the concentrations of MDA and H_2O_2 in Moro and Ren-02, respectively (**Table 1; Figure 3a-b**).

To determine whether SA is essential in scavenging ROS in plants during heat stress, we examined the antioxidant activity of enzymes (CAT and POD) after supplementation of different concentrations of SA in control and heat-stressed plants. Heat stress altered the activity of both CAT and POD. However, the foliar application of SA significantly increased the antioxidant enzyme activities when plants were subjected to heat stress (**Figure 3c-d**). SA application increased the CAT activity in all chili varieties in response to both 1 mM and 2 mM SA, but Moro and Ren-02 showed the maximal activity at 2 mM SA (**Figure 3c**). Similarly, POD activity showed a similar increasing trend upon application of 2 mM SA in Moro and Ren-02 (**Figure 3d**).

The above data indicate that SA treatment may alleviate heat stress by regulating antioxidant machinery, however, all three chili cultivars showed a varied response in their physiological and biochemical traits to tolerate high-temperature stress. Moro and Ren-02 showed a more positive response compared to cv. Tilhari to SA supplementation during heat stress.

Discussion

Heat stress negatively affects plant physiological and biochemical traits. However, foliar application of SA is contemplated as an effective strategy to regulate the physiological, biochemical and other metabolic processes to develop resistance against heat stress (Hasanuzzaman et al., 2017). It helps plants to adapt to heat stress. For example, foliar spray of SA improved heat stress-induced growth inhibition and recovered the plants by improving growth parameters and biomass in the chili plants. Moreover, the growth-promoting effect of SA is dependent upon the cultivar type; for example, SA application showed the highest effects on leaf dry weight in cv. Moro, whereas the chili cultivar Tilhari showed low growth improvement. A similar growth improvement due to SA treatment under heat conditions has been studied in alfalfa cultivars (Wassie et al., 2020). Another study has revealed the biomass recovery in high temperature exposed canola plants after SA co-treatment (Ghani et al., 2021). Similarly, Khanna et al. (2016) demonstrated that SA pretreatment improved the dry biomass and proline contents and developed heat tolerance in spring maize. SA-induced biomass improvement (**Table 1; Figure 1a-f**) of heat-affected chili plants may result from the increased photosynthetic efficiency and/or decreased ROS-induced oxidative damage as stated elsewhere (Jahan et al., 2019).

Photosynthetic pigments (chlorophyll a, b) and carotenoids are essential components of photosynthesis and decipher the basic information about the physiological condition of plants. It is well-documented that chlorophyll and other photosynthetic parameters are very sensitive to heat stress (Ghani et al., 2020). Our study shows that heat stress significantly reduced the chlorophyll a, b, total chlorophyll and carotenoids in all three chili cultivars (**Table 1; Figure 2a–d**). This reduction in photosynthetic pigments might have been due to the overproduction of H_2O_2 and the inactivation of

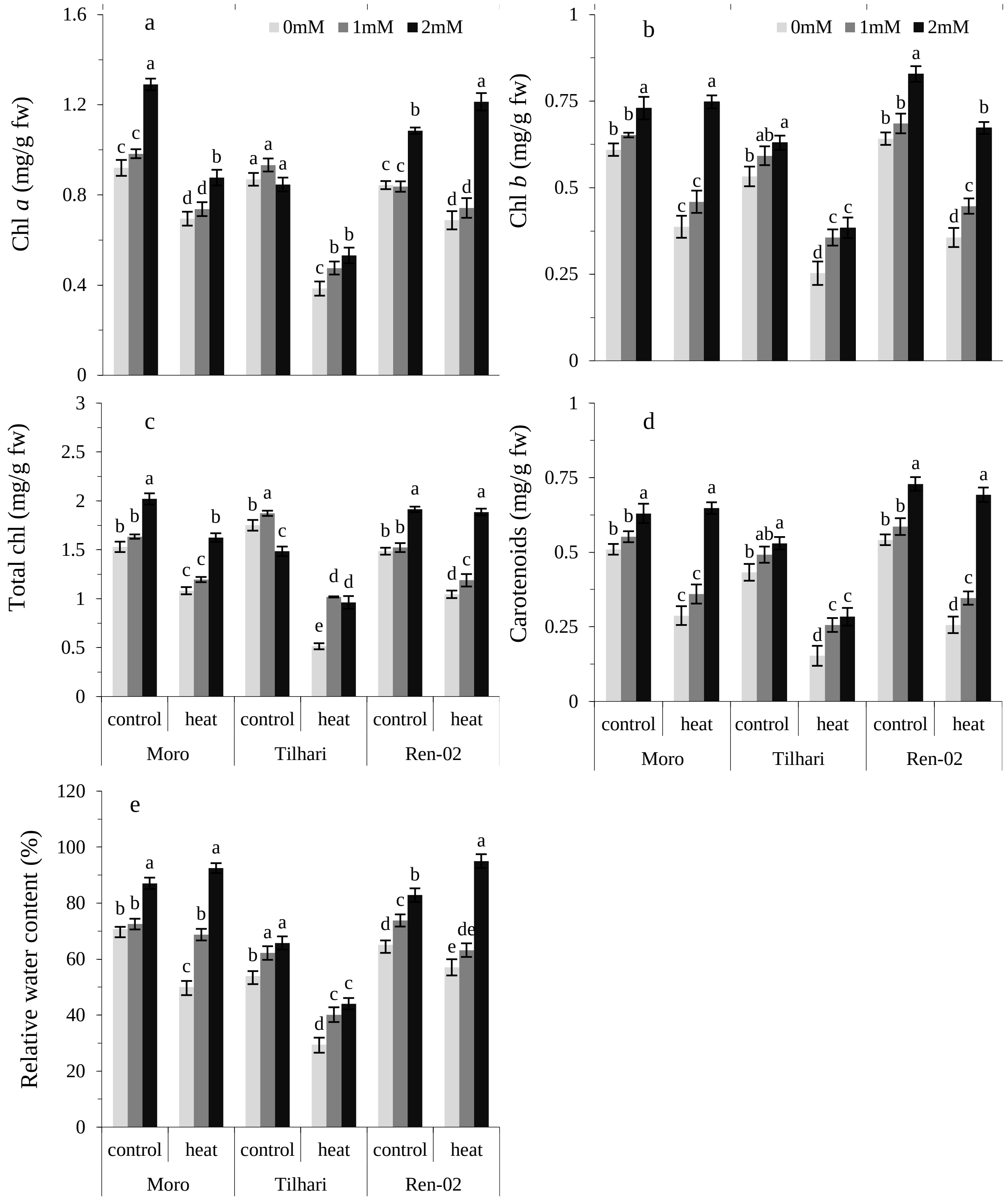

Figure 2. Effects of heat stress and foliar application of salicylic acid (0, 1 and 2 mM) on photosynthetic pigments of three chili cultivars (a) chlorophyll a, (b) chlorophyll b, (c) total chlorophyll, (d) carotenoids, and (e) relative water contents when plants were subjected to high temperature (42 ^{o}C for 72 h). Represented bars are mean ± SE of 3 independent biological replicates (*n* =9). Different letters indicate significant difference between mean values at *P* < 0.05.

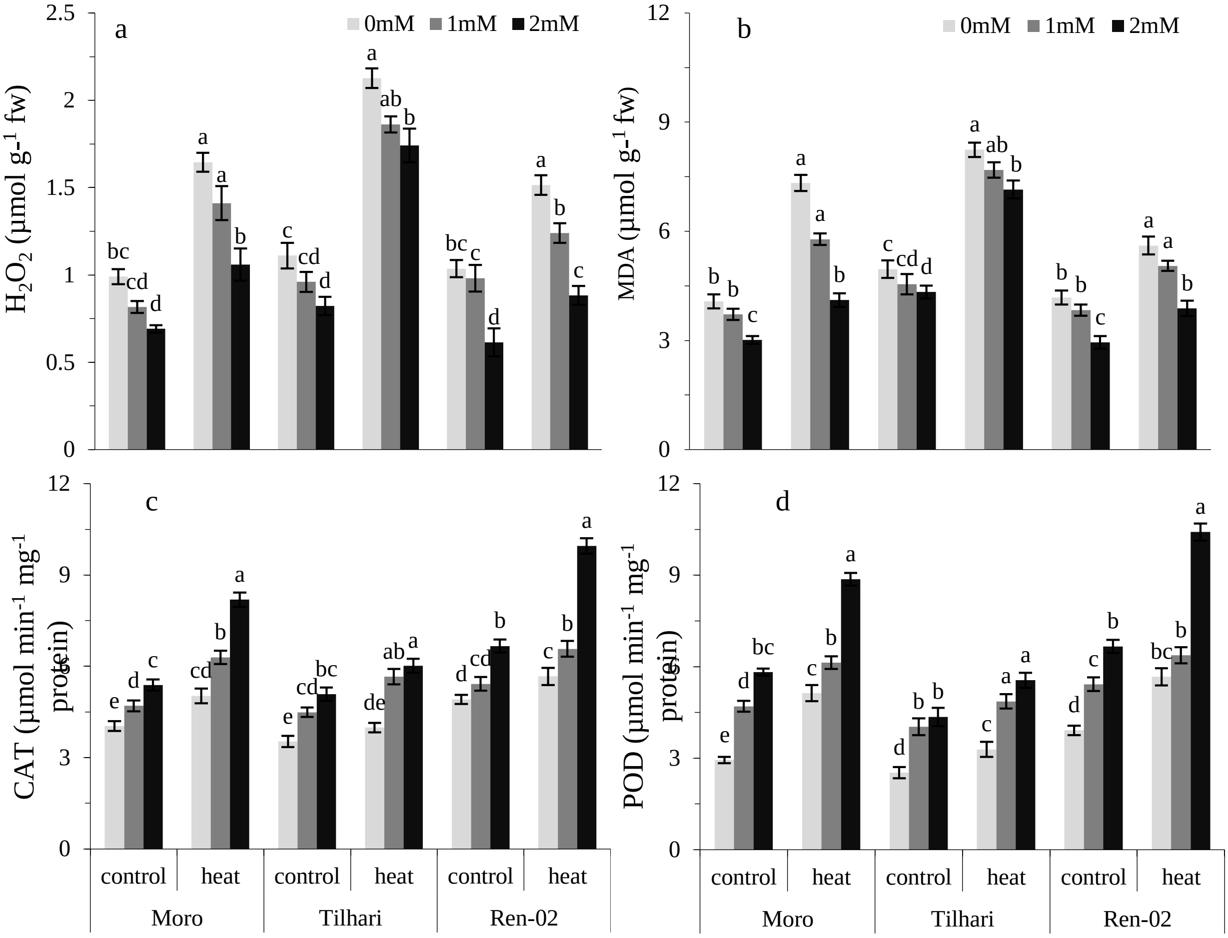

Figure 3. Effects of heat stress and foliar application of salicylic acid (0, 1 and 2 mM) on membrane damage and enzymatic activity of three chili cultivars. (a) Hydrogen peroxide, (b) Malondialdehyde contents, (c) Catalase (CAT) and (d) Peroxidase (POD) activities, when chili plants were kept at 42 °C for 72 h. Data represent as the mean ± SE of three biological replicates (*n* = 9). Different letters indicate significant difference between mean values at *P* < 0.05.

enzymes that play a role in the biosynthesis of chlorophyll (Ashraf and Harris, 2013). However, SA application markedly increased the levels of chlorophyll pigments (a, b, and total), and carotenoid contents in all three chili varieties subjected to heat stress (**Table 1; Figure 2a–d**) as evidenced in cabbage (Lee et al., 2019) and spring maize (Iqbal et al., 2020). SA-induced improvement in chlorophyll contents might have been due to an increase in antioxidants that reduced the hostile effects of high temperature stress and protected the photosynthetic pigments from degradation. Some previous findings also suggested that foliar treatment of SA resulted in increased antioxidants and chlorophyll contents during heat exposure (Ahmad et al., 2017; Munir and Shabbir, 2018; Jahan et al., 2019).

Maintenance of plant osmoregulation is an essential physiological phenomenon for ideal plant growth. RWC is a valuable indicator for the physiological status of water in plants expressing the equilibrium between water absorption and utilization via transpiration (Schonfeld et al., 1988). Our results showed that heat stress reduced the RWC in all three chili cultivars compared to that in the control plants. However, the foliar spray of SA improved the RWC of all chili cultivars (Moro, Tilhari and Ren-02) during heat stress, which might have been due to increased antioxidant activity and regulation of osmotic potential and osmotic adjustment inheat-stressed plants (**Table 1; Figure 2e**). Iqbal et al. (2020) have described that SA treatment meaningfully improved RWC of spring maize under chilling and heat stresses. A similar increment in RWC by SA application has been noticed in pepper plants under heat stress conditions (Kaya et al., 2021).

The measurement of malondialdehyde (MDA) contents is used to estimate the lipid peroxidation in plants (Shen et al., 2014). In the present study, heat stress significantly enhanced H_2O_2 and MDA levels in all three chili cultivars. In contrast, exogenous application of SA produced opposite effects and decreased the concentration of H_2O_2 and MDA (**Table 1; Figure 3a-b**). These results suggest that heat stress induces oxidative damage, while SA plays a protective role by reducing these damages. Under heat stress, three different tri-genomic hybrids of brassica showed reduced growth due to a decrease in relative water content and enzymes' activities. This change might be correlated to elevated ROS levels (Ghani et al., 2021). Further investigations have reported that exogenous application of phytohormone SA significantly reduced the high temperature-induced oxidative damage in various plant species including barley (Habibi, 2012), Indian mustard (Hayat et al., 2009), and mungbean (Saleh et al., 2007). Kaur et al. (2019) also validated such findings and demonstrated that SA pretreatment decreased the MDA and H_2O_2 contents due to the high activity of antioxidants. In another study, co-treatment of SA to the heat-stressed seedlings of alfalfa plants showed lower MDA contents and electrolyte leakage (Wassie et al., 2020).

To protect themselves from oxidative damages, plants activate a complex network of ROS scavenging enzymes, including SOD, CAT, POD, etc. These antioxidant enzymes increase the scavenging of over-accumulated reactive oxygen species and protect the plants from oxidative stress-induced cellular damage. Our data showed that foliar spray of SA to heat-stressed chili plants lead to a substantial rise in the activity of CAT and POD, thereby leading to a decrease in H_2O_2 and MDA contents (**Table1; Figure 3c-d**). Similar outcomes were reported by Afzal et al. (2020), wherein SA stimulated various ROS scavenging pathways including CAT and POD enzymes in different wheat genotypes. Similarly, seedlings of peppermint and corn treated with salicylic acid and melatonin had higher antioxidant activity coupled with improved heat stress tolerance (Haydari et al., 2019).

In conclusion, heat stress negatively affected physiological and biochemical processes in chili plants and promoted growth inhibition, damaged photosynthetic pigments, decreased the relative water content, favored ROS accumulation, and increased membrane damage. Our results showed that exogenous application of SA (particularly 2 mM) reduced ROS and MDA contents by increasing the activity of enzymatic antioxidants, thereby causing more scavenging of ROS, which in turn safeguarded the cellular machinery and assisted plants to flourish in stressful environments. Moreover, this ameliorating effect of SA was more pronounced in Moro and Ren-2. Thus, it can be suggested that the ability of SA to improve the antioxidant system might be a feasible approach to enhance plant growth and yield under stressful environments. Future research to understand the molecular and metabolic processes of SA application to plants in heat stress is required for successful breeding and genetic engineering programs aiming to improve stress tolerance in plants.

Acknowledgments

We are highly thankful to Dr. Muhammad Ahmed Shehzad for providing assistance in statistical analysis.

Authors' Contribution

Hamid Manzoor and Sumaira Rasul conceptualized the study. Data collections were done by Fozia Saeed and Sidra Batool. Data analysis was performed by Sumaira Rasul and Zafar Ullah Zafar. Original draft of the manuscript was prepared by Fozia Saeed and Sidra Batool. Hamid Manzoor and Sumaira Rasul revised the manuscript. The manuscript was approved by all the contributing authors.

References

Abdelaal, K.A., EL-Maghraby, L.M., Elansary, H., Hafez, Y.M., Ibrahim, E.I., El-Banna, M., El-Esawi, M., Elkelish, A. (2020). Treatment of sweet pepper with stress tolerance-inducing compounds alleviates salinity stress oxidative damage by mediating the physio-biochemical activities and antioxidant systems. *Agronomy* 10:26-40.

Abdelrahman, M., Burritt, D.J., Gupta, A., Tsujimoto, H., Tran, L.S.P. (2020). Heat stress effects on source–sink relationships and metabolome dynamics in wheat, *Journal of Experimental Botany* 71:543-554.

Afzal, I., Akram, M., Rehman, H., Rashid, S., Basra, S. (2020). Moringa leaf and sorghum water extracts and salicylic acid to alleviate impacts of heat stress in wheat. *South African Journal of Botany* 129:169-74.

Ahmad, I., Basra, S.M. A., Akram, M., Wasaya, A., Ansar, M., Hussain, S., Iqbal, A., Hussain, S.A. (2017). Improvement of antioxidant activities and yield of spring maize through seed priming and foliar application of plant growth regulators under heat stress conditions. *Semina-Ciencias Agrarias* 38:47-56.

Ananieva, E.A., Christov, K.N., Popova, L.P. (2004). Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. *Journal of Plant Physiology* 161:319-28.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24:1.

Ashraf, M., Harris, P.J. (2013). Photosynthesis under stressful environments: an overview. *Photosynthetica* 51:163-190.

Awasthi, R., Kaushal, N., Vadez, V., Turner, N.C., Berger, J., Siddique, K.H., Nayyar, H. (2014). Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. *Functional Plant Biology* 41:1148-1167.

Bukhat, S., Manzoor, H., Zafar, Z.U., Azeem, F., Rasul, S. (2020). Salicylic acid induced photosynthetic adaptability of *Raphanus sativus* to salt stress is associated with antioxidant capacity. *Journal of Plant Growth Regulation* 39:809-822.

Cakmak, I., Horst, W.J. (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum* 83:463-468.

Chance, B., Maehly, A. (1955). Assay of catalases and peroxidases. *Methods in Enzymology* 2:764–775.

Chavoushi, M., Najafi, F., Salimi, A., Angaji, S. (2019). Improvement in drought stress tolerance of safflower during vegetative growth by exogenous application of salicylic acid and sodium nitroprusside. *Industrial Crops and Products* 134:168-176.

Cohen, I., Zandalinas, S.I., Huck, C., Fritschi, F.B., Mittler, R. (2021). Meta-analysis of drought and heat stress combination impact on crop yield and yield components. *Physiologia Plantarum* 171:66-76.

Conroy, J.P., Virgona, J.M., Smillie, R.M., Barlow, E.W. (1988). Influence of drought acclimation and CO_2 enrichment on osmotic adjustment and chlorophyll *a* fluorescence of sunflower during drought. *Plant Physiology* 86:1108-1115.

FAOSTAT, Green chili production in 2019. (2019). Crops/World Regions/Production Quantity/Green Chillies and Peppers from pick lists. Retrieved 12 December 2022.

Fichman, Y., Mittler, R. (2020). Rapid systemic signaling during abiotic and biotic stresses: Is the ROS wave master of all trades? *Plant Journal* 102:887-896.

Ghani, M.A., Abbas, M.M., Amjad, M., Ziaf, K., Ali, B., Shaheen, T., Awan, F.S., Khan, A.N. (2020). Production and characterisation of tomato derived from interspecific hybridisation between cultivated tomato and its wild relatives. *Journal of Horticultural Science and Biotechnology* 95:506-520.

Ghani, M.A., Abbas, M.M., Ali, B., Ziaf, K., Azam, M., Anjum, R., Iqbal, Q., Nadeem, M., Noor, A., Jillani, U. (2021). Role of salicylic acid in heat stress tolerance in tri-genomic *Brassica napus* L. *Bioagro* 33:13-20.

Gruszecki, W.I., Strzal·ka, K. (1991). Does the xanthophyll cycle take part in the regulation of fluidity of the thylakoid membrane? *Biochimica et Biophysica Acta* 1060:310-314.

Habibi, G. (2012). Exogenous salicylic acid alleviates oxidative damage of barley plants under drought stress. *Acta Biologica Szegediensis* 56:57-63.

Hasan, I., Rasul, S., Bukhat, S., Qureshi, M.K., Aslam, K., Noreen, S., Anjam, M.S., Athar, H.U. R., Manzoor, H. (2020). Salicylic acid supplementation alleviated the adverse effects of salinity stress in *Spinacia oleracea* by improved antioxidative defense system. *Agrochimica* 64:379-395.

Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R., Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences* 14:9643-9684.

Hasanuzzaman, M., Nahar, K., Bhuiyan, T.F., Anee, T.I., Inafuku, M., Oku, H., Fujita, M. (2017). Salicylic acid: An all-rounder in regulating abiotic stress responses in plants; *In* "Phytohormones-Signaling Mechanisms and crosstalk in plant development and stress responses" (Mohamed El-Esawi ed.), pp. 31-75. IntechOpen, London.

Hayat, S., Masood, A., Yusuf, M., Fariduddin, Q., Ahmad, A. (2009). Growth of Indian mustard (*Brassica juncea* L.) in response to salicylic acid under high-temperature stress. *Brazilian Journal of Plant Physiology* 21:187-95.

Haydari, M., Maresca, V., Rigano, D., Taleei, A., Shahnejat-Bushehri, A.A., Hadian, J., Sorbo, S., Guida, M., Manna, C., Piscopo, M. (2019). Salicylic acid and melatonin alleviate the effects of heat stress on essential oil composition and antioxidant enzyme activity in *Mentha piperita* and *Mentha arvensis* L. *Antioxidants* 8:547-558.

Iqbal, H., Yaning, C., Hafeez, H.U., Waqas, M., Ahmed, Z., Raza, S.T., Shareef, M. (2020). Improving heat stress tolerance in late planted spring maize by using different exogenous elicitors. *Chilean Journal of Agricultural Research* 80:30-40.

Jahan, M.S., Wang, Y., Shu, S., Zhong, M., Chen, Z., Wu, J., Sun, J., Guo, S. (2019). Exogenous salicylic acid increases the heat tolerance in tomato (*Solanum lycopersicum* L.) by enhancing photosynthesis efficiency and improving antioxidant defense system through scavenging of reactive oxygen species. *Scientia Horticulturae* 247:421-429.

Kaur, N., Kaur, J., Grewal, S.K., Singh, I. (2019). Effect of heat stress on antioxidative defense system and its amelioration by heat acclimation and salicylic acid pre-treatments in three pigeonpea genotypes. *Indian Journal of Agricultural Biochemistry* 32:106-110.

Kaya, C. (2021). Salicylic acid-induced hydrogen sulphide improves lead stress tolerance in pepper plants by upraising the ascorbate-glutathione cycle. *Physiologia Plantarum* 173(1):8-19.

Khanna, P., Kaur, K., Gupta, A.K. (2016). Salicylic acid induces differential antioxidant response in spring maize under high temperature stress. *Journal of Experimental Biology* 54:386-393.

Lee, H.J., Lee, J.H., Lee, S.G., An, S., Lee, H.S., Choi, C.K., Kim, S.K. (2019). Foliar application of biostimulants affects physiological responses and improves heat stress tolerance in Kimchi cabbage. *Horticulture, Environment, and Biotechnology* 60:841-851.

Miao, Y., Luo, X., Gao, X., Wang, W., Li, B., Hou, L. (2020). Exogenous salicylic acid alleviates salt stress by improving leaf photosynthesis and root system architecture in cucumber seedlings. *Scientia Horticulturae* 272:109577.

Mittler, R., Finka, A., Goloubinoff, P. (2012). How do plants feel the heat? *Trends in Biochemical Sciences* 37:118-125.

Munir, M., Shabbir, G. (2018). Salicylic acid mediated heat stress tolerance in selected bread wheat genotypes of Pakistan. *Pakistan Journal of Botany* 50:2141-2146.

Saleh, A.A.H, Abdel-Kader, D.Z., El Elish, A.M. (2007). Role of heat shock and salicylic acid in antioxidant homeostasis in mungbean (*Vigna radiata* L.) plant subjected to heat stress. *American Journal of Plant Physiology* 2:344-355.

Schonfeld, M.A., Johnson, R.C., Carver, B.F., Mornhinweg, D.W. (1988). Water relations in winter wheat as drought resistance indicators. *Crop Science* 28:526-31.

Shen, C., Hu, Y., Du, X., Li, T., Tang, H., Wu, J. (2014). Salicylic acid induces physiological and biochemical changes in *Torreya grandis* cv. Merrillii seedlings under drought stress. *Trees* 28:961-970.

Szymańska, R., Ślesak, I., Orzechowska, A., Kruk, J. (2017). Physiological and biochemical responses to high light and temperature stress in plants. *Environmental and Experimental Botany* 139:165-177.

Velikova, V., Yordanov, I., Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science* 151:59-66.

Wassie, M., Zhang, W., Zhang, Q., Ji, K., Cao, L., Chen, L. (2020). Exogenous salicylic acid ameliorates heat stress-induced damages and improves growth and photosynthetic efficiency in alfalfa (*Medicago sativa* L.). *Ecotoxicology and Environmental Safety* 191:110206-110215.

Zhao, Y., Li, Y., He, M., Yun, Y., Peng, Y. (2020). Antioxidant responses of the pest natural enemy *Hylyphantes graminicola* (Araneae: Linyphiidae) exposed to short-term heat stress. *Journal of Thermal Biology* 87:102477. https://doi.org/10.1016/j.jtherbio.2019.102477.