European Journal of Biology
Eur J Biol 2023; 82 (2): 167–178
DOI: 10.26650/EurJBiol.2023.1216574

RESEARCH ARTICLE

Physiological and Biochemical Changes of Maize (*Zea mays* 'MV500') in Response to Heat Stress under Levels of Salicylic Acid

Esmail Nabizadeh[1], Narges Dolatmand[2], Masoud Haghshenas[3], Khadijeh Ahmadi[4]

[1]Islamic Azad University, Department of Agronomy, Faculty of Agricultural, Mahabad Branch, Mahabad, Iran
[2]Kurdistan University, Department of Agronomy and Plant Breeding, College of Agriculture, Sanandaj, Iran
[3]University of Mohaghegh Ardabili, Department of Horticulture, Faculty of Agriculture, Ardabil, Iran
[4]Shahed University, Department of Agronomy and Crop Breeding, College of Agriculture, Tehran, Iran

ABSTRACT

Objective: Heat stress is a significant factor leading to decreased crop yield. Exceeding the plant's temperature tolerance threshold in ecosystems often results in significant cellular damage and potentially cellular death. Signaling elicitors may mitigate elevated temperatures' detrimental impact and enhance plant defense mechanisms.

Materials and Methods: The present study investigates the influence of varying temperatures (25, 30, 35, 40, and 45°C) and pre-harvest salicylic acid (SA) application (0, 0.5, 1.5, 2.5, 5, and 10 mM) on the morpho-physiological and biochemical attributes of maize. A factorial-based experiment was set up following a completely randomized design and conducted in a growth room.

Results: The findings demonstrated that a 2.5 mM SA treatment at 35°C produced the largest plant leaf area and total chlorophyll content. The temperature and SA application interplay on carotenoid content were maximum at 5 mM. SA treatment under high-temperature conditions effectively elevated proline content, chl a, chl b, chl total, and malondialdehyde compared to untreated plants. The peak stomatal conductance was also observed with a 2.5 mM SA treatment at 30°C. The maximal catalase and peroxidase activities were recorded at 35°C. Furthermore, 2.5 mM SA at 25°C resulted in the highest levels of soluble proteins and RWC. SA (2.5 mM) applied at 30°C was more efficient at decreasing H2O2 production. The highest proline content was observed with 2.5 mM SA at 45°C.

Conclusion: SA (2.5 mM) treatment can have optimal effects on maize plant growth parameters under high-temperature conditions, potentially mitigating the damaging effects of heat stress.

Keywords: Antioxidant, Biological Yield, Heat Stress, Proline, Soluble Protein.

INTRODUCTION

Globally, maize (*Zea mays L.*) ranks second in production per unit area after wheat.[1] As one of the primary crops in temperate and subtropical regions, maize contributes 20-25% to human food sources, 60-75% to animal feed, and 5% to industrial raw materials. This crop has to endure numerous biotic (like herbivores and pathogens) and abiotic (such as radiation, drought, salinity, and temperature) stresses.[2] Heat stress refers to a temperature increase beyond a specific limit long enough to inflict irreversible harm to plant growth and development. Typically, a rise of 10-15°C above the ideal temperature is deemed heat stress. One of the most critical processes adversely affected by heat stress is photosynthesis.[3] Maize, being a C4 plant, requires a higher optimum temperature for photosynthesis, attributable to a CO_2-concentrating system that curbs Rubisco oxygenase activity.[4]

The plant's survival under stress hinges on its ability to detect the stimulus, respond to the perceived signal, and generate biochemical compounds to adapt to the conditions. Several factors, including calcium, ethylene, jasmonic acid, and salicylic acid (SA), have been identified as plant stress signals.[5] SA acts as a signaling molecule, triggering plant responses to environmental stressors. Recent genetic studies have revealed that over 90% of SA is derived from isochorismate. While the role of isochorismate synthases in SA production is well-established, the enzyme responsible for converting isochorismate to SA remains unidentified. The functionality of SA in protecting against various biotic and abiotic stresses is well-documented.[6]

Corresponding Author: Esmail Nabizadeh **E-mail:** nabizadeh.esmaeil@gmail.com
Submitted: 09.12.2022 • **Revision Requested:** 04.04.2023 • **Last Revision Received:** 24.09.2023 • **Accepted:** 09.11.2023 • **Published Online:** 07.12.2023

This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

Additionally, SA inhibits ethylene production, respiration, and senescence.

As a plant growth regulator, SA regulates several physiological reactions, including photosynthesis, stomatal closure, transpiration, chlorophyll synthesis, and mineral uptake.[7–9] Furthermore, by influencing catalase (CAT) and peroxidase (POD) activity and boosting the accumulation of osmotic solutes such as proline and glycine betaine, SA mitigates various stresses (like heat, cold, salinity, and heavy metals).[10] Also, SA can impact superoxide dismutase (SOD) enzyme activity, converting free oxygen radicals into hydrogen peroxide. While hydrogen peroxide (H_2O_2) can be harmful, it can also serve as a signaling molecule at low concentrations.[11] The increase in root and shoot dry weight and overall plant biomass is likely connected to the photosynthetic efficiency of SA treatments.[12] The photosynthetic efficiency of plants using SA is tied to increased Rubisco activity and chlorophyll content.[13] The increase in leaf quantity and surface area, which are photosynthetic indicators, could be another benefit of SA treatment.[10] Given the role of SA in enhancing plant tolerance to environmental stressors and considering global warming, this experiment examined whether SA could boost the photosynthetic capacity, antioxidant activity, and biological defense system of maize 'MV500' in response to heat stress.

MATERIALS AND METHODS

Experimental Procedure and Treatment Application

This research assessed the impact of varying temperature degrees (25, 30, 35, 40, and 45°C) and the foliar administration of SA at different concentrations (0, 0.5, 1.5, 2.5, 5, and 10 mM). The experiment focused on maize seedlings' morphological, physiological, and biochemical characteristics. The experimental setup was a factorial structure arranged in completely randomized blocks with three replicates in a growth chamber.

The maize seeds used for the experiment were procured from the Seed and Plant Improvement Institute (SPII). The study was carried out as a pot experiment in 2018 within the research greenhouse of the Islamic Azad University of Mahabad, situated at a latitude of 35° 58'N, a longitude of 44° 3'E, and an altitude of 1354 m above sea level in the West Azerbaijan Province, Iran. The experiments were conducted at the Islamic Azad University of Mahabad.

Maize 'MV500' seeds were sown in loamy clay soil in the growth chamber, which had a light/dark cycle of 16/8 h, a light intensity of 14Klux, a relative humidity (RH) of 65±2%, and a day/night temperature of 23±2/16±2°C (GROUC; Iran). The pots were watered daily.

The treatments were initiated after the maize plants had developed three leaves, a stage that lasted for 3 days. The seedlings were subjected to various temperature degrees for 3 h daily (Figure 1). The physical and chemical attributes of the farm soil used in the experiment are presented in Table 1.

Stomatal Conductance, Leaf Area Index (LAI), and Photosynthetic Pigments Evaluation

We used a portable probe (1600-LI) to gauge the stomatal conductance of maize leaves. After the experiment, we quantified the plant leaf area using a leaf area meter (A 300; UK). From each test unit, we picked several mature young leaves. Using a Porometer (Leaf Porometer; SN: LP2402; Decagon, US), we computed their average stomatal conductance (mM (H_2O) $m^{-2}s^{-1}$) early in the morning. To determine the photosynthetic pigments (for instance, chlorophyll a, b, and total chlorophyll), we sampled fully matured leaves, dissolved them in 80% acetone, and centrifuged them. We measured each sample's absorption using a spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) at wavelengths of 663.2, 646.8, and 470 nm for chlorophyll a, chlorophyll b, and total chlorophyll content, respectively. The pigments' quantity was subsequently computed based on µg/g fresh weight per the provided formula.[14]

$$Chlorophyll\,a = (12.25XA_{663.2})\check{}(2.79XA_{646.8}) \quad (1)$$

$$Chlorophyll\,b = (21.21XA_{646.8})\check{}(5.1XA_{663.2}) \quad (2)$$

$$TotalChlorophyll\,(a+b) = (7.15XA_{663.2} + 18.71XA_{646.8}) \quad (3)$$

$$Carotenoid = ((1000XA_{470}) - (1.8XChla) \quad (4)$$

$$- (85.02XChlb))/198 \quad (5)$$

$A_{663.2}$: Absorption in wavelength of 663.2; $A_{646.8}$: Absorption in wavelength of 646.8; A_{470}: Absorption in wavelength of 470.

Cell Membrane Stability (Electrolyte Leakage) Assessment

We transferred 1g of fresh leaf tissue into a falcon containing 20 ml of deionized water to evaluate the cell membrane stability. After 24 h at 25°C, we read the samples' electrolyte leakage (L1) using a conductivity meter (Aqualytic Sensdirect, CD24). We autoclaved the samples for 20 min at 120°C, cooled them down, and read the solution's electrical leakage (L2) again. The cell membrane stability percentage was then calculated as per the formula provided.[15]

$$Electrolyte\ leakage(\%) = (L_1/L_2) \times 100 \quad (6)$$

Relative Water Content (RWC) Measurement

We cut pieces of fully matured leaves into 1 cm^2 and weighed them (FW). Then, we placed the samples in a petri dish filled with distilled water and weighed them in a 4°C incubator after 4 h (TW). Lastly, we put the samples in a 72°C oven for 72 h,

Figure 1. The stages of applying experimental treatments on maize 'Mv500'.

Table 1. Experimental soil analysis results.

pH	N(ppm)	P(ppm)	K(ppm)	Soil Texture (%)	Saturation Percent (%)	Clay (%)	Sand (%)	Silt (%)	**Organic Carbon (%)**
8.09	0.6	7.40	194.3	Loamy Clay	47.44	38	4-30	22	**1.22**

weighed them once more (DW), and calculated their relative water content according to the provided formula.[16]

$$RWC = (FW - DW)/(SW - DW) \times 100 \quad (7)$$

Biological Yield (Biomass) Calculation

After harvest, we measured the leaf, shoot, and stem fresh weights. The plant was then dried in a 70°C oven, and the biomass (dry weight) was measured with 0.001 accuracy.

Enzymatic Extract Preparation

We used the method by Kang and Saltveit[17] with slight modifications. Specifically, we homogenized 0.5 g of leaf fresh weight at 4°C in 3 ml of extraction buffer (50 mM Tris-Hcl buffer, pH 7.5, 3 mM $MgCl_2$, 1 mM Na-EDTA). We then centrifuged the homogenate (Hermle Z 216 MK; Germany) for 20 min at 5000 rpm at 4°C. We used the supernatant as the crude extract to assay CAT, POD, and ascorbate peroxidase (APX) enzyme activity.

Catalase Activity (EC 1.11.1.6) Assessment

We used the method by Aebi.[18] to assay CAT activity. The reaction mixture consisted of 2.5 ml of 50 mM phosphate buffer (pH 7), 0.2 ml of 1% H_2O_2, and 0.3 ml of enzyme extract. We measured the catalase activity as a decrease in absorption at 240 nm and extinction coefficient (0.0436 mM^{-1} cm^{-1}) as per the provided formula.

$$Units\left(\frac{mM}{min}\right) = \frac{\text{dOD}}{\text{min(slope)}} \times \frac{\text{vol of assay (0.0003)}}{\text{Extinction coefficient (0.0436)}}$$

Assessing Peroxidase Activity (EC 1.11.1.7)

The method outlined by Upadhyaya et al.[19] was used to measure POD activity. The reaction mix included 2.5 ml of 50 mM phosphate buffer (pH= 7), 1 ml of 1% H_2O_2, 1 ml of 1% Guaiacol, and 0.1 ml of enzyme extract. One minute was set to monitor the increase in absorbance at 420 nm. The POD activity was then calculated using the extinction coefficient (26.6 mM^{-1} cm^{-1}) in the equation:

$$Units\left(\frac{mM}{min}\right) = \frac{\text{doD}}{\text{min(slope)}} \times \frac{\text{vol of assay(0.0001)}}{\text{Extinction coefficient (26.6)}}$$

Evaluating Ascorbate Peroxidase Activity (EC 1.11.1.11)

The activity of APX enzyme was gauged based on the procedure of Nakano and Asada.[20] This reaction mix contained 2.5 ml 50 mM phosphate buffer (pH 7), 0.1 ml EDTA, 1 mM sodium ascorbate, 0.2 ml of 1% H_2O_2, and 0.1 ml enzyme extract. The APX activity was determined by the decrease in absorption at 240 nm, and the extinction coefficient (2.8 mM^{-1} cm^{-1}) was utilized in the following calculation:

$$Units\left(\frac{mM}{min}\right) = \frac{\text{doD}}{\text{min(slope)}} \times \frac{\text{vol of assay (0.0001)}}{\text{Extinction coefficient (2.8)}}$$

Measuring Protein Content

To estimate the protein levels, a gram of fresh leaf tissue was blended in 5 ml of Tris buffer (0.05 M, pH 7.5). The mixture was centrifuged at 10,000 × g for 25 min at 4°C. The soluble protein concentration was determined using 0.1 ml protein extract and 5 ml of biuret reagent (0.1 g Kumasi Brilliant Blue G250+50 ml of 95% ethanol+100 ml phosphoric acid 85%), which was then diluted to a liter with distilled water. The solution was filtered using a Whatman filter and mixed with a vortex. Absorbance was read at 595 nm by a spectrophotometer (UV/VIS Lambda25 Perkin Elmer) after 2 min, and the concentration was computed based on the bovine serum albumin standard curve.[21]

Quantifying Lipid Peroxidation

In a nutshell, 200 mg of fresh leaf tissue was crushed in 5 ml 0.1% (w/v) TCA. The homogenate was centrifuged (Hermle Z216 MK; Germany) for 5 min at 10,000 × g. Then, 1 ml of the supernatant was added to 4 ml of 20% TCA solution containing 0.5% TBA and boiled for 30 min at 95°C. After cooling in ice, the mixture was centrifuged for 10 min at 10,000 × g. Absorbance was read at 532 nm wavelength. The red complex (malondialdehyde (MDA)-TBA) was targeted for absorption at this wavelength, and non-specific absorption was read at 600 nm and subtracted from the previous value. The extinction coefficient (155 mM^{-1} cm^{-1}) was used to calculate MDA concentration.[22]

Evaluating Hydrogen Peroxide Levels

The method adapted from Velikova et al.[23] was used to measure the H_2O_2 content. A 200 mg leaf tissue sample was homogenized in an ice bath with 3 ml of 0.1% (w/v) TCA. This homogenate was then centrifuged at 12,000 × g for 15 min. One ml of the supernatant was added to 1 ml of 10 mM potassium phosphate buffer (pH= 7.0) and 2 ml of 1 M KI. The absorbance of the supernatant was measured at 390 nm. The H_2O_2 content was determined using the extinction coefficient (0.28 mM^{-1} cm^{-1}).

Proline Measurement Process

The procedure for assessing proline was adapted from Bates et al.[24], but with minor changes. In brief, a 0.1 g of fresh leaf tissue sample was ground up in 10 ml of 3% sulfo-SA. This solution was then spun in a centrifuge (Hermle Z216 MK, Germany) for a quarter of an hour at 4000 revolutions per minute. Following this, 2 ml of the resulting supernatant was combined with 2 mg of ninhydrin reagent and 2 ml of acetic acid. The concoction was then heated in a bath at 100°C for 1 h. The reaction was halted by cooling the mixture in an ice-water bath. Subsequently, 4 ml of toluene was added, which led to the formation of two distinct layers. The proline content was measured using the supernatant. The absorbance was noted at a wavelength of 520 nm, and the proline content was determined using a standard curve.

Statistical Analysis

The data gathered was processed using SAS 9.4 software, and the mean values of the treatments were compared utilizing the Duncan test, with a significance threshold set at 0.05

RESULTS

Growth Metrics

Indicators of photosynthetic capacity (chlorophyll a, chlorophyll b, total chlorophyll content, and carotenoids) were significantly influenced by the treatments ($p<0.01$). The combined effect of SA at a concentration of 2.5 mM and a temperature of 35°C yielded the highest leaf area, chlorophyll a (5.58 mg/g FW), total chlorophyll content (7.76 mg/g FW). The interaction of SA (10 mM) and a temperature of 35°C resulted in the highest chlorophyll b (2.35 mg/g FW), and the maximum carotenoid content (1.84 mg/g FW) was obtained at a concentration of 5 mM SA and temperature of 35°C. The most excellent stomatal conductance was recorded under the joint treatment of SA (2.5 mM) and a temperature of 30°C (Table 2).

Impact of SA and Temperature on RWC

RWC demonstrated a significant correlation between SA and temperature ($p<0.05$). The data revealed a decline in RWC across all SA concentrations as the temperature escalated from 25°C to 45°C. The highest RWC was observed at 25°C when the concentration of SA was increased to 2.5 mM. However, as SA concentrations reached 5 and 10 mM, there was a notable reduction in RWC (Table 3).

Lipid Peroxidation

The stability of the MDA was shown to be significantly impacted by both SA and temperature ($p<0.01$), as well as their combined effect ($p<0.05$). The most stable cell membranes were

observed at different SA levels at 25°C, whereas the least stable was observed in the control treatment at 45°C. As the temperature rose, electrolyte leakage increased across all SA concentrations. However, SA application at 25°C, 30°C, and 35°C did not significantly impact leaf electrolyte leakage. Contrarily, at 40°C and 45°C, electrolyte leakage decreased with up to 2.5 mM of SA application. This trend reversed with 5 and 10 mM SA concentrations, where electrolyte leakage increased (Table 3).

Impact on Biological Yield

Leaf, stem, and plant dry weight, as measures of plant biomass, were significantly influenced by the interaction of temperature and SA ($p<0.01$). The lowest weights for leaf, stem, and plant were recorded when the temperature was at 45°C and the SA concentration was at 10 mM. Elevated levels of SA did not alleviate heat stress, but instead exacerbated it. Conversely, SA at a concentration of 2.5 mM moderated heat stress and resulted in the highest leaf, stem, and plant dry weight at 35°C. This led to an increase of 29%, 36.6%, and 31%, respectively, compared to the control treatment (Table 3).

Antioxidant Enzymes Activity

An increase in APX activity was seen with SA ($p<0.01$). This increase followed the trend of raising SA concentrations to 2.5 mM. The highest APX activity, showing a 10.48% increase compared to the control, was achieved with a SA concentration of 2.5 mM. However, when SA concentration rose from 5 mM to 10 mM, APX activity declined (Figure 2). The effect of temperature on APX activity was less pronounced. APX activity increased at 30°C and 35°C but decreased beyond the plant's tolerance threshold at 40°C and 45°C (Figure 2). Similarly, CAT and POD activity were significantly affected by the interaction of temperature and SA ($p<0.05$). The highest activity levels of CAT and POD were recorded at 35°C. When the temperature was raised from 35°C to 40°C and 45°C, CAT and POD activities decreased (Figure 3).

MDA and H_2O_2

When the temperature increases, MDA content correspondingly escalates. At 45°C, the MDA content was approximately 62.87% greater than at 25°C (Figure 4). SA (2.5 mM) decreased the MDA content by about 14.92% compared to the control. However, when the concentration of SA rose, there was a corresponding rise in MDA content (Figure 4). Reactive oxygen species (ROSs) play a significant role in lipid peroxidation. In this study, the production of ROSs was affected by the interaction of SA and temperature ($p<0.01$). The maximum and minimum levels of H_2O_2 were found in the control group at 45°C and the SA (2.5 mM) group at 30°C, respectively (Figure 4).

Soluble Proteins

Both SA and temperature significantly impacted the content of soluble proteins ($p<0.01$). As the temperature rose by 10-15°C, from 30-35°C, a 60% decrease in soluble protein content was noticed at all tested SA levels. The highest content of soluble proteins was observed when 2.5 mM of SA was applied at 25°C (Figure 5).

Proline

The proline content was significantly influenced by SA, temperature, and the interactive effect of SA and temperature ($p<0.01$). The maximum proline content (47.13 µmol/g FW) was recorded with a SA (2.5 mM) treatment at 45°C. The minimum proline content (10 µmol/g FW) was noticed in the control SA group at 25°C. When the temperature was raised to 40 and 45°C, an increase in SA concentration up to 2.5 mM increased proline content. However, higher concentrations of SA at 5 and 10 mM led to a decrease in proline content (Figure 6).

DISCUSSION

Heat stress notably impacts maize plants' growth and metabolic activities. Such stress instigates physiological alterations in the plants, which eventually induce morphological modifications. Our study observed that these heat stress effects resulted in a decline in the maize plants' morphological and physiological attributes. However, administering SA in varying concentrations, particularly a concentration of 2.5 mM, enhanced the maize plants' growth and physiological features.

As the temperature increases, the photosynthetic system suffers irreversible damage, particularly the photosynthetic pigments. Yet, SA demonstrated its ability to manage heat stress in temperatures exceeding the plant's tolerance threshold. This allowed for an improvement in the photosynthesis mechanism within the bio-kinetic zone. Photosystem II (PSII), lipid permeability, and rubisco activase are critical components of the photosynthetic apparatus mechanisms affected by heat stress.[25] The photosynthetic capacity of a plant fluctuates significantly under heat-stress conditions. Gradual temperature increases and heat shock factors (HSFs)[12] elicit distinct plant responses. Photosynthesis is a crucial factor influencing maize productivity, particularly within an optimal temperature range of 28-37.5°C. In the course of our study, it was found that chlorophyll a, chlorophyll b, total chlorophyll content, and carotenoid levels declined as temperature increased. However, treatment with 2.5 mM of SA increased the amount of chlorophyll a and total chlorophyll. When the SA concentration was increased to 5 and 10 mM, the chlorophyll b and carotenoid amounts, respectively. A temperature of 35°C and SA treatment in varied concentrations produced beneficial outcomes compared to both high and low temperatures. The reduction in photosynthesis is due to damage to the oxygen-releasing complex, resulting from the

Table 2. The effect of temperature stress and salicylic acid treatments on the photosynthetic capacity of maize (*Zea mays L.*).

	Temperatures	LAI (cm^2)	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)	Carotenoid (mg/g FW)	SC (mM (H_2O) m^{-2} s^{-1})
Control	25°C	162.3±2.9 gh†	3.22±0.11 j	1.42±0.15 d	4.69±0.17 h	0.68±0.02 kl	177.7±1.5 e-g
	30°C	177.4±6.6 ef	3.81±0.08 i	1.55±0.12 d	5.41±0.18 g	0.8±0.06 j-l	179±1.001 e-g
	35°C	177.8±1.9 ef	3.91±0.1 hi	1.59±0.15 d	5.55±0.04 g	0.95±0.2 h-j	176.1±2.01 g
	40°C	89.97±4.5 k	0.87±0.06 n	0.25±0.04 e	1.13±0.19 k	0.23±0.01 o	25.3±0.57 j
	45°C	46.13±5.7 m	0.68±0.9 no	0.13±0.03 e	0.82±0.2 kl	0.2±0.012 o	10.37±0.37 k
SA (0.5 mM)	25°C	170.3±6.9 fg	3.94±0.3 hi	1.54±0.17 d	5.52±0.42 g	0.86±0.06 jk	181.3±1.9 e-g
	30°C	180.3±1.5 ef	4.82±0.01d-f	1.76±0.1 cd	6.64±0.1 d-f	1.12±0.1 f-h	184±1.73 de
	35°C	183.1±5.1 de	5.15±0.1 b-d	1.85±0.03b-d	7.1±0.05 b-d	1.29±0.12d-f	181.3±3.3 e-g
	40°C	106.8±3.44 j	1.43±0.15 m	0.38±0.4 e	1.83±0.15 j	0.36±0.02 no	29.33±1.33 ij
	45°C	65.41±6.81 l	0.92±0.04 n	0.2±0.01 e	1.14±0.05 k	0.25±0.01 no	10.73±0.29 k
SA (1.5 mM)	25°C	182.6±2.6 de	4.48±0.14fg	1.86±0.2 b-d	6.4±0.01 ef	1.07±0.07g-i	188.7±2.1 cd
	30°C	198.8±0.7 bc	4.85±0.03c-f	2.06±0.13a-c	6.97±0.2 cd	1.22±0.1 e-g	193.1±4.38 c
	35°C	200.3±1.9 bc	5.23±0.2 a-c	2.21±0.2 ab	7.51±0.4 ab	1.47±0.15 cd	190.3±3.76 c
	40°C	104.3±7.37 j	1.37±0.06 m	0.28±0.01 e	1.67±0.06 j	0.45±0.06mn	34.33±1.45 hi
	45°C	68.9±0.88 l	0.89±0.01 n	0.18±0.01 e	1.08±0.07 kl	0.27±0.22 no	11.77±0.38 k
SA (2.5 mM)	25°C	190.9±5.8 cd	4.92±0.3 c-e	1.86±0.1 b-d	6.84±0.2 c-e	1.21±0.06e-g	200±3.241 ab
	30°C	206.7±3.2 ab	5.15±0.1b-d	2.1±0.05 a-c	7.31±0.1 a-c	1.31±0.05c-f	205.3±3.71 a
	35°C	211.9±2.12 a	5.58±0.18 a	2.12±0.1 a-c	7.76±0.19 a	1.52±0.02 bc	194.7±3.3 bc
	40°C	128.5±3.64 i	1.97±0.79 l	0.4±0.04 e	2.39±0.13 i	0.6±0.01 lm	39.01±1.57 h
	45°C	72.77±2.07 l	0.58±0.5 no	0.09±0.01 e	0.68±0.05 kl	0.22±0.02 o	12.93±0.53 k
SA (5 mM)	25°C	177.6±4.01 ef	4.26±0.2 gh	1.99±0.1a-c	6.31±0.29 f	1.22±0.06e-g	181.7±1.2 e-g
	30°C	205.5±5.6 ab	4.79±0.2 d-f	2.06±0.1 a-c	6.91±0.2 c-e	1.39±0.04c-e	183±1.03 d-f
	35°C	209.1±1.8 ab	5.4±0.17ab	2.2±0.05 ab	7.67±0.19 a	1.84±0.18 a	183±2.65 d-f
	40°C	119.5±1.05 i	0.71±0.1 no	0.2±0.01 e	0.93±0.05 kl	0.24±0.04 no	34±2.082 hi
	45°C	52.67±2.3 m	0.43±0.01o	0.08±0.002e	0.52±0.01 l	0.16±0.012 o	11.7±0.32 k
SA (10 mM)	25°C	155.2±5.01h	2.64±0.19k	1.49±0.35 d	4.17±0.28 h	0.89±0.05i-k	176.7±1.3 fg
	30°C	176.5±4.8 ef	4.63±0.2 ef	2.24±0.4 ab	6.93±0.3 c-e	1.48±0.02 cd	179±0.57 e-g
	35°C	177.1±3.2 ef	5.22±0.1 a-c	2.35±0.19 a	7.64±0.29 a	1.7±0.041 ab	177.3±0.9 e-g
	40°C	83.58±11.9 k	0.68±0.1no	0.18±0.05 e	0.88±0.15 kl	0.22±0.032 o	31.67±1.20 i
	45°C	32.13±6.47 n	0.54±0.01no	0.11±0.01 e	0.66±0.01 kl	0.26±0.01 no	11.07±0.37 k

†: (Mean ± S.d, n=3) Values followed by the same letters in a column are not significantly different according to Duncan tests at 5% level; SA: Salicylic acid, LA: Leaf area, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Total Chl: Total Chlorophyll, S.C: Stomatal conductance.

Table 3. The influence of salicylic acid and temperature on select morpho-physiological parameters of maize (*Zea mays L.*) 'MV500'.

	Temperatures	L.D.W (g per plant)	S.D.W (g per plant)	P.D.W (g per plant)	RWC (%)	MP (%)
Control	25°C	0.486±0.06 ij†	0.163±0.006 gh	0.65±0.061 j	50.07±1.36c-f	24.79±2.73i
	30°C	0.636±0.03 fg	0.25±0.021 d-f	0.886±0.013h	48.33±0.37g-i	34.29±2.18h
	35°C	0.653±0.01 fg	0.26±0.010 d-f	0.913±0.015h	45.43±1.03 k	46.27±1.85g
	40°C	0.39±0.05 kl	0.123±0.01 h-j	0.513±0.052 l	24.5±0.051 n	89.03±0.3b-d
	45°C	0.223±0.01 no	0.08±0.011 j	0.303±0.016no	10.43±0.21 p	96.48±1.11 a
SA (0.5 mM)	25°C	0.54±0.02 hi	0.223±0.01 ef	0.763±0.029i	51.8±0.72 b	22.23±0.93 i
	30°C	0.783±0.01b-e	0.346±0.01 bc	1.13±0.015de	50.93±0.5 b-e	33.63±1.29h
	35°C	0.796±0.01b-d	0.363±0.02 ab	1.16±0.020 cd	47.83±0.9 hi	44.1±2.19 g
	40°C	0.43±0.001 jk	0.13±0.001 h-j	0.56±0.0001kl	26.23±28 m	86.47±1.1 cd
	45°C	0.27±0.001mn	0.09±0.012 ij	0.363±0.01 mn	11.33±0.3 op	94.45±0.7 ab
SA (1.5 mM)	25°C	0.713±0.04 ef	0.24±0.01 d-f	0.953±0.04 gh	53.4±0.46 a	22.9±0.38 i
	30°C	0.86±0.011ab	0.356±0.003 ab	1.21±0.008 bc	51.5±0.15 bc	33.63±1.61h
	35°C	0.893±0.012 a	0.39±0.052 ab	1.28±0.017 ab	48.03±0.9 hi	43.27±1.21g
	40°C	0.466±0.01 i-k	0.15±0.017 hi	0.616±0.016jk	27±0.46 lm	83.81±1.7 de
	45°C	0.273±0.06 mn	0.106±0.01 h-j	0.38±0.045mn	11.83±0.16op	92.5±0.10 ab
SA (2.5 mM)	25°C	0.763±0.03 c-e	0.296±0.008 cd	1.06±0.021 ef	54.5±0.64 a	22.55±1.66 i
	30°C	0.89±0.005 a	0.38±0.010 ab	1.27±0.011 ab	54±0.3201 a	31.23±1.56 h
	35°C	0.92±0.005 a	0.406±0.012 a	1.32±0.0140 a	49.77±0.9 d-g	40.73±1.06 g
	40°C	0.48±0.011 ij	0.21±0.0050 fg	0.69±0.011 j	27.9±0.62 l	74.6±2.080 f
	45°C	0.296±0.003mn	0.123±0.01 h-j	0.42±0.010 m	12.73±0.17 o	84.68±0.76de
SA (5 mM)	25°C	0.746±0.01 de	0.276±0.003de	1.02±0.012 fg	51.77±0.2 bc	25.87±2.11 i
	30°C	0.84±0.04 a-c	0.37±0.031 ab	1.21±0.061 bc	49.5±0.85 e-h	35.53±0.66 h
	35°C	0.876±0.01 a	0.37±0.012 ab	1.24±0.020 b	47.17±0.57 ij	44.9±1.59 g
	40°C	0.336±0.01 lm	0.166±0.008gh	0.503±0.01 l	27.6±0.15 lm	78.53±3.97 f
	45°C	0.22±0.00 no	0.1±0.0050 ij	0.32±0.005 n	12.23±0.15 o	91.2±2.01 a-c
SA (10 mM)	25°C	0.476±0.018 ij	0.143±0.026hi	0.62±0.015jk	51.3±0.05b-d	24.03±1.86 i
	30°C	0.53±0.015 hi	0.24±0.023d-f	0.77±0.031 i	48.73±0.18f-i	35.13±0.29 h
	35°C	0.593±0.08 gh	0.216±0.021fg	0.81±0.017 i	45.87±0.48jk	44.23±1.36 g
	40°C	0.236±0.02 no	0.113±0.003h-j	0.35±0.03 mn	26.23±0.28m	79.79±4.08ef
	45°C	0.163±0.006 o	0.07±0.0101 j	0.233±0.02 o	11.8±0.21 op	91.2±0.89 a-c

†: Data are shown as the means of three replicates ± S.D. Values followed by the same letters in a column are not significantly different according to Duncan tests at a 5% level; SA: Salicylic acid, L.D.W: Leaf Dry Weight, S.D.W: Stem Dry Weight, P.D.W: Plant Dry Weight, RWC: Relative water content, MP: Cell Membrane stability.

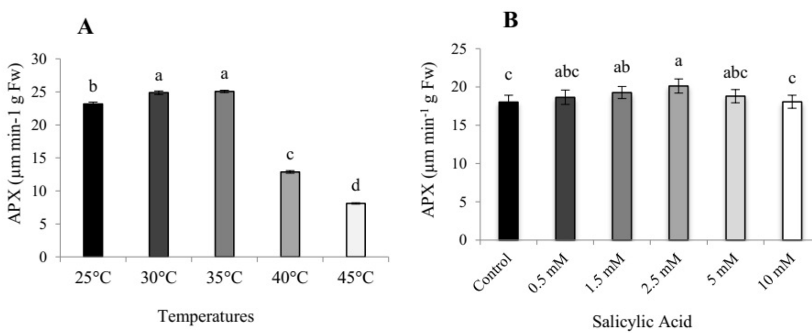

Figure 2. Effect of temperature (A) and salicylic acid (B) on ascorbate peroxidase (APX) activity in maize 'MV500'.

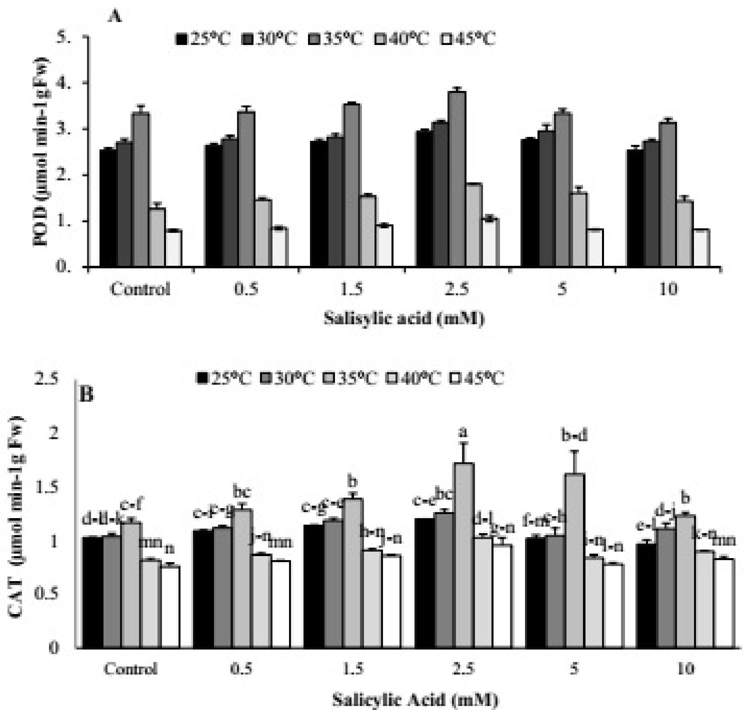

Figure 3. The interaction effect of salicylic acid and temperature on peroxidase (POD) (A) and catalase (CAT) (B) activity in mazie 'MV500'.

limited capacity of the photosynthetic electron transport.[26] An evaluation of 36 genotypes regarding yield traits, phenological traits, plant architectural traits, physiological traits, and stress index under drought, heat, and hybrid stress environments revealed significant losses under heat stress.[27]

An increase in temperature from 25 to 45°C led to a reduction in stomatal conductance. However, treatment with 2.5 mM SA enhanced stomatal conductance under heat-stress conditions. Stomatal closure, a significant factor in heat stress and drought conditions, reduces CO_2 intake into the photosynthetic system. As temperature increases, evaporation and transpiration rates rise, demanding an increased water uptake. The plant responds with stomatal closure if sufficient water is unavailable for absorption. Furthermore, stomatal limitation is attributed to a decrease in Rubisco carboxylation activity and an increase in photorespiration, which results in photosynthetic reduction.[28] SA, a plant growth regulator, positively impacts enzymes involved in photosynthesis, thereby enhancing photosynthetic capacity.[29]

Plant water potential is significantly affected by heat stress. Under such stress, RWC demonstrates a notable decline, with the most prominent increase observed when 2.5 mM of SA was applied at 25°C. As the temperature rises, the evapotranspiration trend escalates, amplifying the water absorption's thermodynamic properties in the root medium. However, when

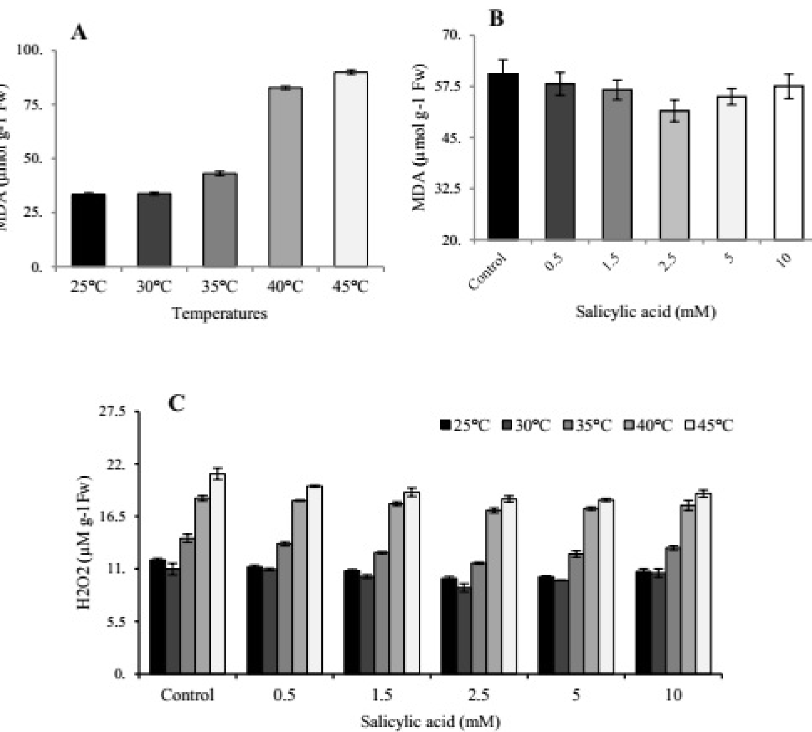

Figure 4. Effect of temperature (A) and salicylic acid (B) on malondialdehyde (MDA) and H_2O_2 (C) in maize ‘MV500’.

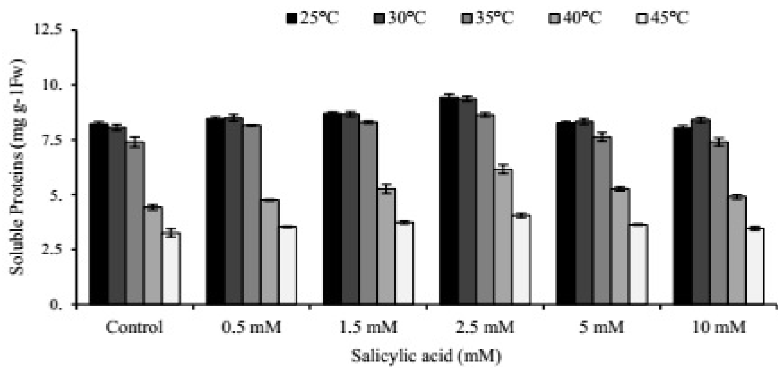

Figure 5. The interaction effect of salicylic acid and temperature on soluble protein content in maize ‘MV500’.

temperatures exceed the plant’s threshold, the balance between the absorbed and evaporated water (known as the Hydrostatic gradient) is disturbed due to the deterioration of cell structures and self-regulation mechanisms and an increase in electrolyte leakage and stomatal closure. This leads the plant to confront drought-like conditions.[30] In this study, it was observed that a temperature of 45°C, without the application of SA, resulted in the highest level of electrolyte leakage. This may be attributed to the enhanced kinetic energy and movement of molecules across cell membranes, breaking chemical bonds in biological membranes and increasing cell membrane fluidity.[30] Heat stress directly affects proteins and unsaturated fatty acids.[31] The damage inflicted on the cell membrane under heat stress conditions compromises the stability of macromolecules and boosts membrane lipid peroxidation. Given that oxidative stress is common under heat stress conditions, this experiment found that heat stress augmented MDA levels.

The application of SA stimulates antioxidant systems, such as CAT, POD, and APX. It also promotes the accumulation of adaptive osmolytes, including glycine betaine and proline, two primary organic osmolytes that amass in response to environmental stressors. Additionally, the application of SA was found to enhance plant water potential, potentially increasing the re-

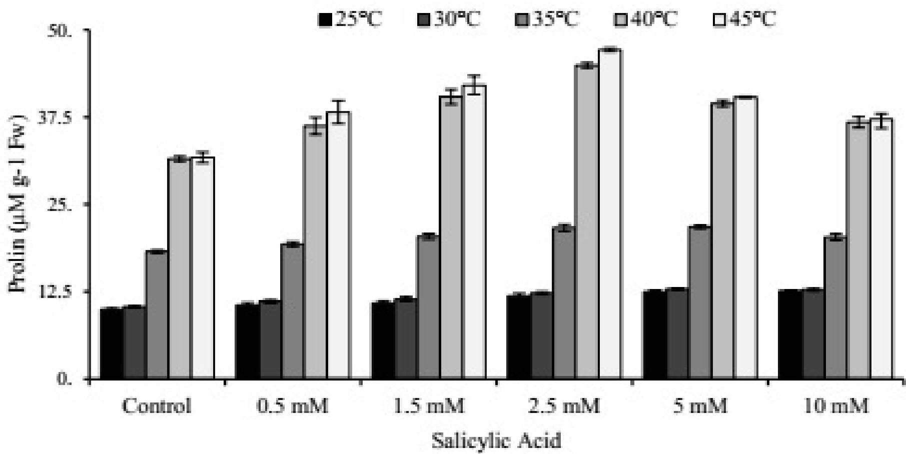

Figure 6. The interaction effect of salicylic acid and temperature on proline content in maize 'MV500'.

sistance of the cell membrane and regulating ROS in plants. This may lead to a decrease in the cell membrane disruption and permeability. In this study, the application of SA increased soluble protein content, cell membrane stability, photosynthetic system efficiency, and antioxidant enzyme activity. As a signaling molecule, SA enhanced gene expression efficacy and HSP-mRNA transcription. Therefore, SA mitigates heat stress in plants and prevents a reduction in soluble protein content. HSFs stimulated the genes responsible for encoding H_2O_2 purification enzymes, such as APX1.[32] The study conducted on rice genotypes observed a significant rise in the activity of antioxidant enzymes, including SOD, APX, and GPX, in both flag leaf and spikelet tissues of the MTU-1010 genotype. Additionally, the MTU-1010 genotype exhibited significantly higher SLW (specific leaf weight) and RWC (relative water content) than the PR-113 genotype across all treatments. This genotype displayed a higher spikelet fertility, a photosynthesis rate, an induced antioxidant system, and improved transpiration, RWC, and SLW, thereby exhibiting greater heat stress tolerance during the flowering stage compared to the PR-113 genotype.[33] Overall, the effect of SA in increasing the activity of antioxidant enzymes under temperature stress is attributed to ROS detoxification. HSFs decrease protein synthesis, particularly as temperatures rise, which triggers the production of a new group of low molecular mass proteins known as heat shock proteins. These proteins act as molecular chaperones within cells.[34] The increase in soluble protein content under SA application may be due to the stimulation of hydrolysis of insoluble proteins, resulting in the accumulation of osmolytes.[35] The protein content decreases as the temperature rises, with the lowest protein content recorded at 45°C. The application of SA increases the soluble protein content, with the highest amount observed at an application of 2.5 mM SA and a temperature of 25°C. Previous reports have also shown that the application of SA increases soluble protein content in Arabidopsis shoots and roots, which aligns with the findings of this research.[35] Furthermore, foliar application of SA has been found to increase carbohydrate content, soluble proteins, free amino acids, and proline content in Basil, supporting the results of this experiment.[36] Proline plays various roles in plants, including regulating osmotic potential, maintaining cell membrane integrity, balancing enzymes/proteins, and the appropriate ratio of $NADP^+$/NADPH and the scavenging of ROS. The proline accumulation under stress conditions depends on the plant's resistance capacity. A disruption of protein synthesis can result in proline accumulation due to a decreased conversion of proline into protein, leading to reduced growth.[37]

CONCLUSION

The exposure to heat stress led to a decrease in biological yield, RWC, CAT, and POD activity. SA induces a differential antioxidant response in spring maize under high-temperature stress. However, compared to the untreated group, the application of SA to adapt to high temperatures effectively increased proline, chlorophyll, and MDA levels. The utilization of SA exhibited the most significant impact on the growth parameters of maize plants under high-temperature conditions, mitigating the detrimental effects of heat stress on maize.

Acknowledgments: This project was performed by the facilities of the Islamic Azad University of Mahabad, Iran.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.N.; Data Acquisition- N.D.; Data Analysis/Interpretation- N.D, M.H., E.N.; Drafting Manuscript- N.D.; Critical Revision of Manuscript- E.N., N.D., M.H., K.A.; Final Approval and Accountability- E.N., N.D., M.H., K.A.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

ORCID IDs of the authors

Esmail Nabizadeh 0000-0002-2449-3984
Narges Dolatmand 0000-0003-1910-825X
Masoud Haghshenas 0000-0001-7379-2107
Khadijeh Ahmadi 0000-0002-2289-9710

REFERENCES

1. Xu N, York K, Miller P, Cheikh N. Co-regulation of ear growth and internode elongation in corn. *Plant Growth Reg.* 2004;44:231–241.
2. Atkinson NJ, Urwin PE. The interaction of plant biotic and abiotic stresses: From genes to the field. *J Exp Bot*. 2012;63:3523–3543.
3. Impa SM, Perumal R, Bean SR, John Sunoj VS, Krishna Jagadish SV. Water deficit and heat stress induced alterations in grain physico-chemical characteristics and micronutrient composition in field grown grain sorghum. *J Cer Sci Technol.* 2019;86:124-131.
4. Crafts-Brandner SJ, Salvucci ME. Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. *Plant Physiol*. 2002;129:1773–1780.
5. Ma M, Wang P, Yang R. UV-B mediates isoflavone accumulation and oxidative-an 460 tioxidant system responses in germinating soybean. *Food Chem*. 2019;275:628-636.
6. Wang LJ, Fan L, Loescher W, et al. Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves. *BMC Plant Biol*. 2010;10:1-10. doi:10.1186/1471-2229-10-34.
7. Sajjad Y, Jaskani MJ, Ashraf MY, et al. Response of morphological and physiological growth attributes to foliar application of plant growth regulators in gladiolus 'white prosperity'. *Pak J Agric Sci*. 2014;51:123–129.
8. Pérez MGF, Rocha-Guzmán NE, Mercado-Silva E, et al. Effect of chemical elicitors on peppermint (*Mentha piperita*) plants and their impact on the metabolite profile and antioxidant capacity of resulting infusions. *Food Chem*. 2014;156:273–278.
9. Karlidag H, Yildirim E, Turan M. Exogenous applications of salicylic acid affect quality and yield of strawberry grown under antifrost heated greenhouse conditions. *J Plant Nutr Soil Sci*. 2009;172:270–276
10. Hayat S, Ali B, Ahmad A. Salicylic acid: Biosynthesis, metabolism and physiological role in plants, In: Salicylic Acid: A Plant Hormone. Springer. 2007; pp:1–14.
11. Dat JF, Lopez-Delgado H, Foyer CH, Scott IM. Effects of salicylic acid on oxidative stress and thermotolerance in tobacco. *J Plant Physiol*. 2000;156:659–665.
12. Souri MK, Tohidloo G. Effectiveness of different methods of salicylic acid application on growth characteristics of tomato seedlings under salinity. *Chem Biol Technol Agric*. 2019;6:26. doi:10.1186/s40538-019-0169-9.
13. Singh B, Usha K. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul*. 2003;39:137–141.
14. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Trans*. 1983;11:591–592.
15. Lutts S, Kinet JM, Bouharmont J. NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance. *Ann Bot*. 1996;78:389–398.
16. Ritchie SW, Nguyen HT, Scott Holaday A. Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. *Crop Sci*. 1990;30:105–111.
17. Kang HM, Saltveit ME. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. *Physiol Plant.* 2002;115:571–576.
18. Aebi H. Catalase *in vitro*. *Methods Enzymol*. 1984;105:121-126.
19. Upadhyaya A, Sankhla D, Davis TD, et al. Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. *J Plant Physiol*. 1985;121:453–461.
20. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*. 1981;22:867–880.
21. Irigoyen JJ, Einerich DW, Sánchez-Díaz M. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa) plants. *Physiol Plant*. 1992;84:55–60.
22. Bradford M. A Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72(1–2):248–254.
23. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Bioph*. 1968;125:189–198.
24. Velikova V, Yordanov I, Edreva A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci*. 2000;15:59–66.
25. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. *Plant Soil*. 1973;39:205. doi:10.1007/BF00018060.
26. Salvucci ME. Association of Rubisco activase with chaperonin-60β: A possible mechanism for protecting photosynthesis during heat stress. *J Exp Bot.* 2008;59:1923–1933.
27. Gupta R. Manganese repairs the oxygen-evolving complex (OEC) in maize (Zea mays L.) damage during seawater vulnerability. *J Plant Nutr Soil Sci*. 2020;20:1387–1396.
28. Yashavanthakumar KJ, Baviskar VS, Navathe S, et al. Impact of heat and drought stress on phenological development and yield in bread wheat. *Plant Physiol Rep.* 2021;26:357–367.
29. Kalaji HM, Schansker G, Brestic M. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynth Res.* 2017;132:13-66.
30. Ali F, Waters DLE, Ovenden B, et al. Heat stress during grain fill reduces head rice yield through genotype-dependent increased husk biomass and grain breakage. *J Cereal Sci.* 2019;90:102820.doi:10.1016/j.jcs.2019.102820.
31. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in

plants: An overview. *Environ Exp Bot*. 2007;61:199–223.

32. Portis AR, Parry MAJ. Discoveries in Rubisco (Ribulose 1, 5-bisphosphate carboxylase/oxygenase): A historical perspective. *Photosynth Res*. 2007;94:121–143.
33. Khan W, Prithiviraj B, Smith DL. Photosynthetic responses of corn and soybean to foliar application of salicylates. *J Plant Physiol*. 2003;160:485–492.
34. Beney L, Gervais P. Influence of the fluidity of the membrane on the response of microorganisms to environmental stresses. *Appl Microbiol Bio*. 2001;57:34–42.
35. Allakhverdiev SI, Kreslavski VD, Klimov VV, et al. Heat stress: An overview of molecular responses in photosynthesis. *Photosynth Res*. 2008;98(1-3):541-550.
36. Reddy RA, Kumar B, Reddy PS, et al. Molecular cloning and characterization of genes encoding Pennisetum glaucum ascorbate peroxidase and heat-shock factor: interlinking oxidative and heat-stress responses. *J Plant Physiol*. 2009;166:1646–1659.
37. Karwa S, Arya SS, Maurya S, Pal M. Physiological characterization of reproductive stage heat stress tolerance in contrasting rice genotypes. *Plant Physiol Rep*. 2020;25:157–162.

How to cite this article

Nabizadeh E, Dolatmand N, Haghshenas M, Ahmadi K. Physiological and Biochemical Changes of Maize (*Zea mays* ‘MV500’) in Response to Heat stress under Levels of Salicylic Acid. Eur J Biol 2023; 82(2): 167–178. DOI:10.26650/EurJBiol.2023.1216574