Asian Journal of Advances in Agricultural Research

Volume 24, Issue 10, Page 17-33, 2024; Article no.AJAAR.124728
ISSN: 2456-8864

Regulation of Physiological and Biochemical Mechanisms Related to Water Stress Tolerance in Barley and Improvement of Growth and Productivity Using Salicylic Acid and Chitosan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajaar/2024/v24i10553

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124728

Original Research Article

Received: 06/08/2024
Accepted: 08/10/2024
Published: 15/10/2024

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Cite as: *El-Wakeel, Sally E., Mohamed Mansour, Ashgan M. Abd El-Azeem, and Aziza A. Aboulila. 2024. "Regulation of Physiological and Biochemical Mechanisms Related to Water Stress Tolerance in Barley and Improvement of Growth and Productivity Using Salicylic Acid and Chitosan". Asian Journal of Advances in Agricultural Research 24 (10):17-33. https://doi.org/10.9734/ajaar/2024/v24i10553.*

ABSTRACT

The present investigation aimed to assess the impacts of three Salicylic Acid (SA) concentrations (T_2=100, T_3=200, T_4=300ppm), three Chitosan concentrations (T_5=150, T_6=225, T_7=300ppm) and T_8= Salicylic Acid (100 ppm) with Chitosan (150 ppm) mixture in comparison to control treatment (T_1) on two barley cultivars Giza126 and Giza 134 under rainfed conditions. Some vegetative and yield traits were recorded, plant height, spike length, number of gains spike^{-1}, number of spikes/m^2, 1000 grain weight, biological and grain yield. A split plot design was performed with three replications. The field experiment was conducted in the northern west coast region of Egypt (Marsa Matruh government) under the rainfed conditions during 2019/2020 and 2020/2021 growing seasons. The results showed a direct relationship between increased foliar application and increased yield attributes. Results indicated that Giza 126 cultivar as affected by T_4 showed the most desirable values for grains spikes^{-1} in first season, spikes/m^2, biological yield and grain yield during the first and second seasons respectively. In addition, barley cultivar Giza 126 as affected by T_3 showed the highest values for grains spikes^{-1} in second season. On the other hand, barley cultivar Giza 134 as affected by T_4 showed the highest values for plant height, spike length and 1000-grain weight in first and second seasons respectively. From results, it can be concluded that the Salicylic Acid (SA) concentration 300 ppm is recommendable for improving the productivity of barley under rainfed conditions of Egypt. To study the potential role of SA and Chitosan on water stress tolerance mechanisms some biochemical and physiological parameters were recorded. Application of SA and Chitosan decreased significantly Reactive Oxygen Species (ROS) measuring by determined the concentrations of MDA and H_2O_2 especially SA with concentrations 300 ppm (T4). For osmolytes (proline, SSC and SPC), all treatments using SA and Chitosan induced significant increase in osmolytes content compared with control under water stress only in the two studied barley genotypes, except for treatment with Chitosan 225 and 300 ppm in Giza 126 for proline content. Similar results were recorded for antioxidant enzymes activity (CAT, POX and PPO) which showed up-regulation for all treatments compared with control under water stress for the two studied genotypes. On contrast, APX antioxidant enzyme recorded the highest activity in control treatment under water stress only. In general, for all studied treatments comparing with control, application of SA_{300} ppm and the mixture between SA_{100} and $Chitosan_{150}$ ppm were the best treatment for induction of up and down regulation of biochemical and physiological component in the barley cells which is known as defense system against water stress damage.

Keywords: Barley; rainfed condition; salicylic acid; chitosan; up-regulation of physiological; biochemical mechanisms.

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is among the most popular and widely used crops due to their economic, high health and nutritional values. Many parts of the world depend on this crop for food, feed and malt-based food products [1,2,3]. It is vulnerable to environmental stresses. Under the dry land condition barley production is better than other cereal crops such as oats and wheat [4,5]. Barley is the main crop grown in a large scale in the North Coastal Region of Egypt and in the newly reclaimed lands [6]. Most of these lands are suffering from water shortage, low levels of soil moisture, where cereals such as wheat and maize cannot grow well. Drought stress can be simply defined as a shortage of water that induces dramatic morphological, biochemical, physiological and molecular changes [7]. All of these changes reduce plant growth and crop production. Improving drought stress tolerance is a very challenging task for barley researchers and more research needed to manage this problem. The progress made in understanding drought tolerance is due to advances in three main research axes: plant breeding, physiology and genetic research. The physiology research focused on the physiological and biochemical metabolic pathways that plants used when exposed to drought stress. To know the differences between the potential and the actual yield in drought-prone environments, it is necessary to select plants with physiological traits conferring drought tolerance. If this quest is to be efficient, both traditional plant breeding methods and molecular methods of improvement need to be explored [8].

Salicylic acid (SA), a phytohormone, is a promising compound that can reduce the sensitivity of plants to environmental stresses

through regulation of the antioxidant defense system, transpiration rates, stomatal movement, and photosynthetic rate[9]. Salicylic acid (SA) as an endogenic phytohormone from phenolic compounds influence many physiological processes such as: seedling growth [10], maintenance of tissue water contents and reduced membrane permeability [11], responsive antioxidative system [12,13,14], tolerance to environmental stresses [15] and activity of photosynthesis pigments [16].

In agriculture, chitosan is used as foliar application to plants, seed treatment, or as a direct soil fertilizer. Studies has shown that chitosan decreases transpiration through partial or full closure of stomata [17]. Chitosan and its derivatives have been shown to be effective in enhancing the crop tolerance to water deficit by mitigating the deleterious effects of water stress on harvest index and yield [18,19]**.** Many studies indicated that chitosan is beneficial in protecting plants against the oxidative stress [20] and enhancing plant growth in different crops**.** [21].

2. MATERIALS AND METHODS

The field experiment was conducted in the northwestern coast region of Egypt (Marsa Matruh government) under the rainfed conditions during 2019/2020 and 2020/2021 growing seasons to study the effects of foliar application by salicylic acid and chitosan on productivity of the two commercial barley cultivars Giza 126 and Giza 134. The treatments were arranged in a split plot design with three replications. The main plots were assigned to varieties, while the sub plots were allocated to growth regulators. Eight treatments were applied: Control, three concentrations of salicylic acid (100ppm, 200ppm, 300ppm), three concentrations of chitosan (150ppm, 225ppm, 300ppm) and salicylic acid 100ppm and chitosan 150ppm mixture.

The sowing of seeds was done manually drilled at the rate of 30 kg Fed^{-1} on 1st December in both seasons. plot size was 4.2m^2. Each treatment was sown in six rows of 3.5 m^2, spaced with 20 cm among rows. Cultivation practices recommended by the Egyptian Ministry of Agriculture under rainfed condition.

Foliar applications of salicylic acid and chitosan were performed in two stages during the two growing seasons: at the tillering stage (40 days after planting) and at the flowering stage (70 days after plant emergence).

The data of temperature, rainfall and relative humidity (%) during the two seasons are showed in Table 1. The experimental site on an average has annual precipitation of about 60.82 and 83.33 mm in the first and second seasons, respectively, with minimum and maximum temperature of 11°C and 24°C**.**

The yearly precipitation at the experimental location is 60.52 mm in the first season and 82.93 mm in the second, with minimum and maximum temperatures of 11°C and 24°C, respectively (Table 1). In comparison to the first season, the averages of the studied traits increased when the rainfull rate increased in the second season.

2.1 Field Data Collection and Statistical Analysis

Plant height, spike length, number of grains $spikes^{-1}$, number of spikes m^{-2}, biological yield, 1000 grain weight and grain yield were recorded for this study. The data collected were statistically analyzed using ANOVA function of SAS program. After performing ANOVA, the differences between the treatment means were compared by LSD test at 5% level of significance [22].

Table 1. Temperature, rainfall and relative humidity (%) during 2019/2020 and 2020/2021 growing seasons at Marsa Matruh location

Month	Temperature				Rain-fed (mm)		Relative humidity (%)	
	2019/2020		2020/2021		2019/2020	2020/2021	2019/2020	2020/2021
	Max	mini	max	mini				
Nov.	24	18	21	17	29.37	0.10	67	63
Dec.	19	15	19	14	3.60	7.82	61	65
Jan.	15	11	18	13	8.50	20.10	62	67
Feb.	17	12	17	12	15.05	7.90	63	68
Mar.	19	13	18	13	4.00	47.01	60	64
Apr.	21	14	22	15	0.30	0.40	55	64

2.2 Biochemical and Physiological Assay

2.2.1 Estimation of reactive oxygen species (ROS)

Lipid peroxidation in barley was assessed by estimating the levels of malondialdehyde (MDA) and H_2O_2. Each sample contained 100 mg of plant material, which was homogenized with 2 ml of 0.1% trichloroacetic acid (TCA) and centrifuged (10000 rpm) for 15 min under cooling at 4°C. Following centrifugation, the supernatant was extracted and used as the malondialdehyde (MDA) and H_2O_2 extract as follows:

a- Malondialdehyde (MDA) content: Approach of [23] is used for MDA quantification. After mixing 500 µl of the supernatant with 1.5 ml of 0.5% thiobarbituric acid (made with 20% TCA), the mixture is incubated for 20 min at 90 °C. The samples are centrifuged for five min at 10,000 rpm after the reaction is stopped in an ice bath. Using a spectrophotometer model UV-160A (Shimadzu, Japan), the absorbance of the supernatant is measured at 532 nm. The non-specific absorbance at 600 nm is then subtracted to adjust the optical density. The extinction value of 155 mM^{-1} cm^{-1} is used to calculate the MDA content, which is reported in nmol MDA per gram of fresh weight (nmol MDA/g FW).

b- Hydrogen peroxide (H_2O_2) concentration: To quantify H_2O_2 content as described by Sarker and Oba [24], 200 µl of plant extract and 800 µl of Na-phosphate buffer (10 mM, pH 7) were combined, and 1 ml of KI (1 M) was subsequently added. The absorbance at 390 nm was measured after the set was incubated for 10 minutes at room temperature. H_2O_2 standard curve was used to calculate the H_2O_2 content.

2.2.2 Estimation of osmolytes

a- Proline content: The proline content of the leaf was measured using the **Bates et al. [25]** method. Frozen leaves weighing 100 mg were crushed. After adding 1.8 ml of 3% sulfo-salicylic acid, the mixture was centrifuged for 10 min at 14,000 rpm. Each sample's 1.5 ml of supernatant is taken out and put into fresh screw-cap glass tubes. After that, 30 ml of acetic acid, 20 ml of 6M ortho-phosphoric acid, and 1.5 ml of ninhydrin solution containing 1.25 g of ninhydrin are added. This mixture is heated for one hour in a water bath to boiling (100 °C). Redness slowly appears in the solution. To create two phases, add 3 ml of toluene to each tube and mix when it has cooled. After gathering the upper phase, the optical density (OD) of this phase is determined at 520 nm using a spectrophotometer. A proline content as µg/g fresh weight was computed.

b- Total soluble sugars content (SSC): The methods described by Dreywood [26] using the Anthrone method was employed to quantify the soluble sugars, fructose and glucose. The process of extracting soluble sugars involves macerating barley leaves in 2 ml of 80% ethanol, then incubating the mixture for 40 min at 75 °C in a water bath, and centrifuging the mixture for five min under cooling. After collecting the supernatant, the pellet was used again in the same manner. To evaporate the alcohol, the two obtained supernatants (4 ml) from separate samples were put in glass screw-cap tubes and placed in a vortex evaporator. The residue was diluted in 10 ml of distilled water (an analytical solution) in each tube. In 75% sulfuric acid, the Anthrone reagent was produced at a concentration of 2 g/L. Anthrone reagent (4 ml) was added to 1 ml of the sample (0.25 ml of the analytical solution + 0.75 ml of distilled water). After the mixture was vortexed, it was heated to 100 °C for eight min. The tubes were then allowed to cool in an ice bath, and the absorbance at 625 nm was determined.

c- Total soluble proteins content (SPC): A proximately 200 mg barley leaf sample was homogenized in 2 ml of chilled Na-phosphate buffer (100 mM, pH 7.5) on ice. Protein extract was the supernatant obtained by centrifugation (8000 rpm/15 min). The amount of soluble proteins was measured photometrically using the Brilliant Blue G-250 reagent, in accordance with the method described by Bradford [27].

2.2.3 Estimation of antioxidant enzymes activity

Each treatment's approximately 0.5 g of fresh barley leaf material was homogenized at 4°C in 3 ml of 50 mM phosphate buffer (pH 7), which contained 50 mM Tris, 1mM EDTA-Na_2, 7.5% PVPP. The mixture was centrifuged under cooling at 12,000 rpm for 30 min and the soluble enzyme activities in the supernatant (enzymes extract) were determined via spectrophotometer at room temperature as follow:

a- **Catalase (CAT) activity:** The activity of catalase (CAT) was measured with the method described by Aebi [28]. The reaction was started by adding 50 µl of enzymes extract to 2ml of

solution assay containing 0.1M Na-phosphate buffer (pH 6.5), 100µl hydrogen peroxide. After mixing the solution, the mixture was incubated 5 min at room temperature and the absorbance was measured at 240 nm every 30 seconds for 3 min. CAT activity was calculated as mM H_2O_2 /g FW/min.

b- **Peroxidase (POX) activity:** The activity of peroxidase (POX) was measured with the method described by Hammerschmidt et al. [29]. Mix 50 µl of the enzyme extract with 2.9 ml of 100mM Na-phosphate buffer (pH 6.0) that contains 0.25% (v/v) guaiacol (2-methoxy phenol) and 100mM H_2O_2 in the reaction assay. For three min, changes in absorbance at 470 nm were noted every 30 seconds. Absorbance/min/g of fresh weight was increased to indicate the level of enzyme activity.

c- **Ascorbate peroxidase (APX) activity:** Ascorbate peroxidase (APX) was determined photometrically with the method described by Sarker and Oba [30]. In summary, the test solution containing Na-phosphate buffer (50 mM, pH 7), H2O2 (1 mM), EDTA (0.2 mM), and ascorbic acid (0.5 mM) was mixed with 50 µl of enzyme extract to initiate the reaction. The reaction was allowed to settle for one min at room temperature, at which point the absorbance was measured at 290 nm.

d- **Polyphenol oxidase (PPO) activity:** Activity of polyphenol oxidase (PPO) was measured as described by Hammerschmidt et al. [29] and evaluated by mixing 1.5ml of 0.1M Na-phosphate buffer (pH 6.5) with 100 µl of enzymes extract well. After that, to start the reaction, 200 µl of 0.01M catechol was added. The change in absorbance/min/g of fresh weight was used to express the absorbance change, which was measured at 495 nm.

All physiological and biochemical data were statistically analyzed using one-way ANOVA with Duncan test. The P value ≤ 0.05 was statistically significant, and the analysis was performed using SXW program.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Analysis of variance**,** data in Table 2 indicated that significant or highly significant differences were obtained for all studied traits among the treatments and the effects due to cultivars except for spike length in the first season. The varieties and treatments × varieties interaction effects were highly significant for all traits except for plant height in the second season and spike length in both growing seasons.

Mean performance of all the studied traits of the interaction among seasons, two varieties are shown in Table 3. Giza 126 achieved the best values for grains spike, spikes/m^2, biological and grain yield in both growing seasons. In contrary Giza134 recorded the highest values for plant height, spike length and100 Grain weight in both growing seasons.

3.2 Effect of Treatments and Seasons Interaction

Mean performance of all the studied traits of treatments and seasons interaction are shown in Table 4. Treatment number 4 [salicylic acid 300ppm] produce desirable results for all studied traits in both seasons, followed by treatment number 3 [salicylic acid 200ppm] for spike length, number of Grains per spike and100 Grain weight and treatment number 8 [salicylic acid 100ppm and chitosan 150ppm] mixture for Plant height, number of spikes per m^2, Biological yield (kg/fed) and Grain yield (kg/fed). While, Treatment number 1 (control treatment) exhibited the lowest values for all traits in the first and second seasons also.

3.3 Effect of Cultivars, Treatments and Seasons Interaction

Data of plant height (cm), spike length(cm), number of grains spike^{-1}, number of spikes m^{-2} ,100 grain weight (g), biological yield (kg/fed) and grain yield (kg/fed) as influenced by the interaction between cultivars and foliar treatments in 2019/2020 and 20/2021seasons are shown in Table 5.

It is clear from the data in Table 5 that Giza 126 cultivar as affected by treatment number 4 [salicylic acid 300ppm] showed its superiority for grains spikes^{-1} in first season (60.0 grain), spikes/m^2 (201.67 and 212.10 spike), biological yield (2174.33 and 2293.33 kg) and grain yield (661.60 and 708.33 kg) during the first and second seasons respectively. In addition, barley cultivar Giza 126 as affected by treatment number 3 [salicylic acid 225ppm] showed the highest values for grains spikes^{-1} in second season (62.37 grain). On the other hand, barley cultivar Giza 134 as affected by treatment

number 4 [salicylic acid 300ppm] showed the highest values for plant height (68.47 and 70.17cm), spike length (6.83 and 7.07cm) and 1000 grain weight (44.24 and 44.94g) in first and second seasons respectively.

3.4 Changes in ROS Parameters

Malondialdehyde (MDA) content: Malondialdehyde (MDA) is one of the end products of lipid peroxidation that indicates the presence of stress. Therefore, it is thought to be a very reliable indicator of a plant's ability to withstand different abiotic stresses especially water deficit. MDA content in the eight studied treatments for the two barley genotypes under water stress condition was investigated. Under water stress conditions as presented in Fig. 1, data showed the role of SA and Chitosan in down-regulations of MDA. There were significant decrease in the content of MDA in all treatments compared to control, except for T7 ($Chitosan_{300}$) in barley Giza 126. Also, SA_{300} (T4) is the most positive treatment exhibiting the lowest MDA content compared with control and the other treatments in both genotypes Giza 126 and Giza 134.

Hydrogen peroxide (H_2O_2) concentration: Hydrogen peroxide (H_2O_2) is one of the most persistent reactive oxygen species (ROS) formed in plant cells during various physiological processes including photosynthesis and photorespiration; under stressful conditions, H_2O_2 play as a signaling molecule. Plants may benefit from this reaction as they build defensive systems against a variety of stressful conditions. It can induce defense genes which required in the induction of systemic acquired resistance. As showed in Fig. 2, SA_{300} (T4) is the most positive treatment playing the major role in down-regulation of H_2O_2 concentrations and exhibiting the lowest level compared with control and the other treatments in Giza 126, while SA_{300} (T4) and the mixture between SA_{100} and $Chitosan_{150}$ (T8) was the best treatments for Giza 134 which recorded the lowest concentration of H_2O_2. On the other hand, control treatment (T1) recorded the highest level of H_2O_2 which highly differentially significant from all other treatments in Giza126 and Giza134.

3.5 Changes in the Osmolytes

Proline content: For proline accumulation, T4 (SA_{100}) up-regulated and accumulated the highest levels of proline under water stress conditions (Fig. 3) in the two studied barley genotypes which differentially significant from control and the other treatments, indicating that this treatment induce water stress tolerance for Giza 126 and Giza134 in north Egypt conditions. For the other treatments, Giza 126 showed no significant differences between control (T1) and Chitosan treatments, except for the lowest concentrations (150 ppm). On the other hand, Giza 134 recorded significant differences for all treatments compared with control, while the treatments recorded partially significant differences between some of treatments when not comparing with control.

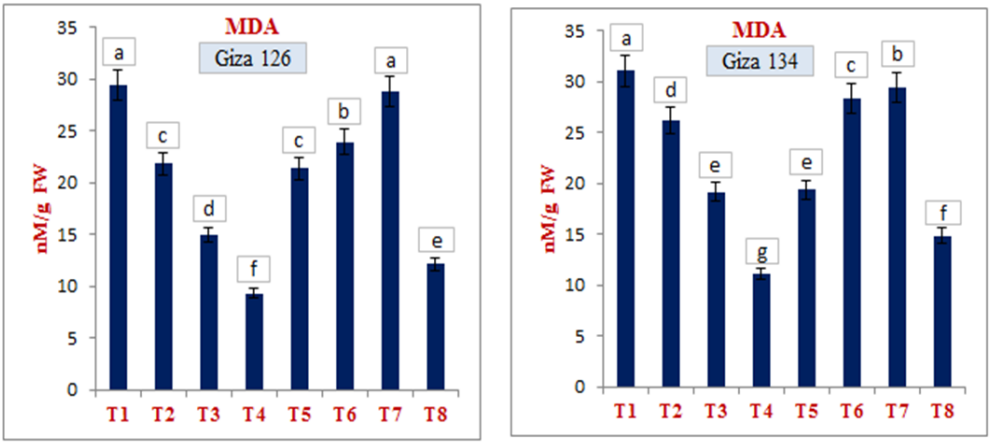

Fig. 1. Effect of SA and Chitosan on down-regulation of malondialdehyde (MDA) level in the two studied barley genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

Table 2. Analysis of variance for the studied traits across the two growing seasons

SOV	df	Plant height		Spike length		Grains spike $^{-1}$		Spikes/m^2		100 Grain weight		Biological yield		Grain yield	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Blocks	2	0.02ns	1.31ns	0.20ns	0.71ns	4.38ns	0.69 ns	7.77ns	7.79ns	0.16*	0.14ns	239.84ns	346.64* ns	60.27ns	144.88ns
Cultivars (C)	1	878.94**	767.20**	1.57ns	1.43*	544.72**	619.20**	2275.63**	4153.38**	21.10**	23.45**	428179.63**	184140.19*	32536.46**	10301.88**
Main Plot Error	2	0.16	0.66	0.30	0.07	1.90	0.59	1.09	3.78	0. 004	0.10	2116.09	3392.06	144.25	22.07
Treatments (T)	7	157.25**	71.43**	2.70**	1.68**	142.72**	125.57**	909.35**	1022.88**	17.71**	14.18**	303829.68**	384960.07**	31234.99**	36679.22**
C x T	7	6.93**	5.94ns	0.16ns	0.13ns	24.20**	29.06**	117.86**	132.15**	1.11**	1.28**	57343.48**	21645.42**	6431.90**	4139.43**
Error	28	1.69**	5.39	0.11	0.16	1.17	3.02	5.68	5.58	0.13	0.13	3426..81	1724.71	248.10	70.60

Table 3. Mean performance of the studied traits as affected by season and cultivars

Cultivars	Plant height (cm)		Spike length (cm)		Grains spike $^{-1}$ (grain)		Spikes/m^2 (spike)		100 Grain weight (g)		Biological yield (kg/fed)		Grain yield (kg/fed)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Giza 126	51.01	57.12	5.51	5.85	53.88	56.43	178.88	192.54	39.84	40.55	1742.33	1894.08	516.49	559.98
Giza 134	59.57	65.11	5.87	6.19	47.14	49.25	165.11	173.93	41.16	41.94	1553.44	1770.21	464.42	530.68
LSD 0.05	0.49	1.01	-	0.33	1.71	0.96	1.30	2.41	0.08	0.39	57.13	72.33	14.91	5.83

Table 4. Mean performance of the studied traits as affected by season and treatments interaction over the two seasons of 2019/2020 and 20/21

Treatment	Plant height (cm)		Spike length (cm)		Grains spike $^{-1}$ (grain)		Spikes/m^2 (spike)		1000 Grain weight (g)		Biological yield (kg/fed)		Grain yield (kg/fed)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
T_1	48.00	55.43	4.23	5.08	41.18	43.53	153.75	163.03	37.87	38.79	1193.33	1320.00	350.00	385.43
T_2	53.08	59.83	5.83	6.17	48.87	51.72	166.00	178.37	39.91	41.12	1600.83	1811.67	467.58	534.03
T_3	57.38	62.50	6.10	6.42	54.17	56.55	171.83	184.48	42.29	42.66	1695.83	1971.67	507.50	578.53
T_4	63.85	65.67	6.50	6.87	56.55	58.19	192.50	204.00	43.01	43.61	1905.08	2145.00	576.18	644.67
T_5	49.68	57.42	5.43	5.68	46.52	50.02	162.02	172.09	39.22	40.23	1530.00	1641.67	444.95	495.42
T_6	54.42	61.23	5.58	5.77	51.23	53.19	168.17	178.49	40.06	40.95	1625.00	1851.67	487.50	549.77
T_7	57.13	62.30	5.83	6.03	52.37	54.17	180.19	191.70	41.78	42.25	1762.17	1937.17	528.65	581.27
T_8	58.78	64.53	6.00	6.13	53.17	55.39	181.50	193.73	39.85	40.35	1870.83	1978.33	561.25	593.55
LSD 0.05	1.53	2.74	0.39	0.48	1.27	2.05	2.81	2.79	0.43	0.43	69.23	49.11	18.62	9.93

T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

Table 5. Mean performance of the studied traits as affected by cultivars, treatments and seasons interaction over the two seasons of 2019/2020 and 2020/2021

Cultivars	Treatments	Plant height (cm)		Spike length (cm)		Grains spike $^{-1}$ (grain)		Spikes/m^2 (spike)		1000 Grain weight (g)		Biological yield (kg/fed)		Grain yield (kg/fed)	
		1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Giza 126	T_1	43.33	50.87	4.33	5.17	40.33	42.00	150.67	162.33	37.41	38.56	1120.00	1293.33	323.33	366.67
	T_2	47.83	55.00	5.50	5.83	52.33	56.40	173.33	189.73	39.41	40.30	1636.67	1790.00	475.67	518.00
	T_3	52.83	58.00	5.70	6.00	58.67	62.37	182.33	195.13	41.25	41.84	1780.00	2023.33	531.33	587.17
	T_4	59.23	62.00	6.17	6.67	60.00	61.33	201.67	212.10	41.78	42.24	2174.33	2293.33	661.60	708.33
	T_5	44.20	52.33	5.37	5.53	48.33	54.04	172.37	186.00	39.18	40.05	1600.00	1723.33	451.67	502.67
	T_6	50.10	58.13	5.50	5.67	56.33	57.33	177.33	192.67	39.87	40.71	1730.00	1956.67	519.00	575.23
	T_7	54.13	59.43	5.67	5.90	57.00	58.33	186.33	200.00	40.94	41.57	1917.67	2011.00	575.30	603.30
	T_8	56.43	61.17	5.83	6.00	58.00	59.67	187.00	202.33	38.83	39.08	1980.00	2061.67	594.00	618.50
Giza 134	T_1	52.67	60.00	4.13	5.00	42.03	45.05	156.83	163.73	38.33	39.02	1266.67	1346.67	376.67	404.20
	T_2	58.33	64.67	6.17	6.50	45.40	47.04	158.67	167.00	40.40	41.94	1565.00	1833.33	459.50	550.07
	T_3	61.93	67.00	6.50	6.83	49.67	50.73	161.33	173.83	43.32	43.48	1611.67	1920.00	483.67	569.90
	T_4	68.47	70.17	6.83	7.07	53.10	55.05	183.33	195.90	44.24	44.97	1761.67	1996.67	528.50	581.00
	T_5	55.17	62.50	5.50	5.83	44.70	46.00	151.67	158.17	39.25	40.40	1460.00	1560.00	438.23	488.17
	T_6	58.73	64.33	5.67	5.87	46.13	49.04	159.00	164.31	40.25	41.19	1520.00	1746.67	456.00	524.30
	T_7	60.13	65.17	6.00	6.17	47.73	50.00	174.04	183.40	42.63	42.93	1606.67	1863.33	482.00	559.23
	T_8	61.13	67.07	6.17	6.27	48.33	51.10	176.00	185.12	40.86	41.61	1635.83	1895.00	490.77	568.60
LSD 0.05		2.17	-	-	-	1.80	2.91	3.98	3.95	0.62	0.61	97.90	69.45	26.34	14.05

T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

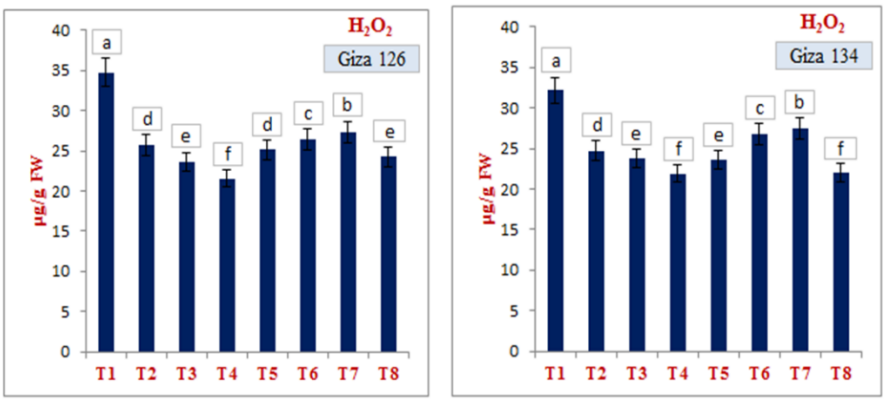

Fig. 2. Effect of SA and Chitosan on down-regulation of H_2O_2 concentration in the two studied barley genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

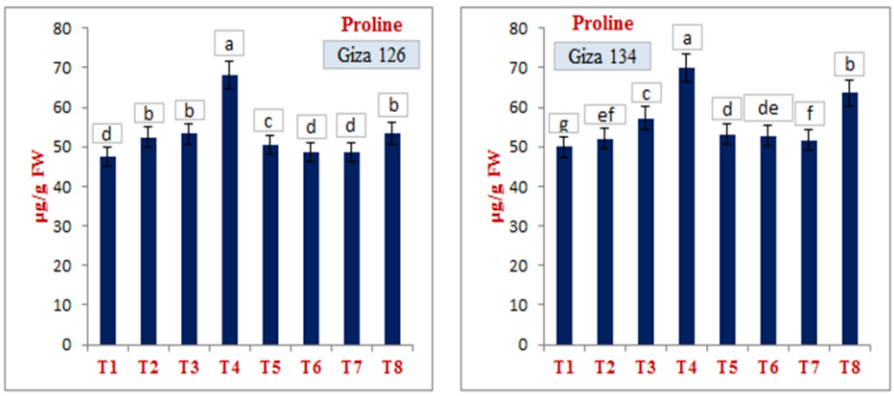

Fig. 3. Effect of SA and Chitosan on accumulation of proline content in the two studied barley genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

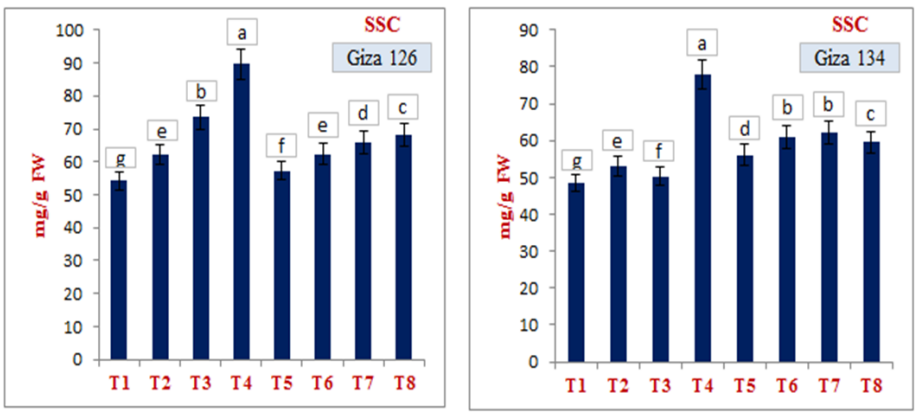

Fig. 4. Effect of SA and chitosan on the soluble sugars content (SSC) in the two studied barley genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

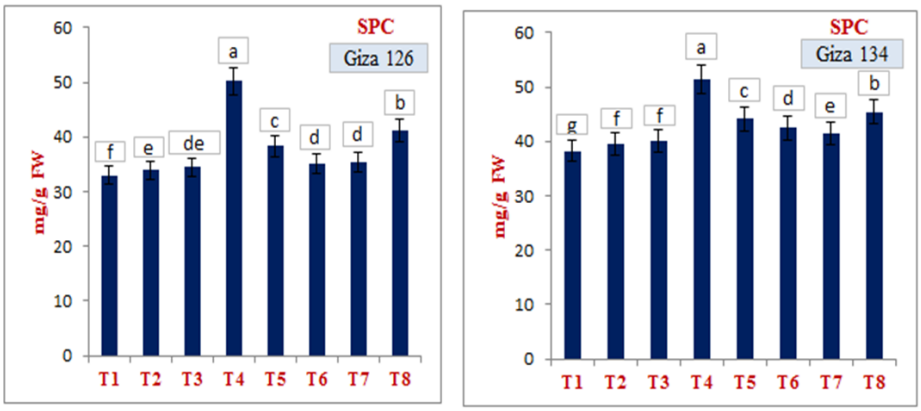

Fig. 5. Effect of SA and Chitosan on total soluble protein content (SPC) in the two studied barley genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

Soluble sugars content (SSC): Data in Fig. 4. showed that, plants under water stress and in presence of SA and Chitosan revealed up-regulation and considerable significant increases in the soluble sugars content (SSC) in the two studied barley genotypes for all treatments, compared to control (T1) those under water stress only. On the other hand, SA_{300} treatment (T4) showed up-regulation of this pathway and recorded the highest concentration for SSC in comparing with control and the other treatments with SA and Chitosan. For Giza 126 genotype, SA treatments induced SSC more than Chitosan treatments, while in Giza 134 Chitosan treatments recorded SSC better than SA treatments, except for SA_{300} ppm.

Soluble protein content (SPC): Soluble protein content was used also to differentiate the tolerance of genotypes under water stress and plant growth regulators. Under water stress conditions only, the two studied genotypes Giza126 and Giza134 have the lowest level of soluble protein which recorded 32.97 and 38.27 mg/g FW, respectively (Fig. 5). Under water stress condition and plant growth regulators, SA_{300} ppm showed the most remarkable increase and up-regulation of SPC comparing with control and the other treatments with SA and Chitosan in the two studied genotypes followed by the application of SA_{100} and $Chitosan_{150}$ (T8).

3.6 Changes in Antioxidant Enzymes Activity

Catalase (CAT) activity: For enzyme activities, under water stress conditions Catalase (CAT) activity increased significantly especially in treatment with SA_{300} ppm in the two studied genotypes Giza 126 and Giza 134 which recorded 95.88 and 113.98 mM H_2O_2/g/FW/min, respectively as presented in Fig. 6. For the other treatments, there is no significant difference were recorded in Giza 126 genotype between control and SA_{100} ppm, SA_{200} ppm and the mixture between SA_{100} ppm and $Chitosan_{150}$ ppm, and between $Chitosan_{225}$ ppm and $Chitosan_{300}$ ppm. Concerning Giza 134, under water stress, all treatments induced significant increases in CAT activity in barley leaves when compared with control, while when comparing treatments with each other there is no significant differences was recorded between SA_{100} and SA_{200} ppm.

Peroxidase (POX)activity: For peroxidase (POX) antioxidant enzyme activity as presented in Fig. 7, the results showed significant and highly significant differences between all treatments in both genotypes comparing with control (T1), except for T7 in Giza 134. On the other hand, no significant differences were recorded between T2, T3 and T8 for Giza 126 while Giza 134 showed no significant differences between (T3 and T4) and (T5 and T6).

Ascorbic peroxidase (APX) activity: For Ascorbic peroxidase (APX) activity, compared with treatment under water stress conditions, control treatment recorded the highest level (Fig. 8). For the other treatments, there were no significant differences was showed between SA_{200} ppm and $Chitosan_{300}$ ppm for Giza 126, while for Giza 134, there is no significant differences were recorded between $Chitosan_{300}$ ppm and Chitosan225.

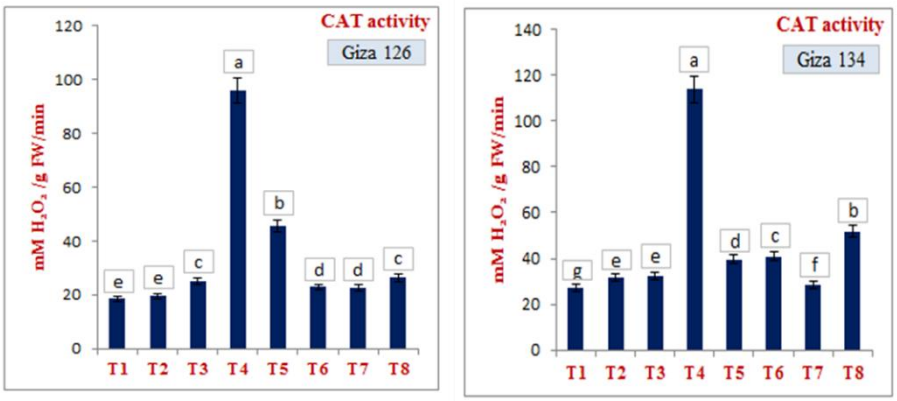

Fig. 6. Effect of Salicylic Acid (SA) and Chitosan on Catalase (CAT) activity in barley leaves for the two studied genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

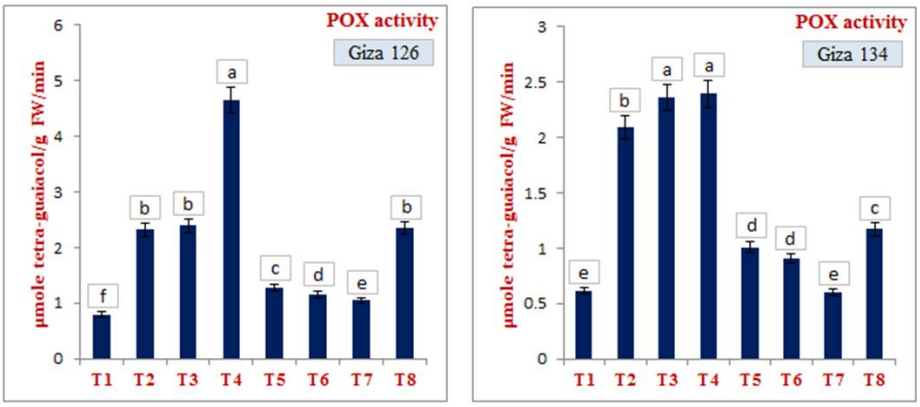

Fig. 7. Effect of Salicylic Acid (SA) and Chitosan on Peroxidase (POX) activity in barley leaves for the two studied genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

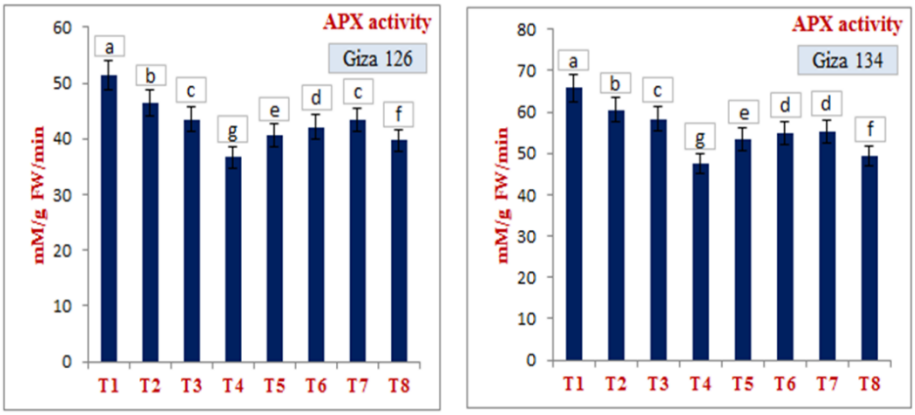

Fig. 8. Effect of Salicylic Acid (SA) and Chitosan on Ascorbic peroxidase (APX) activity in barley leaves for the two studied genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

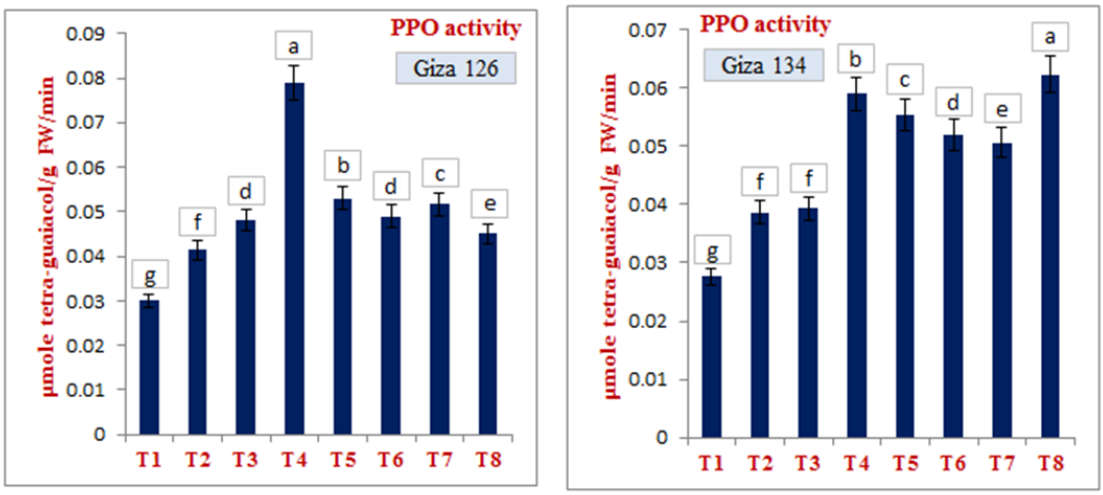

Fig. 9. Effect of Salicylic Acid (SA) and Chitosan on polyphenol oxidase (PPO) activity in barley leaves for the two studied genotypes, Giza 126 and Giza 134 under water stress conditions. T_1=Control, T_2=100ppm, T_3=200ppm, T_4=300ppm of salicylic acid, T_5=150ppm, T_6=225ppm, T_7=300ppm of chitosan and T_8=salicylic acid 100ppm and chitosan 150ppm mixture

Polyphenol oxidase (PPO) activity: Moreover, data presented in Fig. 9 indicated significant activity for antioxidant enzyme polyphenol oxidase (PPO) in barley leaf tissues for all treatments which was increased highly significantly for T4 (SA_{300}) in Giza 126 and T8 (SA_{100} + CH_{150}) in Giza 134 compared to control.

3.7 Discussion

Drought stress is one of the most critical factors in reducing agricultural yield worldwide. Arid regions cover about half of Earth's land area, with Africa, Asia, Latin America, and Europe accounting for 66%, 40%, 24%, and 15% of arid areas, respectively. Approximately 72% of arid regions and 100% of very arid regions are located in developing countries. Therefore, many studies are underway to address the effects of drought stress on crop yields, especially in arid regions [31]. The results in Table 3 showed that Giza 126 exhibited the most desirable values for yield and most components. Noaman et al. [32] showed that Giza 126 considered as one of the most tolerant and adapted barley variety under rainfed condition. In this manuscript, we investigated the effect of foliar application of salicylic acid and Chitosan concentrations on yield, yield components, and other important vegetative traits. The results showed the negative effect of drought stress, especially, on the yield characteristics and growth indicators of the barley plants. In addition, results in Tables 4 and 5 cleared that, Salicylic acid and Chitosan reduced the effects of stress on all studied traits especially grain yield. The results of such studies should be made available to countries with arid and semi-arid climates by FAO in order to encourage farmers to use these novel solutions to reduce the effects of stress and increase yield [31]. Chitosan reduces the effects of drought stress on plants by preventing water from escaping the leaves, closing the pores, and increasing their efficiency, which can produce favorable effects on yield in the face of drought stress [33]. Foliar application of Chitosan reduces the negative effects of drought stress on the plant by controlling the opening and closing of pores [34].

Water stress is the most harmful abiotic stress influencing plant growth and productivity in barley; numerous physiological and biochemical systems, including photosynthesis, water relations, nutrient uptake, oxidative state, osmotic balance, and hormonal balance, are impacted by water deficiencies [35]. So the current study used Giza 126 and Giza 134 barley (*Hordeum vulgare* L.) cultivars to screened for biochemical and physiological parameters under different concentrations of SA and Chitosan under water stress to select the best treatment that are able to avoid harmful of water stress. The results showed highly significant differences among most of treatments comparing with control under water stress conditions and these results are in the same line with many papers

studying barley growth under water stress [36,37].

The results showed significant decrease in the content of MDA and H_2O_2 in barley plants treated with SA and Chitosan under water stress conditions compared to control (under water stress only) and they could be related to decrease of lipid peroxidation in these treatments especially MDA and H_2O_2. Reactive oxygen species (ROS) or lipid peroxidation haves been reported as the early products induced as a result of water stress in plant [38]. These molecules are responsible for most of the oxidative damages in the molecular component of the cell such as DNA, protein, lipid … [39]. Therefore plants under water stress induce active antioxidant systems that preventing the oxidative damage. MDA is a biochemical marker indicating the activity of ROS in plants under water stress conditions and considered the most final product of lipid peroxidation and an important indicator for the oxidative damage which could be occurred to the cellular membranes. H_2O_2 is popular stable lipid peroxidation factor induced in barley plants through physiological processes including photosynthesis, and photorespiration. It plays an important role as a signaling molecule under stressful conditions [40]. Also, Kuzniak and Urbanek [41] indicated that response of H_2O_2 could help plants to improvement their tolerance mechanisms against water stressful factors. It can up-regulate defense genes which required in the induction of systemic acquired resistance (SAR).

Under water stress conditions, the results showed accumulation of osmolytes such as proline, SSC and SPC for all studied treatments, while SA_{300} ppm induced the highest up-regulation in the two studied genotypes Giza 126 and Giza 134. Numerous studies on barley have revealed proline accumulation [42,43]. According to Sallam et al. [36], proline molecule is crucial osmoregulator key for membrane integrity and scavenging free radicals. Proline can also be crucial in activating the de-genotoxicity pathway [44], which increases the tolerance of cultivars that can manufacture more proline when stressed. One of the most significant responses that plants have to mitigate the negative consequences of water scarcity is the accumulation of osmolytes, such as proline, sugars, and proteins [45,46]. They have the ability to modify the osmotic potential, which increases water absorption and increases plants' ability to withstand water stress [47]. Furthermore, by scavenging ROS, it can shield crucial enzymes and other molecular cell components from oxidation [48]. Under water stress, increase of total soluble proteins was due to high amino acids contents as plants accumulate small molecular mass proteins as a result of increase de novo synthesis or inhibition of amino acid degradation [49] and higher protein content might impart better water stress tolerance [50] as it helps in osmotic balance.

Water stress affects plant production by different ways. To avoid water stress by scavenge H_2O_2 and superoxide radical, plants have induced antioxidant enzymatic defenses such as CAT, POX, APX and PPO, when Plants subjected to drought stress they induced defense mechanisms to up-regulate the content of these important components of protective systems and down-regulate of detoxify ROS [51]. The induction of CAT, POX, APX and PPO antioxidant enzymes restricted ROS content, and their increased activities in response to water stress has been widely determined as an indicator for water stress tolerance [52]. This study indicated the rules of CAT, POX, APX and PPO activity under water stress and increasing SA treatments which indicated that these four antioxidant defense enzymes cooperated with each other during water deficit stresses. In the current study, Catalase (CAT) antioxidant enzyme activity showed a positive highly significant increase with increasing the level of SA concentrations under water stress conditions for the two studied genotypes, except for the lower concentrations of SA (100 ppm) in Giza 126, while a significant increase was recorded for treatment with all concentrations of Chitosan and the combinations of SA and Chitosan in the two studied genotypes. The higher activity of CAT in SA treatment with concentration of 300 ppm in Giza 126 and Giza 134 suggests that the H_2O_2 scavenging mechanism is more operative compared to control treatment. Also, the results illustrated the role of SA in up-regulation of peroxidase (POD) activity which well documented that peroxidase (POD) has been implicated in several metabolic processes related to induce tolerance to water stress by participating in lignin synthesis and phenol oxidation [53]. In this study, increasing activity of Polyphenol oxidase may be related to the considerable synthesis of total soluble phenols in the cell, many others phenolic compounds and secondary metabolites which are considered the

substrate for Polyphenol oxidase. Polyphenol oxidases are ubiquitous group of enzymes in angiosperms induced by water stress, and have been implicated in several physiological processes including plant defense against different stresses. They are involved in photoreduction of molecular oxygen, regulation of plastidic oxygen levels and deriving of the phenylpropanoid pathway [54].

4. CONCLUSION

The discussed results showed that Giza 126 cultivar as affected by treatment number 4 [salicylic acid 300ppm] showed the most desirable data for yield and most of its components during the first and second seasons. Also, application of SA_{300} ppm and the mixture between SA_{100} and $Chitosan_{150}$ ppm were the best treatment for induction of up and down regulation of biochemical and physiological component in the barley cells which known as defense system against water stress damage. So. it could be recommended these treatments for increasing barley productivity under ranfed conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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