ANTITRANSPIRANTS APPLICATION FOR IMPROVING THE BIOCHEMICAL CHANGES OF BARLEY UNDER WATER STRESS

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ABSTRACT
Field experiment carried out at Nubaria Agricultural Research station, National Research Centre, Egypt to study the role of antitranspirants application{Salicylic Acid (SA), K_2SO_4 and $MgCO_3$} in alleviating drought-induced changes of some sensitive barley varieties. Foliar application of the antitranspirants compounds registered a positive effect on plant pigment, relative water content, nutrient contents and proline accumulation under water stress condition especially after spraying SA. The barley varieties Tamellalet and Giza 125 highly responded to antitranspirants application especially after foliar application of SA and $MgCO_3$ over remaining varieties. Application of $MgCO_3$ produced the most balanced nutrients for Tamellalet variety under normal irrigation system whereas, under stress condition. The data together provide some indication that Ksar-Megrine and Tombari cultivars has more efficient H_2O_2 scavenging machinery at least in peroxisomes under drought stress. Application of antitranspirants treatments led to significant increase of CAT, POD and GR activity which could alleviate the adverse effect of water stress on barley leaves. Results concluded that antitranspirants application could be effective for barley production in arid and semi-arid areas to reduce the adverse effects of water deficit.

Keywords: SA, K_2, SO_4, $MgCO_3$, chlorophyll, DRIS indices, proline, antioxidant enzymes

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تطبيق استخدام مضادات النتح لتحسين التغييرات الكيميائية الحيوية للشعير تحت ظروف اجهاد الجفاف

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المستخلص
أجريت تجارب حقليه في محطة التجارب الخاصة بالمركز القومي للبحوث الواقعه فى زمام منطقة النوبارية التابع لمحافظة البحيرة بجمهوريه مصر العربيه لدراسة دور الرش الورقى بمضادات النتح والمتمثله في (حمض الساليسيلك و كبريتات البوتاسيوم و كربونات الماغنسيوم) في تقليل التاثيرات السلبية لظروف الجفاف على نمو بعض أصناف الشعير المصرية (صنف:جيزه 125) ، التونسية (صنف: تمبارى)، الجزائرية (صنف:قصر مجرين)، والمغربية (صنف:تاميللات) فى الارض الرملية حديثة الامستصلاح. أوضحت النتائج المتحصل عليها انهناكتأثيرمعنوى من التسميد الورقي بمضادات النتح على محتوى الكلوروفيل فى الاوراق خلال مراحل النمو المختلفة (استطالة الساق ، وخروج البادرات) لبعض أصناف الشعير الحساسة (جيزة 125، تمبارى،قصر ميجرينوتاميللات). وكان هناك ايضاتأثيراً إيجابيا على المحتوى المائى النسبى للاوراق بالاضافه الى تراكم البرولين تحت ظروف نقص المياه والتى كانت واضحه على الاصناف تاميللات وجيزه 125 والتى كانت عالية الاستجابة لمضادات النتح وخاصة فى المعاملات التى تم رشها بحمض الساليسيلك.ايضا هناك تأثير معنوى على المحتوى المعدنى للنبات نتيجة الرش بمضادات النتح تحت ظروف الرى العاديه وتحت ظروف االاجهاد كان افضل حالات الاتزان الغذائي داخل النبات فى المعاملات التى تم رشها بحمض الساليسيلك يلى ذلك المعامله لتي تم رشها بـ كربونات الماغنسيوم ثم معاملات كبريتات البوتاسوم وذلك تحت ظروف الجفاف ونقص المياة مقارنة باضافة الاحتياجات المائية للنبات كان لاستخدام مضادات النتح له تاثير عالى المعنوية على زيادة نشاط الانزيمات المضادة للاكسدة تحت ظروف اجهاد الجفاف مقارنة بظروف الرى المعتاد. حيث وجد ان الرشبتركيز 10 جزء في المليون حمض الساليسيلك و2% كربونات المغنيسيوم ادت الى زيادة نشاط انزيمات البيروكسيديز والكتاليز والجلوتاثيون المختزل تحت ظروف الجفاف.

الكلمات المفتاحية: نقص المياه – الكلوروفيل – مؤشر الدرس– البرولين – الانزيمات المضادة للاكسدة.

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INTRODUCTION

A biotic stress has now been recognized as the biggest and potential threat for agricultural productivity all over the world. Nevertheless, anthropogenic activities in the developmental era have aggravated the degradation of agricultural system and its productivity due to major a biotic stress. Anti-transpiration play important roles in the regulation of plant developmental processes and signaling networks as they are involved either directly or indirectly in a wide range of biotic and a biotic stress responses and tolerance in plants (2).Anti-transpiration are the materials or chemicals which decrease the water loss from plant leaves by reducing the size and number of stomata. Nearly 99 % of the water absorbed by the plant is lost in transpiration. Anti-transpirations were grouped into three kinds, namely film-forming types which coat leaf surface with films that are impervious to water vapor, reflecting materials which reflect back a portion of the incident radiation falling on the upper surface of the leaves and stomatal closing types which affect the metabolic processes in leaf tissues (25). Salicylic acid (SA) is a phenolic compound involved in the regulation of growth and development of plants, and their responses to biotic and a biotic stress factor (21).SA is involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline metabolism, production of glycine, betaine, antioxidant defense system, and plant-water relations under stress conditions and thereby provides protection in plants against a biotic stress (15). Apart from its involvement in the induction of defense-related genes and stress resistance in biotic stressed plants (16)**,** SA has been shown to improve plant tolerance to major drought stress (9). Foliar application of anti-traspirants such as Magnesium Carbonate positively affected all the growth and physiological criteria of the tested plants compared with control treatment and also cause marked reduction in transpiration rate. Generally, under irrigation water shortage, application of anti-transpirants effectively reduced water consumption use and enhanced water productivity. Additionally, most of the growth and yield parameters and seed quality were improved significantly as influenced by spraying anti-transpirants which were responsible for reducing water consumptive use(26). Antioxidant enzymes are very important in scavenging dangerous free oxygen radicals, produced as the usual secondary consequence of environmental stresses, from plant cells. SOD converts the toxic O_2 radicals to H_2O_2 which scavenged to O_2 and water by the antioxidant enzymes such as CAT and POD(24). Glutathione reductase is the last enzyme of the ascorbate/glutathione cycle and plays a principal role in the protection of cells from damage induced by oxidative stress. In drought conditions, GR favors maintenance of the GSH pool, thereby intensifying the antioxidative response of the plant. The effect of mild drought examined on glutathione reductase activity in two species of cotton and concluded that GR activity was greater in drought-stressed plants during recovery strongly which indicates that drought may result in acclimation to greater water deficit and/or cross-tolerance to other stresses later(27).The aim of research work was to study the effect of some antitranspirants compounds on the biochemical changes of barley under water stress condition.

MATERIALS AND METHODS

Field experiments were conducted at the experimental research station of National Research Centre, El-Nubaria region, Boheira Governorate, Egypt (altitude of 27 m above sea level, latitude 30° 72' 66" and longitude 30° 20' 18" E) during the winter growing seasons of 2018/2019 to study the effect of foliar application of anti-transpiration compounds on some biochemical parameters (chlorophyll, proline and enzymes) of barley varieties grown under water stress condition in newly reclaimed sandy soil. The experimental area was classified as arid region with cool winter and hot dry summer prevailing in the experimental area. There was no effective rainfall (low intensity) that can be taken into consideration throughout the two growing seasons. The field capacity and available water of the experimental soil was 14.8 and 10.2 ml /100g soil, respectively. Soil pH was 8.1; Electrical conductivity 1.31 dS m^{-1}, organic matter 0.54%, $CaCO_3$ 3.11% and available N,

P and K were 36.6, 4.8 and 20.7 mg/kg soil, respectively.

1-Experimental design and treatments

The Mediterranean barley varieties were the Egyptian variety (Giza 125), Tunisian variety (Tombari), Algerian varieties (Ksar-Megrine) and Morocco variety (Tamellalet). The seeds of the selected varieties obtained from National gene bank of Tunisia. Drip irrigation regime was applied through two types of in line emitter according to its discharge (4 and 2 liter), which fulfill 75 and 40 % of water holding capacity named as normal and stress condition, respectively. The antitranspirant applied as foliar fertilization of 2% K_2SiO_4, 2% $MgCO_3$ and 10 ppm salicylic acid were randomly distributed within the sub-plots. The net area of each experimental plot was 10.5 m^2. The assigned levels of antitranspirant were foliar sprayed twice during the growth period of barley plant after 40 and 60 days from sowing date. Salicylic acid (HOC_6H_4COOH, Mwt: 138.122) was initially dissolved in absolute ethanol and the final volume of prepared solution was reached, using distilled water. A few drops of Tween-20 as a surfactant agent were added to ensure spreading of the applied solution on the plant leaf surface. All antitranspirant sprayed out in the early morning and the volume of the spraying solution was maintained just to cover completely the barley plant foliage till drip. The field experiment laid out in a split plot design with three replicates. The agricultural practices for barley production under drip irrigation in the growing area were followed according to the recommendations of Ministry of Agriculture and Land Reclamation.

2. Estimation during growth stages

Chlorophyll content

Leaf greenness present in a plant at stem elongation, booting and ear emergency stages determined with the Minolta-SPAD Chlorophyll Meter (Minolta Camera Co., Osaka, Japan). The SPAD-502 chlorophyll meter measures the chlorophyll absorbance in the red and near-infrared regions and calculates a numeric SPAD value which is proportional to the amount of chlorophyll in the leaf (20).

Relative water content

Leaf relative water content (RWC) estimated at ear emergence according to (4).For these 10 fully matured leaves of 5 plants per plot (2 leaves per plant) were selected from the same heights and their fresh weight (FW) recorded. The leaves soaked into distilled water under low lighting conditions for 24 h to measure their saturated weight. After recording turgescence weight (TW), leaves dried at 75 °C for 48 h and their dry weights (D.W) measured. RWC calculated using the following formula: RWC = (FW-DW)/(TW-DW) × 100

Determination of proline

Proline was estimated at booting and ear emergence stages. First, 0.4 g of fresh plant material homogenized in 1.5 ml of distilled water and then incubated in water bath at 100 °C for 30 min. Then, the samples cooled to room temperature (22 °C) and centrifuged for 10 min at 4 000 rpm. Next, 1 ml of a 1% solution of Ninhydrin in 60% acetic acid added to 0.5 ml of the supernatant and incubated at 100 °C for 20 min. After cooling to 22 °C, 3 ml of toluene added and the samples shaken and left in the dark for 24 h for phase separation. One ml of proline extract introduced to a cuvette and the absorbance measured by spectrophotometer at a wavelength of $\lambda = 520$ nm according to (18).

Nutrient content analysis

At ear emergence stage, representative leaves analyzed for the nutrient content (N, P, K) in barley varieties and determined according to (22).

DRIS indices

DRIS indices which are used in the current investigation are quantitative evaluations of the relative degree of imbalance of the nutrients under study and as calculated from the following equations:

$$N, index = +\left[\frac{f(N/P) + f(N/K)}{2}\right]$$

$$P, index = -\left[\frac{f(N/P) + f(K/P)}{2}\right]$$

$$K, index = +\left[\frac{f(K/P) - f(N/K)}{2}\right]$$

where $$f(N/P) = +\left(\frac{N/P}{n/p} - 1\right)\frac{1000}{C.V}$$

when the actual value of N / P > n / p.

or $$f(N/P) = \left(1 - \frac{n/p}{N/P}\right)\frac{1000}{C.V}$$

where

when the actual value of N / P > n / p.

or When the actual value of N / P < n / p, n/p is the mean value for N/P, and CV is the coefficient of variation for high-yielding populations. The other terms of f (N/K) and f (K/P) are derived in a similar way using the means of n/k for N/K and k/p for K/P, respectively in place of n/p. The DRIS indices have positive and negative values which always sum to zero as they measure the relative balance among N, P and K or other elements that might be included. The order of plant nutrient requirements is affected by the value of the index, the most negative index reflects the most required nutrient(30).

Antioxidant enzyme activities

Catalase (CAT): Catalase activity determination carried out according to the method described by [1].The method is based on monitoring the rate of decomposition of H_2O_2 at 30°C. Suitable volume of crude enzyme was added to 1 mL of substrate mixture, which consisted of 0.02 M H_2O_2 in 50 mM phosphate buffer, pH 7.0. The decomposition of H_2O_2 followed as a decline in absorbance at 240 nm for 1 min. One unit of activity defined as the calculated consumption of 1µmole of H_2O_2/min at 30 °C. The extinction coefficient of H_2O_2 taken to be 43.6 M^{-1} cm^{-1}.

Peroxidase assay

Peroxidase (POD) activity determined according to the method of (5), using ABTS as a reducing substrate, in a reaction mixture (1 ml) containing 6 mM H_2O_2, 0.36 mM ABTS, 100 mM sodium acetate buffer (pH 6) and POD concentration which gave linear response over a period of 3 min. The change in absorbance at 405 nm followed at 1 min intervals. One unit of POD activity defined as the amount of enzyme that oxidized 1 mmol ABTS per min at 25°C under the assay conditions.

Glutathione reductase (GR)

The activity of GR determined spectrophotometry at 30°C following the decrease in absorbance at 340 nm according to the method described by (31).The assay reaction mixture contained in a total volume of 1 mL, 50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 0.1 mM NADPH, 0.5 mM oxidized glutathione, and the enzyme solution. One unit of GR activity defined as the amount of enzyme which oxidizes 1 µmole of NADPH per min.

Statistical analysis

Data statistically analyzed using analysis of variance (ANOVA) and the means of varieties included in this trial compared using fisher test run by Least Significant Difference (L.S.D.) at ($P \leq 0.05$) according to (10).

RESULTS AND DISCUSSION

1-Chlorophyll content

Data in Table (1) illustrated the effect of different antitranspirants compounds on the SPAD chlorophyll at different growth stages (stem elongation, booting and ear emergence) of some sensitive barley varieties (Giza 125, Tombari, Ksar-Megrine and Tamellalet) under normal and stress conditions. Data on hand revealed that the highest values under water stress conditions observed at the barley varieties: Giza 125, Tombari and Tamellalet after spraying salicylic acid (SA) and $MgCO_3$ for SPAD chlorophyll at stem elongation, booting and ear emergence, respectively. The application of antitranspirant immediately prior to booting stage may conserve water and improve grain set which could outweigh the photosynthetic limitations (14). Regarding to the reduction percentage of SPAD chlorophyll under water stress, the maximum reduction was found at Tombari and Ksar-Megrine under

control treatments at stem elongation, booting and ear emergence growth stages, respectively. While the minimum reduction recorded at Giza 125 + SA, Tombari + $MgCO_3$ and Tamellalet + SA in same sequences. Farooq *et al*. (8) indicated that the treatment of rice leaves (*Oryza sativa* L.) with SA (100 mg L^{-1}) had a positive effect on photosynthesis and plant growth compared to other treatments of 50 and 150 mg L^{-1} and induced better resistance to drought stress than soaking the seeds in the same SA solutions. Regardless spraying antitranspirant compounds, data indicated that the maximum and minimum values attained at Giza 125 and Tamellalet for SPAD chlorophyll at stem elongation, booting and Ksar-Megrine and Tamellalet for ear emergence, respectively. Also, data noticed that the maximum reduction of SPAD chlorophyll found at Tombari (15%) at stem elongation, Giza125 (13%) at booting stage and Ksar-Megrine (14%) at ear emergence stage, respectively. According to the effect of sprayed antitranspirant compounds, the maximum chlorophyll content observed with after spraying SA (stem elongation), $MgCO_3$; SA (booting) and SA, K_2SO_4 (ear emergence). The minimum reduction of SPAD Chlorophyll found after spraying SA= $MgCO_3$ at stem elongation and booting stages while at ear emergence stage, the lowest ones observed after spraying K_2SO_4 and $MgCO_3$ under water stress condition. Several studies support the stimulatory effect of SA on a number of morphological and physiological processes of plants, including growth, photosynthesis and other metabolic processes. This demonstrates a function as a protective agent in plants, modulating the plant response to drought stress (11).

Table 1. Effect of Antitranspirants on SPAD Chlorophyll of barley varieties under water stress

Variety	Treatment	Stem elongation		Booting		Ear emergence	
		Normal	Stress	Normal	Stress	Normal	Stress
Giza 125	Control	42.1	34.6	47.1	37.7	35.2	30.4
	Salicylic acid	53.3	49.4	49.0	44.2	39.4	35.9
	K_2SO_4	49.0	44.1	49.4	43.8	37.3	35.3
	$MgCO_3$	47.0	43.2	51.2	45.8	36.2	34.0
Tombari	Control	40.8	32.1	42.3	35.4	33.1	29.6
	Salicylic acid	44.8	38.6	55.8	51.1	36.1	33.3
	K_2SO_4	41.8	35.6	45.0	42.1	36.6	34.0
	$MgCO_3$	48.1	42.9	48.9	46.6	35.2	33.9
Ksar-Megrine	Control	42.4	34.2	41.4	32.7	38.1	30.5
	Salicylic acid	49.9	44.2	44.4	40.1	39.6	33.4
	K_2SO_4	46.1	41.1	48.3	42.6	39.6	36.3
	$MgCO_3$	45.1	40.1	47.4	44.6	39.8	35.7
Tamellalet	Control	38.2	33.5	39.5	32.4	37.0	31.1
	Salicylic acid	42.6	39.7	44.4	40.2	40.0	36.8
	K_2SO_4	46.6	42.4	47.8	42.3	38.5	35.4
	$MgCO_3$	43.0	38.8	45.4	40.8	40.2	37.3
LSD (0.05) (V)		0.27	0.28	0.32	0.23	0.28	0.14
(T)		0.44	0.28	0.18	0.27	0.25	0.21
(V * T)		0.60	0.47	0.42	0.42	0.45	0.30

2-Relative water content (RWC)

Data in Table (2) illustrate the effect of sprayed antitranspirants on relative water content (RWC), proline content at booting and ear emergence for sensitive barley varieties. Data on hand indicated that the highest values of RWC attained at Tamellalet, Tombari= Ksar-Megrine after spraying $MgCO_3$, under both normal and stress conditions, respectively. The maximum reduction percentage found at barley Giza 125= Ksar-Megrine (20%) without application of antitranspirant (control), whereas the minimum ones attained at Tamellalet+ K_2SO_4= $MgCO_3$ (5.5%), Tamellalet + SA =Tombari + K_2SO4 (7.7%), respectively. Magnesium Carbonate ($MgCO_3$) is considered to be an antitranspirants that close stomata and thus affect metabolic processes in leaf tissues (12). Regardless spraying antitranspirant compounds, the highest values of the RWC at ear emergence obtained at Tombari (88.4) and Tamellalet (81.0) under both normal and stress conditions, respectively. Whereas, the lowest ones recorded at Giza 126= Ksar-Megrine (74) under stress conditions. Also, it is clear to

mention that the highest and lowest reduction percentage in RWC were 14% (Giza 125) and 8% (Tamellalet). According to the effect of sprayed antitranspirants on the RWC at ear emergence, $MgCO_3$ had a promotive effect on the RWC under both normal and stress condition and gained the highest values under both stress conditions (90.1 and 82.3) which increased by about 8 and 20% relative to the control, respectively.

3-Proline content

Data in Table (2) showed the proline content at booting and ear emergence as affected by normal and stress condition.The control treatment of Tombari variety gained the highest values (3.1 mg/g fresh weight) and the lowest ones (1.2 mg/g fresh weight) observed after spraying SA for Tamellalet under stress condition at booting stage. At ear emergence, the maximum values found at control of Ksar-Megrine (4.1 mg/g fresh weight) and the minimum values were (1.1 mg/g fresh weight) for Tombari after spraying $MgCO_3$. The differences between the highest and lowest values in percentage were 23, 38 and 36, 35% for proline % at booting and ear emergence under normal and stress conditions, respectively. The highest increased percentage under water stress were 32% and 30% at Giza 125 for control treatment at booting and ear emergence growth stage, respectively. Whereas, the lowest percentage for proline content at booting were 11% after spraying SA and 10% at ear emergence stage after spraying K_2SO_4 for Tamellalet variety, respectively. Regardless spraying antitranspirant compounds, the maximum and minimum values of proline content attained for Tombari and Tamellalet at booting stage and Ksar-Megrine and Tombari at ear emergence stage under water stress condition, respectively. Also, data noticed that the maximum increased percentage of proline content found at Tombari (21%) at booting stage and Giza 125 (19%) at ear emergence stage, respectively. Exogenous treatment of drought stressed plants with different levels (0.5 and 1.0 mm) of SA not only caused a decline in the adverse effect of drought in yellow Maize (*Zea mays* L.) plants, but also stimulated physiological traits, productivity and plant resistance to drought stress (7).

Table 2. Relative water content (%) and proline (mg/g fresh weight) of barley under water stress

Variety	Treatment	RWC%		Proline at booting stage		Proline at ear emergence stage	
		Normal	Stress	Normal	Stress	Normal	Stress
Giza 125	Control	83.5	66.8	2.28	3.02	2.13	2.79
	Salicylic acid	90.6	78.6	2.13	2.42	1.72	1.98
	K_2SO_4	85.4	75.1	1.51	1.78	1.59	1.81
	$MgCO_3$	86.6	78.4	1.48	1.66	1.71	1.91
Tombari	Control	85.2	70.6	2.36	3.12	1.90	2.29
	Salicylic acid	90.1	78.6	2.15	2.54	1.78	2.05
	K_2SO_4	87.7	81.1	2.20	2.59	1.12	1.32
	$MgCO_3$	90.4	82.1	2.05	2.34	1.04	1.15
Ksar Megrine	Control	82.3	65.8	2.19	2.75	3.49	4.11
	Salicylic acid	82.3	75.7	1.74	2.02	2.88	3.22
	K_2SO_4	86.3	74.8	1.62	1.98	2.31	2.68
	$MgCO_3$	90.1	80.5	1.93	2.27	2.98	3.34
Tamellalet	Control	81.5	70.5	1.87	2.36	2.57	3.12
	Salicylic acid	87.3	80.6	1.10	1.21	2.50	2.79
	K_2SO_4	89.7	84.8	1.41	1.58	2.42	2.65
	$MgCO_3$	93.3	88.2	1.62	1.90	2.45	2.75
LSD (0.05)	(V)	2.08	1.81	0.65	0.36	0.19	0.41
	(T)	1.56	0.55	0.51	0.20	0.25	0.14
	(V * T)	3.06	1.98	0.97	0.47	0.37	0.46

4-Macronutrients

Table (3) illustrated N, P and K content as affected by sprayed antitranspirant compounds for examined barley varieties under normal and stress conditions. Results indicated that the highest values of N, P and K content under normal condition observed at Tombari + $MgCO_3$, Giza 126 + K_2SO_4, and Tamellalet + K_2SO_4 and under water stress condition, Giza 125+ MgCO3, Tombari + K_2SO_4 and Tamellalet + $MgCO_3$ for N, P and K, respectively. While the smallest ones were

attained at control of Tamellalet, Ksar-Megrine and Giza 126 under normal and stress conditions in same sequences.The rate of reduction in percentage relative to the water stress effect indicated that, the highest reduction was found at control of Tombari=Ksar-Megrine (24.7%) and Tamellalet (28.5%) and Giza 125 (21.8%) for N, P and K content, respectively. Whereas, the lowest reduction in % of N, P and K were attained at Giza 126 + SA (6.2 %), Ksar-Megrine + MgCO3 (4.2%) and Ksar-Megrine + K_2SO_4 (5.0%), respectively.

Table 3. Macronutrient content of barley varieties under water stress

Variety	Treatment	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
		Normal	Stress	Normal	Stress	Normal	Stress
Giza 125	Control	2.17	1.68	0.286	0.206	1.32	1.03
	Salicylic acid	2.85	2.67	0.365	0.329	1.79	1.46
	K_2SO_4	2.51	2.01	0.424	0.365	1.93	1.79
	$MgCO_3$	3.17	2.85	0.381	0.336	1.81	1.62
Tombari	Control	2.35	1.77	0.346	0.252	1.32	1.13
	Salicylic acid	2.43	1.98	0.378	0.328	1.79	1.59
	K_2SO_4	2.84	2.32	0.422	0.394	1.99	1.82
	$MgCO_3$	3.19	2.52	0.370	0.315	1.81	1.61
Ksar-Megrine	Control	2.93	2.21	0.230	0.186	1.42	1.19
	Salicylic acid	2.81	2.45	0.295	0.270	2.19	2.04
	K_2SO_4	3.01	2.55	0.297	0.267	2.16	1.97
	$MgCO_3$	2.24	2.10	0.270	0.257	1.71	1.63
Tamellalet	Control	1.60	1.25	0.293	0.209	1.74	1.36
	Salicylic acid	2.49	2.01	0.333	0.271	2.06	1.78
	K_2SO_4	1.93	1.74	0.329	0.291	2.46	2.18
	$MgCO_3$	2.97	2.66	0.330	0.297	2.52	2.29
LSD (0.05)	(V)	0.782	0.232	0.057	0.087	0.098	0.098
	(T)	0.622	0.313	0.049	0.047	0.166	0.166
	(V * T)	1.180	0.458	0.089	0.112	0.222	0.222

Regarding to the mean values of the N, P and K content by eliminating stress effect, Giza ($MgCO_3$), Tombari (K_2SO_4) and Tamellalet ($MgCO_3$) scored the highest values, while the lowest ones were obtained at control of Tamellalet and Ksar-Megrine, in same sequences. Regardless sprayed antitranspirant compounds, resulted data pointed out that Ksar-Megrine gained the highest N values under both normal and stress conditions, while the lowest value was recorded at Tamellalet variety. But, Tombari (phosphorus) and Tamellalet (K) scored the highest values under normal and stress condition. Whereas the lowest values were observed at Ksar-Megrine (P) and Giza 126 (K). The reduction percentage relative to the stress effect were estimated, the highest and lowest reduction were 21% (Tombari), 17% (Tamellalet) and 14% (Giza 125) and 14% (Giza 125), 10% and 9 % Ksar-Megrine for N, P and K content, respectively.

5-DRIS Indices

Data in Table (4) indicated that the highest absolute total in control treatment under normal irrigation condition, recorded from Tombari variety (175) which recorded highest negative signal K index (-87.4) if compared with other treatments. However, for (N and P) were (34.0, 53.4) considered excessive when the DRIS indices are negative and positive in these case imbalances. Whereas, under stress condition in control treatment, the highest NBI was found in Giza 125 variety (153) with highest negative K index (-76.5), N index (42.3) and P index (34.1). After addition of antitranspirants the nutrient imbalance reduced, the treatment combination (Tamellalet + $MgCO_3$) was most balanced treatment among the studied combinations, with the absolute nutrient index (32.5) which resulted from DRIS indices are (16.2, -5.3, 10.9) for (N, P and K index), respectively under normal irrigation system. Whereas, under stress condition, the most balanced treatment was (Ksar-Megrine + SA), with the absolute nutrient index (11) which resulted from DRIS indices (1.42, 4.03, 5.45) for (N, P and K index), respectively. The negative index the more deficient the nutrients. A positive index indicates that the nutrient levels are above the optimum and more positive index the excessive the nutrients that are relative to normal. If the DRIS index is equal to zero indicating that the nutrient is at optimum level(3).

Table 4. DRIS indices of barley under water stress and antitranspirants application

Varieties	Treatment	Normal irrigation DRIS index N	P	K	NBI	Water stress DRIS index N	P	K	NBI
Giza 125	Control	39.6	27.7	-67.3	134.5	42.3	34.1	-76.5	152.9
	Salicylic acid	30.2	27.2	-57.4	114.7	18.7	27.2	-45.9	91.9
	K_2SO_4	7.43	42.7	-50.2	100.4	0.86	48.4	-49.2	98.4
	$MgCO_3$	31.6	33.3	-64.9	129.8	34.8	20.2	-55.0	110.0
Tombari	Control	34.0	53.4	-87.4	174.8	-10.6	49.1	-38.6	98.2
	Salicylic acid	13.2	36.2	-49.5	98.9	-16.0	47.1	-31.1	94.1
	K_2SO_4	17.5	35.1	-52.6	105.3	-6.0	45.4	-39.4	90.9
	$MgCO_3$	41.1	24.2	-65.3	130.6	11.4	6.81	-18.3	36.5
Ksar-Megrine	Control	72.1	-9.08	-63.0	144.2	33.6	-3.61	-30.0	67.3
	Salicylic acid	23.2	-6.24	-16.9	46.4	1.42	4.03	-5.45	10.9
	K_2SO_4	30.5	-7.58	-22.9	61.1	18.0	10.3	-28.3	56.5
	$MgCO_3$	19.5	7.77	-27.3	54.5	17.9	8.48	-26.4	52.8
Tamellalet	Control	-13.2	26.7	-13.4	53.3	-18.9	21.4	-2.5	42.9
	Salicylic acid	11.2	12.5	-23.7	47.4	7.54	9.31	-16.9	33.7
	K_2SO_4	-18.2	10.5	7.71	36.5	-16.5	9.55	6.92	32.9
	$MgCO_3$	16.2	-5.30	-10.9	32.5	15.2	-5.65	-9.55	30.4

Under normal irrigation, in the treatment of Tamellalet spayed with $MgCO_3$, the N index was decreased from (34.0) to (16.2) in this time the nitrogen increased the nutrient balance in treatment combination in this time the N a positive DRIS index indicates that the nutrient level is above the optimum. Phosphorus index was recorded (53.4) reduced to (-5.3) and a negative DRIS index indicates that the nutrient level is below the optimum in these treatment combinations. Also, and the DRIS index for potassium was recorded (-87.4) increased to (-10.9), the potassium a positive DRIS index indicates that the nutrient level is above or near the optimum. Under water stress, in the control treatment of Giza 125, the N index was decreased from (42.3) to (1.42) in this time the nitrogen a positive DRIS index indicates that the nutrient level is above or near to the optimum. Phosphorus index was recorded (34.1) reduced to (4.03) and a positive DRIS index indicates that the nutrient level is above or near to the optimum in these treatment combinations. Also, and the DRIS index for potassium was recorded (76.5) decrease up to (-5.45), the potassium a negative DRIS index indicates that the nutrient level is below the optimum.This outcome is to be coupled with higher yield with the smaller absolute total for value nutrient index elements agreed with (6). From the DRIS index calculation proved that after addition of antitranspirants the nutrient imbalance reduced and application of SA was most nutrient balanced treatment followed by $MgCO_3$ and K_2SO_4.

6-Antioxidant enzyme activities

6-1Guaiacol peroxidase

Peroxidases (POX) are another group of non-chloroplastic enzymes that detoxify H_2O_2 in the cytosolic part of the cell. They are non-specific in using electron donor for detoxification of H_2O_2 showed the activity of guaiacol peroxidase decreased sharply at 40% watering treatment being 0.125, 0.158 and 0.152 unit/ml for Giza 125, Tombari and Ksar-Megrine cultivars even after application of chemicals enhancers (SA, $MgCO_3$, K_2SO_4) in Fig. (1). However, POX activity showed high activity with Tamellalet cultivar which shoed higher activity in control sample comparing to remining cultivars. Magnesium carbonate and salicylic acid treatments showed highly positive interaction against POX activity under drought stress with Tamellalet cultivar (0.373 and 0.308 unit/ml). Overall the peroxidase assay for Giza 125, Tombari, Ksar-Megrine and Tamellalet cultivars magnesium carbonate revealed high correlation with higher POX activity even in lower levels.

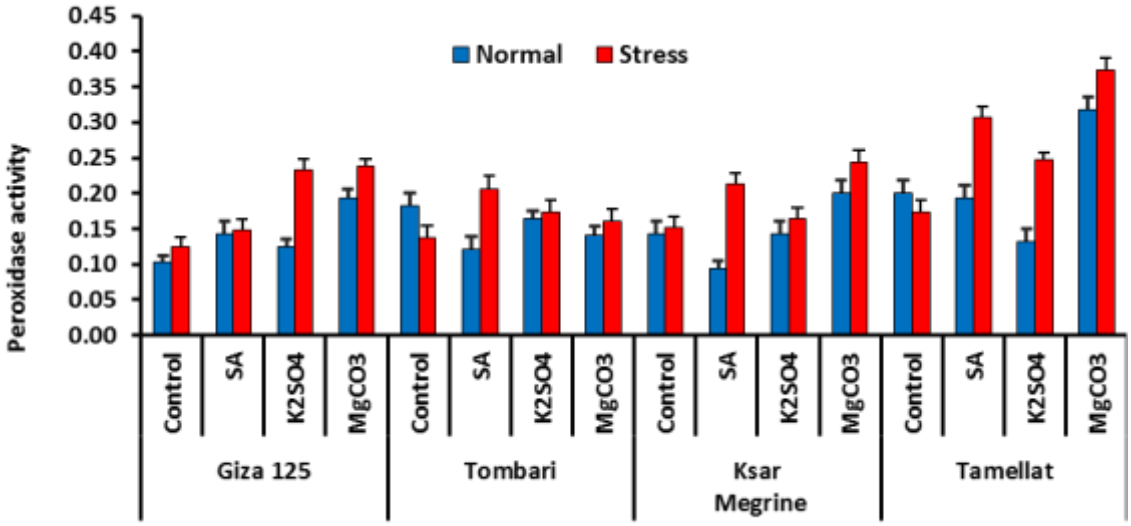

Fig 1. Peroxidase activity of barley as affected by water stress and antitranspirant

Results showing exogenous MgCO3-induced enhanced activities of POX enzyme as observed in previous reports in the different species under salt stress[29]. The results indicate that barley maintained significantly higher POX activity throughout drought stress exposure as compared to normal condition. In spite of the activity increasing of other two antioxidant enzymes (CAT and GR) still the peroxidase enzyme activity was higher under water stress specially Tamellalet cultivars. Peroxidases are non-specific in using electron donor such as guaiacol and pyrogallol depending on the species and even environmental conditions. Keeping this in mind, it becomes difficult to draw any conclusion based on the activity. However, results obtained in this work pinpoint more efficient maintenance of the POX activity in barley.

6-2 Catalase activity

Catalase (CAT) is one of the H2O2 detoxifying enzymes and mostly associated with peroxisomes where it removes H2O2 formed during photorespiration. CAT activity was found higher in Ksar-Megrine cultivar (2020 unit/ml) under drought stress without any enhancers comparing to other three cultivars. Then it increases gradually in Ksar-Megrine and Tombari cultivars (3187, 3183 unit/ml, respectively) specially with salicylic and magnesium carbonate (Fig. 2). While, in Giza 125 and Tamellalet cultivars the CAT activity was low in control sample (1127 unit/ml) without enhancers or after applying chemical enhancers. The data revealed that exogenous application of 10 ppm SA and 2% Magnesium carbonate that helps barley plants to perform well under drought condition by enhancing photosynthetic rate and antioxidative enzyme activity (17). The Giza125 and Tamellalet cultivars showed lower CAT activity under 40% watering treatment even after applying three different enhancers (SA, $MgCO_3$ and K_2SO_4). This differential behavior of these varieties indicates the early adaptive potential of tolerant cultivars in terms of CAT activity. The data together provide some indication that Ksar-Megrine and Tombari cultivars has more efficient H2O2 scavenging machinery at least in peroxisomes under drought stress. Similar results concerning antioxidant protective effects, high activity of CAT is noticed with drought tolerance in tomato cultivars (28).

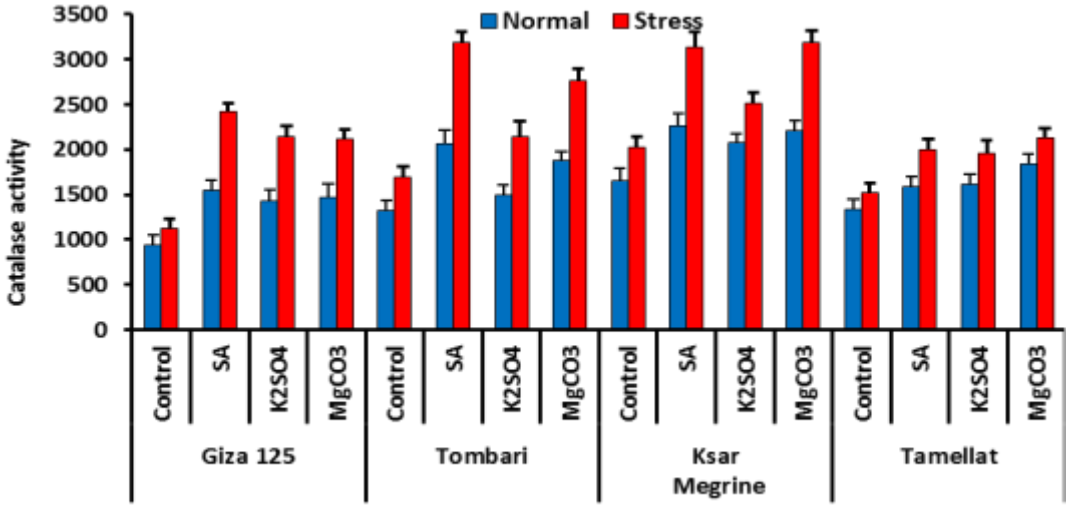

Fig 2. Catalase activity of barley as affected by water stress and antitranspirant

6-3 Glutathione Reductase

Glutathione Reductase (GR) is catalysis the reduction of glutathione disulphide (GSSG) to the sulfhydryl form GSH in chloroplast and use NADPH as a reductant.Under water stress barley cultivars showed high activity all together specially after applying the chemical enhancers, no significant differences observed between for cultivars or the treatment only the difference between growth enhancers under drought condition (SA, K_2SO_4 and $MgCO_3$). Assay data revealed again the effective role of SA to increase the activity of the antioxidant enzymes and growth. the Giza 125 and Tamellalet shows highly GR activity in presence of SA (1.265 and 1.225 unit/ml respectively) after that Tombari and Ksar-Megrine (0.969 and 1.135 unit/ml) (Fig. 3). Also, the control activity level was almost same between all cultivars. its indication for that this enzyme activity affected positively with the exogenous chemical enhancers to improve the growth and tolerance against drought stress in barley system. our results are agreed with the report of (19) who confirm that SA in plants is not limited to pathogen defense signaling, but that SA is also influencing light acclimation processes and the regulation of the redox homeostasis of the cell.These results are in harmony with (23) who reported that the increase in antioxidant enzymes activity under various stress conditions had been linked with cell protection from oxidative damage. High drought tolerance was observed in *Triticum aestivum*L. cv. Yumai 34 seedlings following treatment with exogenous 0.5 mM salicylic acid under drought conditions compared with the stressed plants. This enhanced tolerance is related to the increased transcription of GR, as well as the increased content and biosynthesis of ascorbate–glutathione cycle (13).

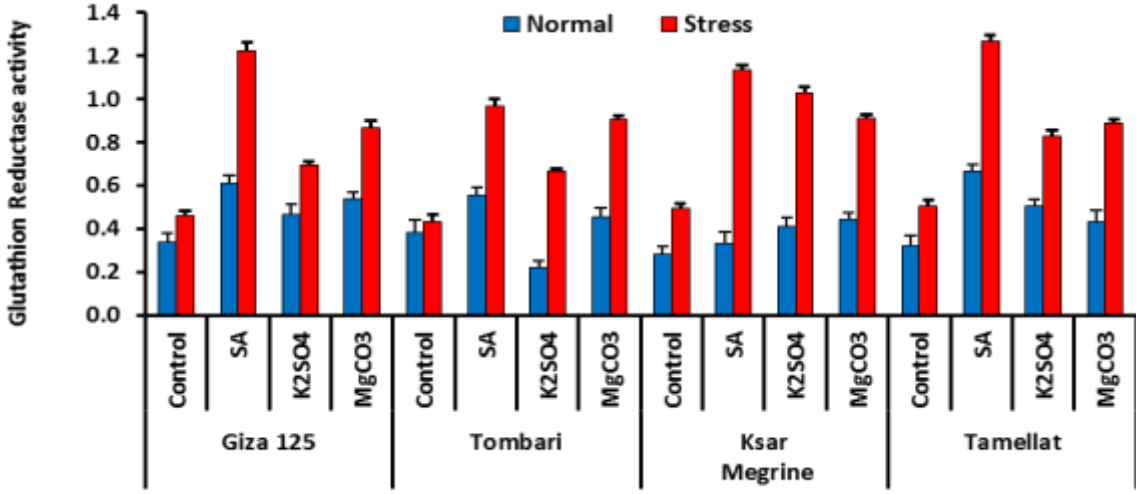

Fig 3. Glutathion reductase of barley as affected by water stress and antitranspirant

The plants adopt certain strategies to overcome the damage caused by water stress through enhanced production of antioxidant enzymes such as; CAT, POD, and GR, and also increased proline accumulation. Antitranspirant treatments applications couldalleviate the adverse effects of water stress enhanced photosynthetic pigments, increased nutrients utilization. Antitranspirants applications could be effective for barley production in arid and semi-arid areas to reduce the adverse effects of water deficit.

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Conflict of interest

All authors declare no conflict of interest in this paper

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