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RESEARCH ARTICLE

Effect of some osmoregulators on photosynthesis, lipid peroxidation, antioxidative capacity, and productivity of barley (*Hordeum vulgare* L.) under water deficit stress

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Abstract

Water deficit stress is an abiotic stress that causes reductions in growth and yield of many field crops around the world. The present research was aimed to elucidate the mitigating efficiency of exogenous application of select osmoregulators and biostimulants, i.e., potassium dihydrogen phosphate, actosol® (humic acid), *Amino more* (amino acids), and *Compound fertilizer*, applied as a spray that reached both foliage and the soil, on growth characteristics, antioxidant capacity, and productivity of barley (*Hordeum vulgare* L. Giza123) under water deficit stress during two successive growing seasons of field experiments in Egypt. Water deficit resulted in stress as estimated by stress indicators and decreased growth and poor health and development as reflected in statistically significant decreases in chlorophyll a and b and major nutrient (NPK) levels in tissues, stem length, number of leaves, and fresh and dry mass as well as yield components such as spike length, grains per spike, biological yield, grain yield, and 1000-grain weight. As a response to water deficit stress, reactive oxygen species (ROS, i.e., superoxide and hydrogen peroxide) levels increased significantly resulting in lipid peroxidation and decreased membrane integrity and significant increases in antioxidant enzymes such as catalase (CAT), polyphenol oxidase (PPO), and peroxidase (POX). All four treatments alleviated the detrimental impacts of water deficit stress as evidenced by statistically significantly increased photosynthetic pigment concentration, tissue NPK levels, growth, and yield parameters compared to the water deficit-stressed control, while the stress responses were significantly reduced. The osmoregulators used either partially restored the growth and yield of osmotic-stressed barley plants or certain treatments enhanced them. All osmoregulators tested mitigated the adverse impacts of water deficit stress on barley plants, but the highest induction was found when plants were treated with actosol®. The beneficial effects of the osmoregulators tested were the strongest overall in the order actosol® > potassium dihydrogen phosphate > *Amino more* > *Compound fertilizer*.

Keywords *Hordeum vulgare* L. · Water deficit stress · Osmoregulators · Antioxidant enzymes · Reactive oxygen species · Electrolyte leakage · Lipid peroxidation

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Abbreviations

ROS	Reactive oxygen species
$O_2^{\cdot-}$	Superoxide
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
CAT	Catalase
POX	Peroxidase
PPO	Polyphenol oxidase

Introduction

Barley (*Hordeum vulgare* L.) belongs to Poaceae family and it is the fourth major cereal crop in terms of World and Egyptian production after maize, wheat, and rice crops (Hafez et al. 2014, Hafez et al. 2016). Abiotic stresses such as water deficit, waterlogging, salinity, and high or low temperatures have been reported to significantly reduce photosynthesis and other biochemical processes related to plant growth and crop productivity (Samarah 2005; Tiwari et al. 2010; Abdelaal et al. 2017; Abdelaal et al. 2018). Drought is a climatic term that means the absence of rain for more than 14 days. Water deficit is a crop management term used to describe production under less than optimum water requirements or when irrigation is not sufficient for reaching field capacity. Water scarcity is the most serious abiotic stress restricting plant productivity throughout the globe (Kotob et al. 2009; Saleem et al. 2016) including areas of Egypt. Under stressful growth conditions, an increment of electrolyte leakage and respiration rate is expected due to oxidative stress, which results in generation of reactive oxygen species (ROS) (Asada 2006). The detrimental effects of abiotic stresses such as water deficit is mainly due to oxidation of cellular biomolecules (i.e., proteins), which is initiated by ROS and free radicals driving the cell to death (Mittler 2002; Helaly et al. 2017). The balance between ROS generation and its scavenging is strongly controlled by the plant antioxidant defense system, particularly under optimal growth conditions (Hameed et al. 2011). Water deficit stress is one of the most common ecological factors which threatens crop plant productivity (Wang et al. 2003) mainly during the flowering and grain-filling phases. Water deficit stress causes significant reduction in stem length, number of branches and leaves of plant as well as leaf area (Abdelaal 2015a). Plants possess many mechanisms to counter the injurious effects of water deficit stress (Vaseva et al. 2012). Various chemicals such as osmoprotectants, growth regulators, and stress signaling molecules are being successfully used against several biotic and abiotic stresses to induce the tolerance (Farooq et al. 2010). Potassium is a macronutrient which plays an important role in growth development of plants and activates more than 60 enzymes (Tisdale et al. 1990). Also, potassium improves water deficit tolerance in plants by maintaining water balance and it is crucial for osmoregulation, photosynthesis, transpiration, stomatal opening and closing as well as protein synthesis (Milford and Johnston 2007; Behairy et al. 2015). The product actosol® is a soil conditioner and fertilizer which contains a mixture of natural humic and fulvic acids. It improves soil structure, enhances plant growth, regulates the carbon cycle and releasing of nutrients, leads to positive effects on plant growth, and induces stress tolerance (Stevenson 1994; Ekinci et al. 2015; Abdelaal 2015b). Application of actosol® and potassium dihydrogen phosphate, respectively, significantly improves growth parameters and chlorophyll concentration (Mahmoud and Youssif 2015). Amino acids, the building blocks of proteins are important for cell growth stimulation and have positive roles in increasing the concentration of osmotic components due to their cell internal function as osmoregulatory (Treichel 1975; Rai 2002; Abd El-Samad et al. 2010). The foliar application of nutrients is readily absorbed by leaves and enhancing the physiological processes and cell growth as well (Robredo et al. 2007) to face the great needs of nutrients during some growth stages particularly at grain-filling stage. However, several experiments have previously conducted to study the harmful effects of water deficit stress on plants; nevertheless, a little information is available concerning water deficit stress around physiological characters and reproductive stage of barley plants. Therefore, the aim of this current research is to study the effect of select osmoregulators, i.e., simple and compound inorganic fertilizers, and complex organic fertilizers that are supposed help plants maintain water balance and for that reason are called osmoregulators, such as potassium dihydrogen phosphate, *Compound fertilizer*, actosol® (humates), as well as *Amino more* (amino acids) on morpho-physiological, biochemical, and yield characters of barley plants (*Hordeum vulgare* L. Giza123) under water deficit stress.

Materials and methods

Field experiments

Field experiments during two successive growing seasons 2014/2015 and 2015/2016 were installed at the Experimental Farm, Faculty of Agriculture, Kafrelsheikh University, Egypt, with the aim to study the effects of four different osmoregulators on growth, morpho-physiological, biochemical characters, and productivity of barley plants (*Hordeum vulgare* L. var. Giza123) under water deficit stress. Meteorological data of the experimental site are presented in Table 1. The used osmoregulators were commercial products with the following names: (1) Potassium dihydrogen phosphate; (2) actosol®; (3) *Amino more*; and (4) *Compound fertilizer*. More details about description, concentrations, and application rate and frequency of the osmoregulators used are

Table 1 Meteorological data of experimental location during both growing seasons (2014/2015 and 2015/2016) as monthly average

Date	Max temp. (°C)	Min temp. (°C)	Precipitation (mm)	Wind (m/s)	Relative humidity (fraction)	Solar radiation (MJ/m^2)	Soil moisture[a] (%, DM)
Nov 2014	26.8	14.7	0.3	2.0	0.7	11.9	15
Dec 2014	20.4	9.0	0.8	2.1	0.7	9.6	–
Jan 2015	20.1	6.0	1.2	2.3	0.7	12.0	–
Feb 2015	22.3	6.0	0.6	2.3	0.7	16.0	–
Mar 2015	26.7	7.9	0.0	2.7	0.6	21.0	–
Apr 2015	27.8	9.7	0.1	2.8	0.6	24.7	–
May 2015	34.6	14.5	0.0	2.6	0.5	28.0	–
Nov 2015	27.3	13.9	0.2	2.1	0.6	12.5	17
Dec 2015	19.9	8.0	1.3	2.0	0.7	10.1	–
Jan 2016	21.4	6.2	0.2	1.8	0.7	11.9	–
Feb 2016	22.3	5.6	0.4	2.2	0.7	15.2	–
Mar 2016	25.5	7.2	0.3	2.5	0.6	20.1	–
Apr 2016	30.0	10.2	0.3	2.5	0.6	25.2	–
May 2016	32.5	14.2	0.3	2.5	0.5	26.2	–

Longitude 30.9375; latitude 31.06679916; elevation 13 m

[a] Soil moisture was measured directly before seed sowing

presented in Table 2. Barley plants were exposed to water deficit stress by not irrigating them beyond sowing and the one-time surface irrigation flooding and draining after germination. Control plots were well watered receiving four more irrigations during the entire growing season, where five irrigations after germination are the standard water requirement for barley in the region. Barley plants under water deficit stress were treated with one of the four above-mentioned osmoregulators to alleviate such abiotic stress by spraying plants twice (Table 1). Barley seeds were sown (120 kg ha^{-1}) on 8th December 2014 (first season) and 11th December 2015 (second season) in a typical clay soil with the following physiochemical characteristics measured according to Horwitz (2005): pH (water) 8.2, electrical conductivity (water extract) 1.8 dS m^{-1}, available N 32.4 ppm, available P 10.5 ppm, available K 289 ppm, soil organic matter (SOM)

Table 2 Description of used osmoregulators and their application rates

No.	Commercial name	Description	Manufacturer	Rate[a]
1	Potassium dihydrogen phosphate	KH_2PO_4, Mr = 136.08 g/mol, grade: ISO	Bioget (Cairo, Egypt)	2 kg ha^{-1} $year^{-1}$
2	actosol®	Liquid product containing, in percent, humates (humic and fulvic acid) 20.00, N 3.50, P (as P_2O_5) 5.00, K (as K_2O) 10.00, Mg 1.00, S 1.50, Ca 0.02, Fe 01.0, Zn 0.10, Mn 0.10, Cu 0.05, B 0.05, and Mo 0.01	Arctech (USA), affiliate in Egypt	10 L ha^{-1} $year^{-1}$
3	*Amino more*	Liquid product containing 15.78 g/100 mL free amino acids (in g/100 mL): alanine (0.92), arginine (1.75), aspartic acid (1.25), glutamic acid (3.00), glycine (0.76), histidine (0.21), isoleucine (0.41), leucine (0.76), lysine (0.74), phenylalanine (0.37), proline (1.48), serine (1.78), threonine (1.33), tyrosine (0.19), and valine (0.93). In addition, 10% K_2O, 1% Fe, 0.05% Zn, 0.05% Mg	Almutahiduwn (Union of Agricultural Development, Teraet El-Gabal 51, Cairo, Egypt)	2.0 L ha^{-1} $year^{-1}$
4	*Compound fertilizer*	White powder containing in %: $FeSO_4 \cdot 7H_2O$ (11.11), H_3BO_3 (11.11), $MgSO_4 \cdot 7H_2O$ (11.11), KH_2PO_4 (33.33), S (11.11), and $ZnSO_4 \cdot 7H_2O$ (22.22).	Bioget (Cairo, Egypt)	2 kg ha^{-1} $year^{-1}$

[a] First application was 2 months from seed sowing and second application was 2 weeks later (half of the osmoregulator amount was dissolved in 1000 L of water at each application to spray the total area of a hectare)

1.9%, sand 17.3%, silt 35.5%, clay 47.2%, and clayey texture. The experimental plot area was 2.5 × 2.5 m^2 and the experimental layout was a randomized complete block design with four replicates. Recommended NP were added at the rate of 110 kg N ha^{-1} as ammonium sulfate (21%) and 125 kg P_2O_5 ha^{-1} as superphosphate (15.5%). Plants were harvested on 4th May 2015 (first season) and 9th May 2016 (second season).

Morpho-physiological characters

Ten plants from each plot were randomly taken at anthesis date to measure stem length, plant fresh and dry mass, number of leaves, chlorophyll a and b, nitrogen (N), phosphorus (P), and potassium (K) contents. A sample of 0.5 g of fresh barley leaves was homogenized with acetone (90% *v*/*v*), filtered, and made up to a final volume of 50 mL. The absorbance was measured spectrophotometrically at 663 and 648 nm according to (Lichtenthaler 1987) to estimate chl a and b content. For N, P, and K determination, plant samples were washed with diluted hydrochloric acid (HCl) solution to remove any adhered substances, then immersed three times in deionized water to remove any residue of HCl. Later, plant samples were dried at ambient temperature in aerated rooms before thermal drying at 70 °C for 48 h. The dried samples were powdered using a grinder and kept in plastic bags for further analysis. For N, P, and K determination, powdered plant samples were digested according to Peterburgski (1968), and N was determined after Block (1968). P was calorimetrically measured according to Jackson (1967), and K was measured by flame photometer using Corning 400 flame photometer (Peterburgski 1968).

Histobiochemical analysis and yield characters

Superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) were visualized in leaf samples as a purple coloration of nitro blue tetrazolium (NBT) and a reddish-brown coloration of 3,3-diaminobenzidine (DAB), respectively. The first seven leaves of barley plants from the top were collected for histobiochemical analysis at 6th March 2015 (for first season) and 17th March 2016 (for second season). The collected leaf blades were vacuum infiltrated with 10 mM potassium phosphate buffer (pH 7.8) containing 0.1 *w*/*v* % NBT (Sigma-Aldrich, Steinheim, Germany) according to Ádám et al. (1989) or 0.1 *w*/*v* % DAB (Fluka, Buchs, Switzerland). NBT- and DAB-treated samples were incubated under day light for 20 min and 2 h, respectively, and subsequently cleared in 0.15 *w*/*v* % trichloroacetic acid in ethanol: chloroform 4:1 *v*/*v* for 1 day (Hueckelhoven et al. 1999; Kiraly et al. 2008). Cleared samples were washed with water and placed in 50% glycerol prior to evaluation. Discoloration of leaf discs resulted by NBT or DAB staining was quantified using a ChemiImager 4000 digital imaging system (Alpha Innotech Corp., San Leandro, USA) in integrated density value units (IDV).

The antioxidant enzyme activities were measured in plant samples as follows: 0.5 g fresh mass of leaf material was homogenized at 0–4 °C in 3 ml of 50 mM TRIS buffer (pH 7.8), containing 1 mM EDTA-Na_2, and 7.5% polyvinylpyrrolidone. The homogenates were centrifuged (12,000 rpm, 20 min, 4 °C), and the total soluble enzyme activities were measured spectrophotometrically in the supernatant (Hafez et al. 2012). The measurements were carried out at 25 °C, using the model UV-160A spectrophotometer (Shimadzu, Japan). Activity of catalase (CAT) was determined spectrophotometrically according to Aebi (1983). Polyphenol oxidase (PPO) activity was determined according to Malik and Singh (1980); the absorbance was recorded at 495 nm. Enzymes activity was expressed as the increase in absorbance min^{-1} g^{-1} fresh mass. Peroxidase (POX) activity was directly determined in the crude enzyme extract according to a typical procedure proposed by (Hammerschmidt et al. 1982). The absorbance was recorded at 470 nm at every 30 s interval for 3 min.

Electrolyte leakage was determined according to Szalai et al. (1996) as follows: 20 discs (1 cm^2) of barely leaves were taken randomly and placed individually into flasks each containing 25 mL deionized water. The samples were shaken for 20 h at ambient temperature to facilitate electrolyte leakage from tissues. Initial electrical conductivity measurements were recorded for each vial using an Acromet AR20 electrical conductivity meter (Fisher Scientific, Chicago, IL). Flasks were then immersed in a hot water bath (Fisher Isotemp, Indiana, PA) at 80 °C (176 °F) for 1 h to induce cell rupture. The vials were again placed on the Innova 2100 platform shaker for 20 h at 21 °C (70 °F). Final conductivity was measured for each flask. Electrolyte leakage percentage was calculated as follows: initial conductivity/final conductivity × 100 according to Szalai et al. (1996), Abdelaal et al. (2014), and Hafez et al. (2014).

Lipid peroxidation was assayed according to Davenport et al. (2003) by measuring the amount of malondialdehyde (MDA). The MDA concentration was estimated by the following formula: MDA (nmol g^{-1}fm) = [6.45 × (A532 − A600) − (0.56 × A450)] × V − 1 W, where V = volume (mL) and W = mass (g).

At harvest (4nd May 2015 and 9th May 2016), the yield components (spike length, number of grains per spike, biological yield (biomass), grain yield/ha, and 1000-grain weight) were determined (Donald and Hamblin 1976). The laboratory analyses were carried out at the (EPCRS) Excellence Center (ISO 9001, ISO 14001 and OHSAS 18001 certified) and the Plant Pathology and Biotechnology Lab (ISO 17025 certified), Department of Agricultural Botany, Kafrelsheikh University.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) procedures according to Gómez et al. (1984) using the MSTAT-C Statistical Software package. The significant difference between the means of treatments was determined at the $p \leq 0.05$ level by Duncan's new multiple range test (Duncan 1955).

Results

Vegetative characters of barley plants under water deficit stress and osmoregulators

Water deficit stress significantly ($p \leq 0.05$) reduced the development of barley plants in both seasons compared to control plants (well watered) (Table 3). In the first growing season, average stem length, number of leaves, and fresh and dry mass of barley plants suffered from water deficit stress—when giving plants only one irrigation during the entire growing season—and were 47.5 cm, 4.2 (plant^{-1}), 4.08 (g plant^{-1}), and 1.45 (g plant^{-1}), respectively, while in the irrigated control, they were 61.0 cm, 5.2 (plant^{-1}), 7.07 (g plant^{-1}), and 2.60 (g plant^{-1}), respectively. Data for the second growing season were consistent with those in first season (Table 3). Each of the four osmoregulators tested significantly enhanced the growth of water deficit-stressed barley plants in both seasons. The osmoregulators were applied as a foliar spray. The plants were sprayed twice during their entire life cycle. Data from first growing season showed that osmotic-stressed barley plants supplied with actosol® had higher average values for stem length (68.5 cm), number of leaves (5.8), and fresh and dry mass (8.16 and 3.03 g plant^{-1}, respectively) than the water deficit-stressed control in both growing seasons. Stem length was also significantly higher even than that in the well-watered control in the first season and so was the number of leaves for the second season. Potassium dihydrogen phosphate caused significant increases compared to the water deficit-stressed control in all parameters in both seasons except the number of leaves in season one. *Amino more* and the *Compound fertilizer* caused significant increases compared to the water deficit-stressed control in all parameters in both seasons except the number of leaves. Compared to the other three osmoregulators, treatment with actosol® caused higher mean values for all parameters in both seasons except for stem length in season two with potassium dihydrogen phosphate (statistically significant and shorter), and these higher values were statistically significant except for stem length in season one with potassium dihydrogen phosphate, and in season two with *Amino more*, for the number of leaves in both seasons for potassium dihydrogen phosphate, for fresh mass in season one and dry mass in both seasons with potassium dihydrogen phosphate, and dry mass in the first season with *Amino more* (Table 3).

Photosynthetic pigments of barley plants under water deficit stress and after treatment with osmoregulators

Photosynthetic pigments estimated by chlorophyll a (Chl a) and b (Chl b) concentrations were statistically significantly lower in osmotic-stressed plants in both seasons compared to the well-watered control (Table 4). Chlorophyll a and b concentrations increased statistically significantly in both seasons after application of each osmoregulator, respectively. *Amino more* application gave the highest Chl a concentration (1.114 and 1.103 mg g^{-1} FM) in both seasons. actosol® application gave the highest Chl a concentration (identical to that caused by *Amino more*, 1.114 mg g^{-1} FM) in the first season. Meanwhile, Chl b concentrations decreased in all treatments compared to control plants (well watered) in the first season. The highest Chl b concentrations were obtained with potassium dihydrogen phosphate (0.486 mg g^{-1} FM) and actosol®

Table 3 Vegetative parameters of barley plants treated with different osmoregulators, i.e., potassium dihydrogen phosphate, actosol®, *Amino more*, and *Compound fertilizer* under water deficit stress during two growing seasons (2014/2015–2015/2016) compared to the irrigated control

Treatments	Stem length (cm)		Number of leaves		Fresh mass (g plant^{-1})		Dry mass (g plant^{-1})	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	61.0 d	63.2 bc	5.2 ab	5.2 b	7.07 ab	8.95 ab	2.60 a	3.42 ab
Water deficit (*D*)	47.5 e	45.0 d	4.2 c	4.5 c	4.08 c	4.65 e	1.45 b	2.33 c
D + potassium[a]	67.8 ab	67.5 a	5.0 abc	5.5 ab	6.48 ab	8.17 bc	2.66 a	3.22 ab
D + actosol	68.5 a	65.2 b	5.8 a	6.0 a	8.16 a	9.89 a	3.03 a	3.70 a
D + *Amino more*	64.8 bc	64.0 b	4.8 bc	4.5 c	6.07 b	7.23 c	2.63 a	3.18 b
D + *Compound fertilizer*	62.0 cd	61.2 c	5.0 abc	5.0 bc	6.95 ab	5.95 d	2.95 a	3.05 b

Means in the same column followed by the same letter are not significantly different according to Duncan's test at 0.05 level

[a] Potassium dihydrogen phosphate

Table 4 Chlorophyll a and b, as well as N, P, and K contents of barley plants treated with different osmoregulators, i.e., potassium dihydrogen phosphate, actosol®, *Amino more*, and *Compound fertilizer* under water deficit stress during two growing seasons (2014/2015–2015/2016) compared to the well-watered control

Treatments	Chl a (mg g^{-1} FM)		Chl b (mg g^{-1} FM)		N (%)		P (%)		K (%)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	1.065 d	1.069 d	0.499 a	0.442 e	2.4 d	2.4 d	0.57 b	0.54 bc	2.2 d	2.3 c
Water deficit (*D*)	1.045 e	1.034 e	0.335 d	0.414 f	2.1 e	2.2 e	0.48 d	0.48 d	1.9 e	2.0 d
D + potassium[a]	1.089 b	1.093 b	0.486 ab	0.486 b	2.6 b	2.6 b	0.66 a	0.56 b	2.5 b	2.4 b
D + actosol	1.114 a	1.094 b	0.469 b	0.498 a	2.8 a	2.7 a	0.54 c	0.60 a	2.5 a	2.6 a
D + *Amino more*	1.114 a	1.103 a	0.474 b	0.480 c	2.6 bc	2.6 b	0.55 bc	0.54 c	2.5 b	2.5 b
D + *Compound fertilizer*	1.083 c	1.079 c	0.430 c	0.470 d	2.5 cd	2.5 c	0.53 c	0.54 c	2.3 c	2.4 b

Means in the same column followed by the same letter are not significantly different according to Duncan's test at 0.05 level

FM fresh mass

[a] Potassium dihydrogen phosphate

(0.498 mg g^{-1} FM) treatments in the first and second seasons (Table 4), and these values were elevated compared to both controls.

NPK contents of barley plants under water deficit stress and osmoregulators

Nitrogen phosphorous and potassium (NPK) levels in the biomass were influenced by water deficit stress and foliar sprays of different osmoregulators, especially actosol® and potassium dihydrogen phosphate. NPK levels were lower in water deficit-stressed plants in both seasons. Application of the osmoregulators increased NPK contents in barley plants compared to osmotic-stressed plants. The highest concentrations of N (2.8% and 2.7% in seasons 1 and 2, respectively) and K (2.5% and 2.6%, in seasons one and two, respectively) were recorded with actosol® treatment, and these values are higher than those of the controls and the treatments with other osmoregulators. As for P, the highest levels were found with potassium dihydrogen phosphate treatment (0.66%) in the first season and with actosol® treatment (0.60%) in the second season (Table 4). The above differences were all statistically significant.

Histochemical and biochemical characters of barley plants under water deficit stress and with osmoregulators

Histochemical analysis of reactive oxygen species

The reactive oxygen species (ROS), superoxide ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2), were visualized (purple and brown discoloration, respectively) in barley leaves by histochemical staining. The discoloration increased in osmotic-stressed barley plants in comparison with control plants (well watered) and plants treated with osmoregulators (Fig. 1). The levels of $O_2^{\cdot-}$ and H_2O_2 were expressed as integrated density values (IDV) and ROS levels significantly increased in water deficit-stressed barley plants. Treatment with osmoregulators greatly reduced the concentration of ROS (Fig. 2). Potassium dihydrogen phosphate and actosol® application diminished production of $O_2^{\cdot-}$ (29.9 and 31.6 in both seasons) and (34.8 and 30.7 in both seasons) respectively, compared to the osmotic-stressed plants (48.1 and 54.9 in both seasons). The same treatments reduced H_2O_2 production (23.9 and 22.5 in both seasons) and (27.8 and 26.5 in both seasons) respectively, compared to the osmotic-stressed plants (39.2 and 36.5 in both seasons).

Activities of antioxidant enzymes

Exposing barley plants to water deficit stress caused significant increases in both seasons in the activities of antioxidant enzymes CAT (+ 79%, + 64%), POX (+ 88%, + 65%), and PPO (+ 109%, + 101%), compared to the well-watered control. Plants treated with different osmoregulators under water deficit stress had significantly lower levels of the antioxidant enzyme activities than the water deficit-stressed plants, but the levels were still significantly higher than in the well-watered control, except for POX in response to *Amino more* in season 2 (Fig. 3). Foliar application of the potassium dihydrogen phosphate, actosol®, *Amino more*, and *Compound fertilizer*, respectively, reduced the activity of CAT, POX, and PPO in leaves of osmotic-stressed plants, but the changes in enzyme activities were not statistically significant between every osmoregulator. actosol® reduced CAT activity the least

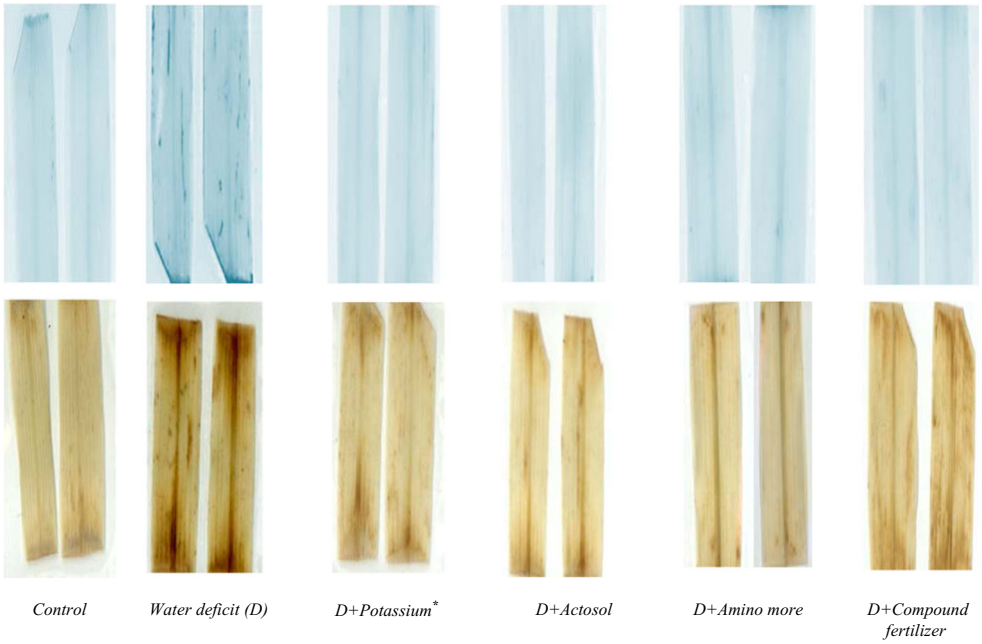

Fig. 1 Histochemical staining for superoxide, $O_2^{\cdot-}$ (nitro blue tetrazolium, upper row) and hydrogen peroxide, H_2O_2 (diaminobenzidine, lower row) in leaves of barley plants treated with different osmoregulators, i.e., potassium dihydrogen phosphate, actosol®, *Amino more*, and *Compound fertilizer* under water deficit stress during second growing season (2015/2016) compared to the control plants (well watered). *Potassium dihydrogen phosphate

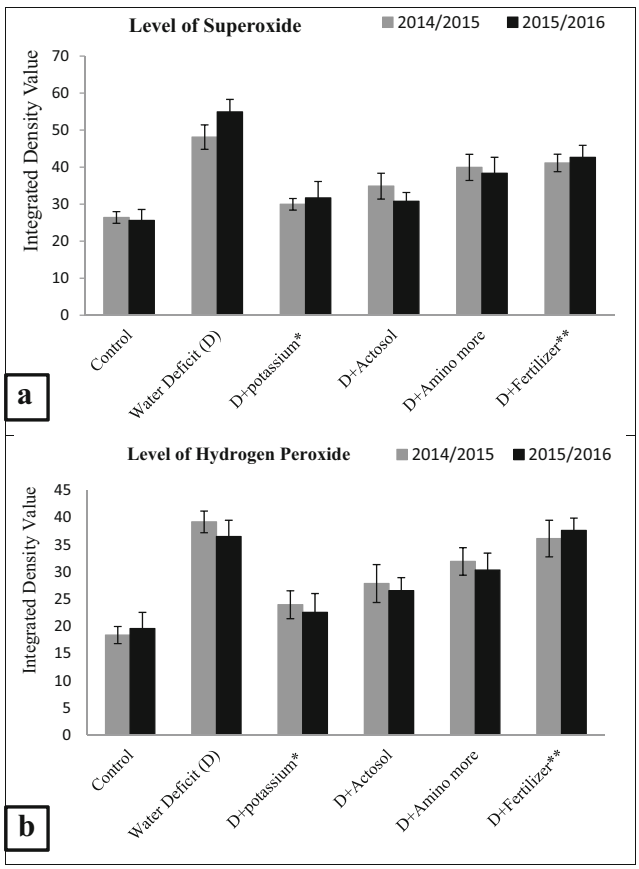

Fig. 2 Quantification of staining for superoxide, $O_2^{\cdot-}$ (**a**) and hydrogen peroxide, H_2O_2 (**b**) in leaves of osmotic-stressed barley plants treated with different osmoregulators, i.e., potassium dihydrogen phosphate, actosol®, *Amino more*, and *Compound fertilizer* under water deficit stress during growing seasons (2014/2015–2015/2016) compared to control plants (well watered). (*Potassium dihydrogen phosphate; ***Compound fertilizer*)

(189.2 and 193.4 mM H_2O_2 g^{-1} FM min^{-1}) in water deficit-stressed plants compared to well-watered plants (105.6 and 117.6 mM H_2O_2 g^{-1} FM min^{-1}) in two seasons. The same trend in changes in response to treatments with different osmoregulators was observed for all three enzymes, i.e., CAT, POX, and PPO (Fig. 3). The lowest activities, i.e., strongest reduction in any treatment were recorded in the *Amino more* and *Compound fertilizer* treatments, and these values were statistically significantly different from each other for CAT in year 2, and for PPO in year 1 (Fig. 3). For example, *Compound fertilizer* reduced, in both seasons, CAT (− 24%, − 19%), PPO (− 33%, − 38%), and POX (− 29%, − 30%), while *Amino more* reduced CAT in season 2 by 17% and this value is not significantly different from that for *Compound fertilizer*.

Electrolyte leakage and lipid peroxidation

Electrolyte leakage (EL) significantly increased in plants when growing under water deficit stress (+ 69%, + 44%, in seasons 1 and 2) (Fig. 4). All osmoregulators tested significantly decreased electrolyte leakage from detached barley leaves compared to well-watered plants and water deficit-stressed plants, the latter showing the highest electrolyte leakage values (43.2 and 41.3 μS cm^{-2} in year 1 and 2, respectively). All four treatments reduced EL compared to the water deficit-stressed control and to or below the level found in well-watered control. The best treatment was potassium dihydrogen phosphate, where reduction in electrolyte leakage was − 51% and − 43% in seasons 1 and 2, respectively, compared to the water deficit-stressed control, and by − 15% and − 17% compared even to the well-watered control (25.6 and 28.6 μS cm^{-2}) in seasons 1 and 2, respectively. The other three

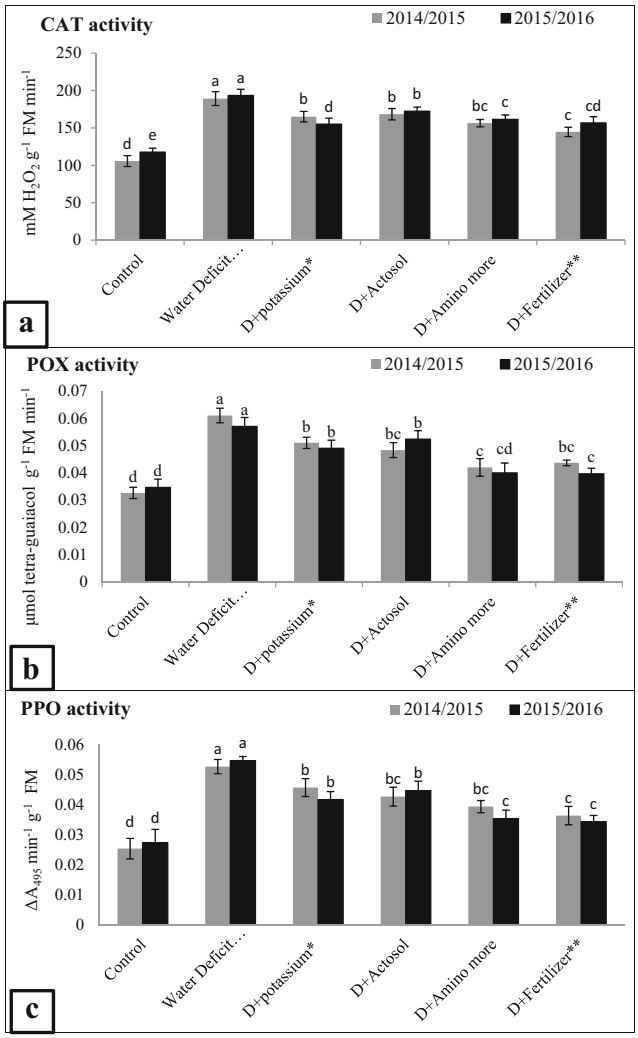

Fig. 3 Activities of catalase, CAT (**a**); peroxidase, POX (**b**); and polyphenol oxidase, PPO (**c**) enzymes in leaves of water deficit-stressed barley plants treated with different osmoregulators, i.e., potassium dihydrogen phosphate, actosol®, *Amino more*, and *Compound fertilizer* under osmotic stress during growing seasons (2014/2015–2015/2016) against control plants (well watered). (*Potassium dihydrogen phosphate; ***Compound fertilizer*). Means in the same year with the same letter over the bar are not significantly different according to Duncan's test at 0.05 level. FM, fresh mass

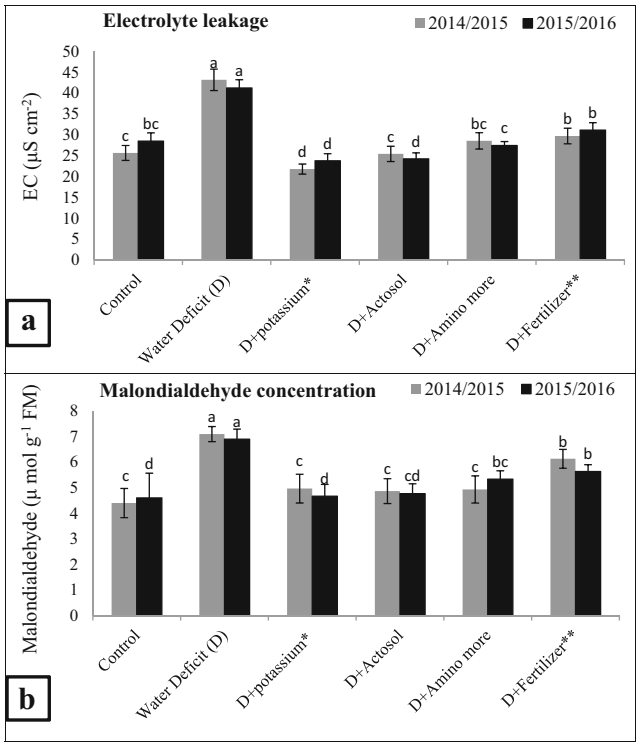

Fig. 4 Electrolyte leakage (**a**) and malondialdehyde (**b**) in leaves of osmotic-stressed barley plants treated with different osmoregulators, i.e., potassium dihydrogen phosphate, actosol®, *Amino more*, and *Compound fertilizer* under water deficit stress during growing seasons (2014/2015–2015/2016) compared to control plants (well watered). (*Potassium dihydrogen phosphate; ***Compound fertilizer*). Means in the same year with the same letter over the bar are not significantly different according to Duncan's test at 0.05 level. FM, fresh mass

osmoregulators were less effective in the order *Compound fertilizer* < *Amino more* < actosol® (Fig. 4).

Malondialdehyde (MDA) concentration significantly increased in barley plants growing under water deficit stress. MDA content was 7.1 and 6.9 μmol g^{-1} FM in two seasons, respectively, for osmotic-stressed plants, while control plants had an MDA content of 4.4 and 4.6 μmol g^{-1} FM, in both seasons, respectively; this represents a + 59% and + 50% change for seasons 1 and 2, respectively. All four treatments caused significant reduction of MDA compared to the water deficit-stressed control. However, MDA content in leaves of osmotic-stressed barley plants, when supplied with potassium dihydrogen phosphate and actosol© were similar to or restored to values those measured in control plants (not significantly different from well-watered control). MDA content was (4.9 and 4.6 μmol g^{-1} FM) in the treatment with potassium dihydrogen phosphate and (4.8 and 4.7 μmol g^{-1} FM) in treatment of actosol® in seasons 1 and 2, respectively (Fig. 4). However, plants receiving *Compound fertilizer* still had statistically significantly higher MDA contents (6.13 and 5.63 μmol g^{-1} FM in years 1 and 2, respectively) than the well-watered control.

Yield of barley plants under water deficit stress and osmoregulators

Water deficit stress significantly ($p \leq 0.05$) reduced the spike length, number of grains per spike, biological yield, grain yield, as well as weight of 1000 grains (Table 5). Application of osmoregulators caused a recovery or even an increase in yield components of osmotic-stressed barley plants in both seasons. The highest values in spike length were obtained with actosol® treatment (9.00 and 8.75 cm, in two seasons) and with potassium dihydrogen phosphate treatment (9.00 cm) in the second season only. Moreover, the highest values in grains per spike were obtained with actosol®

Table 5 Yield parameters of barley treated with different osmoregulators, i.e., potassium dihydrogen phosphate, actosol®, *Amino more*, and *Compound fertilizer* under water deficit stress during two growing seasons (2014/2015–2015/2016) compared to the well-watered control

Treatments	Spike length (cm)		Grains per spike		Biological yield (ton/ha)		Grain yield (ton/ha)		Weight of 1000 grains (g)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	8.75 a	7.75 b	64.5 a	57.0 b	5.3 e	5.4 d	2.2 e	2.3 d	53.78 c	55.82 c
Water deficit (*D*)	6.50 c	6.00 c	42.0 c	45.0 c	4.4 f	4.4 e	1.7 f	1.7 e	47.94 d	50.60 d
D + potassium[a]	7.75 b	9.00 a	54.0 b	61.5 ab	6.5 b	6.1 b	3.2 b	3.0 c	63.61 a	62.04 a
D + actosol	9.00 a	8.75 a	66.0 a	64.5 a	6.8 a	6.3 a	3.4 a	3.3 a	64.51 a	63.01 a
D + *Amino more*	7.50 b	8.00 b	58.5 ab	58.5 b	6.3 c	6.1 b	3.0 c	3.2 b	62.15 b	60.36 b
D + *Compound fertilizer*	7.50 b	7.50 b	58.5 ab	57.0 b	6.2 d	5.9 c	2.9 d	3.0 c	61.53 b	60.34 b

Means in the same column followed by the same letter are not significantly different according to Duncan's test at the 0.05 level

[a] Potassium dihydrogen phosphate

treatments (66.00 and 64.50) in both seasons. Also, actosol® treatment caused the highest biological yield (6.8 and 6.3 ton/ha, in both seasons) and grain yield (3.4 and 3.3 ton/ha, in both seasons) compared to control (well watered) and water deficit stress treatments. However, all tested osmoregulators caused higher values for barley yield and yield components, not only in comparison with osmotic-stressed plants but also the well-watered control plants. Furthermore, the highest values of 1000 grains weight were recorded with actosol® treatment (64.51 and 63.01 g) and potassium dihydrogen phosphate (63.61 and 62.04 g) in seasons 1 and 2, respectively (Table 5).

Discussion

Water deficit resulted in stress as estimated by stress indicators and decreased growth and poor health and development as reflected in decreased chlorophyll and b content and major nutrient (NPK) levels in tissues, stem length, number of leaves, and fresh and dry mass as well yield components such as spike length, grains per spike, biological yield, grain yield, and 1000-grain weight in barley. As a response to water deficit stress, reactive oxygen species (superoxide and hydrogen peroxide) levels increased resulting in lipid peroxidation as estimated by malondialdehyde concentration and decreased membrane integrity and increases in antioxidant enzymes such as CAT, PPO, and POX.

The four treatments to alleviate the water deficit stress included simple and complex inorganic fertilizers and their combinations with organic fertilizers and biostimulants. They all act, in part, as osmoregulators, helping plants maintain turgor and take up nutrients under lower water potential conditions. They were applied as a spray that reached both the foliage and the soil.

Water deficit stress can be mitigated by treatments that supply osmoprotectants, stimulate the uptake and/or production of osmoprotectants, and improve water use efficiency (Barati et al. 2015; Singh et al. 2015). Furthermore, such treatments may indirectly help plants by suppressing their pests and diseases (Kesba and Al-Shalaby 2008). The positive effect of actosol® to enhance stem length and number of leaves may be due to that it contains certain necessary nutrients for growth and promote soil structure and increases the metabolic activity of microorganisms. These results are in agreement with those reported by (Kazemi 2013). The decrease in fresh and dry mass of water deficit-stressed barley plants could be a result to the effect of water on stimulating and regulating the photosynthetic enzymes which influences both fresh and dry matter production (Abdalla and El-Khoshiban 2007). Potassium is an essential macronutrient with roles in transpiration, water movement, and translocation of other nutrients and organics in xylem and phloem tissues (Cao and Tibbitts 1992). Potassium dihydrogen phosphate application enhances photosynthesis and consequently increases carbohydrate content, which increases carboxylation efficiency under water deficit stress conditions leading to an increase of dry mass of plants (Marschner 2012). The detrimental impacts of water deficit stress could be attributed to the decrease in photoassimilation and oxidative damage to the chloroplasts as well as disorganization of thylakoid membranes and chlorophyll degradation under water deficiency conditions (Siddiqui et al. 2015).

The positive effects of osmoregulators, especially actosol®, may be due to increasing the availability of nutrient elements for plant growth and production (Abd El-Aal et al. 2005; Kotob et al. 2009; Kazemi 2013). Furthermore, actosol® may improve nutrients uptake and their mobility in soil through the stimulation of microbiological activity (Denre et al. 2014). Humates regulate the osmotic pressure and thus

assisting movement of the water from soil to the plant (Alobaidy 2008; ARCTECH Inc. 2015). These results are in agreement with the findings of others (Albayrak and Camas 2005; Ali and Elbordiny 2009; Shahryari et al. 2009; Mahmoud and Youssif 2015; Bettoni et al. 2016; Bettoni et al. 2017; Al-Fraihat et al. 2018). The increase in potassium content can prevent photosynthesis inhibition (Egilla et al. 2005), enhancing the total dry mass accumulation of osmotic-stressed plants; this may be related to stomatal regulation by K^+ and corresponding higher rates of photosynthesis (Marschner 2012). Potassium can also alleviate the damage caused by ROS under water deficit stress (Abd El-Aal et al. 2005; Cakmak 2005). Correspondingly, actosol® inhibition of ROS accumulation may be mediated by enhancement of the enzymatic antioxidants and non-enzymatic antioxidants (Laspina et al. 2005; Fallahi et al. 2017). The beneficial effects of potassium dihydrogen phosphate on enzyme activities could result from enhancing CO_2 fixation and transport of photosynthates throughout plant organs (Cakmak 2005). The harmful effects of water deficit stress also derive from cellular dehydration. The beneficial effect of potassium dihydrogen phosphate on reduction of electrolyte leakage maybe attributed to potassium, which plays an important role in improving the function and stability of cell membrane and regulating water deficit stress. Our results with applications of potassium dihydrogen phosphate were consistent with those reported by others (Kant and Kafkafi 2002; Kaya et al. 2007). At the genetic and molecular levels, potassium transporters play a role in ion homeostasis in barley and related grain crops (Song et al. 2014; Shahzad et al. 2015; Wu et al. 2015; Zhang et al. 2018). A high-affinity potassium transporter not only plays important roles in K acquisition and translocation but also positively regulates responses to drought stress in rice (Chen et al. 2017) and has been studied in high salt tolerant barley accessions (Qiu et al. 2011; Han et al. 2018).

Also, the adverse effect of water deficit stress and the resulting of dehydration of protoplasm, decreasing relative water content (Hussain et al. 2008), causes oxidative stress and membrane damage. The beneficial role of potassium dihydrogen phosphate and actosol® may be mainly attributed to improving cell membrane stability and water deficit adjustment ability and consequently decreased malondialdehyde concentration. Electrolyte leakage was alleviated by humic acid and potassium application as was found for bean as well (Aydin et al. 2012).

Intracellular free amino acid levels are part of the water deficit, draught, and salinity stress response (Rai 2002; Sarwat and EI-Sherif 2007; Alobaidy 2008; Abd El-Samad et al. 2010; Singh et al. 2015) and external application of amino acids can support the stressed plant, especially in combination with mineral nutrients alleviates the stress as we have found.

Water deficit stress can reduce yield components by causing reduction in leaf area expansion, which in turn, will lead to reductions in all other yield components (Samarah 2005; McGranahan and Poling 2018). Each one of the four biostimulant osmoregulators contributes to osmotic balance by supplying low molecular weight osmolytes such as ions or amino acids, and by supporting photosynthesis that can add to the osmotically active metabolite pool in the sap (Rai 2002). In agreement with our findings, it was reported that osmoregulators improve growth characters and relative water content correspondingly, increase yield and yield components (Clarke and McCaig 1982). Similar results were recorded in some crops under drought and salinity stresses by (Ekinci et al. 2015; Mahmoud and Youssif 2015; Helaly et al. 2017).

Conclusion

The current study aimed to evaluate the role of select osmoregulators, i.e., actosol®, potassium dihydrogen phosphate, *Amino more*, and *Compound fertilizer* to mitigate the detrimental impacts of water deficit stress in order to help crop production where water scarcity is a problem around the world. When barley plants grew with only one irrigation after germination, the growth dynamics and yield were significantly affected compared to control (well watered).

Water deficit resulted in stress as indicated decreased growth and poor health and development as reflected in decreased chlorophyll and b content, and major nutrient (NPK) levels in tissues, stem length, number of leaves, and fresh and dry mass as well yield components such as spike length, grains per spike, biological yield, grain yield, and 1000-grain weight in barley. As a response to water deficit stress, reactive oxygen species (superoxide and hydrogen peroxide) levels increased resulting in increased malondialdehyde levels and decreased membrane integrity and increases in antioxidant enzymes such as CAT, PPO, and POX.

In addition, levels of reactive oxygen species, i.e., superoxide and hydrogen peroxide considerably increased causing increased malondialdehyde levels from lipid peroxidation of membranes, electrolyte leakage, and elevation in the activities of antioxidant enzymes (catalase, peroxidase and polyphenol oxidase). Spraying barley plants twice during the entire growth season with any of the tested osmoregulators substantially enhanced the barley plants' growth by diminishing the generation of superoxide and hydrogen peroxide and also reduced electrolyte leakage and malondialdehyde concentration, thus maintaining homeostasis in plant cells resulting in better growth, and the yield in certain treatments was even better than in the well-watered control plants. actosol®, followed by potassium dihydrogen phosphate, was the best treatment overall that

alleviated the adverse effects of water deficit in barley (*Hordeum vulgare* L. Giza123) at the Experimental Farm, Faculty of Agriculture, Kafrelsheikh University, Egypt.

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References

Abd El-Aal FS, Shafeek MR, Ahmed AA, Shaheen AM (2005) Response of growth and yield of onion plants to potassium fertilizer and humic acid. J Agric Sci Mansoura Univ 30(1):441–452

Abd El-Samad HM, Shaddad MAK, Barakat N (2010) The role of amino acids in improvement in salt tolerance of crop plants. J of Stress Physiol and Bioch 6(3):25–37

Abdalla M, El-Khoshiban N (2007) The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticum aestivum* cultivars. J Appl Sci Res 3(12):2062–2074

Abdelaal K (2015a) Effect of salicylic acid and abscisic acid on morpho-physiological and anatomical characters of faba bean plants (*Vicia faba* L.) under drought stress. J Plant Prod Mansoura Univ 6(11): 1771–1788

Abdelaal K (2015b) Pivotal role of bio and mineral fertilizer combinations on morphological, anatomical and yield characters of sugar beet plant (*Beta vulgaris* L.). Middle East J Agric Res 4(4):717–734

Abdelaal K, Hafez Y, Badr M, Youseef W, Esmaeil S (2014) Biochemical, histological and molecular changes in susceptible and resistant wheat cultivars inoculated with stripe rust fungus *Puccinia striiformis* f. sp. *tritici*. Egyp J Biol Pest Control 24:421–429

Abdelaal K, Hafez Y, Sabagh AEL, Saneoka H (2017) Ameliorative effects of Abscisic acid and yeast on morpho-physiological and yield characteristics of maize plant (*Zea mays* L.) under drought conditions. Fresenius Environ Bull 26(2):7372–7383

Abdelaal K, Omara I, Hafez Y, Esmail S, Sabagh AEL (2018) Anatomical, biochemical and physiological changes in some Egyptian wheat cultivars inoculated with *Puccinia graminis* f. sp. *tritici*. Fresenius Environ Bull 27(1):296–305

Ádám A, Farkas T, Somlyai G, Hevesi M, Király Z (1989) Consequence of $O_2^{\cdot-}$ generation during a bacterially induced hypersensitive reaction in tobacco: deterioration of membrane lipids. Physiol Mol Plant Pathol 34:13–26

Aebi HE (1983) Catalase. "Methods of Enzymatic Analysis", 3rd ed. Verlag Chemie, Weinheim, pp 273–286

Al-Fraihat AH, Al-Tabbal JA, Abu-Darwish MS, Alhrout HH, Hasan HS (2018) Response of onion (*Allium cepa*) crop to foliar application of humic acid under rain-fed conditions. Int J Agric Biol 20(5):1235–1241

Albayrak S, Camas N (2005) Effects of different levels and application times of humic acid on root and leaf yield and yield components of forage turnip (*Brassica rapa* L.). J Agron 4(2):130–133

Ali LKM, Elbordiny MM (2009) Response of wheat plants to potassium humate application. J Appl Sci Res 5(9):1202–1209

Alobaidy MG (2008) Effect of putrescine and humic acid on cotton plant growing under salinity stress conditions. M Sc in Agric Sci (Plant Physiology), Cairo University, Egypt

ARCTECH Inc (2015) Technical Bulletin # 5 humic acid: a review of characteristics, properties, analytical methods and applications EPA Dialogue Committee Response, 2015(1): https://www.epa.gov/sites/production/files/2015-2003/documents/9545955.pdf

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141(2):391–396

Aydin A, Kant C, Turan M (2012) Humic acid application alleviate salinity stress of bean (*Phaseolus vulgaris* L.) plants decreasing membrane leakage. African J Agric Res 7(7):1073–1086

Barati V, Ghadiri H, Zand-Parsa S, Karimian N (2015) Nitrogen and water use efficiencies and yield response of barley cultivars under different irrigation and nitrogen regimes in a semi-arid Mediterranean climate. Arch Agron Soil Sci 61(1):15–32

Behairy AG, Mahmoud AR, Shafeek MRA, H A, Hafez MM (2015) Growth, yield and bulb quality of onion plants (*Allium cepa* L.) as affected by foliar and soil application of potassium. Middle East J Agric Res 4(1):60–66

Bettoni MM, Mogor AF, Pauletti V, Goicoechea N (2017) The interaction between mycorrhizal inoculation, humic acids supply and elevated atmospheric CO_2 increases energetic and antioxidant properties and sweetness of yellow onion. Hortic Environ Biotechnol 58(5):432–440

Bettoni MM, Mogor AF, Pauletti V, Goicoechea N, Aranjuelo I, Garmendia I (2016) Nutritional quality and yield of onion as affected by different application methods and doses of humic substances. J Food Compos Anal 51:37–44

Block RJ (1968) A manual of paper chromatography and paper electrophoresis. Academic Press, New York

Cakmak I (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J Plant Nutr Soil Sci 168(4):521–530

Cao WX, Tibbitts TW (1992) Potassium content effect on growth, gas exchange and mineral accumulation in potatoes. J Plant Nutr 15(9): 1359–1371

Chen G, C Liu, Z Gao, Y Zhang, H Jiang, L Zhu, D Ren, L Yu, G Xu and Q Qian (2017). *OsHAK1*, a high-affinity potassium transporter, positively regulates responses to drought stress in rice. Front Plant Sci 8

Clarke JM, McCaig TN (1982) Excised-leaf water retention capability as an indicator of drought resistance of *Triticum* genotypes. Can J Plant Sci 62(3):571–578

Davenport SB, Gallego SM, Benavides MP, Tomaro ML (2003) Behaviour of anti-oxidant defense system in the adaptive response to salt stress in (*Helianthus annuus* L.) cell. Plant Growth Reg 40: 81–88

Denre M, Ghanti S, Sarkar K (2014) Effect of humic acid application on accumulation of mineral nutrition and pungency in garlic (*Allium sativum* L.) cultivars. Int J Biotechnol Mol Biol Res 5(2):7–12

Donald CM, Hamblin J (1976) The biological yield and harvest index of cereals as agronomic and plant breeding criteria. Adv Agron 28: 361–405

Duncan B (1955) Multiple ranges and multiple F-test. Biometrics 11:1–42

Egilla JN, Davies FT, Boutton TW (2005) Drought stress influences leaf water content, photosynthesis, and water-use efficiency of *Hibiscus rosa-sinensis* at three potassium concentrations. Photosynthetica 43(1):135–140

Ekinci M, Esringu A, Dursun A, Yildirim E, Turan M, Karaman MR, Arjumend T (2015) Growth, yield, and calcium and boron uptake of tomato (*Lycopersicon esculentum* L.) and cucumber (*Cucumis sativus* L.) as affected by calcium and boron humate application in greenhouse conditions. Turk J Agric For 39(5):613–632

Fallahi H-R, Ghorbany M, Aghhavani-Shajari M, Samadzadeh A, Asadian AH (2017) Qualitative response of roselle to planting methods, humic acid application, mycorrhizal inoculation and irrigation management. J Crop Improv 31(2):192–208

Farooq M, Wahid A, Lee DJ, Cheema SA, Aziz T (2010) Comparative time course action of the foliar applied Glycinebetaine, salicylic acid, nitrous oxide, brassinosteroids and spermine in improving drought resistance of rice. J Agron Crop Sci 196(5):336–345

Gómez KA, Gómez AA, I International Rice Research (1984) Statistical procedures for agricultural research. John Wiley & Sons, New York

Hafez YM, Abdelaal KAA, Eid ME, Mehiar FF (2016) Morpho-physiological and biochemical responses of barley plants (*Hordeum vulgare* L.) against barley net blotch disease with application of non-traditional compounds and fungicides. Egypt J Biol Pest Control 26(2):261–268

Hafez YM, Bacso R, Kiraly Z, Kuenstler A, Kiraly L (2012) Up-regulation of antioxidants in tobacco by low concentrations of H_2O_2 suppresses necrotic disease symptoms. Phytopathology 102(9):848–856

Hafez YM, Mourad RY, Mansour M, Abdelaal KAA (2014) Impact of non-traditional compounds and fungicides on physiological and biochemical characters of barely infected with *Blumeria graminis* f. sp. *hordei* under field condtitions. Egypt J Biol Pest Control 24(2):445–453

Hameed A, Bibi N, Akhter J, Lqbal N (2011) Differential changes in antioxidants, proteases, and lipid peroxidation in flag leaves of wheat genotypes under different levels of water deficit conditions. Plant Physiol Biochem 49(2):178–185

Hammerschmidt R, Nuckles EM, Kuc J (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol Plant Pathol 20(1):73–82

Han Y, Yin S, Huang L, Wu X, Zeng J, Liu X, Qiu L, Munns R, Chen Z-H and Zhang G (2018) A sodium transporter *HvHKT1;1* confers salt tolerance in barley via regulating tissue and cell ion homeostasis. Plant Cell Physiol

Helaly M, Mohammed Z, El-Shaeery N, Abdelaal K, Nofal I (2017) Cucumber grafting onto pumpkin can represent an interesting tool to minimize salinity stress. Physiological and anatomical studies. Middle East J Agric Res 6(4):953–975

Horwitz W (2005). Official methods of analysis of AOAC International. Gaithersburg, Maryland, AOAC International

Hueckelhoven R, Fodor J, Preis C, Kogel K-H (1999) Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. Plant Physiol (Rockville) 119(4): 1251–1260

Hussain M, Malik MA, Farooq M, Ashraf MY, Cheema MA (2008) Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. J Agron Crop Sci 194(3):193–199

Jackson ML (1967) Soil chemical analysis. Prentice-Hall, New Delhi

Kant S and Kafkafi U (2002) Potassium and abiotic stresses in plants. Potassium for sustainable crop production. N. S. Pasricha and S. K. Bansal. Gurgaon, India, Potash Institute of India: 233–251

Kaya C, Tuna AL, Ashraf M, Altunlu H (2007) Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. Environ Exp Bot 60(3):397–403

Kazemi M (2013) Vegetative and reproductive growth of tomato plants affected by calcium and humic acid. Bull Env Pharmacol Life Sci 2(11):24–29

Kesba HH, Al-Shalaby MEM (2008) Survival and reproduction of *Meloidogyne incognita* on tomato as affected by humic acid. Nematology 10:243–249

Kiraly L, Hafez YM, Fodor J, Kiraly Z (2008) Suppression of tobacco mosaic virus-induced hypersensitive-type necrotization in tobacco at high temperature is associated with downregulation of NADPH oxidase and superoxide and stimulation of dehydroascorbate reductase. J Gen Virol 89:799–808

Kotob S, El Shall S and Walla DS (2009) Applications of actosol® humic acid products in Egypt. ASA-CSSA-SSSA Symposium 98—microbial and humic amendments: advances in understanding their effects on soils and plants: I, November 1–5, 2009. Pittsburg, PA, USA

Laspina NV, Groppa MD, Tomaro ML, Benavides MP (2005) Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. Plant Sci 169(2):323–330

Lichtenthaler HK (1987) [34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology, Academic Press 148:350–382

Mahmoud H, Youssif S (2015) Response of garlic (*Allium sativum* L.) to natural fertilizers and ores under Ras Sudr conditions. Middle East J Appl Sci 5(4):1174–1183

Malik CP, Singh MB (1980) In: Plant Enzymology and Histoenzymology. Kalyani Publishers, Indian and printed in Navin, Shanndara. Delhi pp 54–56

Marschner P (2012) Marschner's mineral nutrition of higher plants, 3rd edn. Elsevier/Academic Press, Amsterdam; Boston

McGranahan DA, Poling BN (2018) Trait-based responses of seven annual crops to elevated CO_2 and water limitation. Renew Agric Food Syst 33(3):259–266

Milford GFJ and Johnston AE (2007). Potassium and nitrogen interactions in crop production. Proceedings (International Fertiliser Society), no. 615., York, International Fertiliser Society

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7(9):405–410

Peterburgski A (1968) Handbook of agronomic chemistry (in Russian). Kolos Publishing House, Moscow, pp 29–68

Qiu L, Wu D, Ali S, Cai S, Dai F, Jin X, Wu F, Zhang G (2011) Evaluation of salinity tolerance and analysis of allelic function of HvHKT1 and HvHKT2 in Tibetan wild barley. Theor Appl Genet 122(4):695–703

Rai VK (2002) Role of amino acids in plant responses to stresses. Biol Plant 45(4):481–487

Robredo A, Pérez-López U, de la Maza HS, González-Moro B, Lacuesta M, Mena-Petite A, Muñoz-Rueda A (2007) Elevated CO_2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. Environ Exp Bot 59(3):252–263

Saleem MF, Raza MAS, Ahmad S, Khan IH, Shahid AM (2016) Understanding and mitigating the impacts of drought stress in cotton—a review. Pak J Agric Sci 53(3):609–623

Samarah NH (2005). Effects of drought stress on growth and yield of barley. Agronomy for sustainable development, Springer Verlag/ EDP Sciences/INRA 25 (1): 145–149

Sarwat MI, EI-Sherif MH (2007) Increasing salt tolerance in some barley genotypes (*Hordeum vulgare*) by using kinetin and benzyladenin. World J Agric Sci 3(5):617–629

Shahryari R, Gadimov A, Gurbanov E and Valizade M (2009). Applications of potassium humate to wheat for organic agriculture in Iran. Asian Journal of Food and Agro-Industry (Special Issue): S164-S168

Shahzad K, Rauf M, Ahmed M, Malik ZA, Habib I, Ahmed Z, Mahmood K, Ali R, Masmoudi K, Lemtiri-Chlieh F, Gehring C, Berkowitz GA, Saeed NA (2015) Functional characterisation of an intron retaining K+ transporter of barley reveals intron-mediated alternate splicing. Plant Biol 17(4):840–851

Siddiqui MH, Al-Khaishany MY, Al-Qutami MA, Al-Whaibi MH, Grover A, Ali HM, Al-Wahibi MS, Bukhari NA (2015) Response of different genotypes of faba bean plant to drought stress. Int J Mol Sci 16(5):10214–10227

Singh M, Kumar J, Singh S, Singh VP, Prasad SM (2015) Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. Rev Environ Sci Bio-Technol 14(3):407–426

Song ZZ, Yang SY, Zuo J, Su YH (2014) Over-expression of *ApKUP3* enhances potassium nutrition and drought tolerance in transgenic rice. Biol Plant 58(4):649–658

Stevenson FJ (1994) Humus chemistry genesis, composition, reactions, 2nd edn. John Wiley & Sons, New York

Szalai G, Janda T, Paldi E, Szigeti Z (1996) Role of light in the development of post-chilling symptoms in maize. J Plant Physiol 148(3–4): 378–383

Tisdale SL, Nelson WL, Beaton JD (1990) Soil fertility and fertilizers. Macmillan, New York, N.Y., pp 60–62

Tiwari JK, Munshi AD, Kumar R, Pandey RN, Arora A, Bhat JS, Sureja AK (2010) Effect of salt stress on cucumber: Na+-K+ ratio, osmolyte concentration, phenols and chlorophyll content. Acta Physiol Plant 32(1):103–114

Treichel S (1975) Effect of NaCl on concentration of proline in different halophytes. Zeitschrift Fur Pflanzenphysiologie 76(1):56–68

Vaseva I, Akiscan Y, Simova-Stoilova L, Kostadinova A, Nenkova R, Anders I, Feller U, Demirevska K (2012) Antioxidant response to drought in red and white clover. Acta Physiol Plant 34(5):1689–1699

Wang WX, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218(1):1–14

Wu H, Zhu M, Shabala L, Zhou M, Shabala S (2015) K+ retention in leaf mesophyll, an overlooked component of salinity tolerance mechanism: a case study for barley. J Integr Plant Biol 57(2):171–185

Zhang H, Xiao W, Yu W, Yao L, Li L, Wei J and Li R (2018). Foxtail millet *SiHAK1* excites extreme high-affinity K+ uptake to maintain K+ homeostasis under low K+ or salt stress. Plant Cell Reports