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RESEARCH ARTICLE

Exogenous application of ascorbic acid and putrescine: A natural eco-friendly potential for alleviating NaCl stress in barley (*Hordeum vulgare*)

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ABSTRACT

A pot experiment was performed in the green house of Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the winter seasons of 2019 and 2020 to investigate the effect of exogenous application of ascorbic acid (AsA) and putrescine (Put) in ameliorating the growth parameters of barley (*Hordeum vulgare* L.) plant under saline conditions (9.3 and 14 dS m^{-1}). Two concentrations of either AsA (100 and 300 ppm) or Put (100 and 200 ppm) were foliar-sprayed individually or in combination with both salt concentrations. Vegetative, yield, and anatomical characters, leaf photosynthetic pigments, and grain crude protein declined in response to stress, while electrolyte leakage (EL), proline, glycine betaine (GB), total carbohydrates and antioxidant enzymes increased under same conditions. The maximum increments in vegetative characters were notable at concentrations of either AsA at 300 ppm or Put at 100 ppm. Yield characters were enhanced at 300 ppm AsA and both concentrations of Put. Improvement in anatomical features of leaf and stem was achieved with the combination of either AsA at 300 ppm or Put at 100 ppm with salinity at 14 dS m^{-1}. AsA was more effective in enhancing photosynthetic pigments and crude protein individually or in combination with salinity. Combinations of either AsA or Put with salinity induced decrements in EL, GB and antioxidant enzymes and increments in proline and total carbohydrates. In conclusion, foliar application of AsA and Put could be considered an eco-friendly approach to alleviate the adverse effects of salinity on morphological and physiological characters of barley.

Keywords: Salinity; ascorbic acid; putrescine; barley; anatomy; biochemical

INTRODUCTION

Salinity is a crucial problem affecting around 20% of the total world's irrigated agricultural land (EL-Sharkawy et al., 2017). In Egypt, around 33% of cultivated lands are affected by salinity distributed as follows: 60% in Northern Delta, 20% in Southern Delta and Middle Egypt, and 25% in Upper Egypt (Mohamed et al., 2007; El-Sharkawy et al., 2017). As a semi-arid region, soil salinity in Egypt is attributed mainly to low precipitation and higher temperatures which increase surface evaporation and hence salt concentration, additionally, poor drainage systems and irrigation with either poor quality or sea water contributes more to soil salinity (El-Hendawy, 2004; Mohamed et al., 2007).

Soil or water salinity is a severe type of abiotic stress limiting crop production and affecting plant growth and development. Primary effects of salinity on plants include water stress caused by hyperosmotic pressure, salt stress due to excessive accumulation of ions, and nutrient imbalance (Ellouzi et al., 2013; El-Sharkawy et al., 2017). Secondary consequences of salinity involve oxidative damage caused by accumulation of reactive oxygen species (ROS) resulting in plant cell damage on the molecular and biochemical levels (El-Hendawy, 2004). Plants exhibit several tolerance mechanisms to counteract osmotic and ionic stresses and regain cell homeostasis; where, accumulation of osmolytes such as proline, glycine betaine, polyamines, carbohydrates and polyols is the most important mechanism by which plants adapt to salt and water stresses (Ashraf and Harris, 2004; Sairam and Tyagi, 2004; Mohamed et al., 2007). Moreover, concentrations of antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) increase in salt tolerant plants and act mainly in scavenging ROS and reducing oxidative damage (Ashraf and Harris, 2004; Sairam and Tyagi, 2004; Mohamed et al., 2007).

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An important approach to ameliorate hazardous effects of salinity is the exogenous application of some natural or chemical agents including natural extracts, organic substances, growth regulators, macro- and micronutrients, vitamins (*e.g.* ascorbic acid) and osmoprotectants (*e.g.* polyamines, glycine betaine and proline) (Ahmed et al., 2013; Rademacher, 2015). Ascorbic acid (AsA) or vitamin C is an important antioxidant regulating many plant biological processes; cell division and differentiation, photosynthesis, respiration and other metabolic activities (Bakry et al., 2013; Mittal et al., 2018). Moreover, AsA is a major non-enzymatic antioxidant defense mechanism by which plants counteract the adverse effects of abiotic stress including salinity and further oxidative damage, where, it controls accumulation of ROS (El-Bassiouny and Sadak, 2015; Moori And Eisvand, 2017, Mittal et al., 2018). Growth and yield as well as biochemical characters for different plant species were found to be improved in general and under stress conditions after the exogenous application of AsA (Darvishan et al., 2013; El-Bassiouny and Sadak, 2015; Moori and Eisvand, 2017; Mittal et al., 2018). Putrescine (Put), a polyamine, is involved in growth regulation during the different developmental stages of plant with a favorable effect in mitigating the impact of salinity through regaining cell osmotic pressure and scavenging of ROS (Zhong et al., 2016; Gul et al., 2018). Exogenous application of Put was found to improve the biometric characteristics of different plants and to enhance the chemical constituents under salt stress conditions (Ahmed et al., 2013; Gerami et al., 2019; Ghalati et al., 2020).

Another approach to overcome salinity stress is the cultivation of halophytes (salinity tolerance $\geq$ 4 dS m^{-1}) (Mohamed et al., 2007). Whereas most plants are classified as glycophytes (salinity tolerance $\leq$ 4 dS m^{-1}), barley (*Hordeum vulgare* L.) is considered a marginal halophyte with ability to tolerate 5 g L^{-1} NaCl (equiv. to 7.8 dS m^{-1}) (Sairam and Tyagi, 2004; El-Sharkawy et al., 2017). Moreover, barley is considered a salt excluder, limiting the Na^+ transport from shoots to leaves (Munns et al., 2006; Abdi et al., 2016; El-Sharkawy et al., 2017). Barley ranks as the fourth most important cereal crop after wheat, maize and rice with wide range distribution around the world and concentration in temperate regions, moreover, barley can tolerate different stress conditions such as drought and salinity (El-Sharkawy et al., 2017).

The main objective of this investigation is to test the effect of exogenous foliar application of AsA and Put in ameliorating yield and biochemical characteristics of barley under saline conditions in order to achieve efficient use of saline soils in Egypt.

MATERIAL AND METHODS

Experimental procedures

The present investigation was carried out at Faculty of Agriculture, Cairo University, Giza, Egypt during the two consecutive winter growing seasons of 2018/2019 and 2019/2020. A pot experiment was conducted in the green house of Agricultural Botany Department to evaluate the effect of foliar spray with different concentrations of AsA (100 and 300 ppm) and Put (100 and 200 ppm) on barley plants irrigated with salty water (NaCl) at concentrations of 9.3 and 14 dS m^{-1}, whereas control plants were irrigated with tap water. Barley grains (Giza 123) were obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. The grains were planted on 20th and 22th Oct. of the years 2018 and 2019, respectively, in plastic pots (25 cm in diameter) filled with sandy loam soil (Table 1). Initially, ten seeds per pot were sown, 6 days after emergence; the seedlings were thinned to 3 seedlings per pot. The experiment was arranged in split plot design with three replicates, each contained 10 pots.

Plant seedlings were irrigated with tap water for two weeks after sowing; afterwards, plants were irrigated with either tap water (control) or water at the aforementioned salinity concentrations along the period of the experiment.

The concentrations of AsA and Put were applied twice, the first application was four weeks after sowing and the second one was two weeks after the first.

Plant characteristics

Biometric measurements

Biometric measurements were taken for random samples of 9 plants per treatment (3 per replicate) after 65 days

Table 1: Physical and chemical characteristics of soil used for barley cultivation during the two growing seasons.

Chemical characteristics										
Soil characters	**PH**	**EC**	**TDS**	**Soluble anions (meq L^{-1})**			**Soluble cations (meq L^{-1})**			
	(1:1)	**dS m^{-1}**	**mg L^{-1}**	**CO_3^{2-}**	**HCO_{3-}**	**Cl^-**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**K^+**
Soil	8.23	0.44	281.6	0.00	1.30	2.99	3.40	1.20	1.52	0.40
Physical characteristics										
Soil characters			**Texture**	**Sand %**		**Slit %**			**Clay %**	
Soil			Sandy loam	65.10		18.98			15.92	

from sowing date as follows: plant height (cm), number of leaves/plant and shoot dry weight/plant (g).

Yield characters

Yield characters were recorded after spike maturation as follows: number of spikes/plant, main spike length (cm), number of grains/plant, grain weight/spike (g) and seed index (weight of 1000 grains in g).

Microscopic measurements

A microscopic study was carried out only on plants which showed remarkable response to salinity stress and treatments with either AsA or Put. Specimens of stem and leaves were taken during the second season from the internode of median portion of main stem and its corresponding leaf after 65 days from sowing date. Specimens were prepared and sectioned to a thickness of 20 μ and finally double-stained with crystal violet-erthrosin, and mounted in Canada balsam (Nassar and El-Sahhar, 1998). The transverse sections of each of leaf and stem were photomicrographed and different microscopic measurements were calculated.

Biochemical constituents

Photosynthetic pigments

Photosynthetic pigments (chlorophyll a, b and total carotenoids) were determined colorimetrically in fresh leaves according to Mornai (1982) using spectrophotometer at wavelengths 663, 647 and 470 nm for chlorophyll a (chl a), chlorophyll b (chl b) and total carotenoids, respectively. The concentrations of these pigments were calculated by means of Mornai's formula and expressed as mg g^{-1} fresh weight (FW).

Electrolyte leakage (EL)

EL was measured in fresh leaves according to the method described by Blum and Ebercon (1981) where, electric conductivity was recorded twice; EC_1 at room temperature and EC_2 at 121°C, then calculated as a percentage using the equation: $(EC_1/EC_2) \times 100$.

Crude protein

Total N content in grains was analyzed using the Kjeldahl method (Peach and Tracey, 1956); the calculated nitrogen percentage was multiplied by 6.25 to estimate the crude protein percentage (Pomeranz and Clifton, 1987).

Proline

Proline was extracted from dried leaves and determined colorimetrically using spectrophotometer at 520 nm according to Bates et al. (1973) and expressed in units of mg g^{-1} dry weight (DW).

Glycine betaine (GB)

Glycine betaine (GB) was determined in leaves using high performance liquid chromatography (HPLC) following the method of Naidu (1998) and expressed in units of μmol g^{-1} FW.

Total carbohydrates

Total carbohydrates were determined colorimetrically in dried leaves according to the phenol-sulfuric acid method described by Herbert et al. (1971) and expressed in terms of g 100 g^{-1} DW.

Antioxidant enzymes

Peroxidase

Peroxidase was determined following the method of Quessada and Macheix (1984) and expressed in units of mg^{-1} protein.

Catalase

Catalase was determined in units of mg^{-1} protein as described by Aebi (1984).

Statistical analysis

Test of normality distribution was carried out according to Shapiro and Wilk (1965), by using SPSS v. 17.0 (2008) computer package. Combined analysis of variance of a split plot design arranged in Randomized Complete Block Design (RCBD) across the two seasons was computed after carrying out Bartlett's test according to Snedecor and Cochran (1994). Estimates of Duncan's Multiple Range Test were calculated using MSTAT-C (1991) software to test the significance of differences between means according to Waller and Duncan (1969).

RESULTS

Biometric measurements

Results showed significant differences ($p \leq 0.01$) in all biometric traits of barley plants subjected to salinity stress. Plant height and number of leaves/plant decreased significantly with both salinity concentrations (9.3 and 14 dS m^{-1}) compared with control plants, while shoot dry weight/plant showed a significant decrease only with the higher concentration of salinity (14 dS m^{-1}) (Table 2).

Data on treatments with different concentrations of AsA (100 and 300 ppm) and Put (100 and 200 ppm) revealed significant differences ($p \leq 0.01$) in all studied traits. Mean values of plant height, number of leaves/plant and shoot dry weight/plant clarified a significant increment in plants treated with both concentrations of AsA and Put at concentration 100 ppm compared with control plants, while mean value for number of leaves/plant was insignificant with that of control plants when plant were treated with Put at 200 ppm (Table 2). The highest increment in plant height and number of leaves/plant was noted for plants

Table 2: Biometric and yield traits of barley as affected by salinity and sprayed with different concentrations of ascorbic acid (AsA) or putrescine (Put) under two salinity concentrations (data are combined across 2019 and 2020 seasons)

Salinity (dS m^{-1})	Treatment	Plant height (cm)	Number of leaves/plant	Shoot dry weight (g)	Spike length (cm)	Number of spikes/ plant	Number of grains/ spike	Grain weight/ spike (g)	1000 Grain weight (g)
Control		55.6±1.3	14.0±0.4	0.9±0.1	14.0±0.4	2.9±0.2	28.1±1.5	1.1±0.1	36.2±1.1
9.3 dS m^{-1}		48.6±0.6	9.8±0.3	0.7±0.1	12.7±0.4	2.2±0.3	21.8±1.0	0.8±0.1	28.0±0.8
14 dS m^{-1}		41.8±1.1	9.0±0.3	0.4±0.1	10.4±0.5	1.7±0.2	17.0±0.9	0.6±0.03	21.2±1.5
$LSD_{0.05}$		2.19	0.63	0.19	0.89	0.66	1.7	0.12	3.36
	Control	44.7±1.9	10.1±0.6	0.5±0.1	11.5±0.5	1.7±0.3	19.3±1.9	0.7±0.1	25.0±1.5
	AsA 100 ppm	48.3±0.9	11.2±0.6	0.7±0.1	12.1±0.5	2.1±0.2	22.7±1.7	0.9±0.1	28.6±1.4
	AsA 300 ppm	50.7±1.3	12.3±0.3	0.7±0.1	12.8±0.5	2.3±0.2	24.3±1.7	0.9±0.1	31.0±1.0
	Put 100 ppm	50.3±1.4	11.1±0.4	0.7±0.1	12.6±0.5	2.4±0.5	22.2±1.4	0.9±0.1	29.5±2.0
	Put 200 ppm	49.4±0.9	9.9±0.4	0.8±0.1	12.8±0.7	2.7±0.4	23.0±0.6	0.9±0.1	28.1±1.5
$LSD_{0.05}$		2.18	0.98	0.13	0.72	0.49	2.72	0.13	2.72
0	Control	51.1±1.5	13.2±1.1	0.7±0.1	13.5±0.3	2.3±0.3	23.9±2.3	1.0±0.2	33.7±0.9
	AsA 100 ppm	52.2±1.8	15.2±1.1	0.9±0.02	13.4±0.3	2.7±0.1	28.7±3.1	1.1±0.1	36.2±1.2
	AsA 300 ppm	59.6±1.8	14.8±0.2	0.8±0.1	13.9±0.3	2.8±0.3	31.5±2.0	1.2±0.1	37.7±0.2
	Put 100 ppm	56.8±1.8	14.2±0.2	0.8±0.1	14.9±0.7	3.2±0.6	28.8±1.8	1.1±0.1	36.4±3.2
	Put 200 ppm	58.3±1.4	12.7±0.2	1.0±0.1	14.2±0.8	3.3±0.2	27.8±0.3	1.2±0.1	37.1±1.4
9.3 dS m^{-1}	Control	45.5±1.8	9.5±0.5	0.6±0.1	11.7±0.6	1.7±0.4	18.9±1.8	0.7±0.1	24.3±1.1
	AsA 100 ppm	49.4±0.2	9.2±0.4	0.7±0.1	12.5±0.3	2.2±0.2	23.1±0.6	0.8±0.1	28.4±0.8
	AsA 300 ppm	49.7±0.5	11.3±0.2	0.8±0.1	13.7±0.5	2.3±0.2	22.5±1.8	0.9±0.03	31.3±0.7
	Put 100 ppm	51.3±0.9	10.5±0.3	0.7±0.1	12.8±0.3	2.2±0.7	20.9±1.3	0.9±0.1	30.0±1.3
	Put 200 ppm	46.8±0.3	8.4±0.5	0.8±0.1	12.8±0.6	2.5±0.6	23.4±1.2	0.9±0.1	25.9±1.5
14 dS m^{-1}	Control	37.5±2.3	7.7±0.3	0.3±0.1	9.3±0.6	1.2±0.2	15.3±1.7	0.5±0.04	17.0±2.4
	AsA 100 ppm	43.1±0.7	9.2±0.2	0.4±0.1	10.3±0.9	1.5±0.3	16.2±1.3	0.6±0.01	21.3±2.1
	AsA 300 ppm	42.8±1.5	10.7±0.4	0.5±0.1	11.0±0.8	1.7±0.1	19.0±1.3	0.7±0.04	24.2±2.2
	Put 100 ppm	42.9±1.7	8.7±0.7	0.5±0.1	10.0±0.5	2.0±0.3	16.9±1.2	0.7±0.04	22.2±1.4
	Put 200 ppm	42.9±1.7	8.7±0.4	0.5±0.1	11.4±0.7	2.2±0.4	17.8±0.5	0.7±0.1	21.2±1.7
$LSD_{0.05}$		3.77	NS	NS	NS	NS	NS	NS	NS

Values are means ± SE (n= 9), LSD0.05 = Least significant difference at 0.05 level of probability, NS= Non significant at ≤ 0.05 and 0.01 levels of probability according to two-way ANOVA combined analysis.

treated with AsA at concentration 300 ppm while the maximum increase of shoot dry weight was recorded for plants treated with Put at 200 ppm.

Regarding the effect of AsA and Put on plants treated with salt (9.3 and 14 dS m^{-1}), data showed no significant differences among biometric traits except for plant height (Table 2). Application of AsA at 100 and 300 ppm and Put at 100 ppm induced a significant increase in plant height of salt treated plants with the highest increment exhibited by plants sprayed with Put at 100 ppm (Table 2).

Yield characters

Combined analysis of yield data over the two growing seasons showed significant differences ($p \leq 0.01$ and 0.05) in all studied traits in salt stressed plants. A significant decrease in all yield characters was recorded for plants treated with salt at concentrations of 9.3 and 14 dS m^{-1} in comparison with control plants with no significant differences between the two aforementioned salinity concentrations in number of spikes/plant (Table 2).

Regarding data for treatments with different concentrations of AsA and Put, significance differences ($p \leq 0.01$ and 0.05)

were noted for all yield characters in treated plants. Plants treated with AsA at 300 ppm and Put at 100 and 200 ppm exhibited a significant increment in all yield traits compared with control plants, with the maximum increase recorded for plants treated with AsA at 300 ppm (Table 2).

Results in (Table 2) showed no significant difference in yield characteristics of salt treated plants sprayed with AsA or Put at any given concentration.

Anatomical studies

Microscopic measurements of certain histological features were recorded in transverse sections through the median portion of the main stem and the corresponding leaf blade on the same portion in response to salinity concentrations at 9.3 and 14 dS m^{-1} in combination with either 300 ppm AsA or 100 ppm of Put (Tables 3 and 4, and Figs. 1 and 2).

Leaf structure

(Table 3 and Fig. 1) showed that salinity adversely affected leaf anatomical structure. A prominent decrease was noted in midrib and lamina thicknesses, length and width of vascular bundle, and meta-xylem vessel diameter with increasing salinity levels with the highest decrement recorded at 14 dS m^{-1} by 29.2, 40.7, 24.0, 28.3 and 29% for the previous characters, respectively in comparison with the control plants.

Treatment with AsA at 300 ppm induced a notable improvement in leaf histological features for salt stressed plants at 9.3 and 14 dS m^{-1} (Table 3 and Fig. 1). An increment of 7.1, 41.0, 30.4, 22.4 and 23.9% was recorded for midrib thickness, lamina thickness, length and width of vascular bundle, and meta-xylem vessel diameter, respectively in plants treated with AsA at 300 ppm and 14 dS m^{-1} salinity concentration compared with untreated plants under the same level of salinity. However, these values remain lower than those of the control plants.

Regarding the effect of Put on salt stressed plants, treatment at 100 ppm caused a slight enhancement in some leaf anatomical features in plants under 14 dS m^{-1} salinity concentration with increments of 4.4, 6.4, 7.1,14.2 and 9.2% for midrib and lamina thicknesses, length and width of vascular bundle, and meta-xylem vessel diameter, respectively(Table 3, Fig. 1). However, this increment remains less than that induced by AsA.

Stem structure

Histological features of barley stem exhibited a prominent decrement with increasing salinity concentrations up to 14 dS m^{-1} (Table 4 and Fig. 2). Stem diameter, stem wall

Table 3: Histological measurements of barley leaf as affected by salinity and sprayed with different concentrations of ascorbic acid (AsA) and putrescine (Put) under two salinity levels

	Histological characters (µm)						
	Control	9.3 dS m^{-1}	9.3 dS m^{-1} + AsA 300 ppm	9.3 dS m^{-1} + Put 100 ppm	14 dS m^{-1}	14 dS m^{-1} + AsA 300 ppm	14 dS m^{-1} + Put 100 ppm
Midrib thickness	461.2±15.7	374.0±1.4	396.4±8.8	385.4±9.9	326.6±16.2	349.9±14.6	340.9±10.1
Lamina thickness	202.8±12.4	163.4±16.9	184.1±21.5	169.0±11.4	120.2±5.1	169.5±15.8	127.9±10.3
Dimensions of vascular bundle							
Length	92.2±7.9	80.9±4.7	95.9±0.75	83.3±5.8	70.1 ±0.25	91.4 ±10.6	75.1±7.1
Width	123.6±6.7	107.5±2.7	106.8±3.4	102.6±2.1	88.6±8.1	108.5±18.1	101.2±0.85
Meta-xylem vessel diameter	25.3±7.3	24.5 ±5.2	18.5±2.9	22.4±2.6	18.0±2.3	22.3±0.45	19.6±1.9

Values in each column are means ±SE, n=3.

Table 4: Histological measurements of barley stem as affected by salinity and sprayed with different concentrations of ascorbic acid (AsA) and putrescine (Put) under two salinity levels

	Histological characters (µm)						
	Control	9.3 dS m^{-1}	9.3 dS m^{-1} + AsA 300 ppm	9.3 dS m^{-1} + Put 100 ppm	14 dS m^{-1}	14 dS m^{-1} + AsA 300 ppm	14 dS m^{-1} + Put 100 ppm
Stem diameter	3745±90	2315±55	3370±60	3530±50	1815±105	3380±40	3180±50
Stem wall thickness	360±15	320±45	343±5	347.5±12.5	300±20	350±15	310±20
Epidermis thickness	14.3±1.5	16.3±1.5	14.3±1.3	17.1±2.2	10.1±0.55	11.6±0.7	15.3±0.1
Hypodermis thickness	58.3±1.5	86±2.0	68.3±1.9	70±5.0	30.3±1.9	48.6±2.7	54.3±3.0
Dimensions of vascular bundle							
Length	125.6±1.5	143.8±2.9	113.5±1.0	108.9±2.8	115.7±2.9	131.2±2.4	118.5±2.2
Width	125.2±0.9	122.7±2.6	106.4±2.0	109.3±7.5	103.8±2.3	113.1±1.0	116.4±2.0
Xylem tissue thickness	72.2±3.5	82.9±1.2	71.1±2.4	64.35±2.0	69.5±3.2	74.8±1.1	74.3±2.0
Phloem tissue thickness	31.8±1.9	39.9±3.1	29.1±2.0	22.9±2.1	18.2±2.1	29.8±2.0	29. 5±2.0
Xylem vessel diameter	33.1±2.2	19.2±1.4	29.8±2.9	25.3±1.1	22.6±0.45	26.4±2.5	27.6±0.7
Pith diameter	3025±55	1650±90	2695±75	2850±60	1250±90	2630±40	2490±100

Values in each column are means ±SE, n=3.

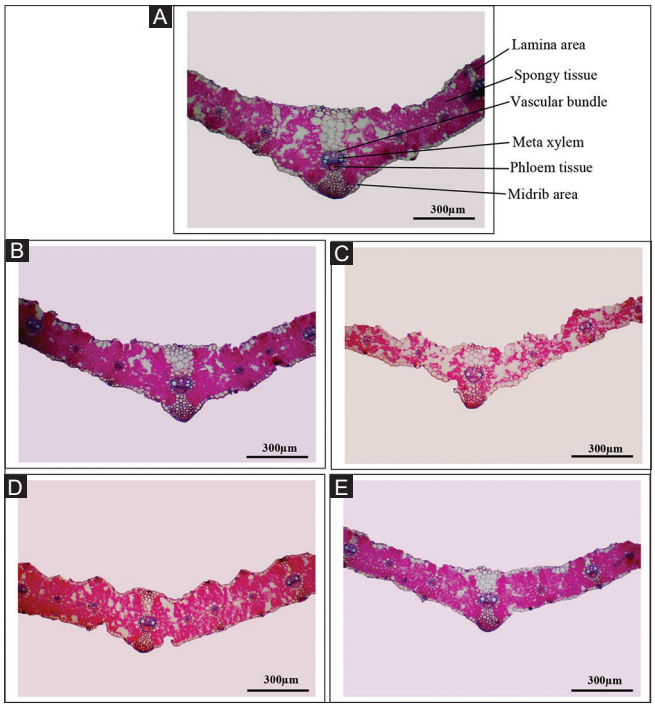

Fig 1. Anatomical structure of barley leaf as affected by salinity at 9.3 dS m^{-1} and 14 dS m^{-1}, and sprayed with either ascorbic acid (AsA) or putrescine (Put) at concentrations of 300 and 100 ppm, respectively under salinity level of 14 dS m^{-1}. A. Control, B. Salinity at 9.3 dS m^{-1}, C. Salinity at 14 dS m^{-1}, D. Salinity at 14 dS m-1; AsA at 300 ppm, E. salinity at 14 dS m^{-1}; Put at 100 ppm.

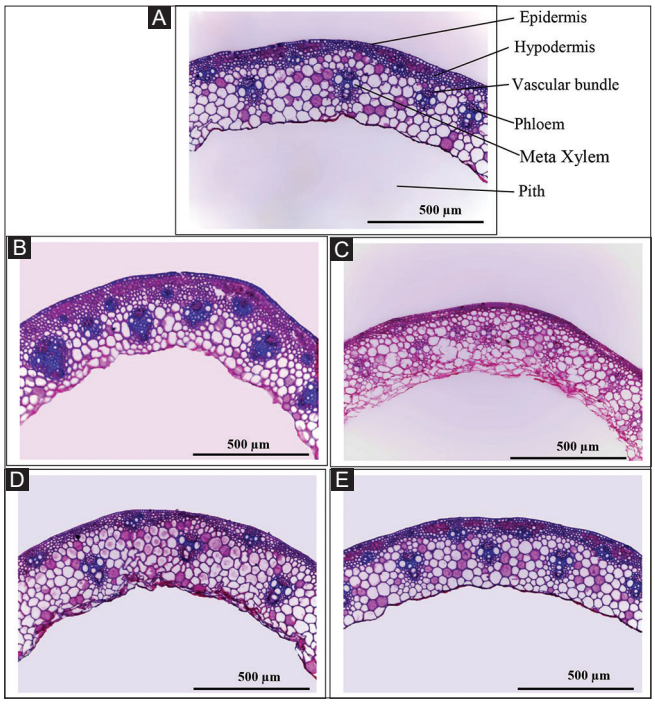

Fig 2. Anatomical structure of barley stem as affected by salinity at 9.3 dS m^{-1} and 14 dS m^{-1}, and sprayed with either ascorbic acid (AsA) or putrescine (Put) at concentrations of 300 and 100 ppm, respectively under salinity level of 14 dS m^{-1}. A. Control, B. Salinity at 9.3 dS m^{-1}, C. Salinity at 14 dS m^{-1}, D. Salinity at 14 dS m^{-1}; AsA at 300 ppm, E. salinity at 14 dS m^{-1}; Put at 100 ppm.

thickness and pith diameter decreased by 51.5, 16.7 and 58.7%, respectively in plants treated with salt at 14 dS m^{-1} compared with control plants. An increase in epidermis and hypodermis thicknesses was observed at 9.3 dS m^{-1} with increased looseness of hypodermal cells, while they decreased at 14 dS m^{-1} by 29.4 and 48%, respectively compared with control plants. Similarly, vascular bundle length, xylem and phloem thicknesses exhibited a slight increase at 9.3 dS m^{-1} then decreased by 7.9, 3.7, 42.6%, respectively at 14 dS m^{-1} less than control plants. Moreover, the highest decrement in xylem vessel diameter was noted in salt treated plants at 9.3 dS m^{-1}, with reduction of 42% less than control plants.

An improvement in most of stem microscopic features was noted in salt treated plants (9.3 and 14 dS m^{-1}) after the application of AsA at 300 ppm (Table 4, Fig. 2). The highest increment in stem diameter (86.2%), stem wall, epidermis, and hypodermis thicknesses (16.7, 14.8, and 60.4%), vascular bundle length and width (13.5 and 8.9%), xylem and phloem thicknesses (7.7 and 64%), xylem vessel and pith diameters (16.9 and 110.4%) was recorded for plants treated with salt at 14 dS m^{-1} and AsA at 300 ppm compared with those of plants treated with salt at 14 dS m^{-1} only.

Similarly, Put at 100 ppm enhanced most of stem histological features of salt stressed plants at 9.3 and 14 dS m^{-1} (Table 4, Fig. 2). Increments of 75.2, 3.3, 51.5, 79.2, 2.5, 12.1, 6.9, 62.9, 22.1 and 99.2% were observed for stem diameter, stem wall, epidermis, and hypodermis thicknesses, vascular bundle length and width, xylem and phloem thicknesses, xylem vessel and pith diameters, respectively for Put-sprayed salt stressed plants at 14 dS m^{-1} higher than those plants treated with salt at 14 dS m^{-1} only.

Biochemical constituents

Significant differences ($p \leq 0.05$) were observed in data of all studied biochemical constituents of barley in response to different treatments (Fig. 3). Both salinity concentrations induced significant decrements in photosynthetic pigments (chl a, b and total carotenoids) in leaves as well as crude protein in grains. Application of AsA at 100 and 300 ppm had the best effect on the aforementioned constituents compared with control with the maximum mean values obtained at 300 ppm, while, both concentrations of Put had no sound effect. In interaction with salinity, both concentrations of AsA significantly enhanced chl a, while Put was effective only at concentration of 100 ppm. Moreover, chl b and carotenoids exhibited a significant increase when plants were treated with either AsA or Put at 100 ppm under both salinity levels. In comparison with individual salinity concentrations, increments in crude protein were

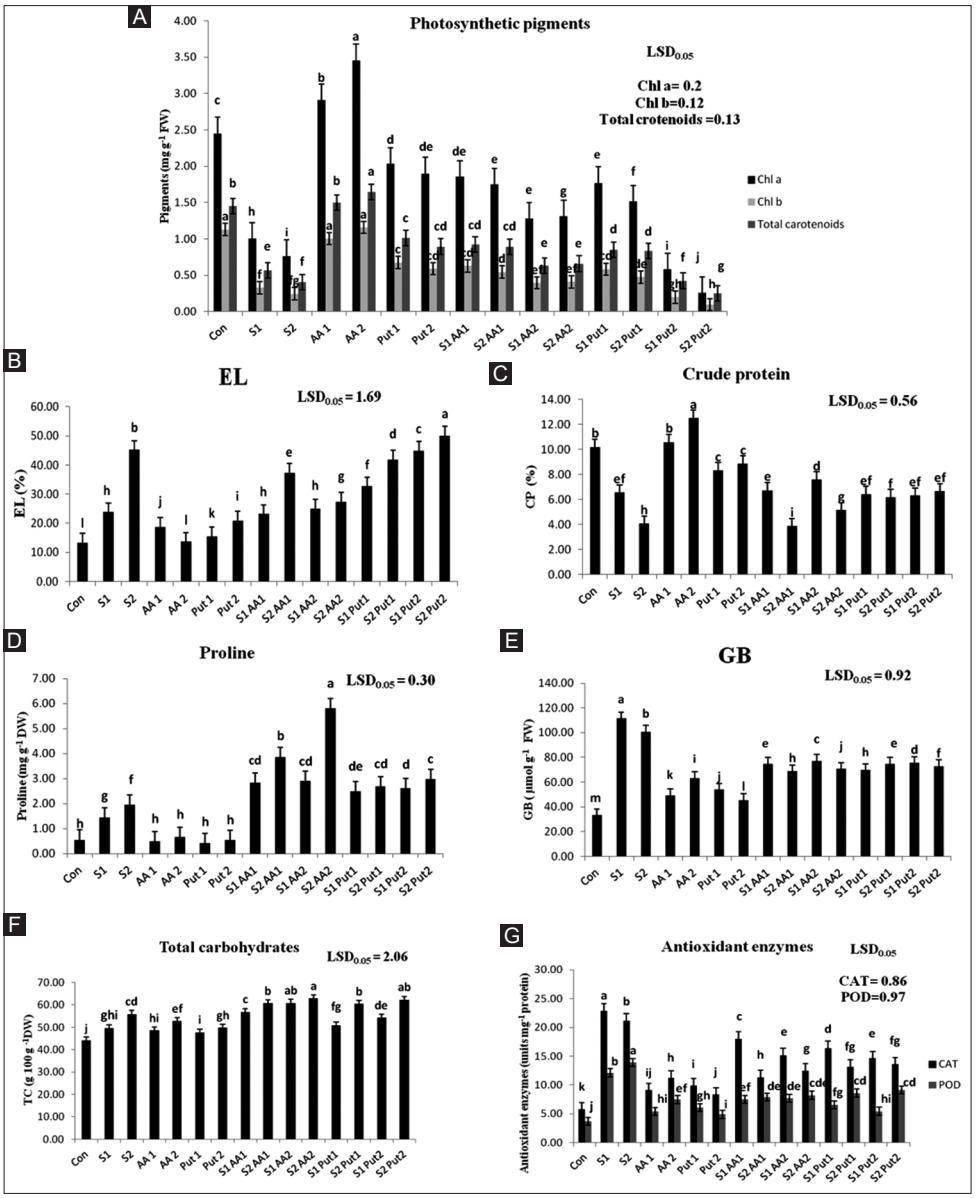

Fig 3. Biochemical constituents of barley as affected by salinity and sprayed with different concentrations of ascorbic acid (AsA) and putrescine (Put) under two salinity concentrations. A. Photosynthetic pigments, B. Electrolyte leakage (EL), C. Crude protein, D. Proline, E. Glycine betaine (GB), F. Total carbohydrates, G. Antioxidant enymes (Catalase; CAT, Peroxidase; POD). Con=control, S1= salinity at 9.3 dS m^{-1}, S2= salinity at 14 dS m^{-1}, AA1= AsA at 100 ppm, AA2= AsA at 300 ppm, Put 1= Put at 100 ppm, Put 2= Put at 200 ppm, S1AA1= salinity at 9.3 dS m^{-1}; AsA at 100 ppm, S1AA2= salinity at 9.3 dS m^{-1}; AsA at 300 ppm, S2AA1= salinity at 14 dS m^{-1}; AsA at 100 ppm, S2AA2= salinity at 14 dS m^{-1}; AsA at 300 ppm, S1 Put1= salinity at 9.3 dS m^{-1}; Put at 100 ppm, S1AA1= salinity at 9.3 dS m^{-1}; Put at 100 ppm, S1 Put 2= salinity at 9.3 dS m^{-1}; Put at 200 ppm, S2 Put1= salinity at 14 dS m^{-1}; Put at 100 ppm, S2 Put2= salinity at 14 dS m^{-1}; Put at 200 ppm. Means with different letters are significantly different ($P \leq 0.05$) according to the Duncan's multiple range test.

observed in grains of plants treated with 300 ppm AsA under both salinity levels and those treated with both concentrations of Put under salinity level of 14 dS m^{-1}. Despite the enhancing effect of AsA and Put on the studied constituents under both salinity levels, all mean values remain below those recorded for control.

On the other hand, significant increments were recorded for EL, proline, GB, total carbohydrates and antioxidant enzymes (CAT and POD) in leaves under both salinity levels. No sound changes were observed in EL and proline when plants were treated with both concentrations of AsA and Put in comparison with control, while slight increments were noted for the remaining constituents under the same conditions. Regarding salinity interaction, the aforementioned constituents exhibited different behaviors. EL decreased significantly in plants treated with both concentrations of AsA at salinity level of 14 dS m^{-1}

compared with those treated with 14 dS m^{-1} only, while Put at 100 ppm induced a significant decrement under the same salinity level; however, mean values remain higher than that of control. Significant increments in proline were noted in plants treated with both concentrations of AsA and Put under both salinity levels when compared to control and those treated with salinity only, with the maximum mean value recorded for treatment with AsA at 300 ppm under salinity level of 14 dS m^{-1}.

Mean values of total carbohydrates in leaves increased significantly with both concentrations of AsA and Put under the two salinity levels except for Put at 100 ppm with salinity at 9.3 dS m^{-1}. GB, CAT and POD showed same behavior, where, mean values significantly decreased in plants treated with both concentrations of AsA and Put under both salinity levels compared with salinity levels only; however, they remain higher than control value.

DISCUSSION

Barley is considered a salt tolerant cereal that could tolerate salinity concentrations up to 250 mM (~ 25 dS m^{-1}) NaCl (Munns et al., 2006). However, increasing salinity levels could sharply affect plant growth and performance due to its deleterious effect on cell division and elongation (Agami, 2014) attributed mainly to disturbance of cell homeostasis and osmotic potential by excessive salt (Desoky and Merwad, 2015; Pakar et al., 2016). A notable declination in biometric characters was reported in the current study which accords with the findings of several authors on salt stressed barley. For example, shoot dry matter of barley was found to decrease significantly in plants subjected to salinity at 13 dS m^{-1} while plant height was not affected (Endris and Mohammed, 2007). Moreover, salt concentration at 100 mM (10 dS m^{-1}) decreased shoot length, number of leaves, and biomass production of two different barley species (Degl'Innocenti et al., 2009). In addition, Pakar et al. (2016) reported that salinity concentration up to 15 dS m^{-1} significantly reduced plant height and dry weight of barley, while, El-Sharkawy et al. (2017) found a decrement in shoot dry weight of barley with salt treatments at 10 and 15 dS m^{-1} for two different genotypes (sensitive and tolerant). Many authors as well emphasized the role of AsA in enhancing plant growth and development due to its role in regulation of cell division (Desoky and Merwad, 2015). In that concern, foliar application of corn with AsA at 150 ppm increased stem and leaf dry weights and leaf fresh weight as reported by Dolatabadian et al. (2010). Moreover, Bakry et al. (2013) found that application of AsA at 300 ppm significantly increased plant height in wheat cultivars, while, Hussein and Alva (2014) reported a significant increment only in number of leaves of millet treated with AsA at 150 ppm. Similarly, Desoky and Merwad (2015) found that application of AsA at 0.1 and 0.2% induced significant increase in plant height and dry weight of shoots in wheat. In a similar manner, Put was found to be an important growth regulator; Hassanein et al. (2013) indicated a significant increase in shoot fresh and dry weights of wheat when sprayed with Put at 2.5 mM. Moreover, Magda et al. (2014) found that application of Put at levels up to 100 ppm induced a significant increment in plant height, number and dry weight of both leaves and tillers in barley. Also, Hassan and Bano (2016) stated that foliar spray of wheat with Put at 0.24 g/l caused a significant increase in plant height. The previous records on AsA and Put effect on plant vegetative performance generally accords with the results of the current investigation. Studying the combined effect of either AsA or Put and salinity, this study reported a significant increment only in plant height while other parameters were not improved. In this concern, many authors recorded improvement in some characters but not the others; Hussein and Alva (2014) found that application of AsA at 150 ppm in salt stressed millet plants caused no significance difference in morphological traits except for leaf area and stem dry weight. Additionally, Agami (2014) indicated that pre-soaking of barley seeds in 1 mM AsA before salt treatment at 100 and 200 mM NaCl had favorable effects on shoot length but had no effect on leaf number. Shoot length of turnip was significantly improved in plants treated with NaCl (50 and 100 mM) and AsA (50 and 100 mM) (Mittal et al., 2018). In a similar manner, application of Put at 2 ppm caused a significant increase in plant height of cotton plants treated with a salt mixture at 9000 ppm, while no significant difference was noted for leaf number (Ahmed et al., 2013).

Consequently, increasing salinity levels could as well adversely affect yield production in cereal crops in general with barley being among the most tolerant cereals; however, salinity could also induce reproductive disorders in barley which would in turn affect yield production (Munns et al., 2006, Bybordi, 2010). Such disorders could be attributed to the disturbance of photosynthetic apparatus which in turn affects carbohydrate metabolism and production of assimilates (Ola et al., 2012, Pakar et al., 2016). The current study communicates a significant decrement in yield parameters of barley in response to salinity stress which agrees with the significant decrease in number of tillers, number and weight of kernels/spike of barley reported by Endris and Mohammed (2007). In a similar manner, grain number and yield, and harvest index for barley decreased significantly with increasing salinity levels up to 15 dS m^{-1} (Pakar et al., 2016). Grain yield was also reduced significantly in different genotypes of barley subjected to salinity at 10 and 18 dS m^{-1} (Mahlooji et al., 2018). Similar to the findings of this study, reporting the

positive effects of AsA on yield parameters, a significant increase was recorded in grain weight (Dolatabadian et al., 2010), seed index and seed yield (Darvishan et al., 2013) of corn at 150 ppm. Moreover, Bakry et al. (2013) reported a significant increase in all yield characters of wheat cultivars with the application of AsA at 300 ppm, while, Desoky and Merwad (2015) found that AsA at 0.2% caused a significant increase only in grain yield/plant and seed index of wheat. Similarly, foliar spraying with Put at 2.5 mM was effective in increasing number of spikes/plant, weight of grains/plant and seed index of wheat (Hassanein et al., 2013). Additionally, Application of Put up to 100 ppm caused a significant increment in number and dry weight of spikes/plant, grain yield/plant in barley (Magda et al., 2014). Furthermore, Hassan and Bano (2016) found that grain number and seed index of wheat in a pot experiment increased significantly with the foliar spraying of Put at 0.24 g/l. In accordance with this study, Mohsen et al. (2013) reported that pre-soaking of faba bean seeds in 50 ppm AsA had no significant effect on yield parameters for salt stressed plants (150 mM NaCl), while, Ahmed et al. (2013) found that application of Put up to 2 ppm to cotton plants treated with a salt mixture at 9000 ppm was not significant for all yield characteristics except for seed index.

Besides affecting the morphological traits of barley, a clear declination in anatomical features of leaf and stem was observed with increasing salinity concentrations. The prominent decrement in leaf lamina thickness with increasing levels of salinity indicates a corresponding decrease in mesophyll tissue which could be attributed to the decrease in translocation of nutrients and assimilates caused by salinity which in turn limits cell division and expansion (Ola et al., 2012; Atabayeva et al., 2013). Moreover, the decrease in vascular bundle area could help in preserving water content in leaf through the reduction of transpiration rate and consequently preserving the photosynthetic apparatus (Abd Elbar et al., 2019). Additionally, the decrement in vessel diameter caused by abiotic stress could decrease water translocation on one hand but on the other hand could help protecting the water column from embolism (Abd Elbar et al., 2019). In accordance with this study, a gradual decrement in midvein, lamina and mesophyll thicknesses as well as vascular bundle and xylem vessel diameters was recorded under different salinity (NaCl) levels as reported by Ola et al. (2012) on kallar grass, Atabayeva et al. (2013) and Agami (2014) on barley. AsA was found to play an important role in cell division and enlargement in addition to counteracting the inhibitory oxidative stress through enhancement of antioxidant enzymes (Agami, 2014). In this concern, application of AsA was reported by Agami (2014) and Desoky and Merwad (2015) to enhance leaf histological features of salt stressed barley and wheat plants which is in general agreement with the findings of this study. As an important polyamine, Put is important for plant growth processes including cell division and expansion, differentiation of vascular tissues, and shoot development (Ola et al., 2012). Badawy et al. (2015) illustrated the positive effects of Put on *Antirrhinum majus* leaves causing an increment in spongy tissue, midrib, and xylem and phloem thicknesses. Similarly, Yuan et al. (2015) elaborated the role of Put as an important polyamine acting as active oxygen scavenger, reducing accumulation of Na and Cl ions and protecting cell membrane, however, they did not report sound changes in the improvement of leaf histological features subjected to salinity except for improved tightness of mesophyll tissue.

Similarly in stem anatomy, increasing salinity levels could decrease plant ability to absorb water which adversely affects cell division and expansion and in turn growth rate leading to drastic reduction in stem diameter and related histological features (Ola et al., 2012). In *Cynodon dactylon*, stem epidermal cell area and hypodermal sclerenchyma increased prominently with increasing salinity levels in the salt range type while in normal (non-saline) type, they increased to a certain extent with increasing salinity levels, then declined drastically (Hameed et al., 2010) which accords with the results of this study reporting an increase in epidermal and hypodermal thicknesses at salinity concentration of 9.3 dS m^{-1} followed by a clear declination at 14 dS m^{-1}. The aforementioned increment in stem epidermis and sclerenchyma is considered an important adaptive mechanism to decrease water loss at higher salinity levels and also provide rigidity to the stem (Hameed et al., 2010; Ola et al., 2012; Younis et al., 2014; Parida et al., 2016). In accordance with this research, Hameed et al. (2010) reported an increase in vascular bundle area (and corresponding xylem and phloem areas) at lower salt levels followed by a declination in the later features with increasing salinity levels in aforementioned ecotypes of *Cynodon dactylon*. In addition, Dolatabadian et al. (2011) indicated a prominent increment in vascular cell tissue thickness of soybean stem with increasing salinity accompanied by increase in xylem vessel diameter. This later increase could be attributed to increase in vessel lignification induced by salt stress which is also considered an adaptive mechanism. Moreover, this lignification could result in slower flow rate of water which serves in water transport after clogging of larger vessels (Abd Elbar et al., 2019). On the other hand, a prominent decrement in vascular area was found by Ola et al. (2012) and Younis et al. (2014). Finally, El-Rodeny and El-Okkiah (2012) indicated poor development of vascular cylinder with no changes in xylem or phloem thickness but significant decrement in metaxylem vessel diameter while Parida et al. (2016) confirmed a steady water flow and better water translocation as no changes

occurred in xylem vessel diameter with higher salt levels. Improvement in histological features of barley stem was recorded with the application of either AsA or Put to salt stressed plants. In this concern, Abou-Leila et al. (2012) reported increments in number of xylem rows as well as number of vessels/bundle in stems of salt stressed Jatropha plants after the application of AsA. Similarly, El-Afry et al. (2018) found an improvement in all histological aspects of flax stem at 8 dS m^{-1} sprayed with 400 ppm AsA. According to Badawy et al. (2015), application of Put at 200 ppm had a favorable effect on xylem, phloem and pith thicknesses of *Antirrhinum majus* stem. Under stress conditions, Put at 0.2 mM improved stem, xylem and phloem areas of water stressed thyme plants (Abd Elbar et al., 2019) while Put did not show any significant effect in stem anatomy of salt stressed radish seedlings (Çavuşoğlu et al., 2008).

Salinity also negatively affects plant cell structure and its biochemical constituents. In response, plants exhibit different tolerance mechanism to counteract these effects. Photosynthetic pigments are the main products of chloroplast in plant cells (Sadak et al., 2013). Salinity majorly contributes to the loss of chloroplast membrane and instability of its structure (Sadak et al., 2013; Mahlooji et al., 2018). In this concern, photosynthetic efficiency declines with salinity stress as a result of pigment photo-oxidation and accumulation of ROS in chloroplasts (Shu et al., 2015; Mahlooji et al., 2018). Consequently, ROS will lead to chloroplast breakdown, either through inhibition of chlorophyll biosynthesis or activation of chlorophyllase enzyme which catalyzes chlorophyll degradation (Sadak et al., 2013; Hassan and Bano, 2016; Zhong et al., 2016; Abd Elbar et al., 2019; Farooq et al., 2020). In consistency with the findings of this study, many author reported the negative effects of salinity on photosynthetic pigments in leaves of some cereal crops (Sadak et al., 2013; Agami, 2014, Moharramnejad et al., 2015; Abdi et al., 2016; Hassan and Bano, 2016; Mahlooji et al., 2018). As an antioxidant, AsA has a direct role in scavenging and detoxifying ROS (EL-Afry et al., 2018; Farooq et al., 2020), in addition to its role in enhancing photosynthetic efficiency and inhibiting activity of chlorophyllase (Mittal et al., 2018). Moreover, Put plays an important role in stabilizing membranes and thylakoid structure (decreases lipid peroxidation) in addition to its ROS scavenging effect (Shu et al., 2015; Hassan and Bano, 2016). Regarding this, in accordance with this study, exogenous application of AsA and Put was found to have enhancing effects on photosynthetic pigments in addition to alleviating adverse effects of salinity as reported by Sadak et al. (2013) on wheat, Agami (2014) on barley, Shu et al. (2015) on cucumber, Hassan and Bano (2016) on wheat, EL-Afry et al. (2018) on flax, Gul et al. (2018) on maize, Mittal et al. (2018) on turnip, Gerami et al. (2019) on stevia, Yuan et al. (2018) and Wu et al. (2019) on cucumber and Ghalati et al. (2020) on guava.

EL is a good indicator of cell membrane integrity (Mahlooji et al., 2018; Ghalati et al., 2020). Accumulation of ROS induced by salinity affects osmotic potential and leads to the loss of K^+ from cells destabilizing the structure of cell membrane and increasing the rate of electrolyte leakage (EL-Sharkawy et al., 2017; Mahlooji et al., 2018). In accordance with the previous, this study reports an increase in electrolyte leakage with increasing salinity levels. While Put directly affects the stability of cell membrane and counteracts salinity effect, AsA acts indirectly in reducing oxidative damage by ROS, hence, reducing lipid peroxidation of plasma membrane (Shalata and Neumann, 2001; Ghalati et al., 2020). In this study, AsA was effective in reducing EL with high salt concentration (14 dS m^{-1}) which is consistent with the findings of Agami (2014) on barley. Similarly, Put at 100 ppm was effective at the higher salinity level, according with Ghalati et al. (2020) on guava where Put at 500 ppm was effective in reducing EL at salinity level of 10 dS m^{-1}.

Proteins are important constituents of many membrane structures and are involved in many biological processes of plant cell including ion and metabolite transport (Komatsu et al., 2007). Under saline conditions, decrements in protein content of plant cells could be attributed to the declination in protein biosynthesis as well as degradation of proteins due to activity of proteases (Haddadi et al., 2016; Mittal et al., 2018). In consistency with the previous conclusion, the results of this study reported a highly significant decrement in crude protein content. Similar decrements were reported by Rahdari and Hoseini (2013) on wheat, Haddadi et al. (2016) on *Mentha aquatica*, Gul et al. (2018) on maize, and Mittal et al. (2018) on turnip. Alone or under stress, AsA could increase protein content as it enhances the expression of new proteins (Mittal et al., 2018). Similarly, improved protein content was reported after the exogenous application of Put due to its direct role in protein synthesis and stabilizing membranes (Hanafy Ahmed et al., 2010). In accordance, enhancement in protein content was found in this study either with the application of AsA or Put alone or under saline conditions. Similar increments in protein content were concluded by Shummu et al. (2012) on tomato, Rahdari and Hoseini (2013), Sadak et al. (2013) and Hassan and Bano (2016) on wheat and Mittal et al. (2018) on turnip.

Decrease in protein content was reported to be accompanied by increase in free amino acids (Hanafy Ahmed et al., 2010; Abd Elhamid et al., 2014; El-Bassiouny and Sadak, 2015; Mohamed et al., 2018). Proline is the most abundant amino acid associated with salt stress; playing an important role in osmo-regulation, scavenging ROS, stabilizing

membrane structures and integrity, and protecting protein configurations (Agami, 2014; Abd Elhamid et al., 2014; Abdi et al., 2016; Haddadi et al., 2016; Parida et al., 2016; Mohamed et al., 2018). Furthermore, the previous authors reported increment in proline level with increasing salinity concentration which is consistent with the results of this study. AsA is important for the biosyntehsis of proline (Desoky and Merwad, 2015; El-Bassiouny and Sadak, 2015), while, Put and proline metabolic pathways are interconnected in response to salinity stress (Ghalati et al., 2020). Increments in proline associated with the application of either AsA or Put under saline conditions was found in this study, in agreement with the findings of Abd Elhamid et al. (2014), Abbasi and Faghani (2015), Desoky and Merwad (2015) and Hassan and Bano (2016) on wheat, Ahmed et al. (2013) on cotton, Agami (2014) on barley and EL-Afry et al. (2018) on flax.

Glycine Betaine (GB) is a zwitterionic, quaternary amine which is associated with abiotic stress, specifically in poaceae members such as maize and barley (Sairam and Tyagi, 2004; Giri, 2011; Ahmad et al., 2013; Annunziata et al., 2019). As an osmoprotectant, GB acts majorly in osmotic regulation and protection of macromolecule structures in addition to its effect in scavenging ROS (Giri, 2011; Moharramnejad et al., 2015; Mogazy et al., 2020). Moreover, GB could indirectly reverse impairment of photosystem II and enhance chlorophyll components under salinity (Giri, 2011; EL-Sharkawy et al., 2017). In this study, highly significant increase was notable under both salinity levels which agrees with the findings of Abbasi and Faghani (2015) on wheat, Estaji et al. (2019) on cucumber and Hadia et al. (2020) on wheat. Due to their interconnected effects in scavenging ROS and preserving sub-cellular structures under salinity stress, AsA and Put are suggested to enhance GB content and add to its effect. In this study, level of GB increased in plants treated with either AsA or Put alone in comparison with control, while it decreased in combinations of AsA or Put with salinity in comparison with salinity alone, nevertheless, still higher than that of control. This decrement could suggest a reversible effect to the harm induced by salinity and better tolerance of plants. In this concern, increments in GB with the application of AsA alone or in combination with salinity or water stress were reported by Abbasi and Faghani (2015) on wheat and Farooq et al. (2020) on safflower, while Ebeed et al. (2017) found that application of Put enhanced accumulation of GB in water stressed wheat.

Carbohydrates play an important role in osmotic adjustment (Sadak et al., 2013; El-Bassiouny and Sadak, 2015; Abdi et al., 2016; Zhong et al., 2016). Moreover, they act as osmoprotectants and free radical scavengers alleviating the adverse effects of salinity stress (Agami, 2014; Mohamed et al., 2018). Besides, salinity impairs carbohydrate metabolism and translocation which results in accumulation of starch and sugars (Zhong et al., 2016). This study communicates an increment in total carbohydrates content under salinity stress which agrees with results found by El-Bassiouny and Sadak (2015), Hassan and Bano (2016), Zhong et al. (2016) and Mohamed et al. (2018). AsA enhances carbohydrate biosynthesis and increase endogenous one (Sadak et al., 2013; El-Bassiouny and Sadak, 2015), while, Put improves carbohydrate metabolism and translocation (Zhong et al., 2016; Gul et al., 2018; Yuan et al., 2018). In this study, application of either AsA or Put alone or under salinity increased total carbohydrates which accords with the findings of Sadak et al. (2013) on wheat and Agami (2014) on barley.

Antioxidant enzymes (*e.g.* CAT and POD) in plant cell are important adaptive mechanisms to alleviate salinity stress (Haddadi et al., 2016; EL-Sharkawy et al., 2017). Catalase (CAT) and peroxidase (POD) are free radical scavengers counteracting the negative effects of oxidative damage induced by salinity (Abd Elhamid et al., 2014; EL-Afry et al., 2018). In this concern, in the current study, levels of CAT and POD significantly increased in response to salinity, which is in general agreement with Hassan and Bano (2016), EL-Afry et al. (2018), Gul et al. (2018), Gerami et al. (2019). As mentioned previously, AsA and Put are major ROS scavengers which add to the effect of antioxidant enzymes. In this study, either AsA or Put in combination with salinity reduced the content of CAT and POD in comparison with salinity alone which suggests an improvement against the deleterious effect of ROS (El-Bassiouny and Sadak, 2015; EL-Afry et al., 2018; Gul et al., 2018; Ghalati et al., 2020). In contrast, other authors reported increments in CAT and POD with the application of AsA in reponse to salinity (Shummu et al., 2012; Agami, 2014; Hassan and Bano, 2016; Gerami et al., 2019).

CONCLUSION

Salinity stress induced hazardous changes in barley vegetative, yield, anatomical, and biochemical characteristics. In this study, salinity up to 14 dS m^{-1} negatively affected vegetative and yield traits. Foliar application of either AsA or Put alone or in combination with salinity resulted in various plant responses. The maximum increments in vegetative traits were exhibited by plants treated with AsA at 300 ppm and Put at 100 ppm, while yield characters were enhanced at AsA 300 ppm and both concentrations of Put. Similarly, salinity caused drastic reduction in anatomical features of barley, while, notable improvement was clear in transverse sections of stems and leaves of plants treated with AsA at 300 ppm and Put at 100 ppm, with better

results achieved at 300 ppm AsA. On the biochemical level, salinity significantly reduced photosynthetic pigments and crude protein contents, while it increased EL, proline, GB, total carbohydrates and antioxidant enzymes. Sound improvements were clear in the biochemical constituents with the application of either AsA or Put alone or in combination with salinity. In general, AsA and Put proved good potential for alleviating salt stress in barley.

Authors' contributions

Engy A. Seleem: Research hypothesis, Methodology and experimental procedures, Data collection and analysis, Result interpretation, Manuscript writing.

Hend Mohammad Saad Ibrahim: Research hypothesis, Methodology and experimental procedures, Data collection and analysis, Result interpretation, Manuscript writing.

Zeinab K. Taha: Research hypothesis, Methodology and experimental procedures, Data collection and analysis, Result interpretation, Manuscript writing.

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