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ORIGINAL ARTICLE

Plant growth regulators ameliorate the ill effect of salt stress through improved growth, photosynthesis, antioxidant system, yield and quality attributes in *Mentha piperita* L.

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Abstract
The roles of plant growth regulators (PGRs) in plants are well documented. However, there is a little information regarding their roles in alleviating salt stress in plants, particularly peppermint. This necessitated the evaluation of the efficiency of three selected PGRs in counteracting the ill effect of salt stress by conducting a pot experiment on peppermint (Lamiaceae). Three uniform size suckers were transplanted in each pot containing proper nutrients. Thirty day old plants were subjected to 4 levels of salinity, viz. 0, 50, 100 or 150 mM NaCl. Salt stress was given at 30 days after their transplantation (DAT). Plants were sprayed twice, i.e., at 60 and 75 DAT with 10^{-6} M each of gibberellic acid (GA_3), salicylic acid (SA) or triacontanol (Tria). The sampling was made at 100 DAT and harvesting at 120 DAT. The graded levels of salinity decreased growth, photosynthesis, carbonic anhydrase (CA) activity, NPK content, peltate glandular trichome (PGT) density, essential oil (EO) and menthol content and herb, EO and menthol yield, but increased catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) activities and proline content linearly. Spray of PGRs particularly SA improved all parameters under both salt and salt free conditions. The maximum values for yields of herb, EO and menthol were noted with 0 mM NaCl × SA. However, antioxidants, proline content, PGT density and EO content were found to be maximum with 150 mM NaCl × SA.

Keywords Salt stress · PGRs · SA · Growth · Photosynthesis · Menthol

Introduction

Mentha piperita L. (peppermint) is an important medicinal and aromatic plant (MAP) belonging to the family Lamiaceae. Its oil and leaves are used in traditional medicines and pharmaceutical products as also flavouring agent. Peppermint oil due to presence of terpenoids like menthol, menthone, menthyl acetate, menthofuran, isomenthone, pulegone and limonene has therapeutic, antifungal, anticholinestearic, antimicrobial and anti-oxidant activities (Lawrence 2006; Santoro et al. 2013; Barros et al. 2015). The previous studies revealed that salinity influenced osmotic and ionic imbalance in plants adversely leading to poor growth, yield and secondary metabolite products like essential oil and its components (Aziz et al. 2008; Khorasaninejad et al. 2010; Olfa et al. 2016). Ill effects of salinity may be overcome to a great extent by evolution of salt resistant varieties, balanced manuring and judicious fertilizer and PGRs application (Ondrasek et al. 2011; Jayakannan et al. 2015). Of these factors, PGR application is of quite importance. Gibberellins are natural diterpenes that are significant for their role particularly in seed germination, vegetative growth, bolting and induction of flowering, or fruit development (Sun and Gubler 2004). The hormone may induce or increase the endogenous levels of SA under abiotic stress conditions (Alonso-Ramírez et al. 2009). SA is an endogenous signalling molecule and is involved in stress tolerance of plants by regulating physiological and biochemical processes including photosynthesis and antioxidant metabolism (Gunes et al. 2007; Horvath et al. 2007; Nazar et al. 2011; Palma et al. 2013). It helps in tolerating plant salinity stress by altering nutrient uptake, membrane functioning, water relations, inhibition of ethylene biosynthesis and lipid peroxidation (Raskin 1992; Aldesuquy et al. 1998; Rajasekaran and Blake 1999; Srivastava and Dwivedi 2000). Tria is a saturated

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primary alcohol and has plant growth promoting properties (Ries et al. 1977). Tria regulates of photosynthesis, water and mineral nutrient uptake by influencing the enzymes which regulate growth and metabolic processes in plants (Chen et al. 2002; Naeem et al. 2011). Tria can also up-regulate crop growth under stressful environments (Muthuchelian et al. 1996; Radhakrishnan and Kumari 2008). Keeping the importance of such hormones in view, the present study was undertaken with the hypothesis whether application of the three selected PGRs at crucial stage of growth can counteract the negative effect of salinity in peppermint. The present investigation may be helpful in making a normal crop to the moderate tolerant one.

Materials and methods

Plant material, PGRs and salt treatment

This pot experiment was conducted on peppermint during summer season in a net house of the Department of Botany, Aligarh Muslim University, Aligarh (27°52′N latitude, 78°51′E longitude, 187.45 m altitude). During this experimental period, there were a 12 h photoperiod of natural daylight, maximum and minimum temperature of 37.5 and 15 °C, respectively and relative humidity of 59.5% on average, with the total rainfall being 125.00 mm. Prior to transplantation, soil samples were randomly collected from each pot filled with a homogenous mixture of 4 kg soil and 1 kg organic manure (4:1) for their analysis. Physio-chemical characteristics of the experimental soil were, texture-sandy loam, pH (1:2) 7.6, E.C. (1:2) 0.58 dS m^{-1}, available N, P and K 98.5, 78.5 and 148.1 mg kg^{-1} soil, respectively. The soil was maintained at sufficient moisture to ensure optimum growth of plants. A recommended basal dose of N, P and K (N_{80} P_{40} K_{40}) kg ha^{-1} (Singh et al. 2012), i.e., 36 mg N + 17.9 mg P_2O_5 and 17.9 mg K_2O kg^{-1} soil (Gupta 2004) was applied in the form of urea, single superphosphate and muriate of potash, respectively at the time of transplanting. Healthy peppermint suckers of uniform size and age obtained from the Central Institute of Medicinal and Aromatic Plants, Lucknow (India) were transplanted at the rate of 3 suckers pot^{-1}. Plants were subjected to four graded levels of salt stress (0, 50, 100 or 150 mM NaCl) at 30 days after transplanting (DAT). Two sprays of dilute solution (10^{-6} M) each of the three PGR's (GA_3, SA or Tria) were applied to the foliage of plants using a hand sprayer at the interval of 15d with the first spray giving at 60 DAT. The control plants were sprayed with the double distilled water (DDW). The experiment was conducted according to a factorial randomized design. In all, there were 16 treatments. Each treatment was replicated five times. The standard cultural practices were adopted for growing the crop. The performance of the crop in respect of growth, physio-biochemical, yield and quality-parameters were studied at 100 DAT. Harvesting of the crop was done at 120 DAT.

Determination of growth parameters

Shoot and root length of the plants were determined in cm and fresh weight of the plants in g. After drying at 80 °C for 48 h in an oven, dry weights of per plant were obtained in g. After counting leaves, leaf area of the upper third fully expanded leaf of each plant (cm^2) was determined by a leaf area meter (ADC Bioscientific, Hoddesdon, Herts, UK).

Determination of physiological and biochemical parameters

SPAD chlorophyll

Estimation of leaf chlorophyll was accomplished with the help of the SPAD-502 (KMS Inc. Japan).

Leaf gas exchange parameters

Gas exchange parameters were determined using an infrared gas. Net photosynthetic rate (P_N) and its related attributes [stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E)] were evaluated by a portable system (LI-COR 6400, LICOR, Lincoln, NE, USA). The conditions as temperature, RH, CO_2 concentration, and photon flux density in the leaf chamber of the system were at 25 °C, 85%, 600 µmol mol^{-1}, and 800 µmol m^{-2} s^{-1}, respectively.

Carbonic anhydrase activity

Leaf carbonic anhydrase (CA) activity in the leaves was measured following the method described by Dwivedi and Randhawa (1974). Leaves (200 mg) were cut into small pieces in 10 mL cysteine hydrochloride solution (0.2 M). These leaf samples were blotted and transferred in a test tube, followed by the addition of 4 mL phosphate buffer (pH 6.8), 4 mL $NaHCO_3$ (0.2 M in 0.02 M NaOH), 0.2 mL bromothymol blue (0.002%). This reaction was titrated against 0.5N HCl using methyl red as an indicator. The activity of the enzyme was expressed as mM CO_2 kg^{-1} (FM) s^{-1}.

Catalase, peroxidase, and superoxide dismutase activities

Leaf enzyme extract

For the estimation of activities of antioxidant enzyme, leaf extract was prepared by homogenizing 2 g ice cold leaves in a mixture of 6 mL phosphate buffer of pH 7.0 (50 mM),

1 mL ethylenediaminetetraacetic acid (1 mM), 1 mL phenylmethanesulfonylflouride (1 mM), 1 mL Triton X-100 (0.5% v/v) and 1 mL polyvinyl pyrollidone (2% w/v) in a pre-chilled mortar and pestle. The homogenates were centrifuged at 12,000×*g* for 20 min at 4 °C. The supernatant was used for determination of enzyme activities.

Catalase activity

The catalase activity was assayed by measuring the initial rate of H_2O_2 disappearance using the method of Aebi (1984). Leaf enzyme extract at 0.1 mL was mixed with 3 mL H_2O_2 (30 mM accomplished by diluting 0.34 mL 30% H_2O_2 to 100 mL with the phosphate buffer of pH 7). The decomposition of H_2O_2 was followed by decline in optical density (OD) at 240 nm. The absorbance was noted with the help of a spectrophotometer (Spectronic UV-1700 Shimadzu, Japan) at the room temperature (= 20 °C) with the interval of 30 s using a blank containing buffer solution instead of H_2O_2 along with the enzyme extract. The activity of the enzyme was expressed as μM H_2O_2 (decomposed) kg^{-1} FM s^{-1}.

Peroxidase activity

The activity of peroxidase was determined by the method of Chance and Maehly (1955). The reaction mixture in a total volume of 3 mL was prepared by taking 2.10 mL DDW, 0.32 mL phosphate buffer of pH 6.0 (100 mM), 0.16 mL H_2O_2 (0.5%), 0.32 mL pyrogallol solution (5% w/v) and 0.1 mL leaf enzyme extract. The absorbance of the reaction mixture was read at the interval of 20 s at 420 nm with the help of the spectrophotometer using a blank. The assaying period was approximately 3 min and the time interval between two successive readings was 20 s and the temperature was 20 °C. the blank was prepared using the buffer solution instead of the leaf enzyme extract, along with the other ingredients of the mixture. The activity of the enzyme was expressed as μM pyrogallol (oxidised) kg^{-1} FM s^{-1}.

Superoxide dismutase activity

The activity of the enzyme was determined by the method of Kono (1978). The reaction mixture in a total volume of 3 mL was prepared by taking 1.2 mL sodium bicarbonate of pH 10.2 (50 mM), 0.3 mL NBT (24 μM), 0.2 mL $EDTA^{-Fe++}$ (0.1 mM) and 0.1 mL leaf enzyme extract. Just after the addition the enzyme extract, the absorbance was recorded for 2 min with the help of the spectrophotometer at 560 nm using a blank containing buffer solution instead of the leaf enzyme extract along with the above solutions. Using the unit of the superoxide dismutase (the amount of the enzyme required for causing 50% reduction of NBT at 25 °C) the activity of the enzyme was expressed as mM NBT (reduced) kg^{-1} FM s^{-1}.

Proline content in leaves

The proline content was measured in fresh leaves following the method of Bates et al. (1973). Leaves at 0.5 g were homogenized with the help of a pestle and mortar with 10 mL 3% sulfosalicylic acid. The homogenate was filtered through Whatman No. 2 filter paper and the filtrate was collected into a test tube. The final volume of the filtrate was made to 10 mL by adding 3% aqueous sulfosalicylic acid (if required). From this extract filtrate, 2 mL were mixed with glacial acetic acid and acid ninhydrin each at 2 mL in a test tube. This mixture was heated on a boiling water bath for 1 h. The reaction was terminated by transferring the test tube to ice bath followed by the addition of 4 mL toluene with vigorous shaking for 15–20 s. The chromophore containing toluene was aspirated from the aqueous phase and was warmed to room temperature. The absorbance of the red colour was read at 520 nm against a toulene blank on the spectrophotometer. The amount of proline in the sample was calculated using a standard curve prepared from pure proline ranging from 0.1 to 36 μmol and expressed on fresh mass basis of the sample. The proline content is expressed as μmol g^{-1} FM.

Leaf nitrogen, phosphorus and potassium contents

The oven-dried leaf powder at 100 mg was transferred into a 50 mL Kjeldahl flask followed by the addition of 2 mL analytical reagent grade concentrated sulphuric acid. After heating the contents for about 2 h followed by cooling for about 15 min at room temperature, 0.5 mL hydrogen peroxide (30%) was added drop by drop and the solution was heated again till the colour of the solution changed from black to light yellow. The heating and cooling procedure was repeated until the content of the flask became colourless. The peroxide-digested material was transferred from the Kjeldahl flask into a 100 mL volumetric flask with three washings each with 5 mL DDW. The volume of the flask was maintained by DDW. The digested material was used for estimation of N, P and K content. Leaf N and P contents were estimated according to the method of Lindner (1944) and Fiske and Subba Row (1925), respectively. Leaf K content was analyzed using flame-photometer (Hald 1946).

Determination of secondary metabolites

Peltate glandular trichome diameter and density

Peltate glandular trichome (PGT) diameter and density were determined with the help of scanning electron

microscope (SEM) of make JEOL, JSM-6510 LV, Japan. Leaves were coated with gold prior to scanning. The diameter of the PGTs was determined in micrometres (µm) using SEM. The calculations were done according to Maffei et al. (1986). The PGT diameter was expressed as µm and density as mm^{-2}.

Essential oil content and menthol content

Essential oil (EO) content was determined by the hydro distillation method of Guenther (1972). Fresh leaves at 50 g allowed distilling for 3 h distillation and the EO content was expressed as % (v/w). The menthol content of EO was determined by gas chromatography. Menthol was calculated by knowing its peak area given in the chromatogram. The menthol content (%) was determined by dividing the menthol peak area by the total chromatogram and the resulting quotient was multiplied by 100 (Adams 2007).

The details for the estimation of menthol content by GC were as follows: GC, Nucon 5700, New Delhi, India was equipped with an AT-1000 stainless steel column, a flame ionization detector and an integrator. N was used as the carrier gas. The flow rates of N, H and O were maintained at 0.5, 0.5, and 5 mL s^{-1}, respectively. The temperature of the detector was 250 °C; oven, 160 °C and injector, 250 °C. The sample size was 2 µL invariably.

Statistical analyses

The data were statistically analysed using the SPSS 16.0 for Windows Software Package and the replicate means were separated by Duncan's multiple range tests.

Results

Growth characters

The data (Table 1) revealed that increasing levels of salinity decreased shoot and root length, leaf number, leaf area, shoot and root fresh weight and shoot and root dry weight linearly. A decrease of 16.12%, 20.89% and 25.06% in fresh weight per plant, for e.g., was noted with 50, 100 and 150 mM NaCl respectively over the water-sprayed control. A reverse relationship was observed between salinity levels and parameters namely chlorophyll content, P_N, g_s, C_i, E, CA activity and leaf nutrient such as N, P and K contents (Table 2). However, increasing salt levels enhanced CAT, POX, SOD and proline content continuously (Table 3). An increase of 23.77%, 32.10% and 40.03%, for e.g., in CAT activity was observed with 50, 100 and 150 mM NaCl, respectively, over the no salt treatment. The graded levels of salinity declined EO Yield, menthol content and yield and PGT diameter progressively, the lower level of salinity (50 mM NaCl) decreased both EO content and PGT density; however, 100 mM NaCl improved these parameters to some

Table 1 Effect of salt stress on growth characters (means of five replicates)

Stress treatments (mM NaCl)	Shoot length per plant (cm)	Root length per plant (cm)	Leaves per plant	Area per leaf (cm^2)	Shoot fresh weight per plant (g)	Root fresh weight per plant (g)	Shoot dry weight per plant (g)	Root dry weight per plant (g)
0	60.73	56.65	224.20	6.00	72.63	28.81	24.28	4.89
50	50.68	47.66	192.10	5.02	60.92	24.10	19.86	4.63
100	48.05	44.77	175.15	4.74	57.46	25.76	19.02	4.79
150	45.59	42.62	171.20	4.49	54.43	22.08	17.10	4.07
CD at 5%	0.45	0.58	2.17	0.12	0.51	0.49	0.48	0.58

Table 2 Effect of salt stress on physio-biochemical parameters (means of five replicates)

Stress treatments (mM NaCl)	Chlorophyll content (SPAD)	P_N (µM CO_2 m^{-2} s^{-1})	g_s (M H_2O m^{-2} s^{-1})	E (M H_2O m^{-2} s^{-1})	C_i (M CO_2 m^{-2} s^{-1})	CA activity (M CO_2 kg^{-1} s^{-1})	N content (%)	P content (%)	K content (%)
0	54.76	6.10	0.107	2.323	3.12	32.00	2.45	0.31	1.93
50	45.69	5.12	0.091	1.953	2.60	26.36	2.18	0.29	1.79
100	42.90	4.88	0.087	1.856	2.46	24.81	2.08	0.28	1.71
150	40.51	4.64	0.083	1.765	2.33	23.52	2.03	0.27	1.65
CD at 5%	0.55	0.007	0.003	0.013	0.005	1.175	0.11	0.02	0.10

Table 3 Effect of salt stress on antioxidant enzyme activities, proline content and yield and quality attributes (means of five replicates)

Stress treatments (mM NaCl)	CAT Act. (μM H_2O_2 kg^{-1} FM s^{-1})	POX Act. (μM pyrogallol kg^{-1} FM s^{-1})	SOD Act. (mM NBT kg^{-1} FM s^{-1})	Proline con. (μM proline g^{-1} FM)	Herb yield per plant (g)	PGT density (mm^{-2})	PGT diameter (μm)	EO content (%)	EO yield per plant (mL)	Menthol content (%)	Menthol yield per plant (mL)
0	212.99	142.66	51.40	31.89	72.63	34.40	17.88	0.66	0.49	37.16	0.186
50	262.95	173.36	62.99	38.64	50.68	28.48	17.20	0.55	0.34	32.86	0.112
100	281.36	185.18	67.54	41.56	48.05	29.96	16.70	0.58	0.34	30.62	0.100
150	298.24	196.30	71.54	44.04	45.59	35.49	16.19	0.67	0.39	28.20	0.103
CD at 5%	1.10	0.88	1.06	1.47	0.51	0.671	0.385	0.003	0.004	0.178	0.002

extent over 50 mM NaCl (Table 3). The highest salt level (150 mM NaCl) gave slightly higher values even than the no salt treatment.

Foliar application of PGRs in order of SA > GA_3 > Tria was found to be effective in enhancing almost all growth parameters (Table 4). The spray of SA improved for instance shoot fresh weight per plant by 46.05%, GA_3 by 40.42% and Tria by 33.22% over the water-sprayed control. However, foliar sprays of PGRs did not vary in their effect for root dry weight (Table 4). An almost similar trend observed for physio-biochemical parameters in respect of the effects of foliar PGR treatments (Tables 5, 6). The spray of SA enhanced, for e.g., CAT activity, by 31.46%, GA_3 by 21.80%, and Tria by 30.60% over the water-sprayed control (Table 6). Similarly foliar application of PGRs in order of SA > GA_3 > Tria improved all yield and quality parameters, except PGT diameter (Table 6). For e.g., the spray of SA increased EO content by 80.00%, EO yield by 154.55%, menthol content by 53.84%, menthol yield by 53.84% and PGT density by 95.05% over the water-sprayed control (Table 6).

Among interactions 0 mM NaCl × SA excelled others for all growth parameters studied (Fig. 1). This interaction increased for example fresh weight per plant by 121.15% over the lowest value giving interaction 150 mM NaCl × water (Fig. 1). In respect of physio-biochemical parameters interaction 0 mM NaCl × SA proved best for chlorophyll content, P_N, g_s, C_i, E, CA activity and leaf nutrient such as N and K contents, while 150 mM NaCl × SA for CAT, POX, SOD and proline content (Figs. 2, 3). The interaction 0 mM NaCl × SA increased, for e.g., P_N by 89.50% over the least value giving interaction 150 mM NaCl × water. The effect of interaction was not found to be significant on CA, SOD and proline and leaf P content (Figs. 2, 3). Of interactions, 0 mM NaCl × SA excelled others for EO yield, menthol content and yield and PGT diameter (Fig. 3). However, the perusal of SEM images (Fig. 4) and gas chromatogram (Fig. 5) revealed that interaction treatment 150 mM NaCl × SA proved to be best for EO content and PGT density (Fig. 3). Interaction 0 mM NaCl × SA gave, for e.g., 453.85% higher EO yield over the lowest value giving interaction.

Discussion

This study is an attempt to understand the effect of spray of PGRs under salt stress on the performance of peppermint through the study of various phenomena using growth, biochemical analysis, scanning electron microscopy and gas chromatography. In the present study, salt stress treatments decrease growth characteristics, photosynthetic parameter, leaf nutrients as also yield and quality attributes while increased the contents of antioxidant enzymes and proline

Table 4 Effect of plant growth regulators on growth characters (means of five replicates)

PGR spray treatments (10^{-6} M)	Shoot length per plant (cm)	Root length per plant (cm)	Leaves per plant	Area per leaf (cm^2)	Shoot fresh weight per plant (g)	Root fresh weight per plant (g)	Shoot dry weight per plant (g)	Root dry weight per plant (g)
Water	39.98	37.50	147.15	3.88	47.23	22.23	15.50	4.30
GA_3	55.09	51.35	206.00	5.48	66.32	26.14	21.85	4.82
SA	57.20	53.66	214.15	5.68	68.98	27.17	22.98	4.60
Tria	52.78	49.18	195.35	5.21	62.92	25.21	20.83	4.68
CD at 5%	0.45	0.58	2.17	0.12	0.51	0.49	0.48	NS

Table 5 Effect of plant growth regulators on physio-biochemical parameters (means of five replicates)

PGR spray treatments (10^{-6} M)	Chlorophyll content (SPAD)	P_N (μM CO_2 m^{-2} s^{-1})	g_S (M H_2O m^{-2} s^{-1})	E (M H_2O m^{-2} s^{-1})	C_i (M CO_2 m^{-2} s^{-1})	CA activity (M CO_2 kg^{-1} s^{-1})	N content (%)	P content (%)	K content (%)
Water	35.24	4.14	0.074	1.560	2.05	20.17	1.82	0.26	1.55
GA_3	49.48	5.53	0.098	2.110	2.82	28.81	2.26	0.30	1.83
SA	51.95	5.76	0.102	2.200	2.94	30.29	2.37	0.31	1.88
Tria	47.20	5.31	0.095	2.026	2.69	27.43	2.28	0.29	1.82
CD at 5%	0.55	0.005	0.003	0.013	0.005	1.175	0.11	0.02	0.10

(Figs. 1, 2, 3). The decrease in fresh weight of peppermint plants resulted from the application of NaCl particularly at 150 mM over the no salt treatment can be attributed to reduced shoot and root length, leaf number and area per leaf. The reduction in these growth characters may be attributed to hyperosmotic stress caused by NaCl leading to a water potential imbalance between the apoplast and symplast. The resulted low water potential would decrease turgor pressure hence diminished growth (Bohnert et al. 1995; Timperio et al. 2008). Moreover the reduction in root length may cause comparatively less nutrient and water absorption in salt treated plants resulting in suboptimal metabolism which may cause poor plant growth including root growth as depicted in root fresh weight. The decrease in dry weight of shoots may be correlated with the poor fresh weight of treated plants. Our findings on growth in respect of salt stress broadly corroborate with the findings of Li et al. (2014) on peppermint and Gengmao et al. (2014) on red sage. The salt stress not only decrease growth characters but also photosynthetic parameters (Fig. 2). The decrease in P_N due to application of salt particularly 150 mM NaCl over no salt treatment may be attributed to decreased chlorophyll content, g_s and E of NaCl treated plants. The suppressed photosynthesis by salt treatment is in accordance with the result of Khan (2003) on wheat and Babar et al. (2014) on fenugreek. Salinity stressed plants especially 150 mM NaCl treated ones exhibited a decreasing trend in respect of leaf nutrient contents (Fig. 2). This decrease in leaf nutrients may be correlated with the reduced root length of treated plants on one hand and salinity mediated ionic imbalance on the other. The effect of salt on leaf nutrients are in accordance with Yildirim et al. 2008. The salt stress on the other hand increased CAT, POD and SOD activities as also proline content (Table 3). The increase in antioxidant enzyme activities and proline content may be the result of the protective mechanism. Application of NaCl increases the synthesis of harmful reactive oxygen species (ROS) through salt effected ionic imbalance in the cell. To overcome the effect of these ROS, antioxidant enzymes and proline are synthesized in an ample quantity within the cell as a result of stress adaptation (Alscher et al. 2002; Sperdouli and Moustakas 2012). These results agree with those of Tuna et al. (2008) in maize plants, Sairam and Srivastava (2002) in *Cassia angustifolia* plants, Agarwal and Pandey (2004) in wheat. Salt stress especially 150 mM NaCl increased EO content and PGT density (studied for the first time in respect of the salinity) but decreased herb yield, EO yield as also menthol content and its yield (Table 3.). The decrease in these attributes could be because of the observed reduction in growth characters, photosynthesis and nutrient contents. The high density of PGTs of treated plants would naturally affect the EO content hence higher value of the oil content. Most of these findings are in agreement of Tabatabaie and Nazari (2007), Aziz et al. (2008) and Khorasaninejad et al. (2010).

Exogenously leaf-applied PGRs improved growth, biochemical, yield and quality parameters (Tables 4, 5, 6). The alleviation in growth of plants due to PGRs especially SA may be traced to the fact that PGRs increase membrane

Table 6 Effect of plant growth regulators on antioxidant enzyme activities, proline content and yield and quality attributes (means of five replicates)

PGR spray treatments (10^{-6} M)	CAT Act. (μM H_2O_2 kg^{-1} FM s^{-1})	POX Act. (μM pyrogallol kg^{-1} FM s^{-1})	SOD Act. (mM NBT kg^{-1} FM s^{-1})	Proline con. (μM proline g^{-1} FM)	Herb yield per plant (g)	PGT density (mm^{-2})	PGT diameter (μm)	EO content (%)	EO yield per plant (mL)	Menthol content (%)	Menthol yield per plant (mL)
Water	224.35	148.36	53.43	33.34	47.23	22.31	17.21	0.45	0.22	24.76	0.069
GA_3	273.26	180.59	65.67	40.42	66.32	32.13	17.42	0.62	0.41	34.25	0.129
SA	294.93	194.86	70.86	43.61	68.98	43.52	16.61	0.81	0.56	38.09	0.183
Tria	262.99	173.68	63.50	38.77	62.92	30.38	16.72	0.58	0.37	31.73	0.119
CD at 5%	1.10	0.88	1.06	1.47	0.51	0.671	0.385	0.003	0.004	0.178	0.002

permeability, cell division and cell enlargement. The favourable response of plants to PGRs has also been reported by Yildirim et al. (2008), Babar et al. (2014) and Özkan and Baydar (2016). The improvement in P_N resulted from the application of PGRs particularly SA may be ascribed to the enhancement in photosynthetic attributing characters like chlorophyll content, g_s and E of treated plants. These findings may be well documented with support of Popova et al. (1997), Stevens et al. (2006), Ashraf et al. (2010) and Pazoki (2015). The improvement in nutrient contents are of PGR-treated plants particularly SA treated plants may be attributed to the enhanced membrane permeability on one hand and the longer root length on the other hence higher values for nutrient contents. These findings on nutrient content more or less match with those of Gunes et al. (2005) and Yildirim et al. (2008). The explanation for the improvement in activities of antioxidant enzymes as also proline content due to hormones especially SA is not far to seek. The PGRs particularly SA is supposed to act as secondary messengers for the metabolism responsible for the improved synthesis of antioxidant enzymes and osmolytes as well. The enhanced activities of the antioxidant enzymes and proline content due to PGR application, especially SA has been observed by other workers also including Senaratna et al. (2000) and Borsani et al. (2001). Plants treated with PGRs particularly SA gave higher value not only for herb yield and PGT density but also for EO content and yield as well as menthol content and yield (Figs. 4, 5). The improvement in these secondary metabolite parameters may be related to the higher values for growth and photosynthetic characters and nutrient contents of treated plant. The higher density of PGTs of treated plants may result in higher EO content. These results might be associated with the gene mediated improved metabolism. The role of SA in increasing secondary metabolites of plants has been well documented in various literatures (Zheljazkov 2010; Shabrangi and BeigiJazi 2014; Saharkhiz and Goudarzi 2014). The beneficial effect of foliar application of PGRs especially SA on the performance of peppermint under stress conditions (Figs. 1, 2, 3) may be explained on the same as discussed above. Our findings broadly corroborate with the findings of Shakirova et al. (2003), Arzhe et al. (2015), Pazoki (2015) and Zohra et al. (2016).

Conclusion

Based on the results of the present study, it could be concluded that peppermint plants were deleteriously affected by NaCl stress. The severity of the salt damage increased with the elevating concentration of NaCl. However, exogenously leaf applied PGRs ameliorated most of the parameters studied under stress and stress free conditions. The amelioration was more pronounced when plants were free

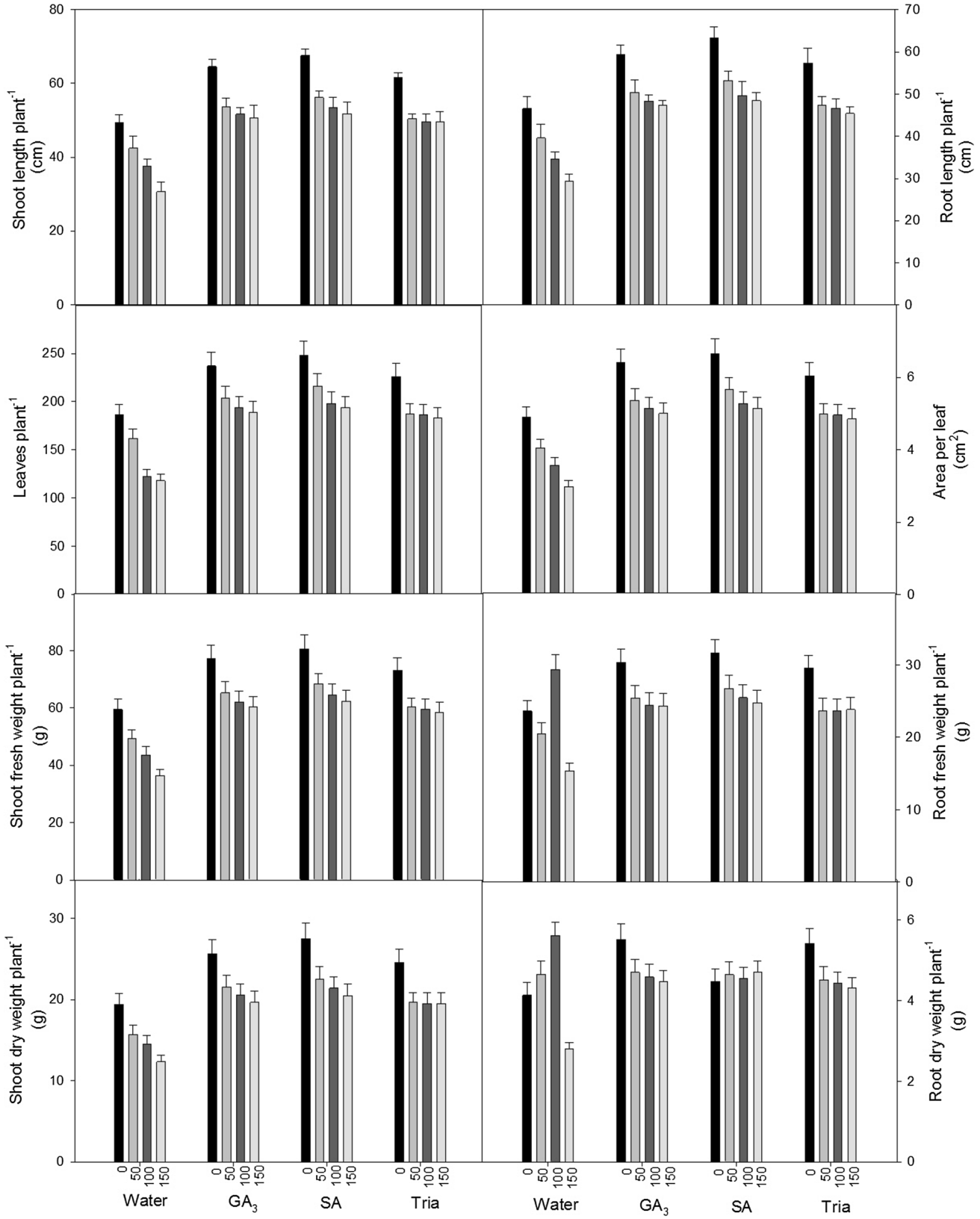

Fig. 1 Salt mediated effect of plant growth regulators on growth parameters

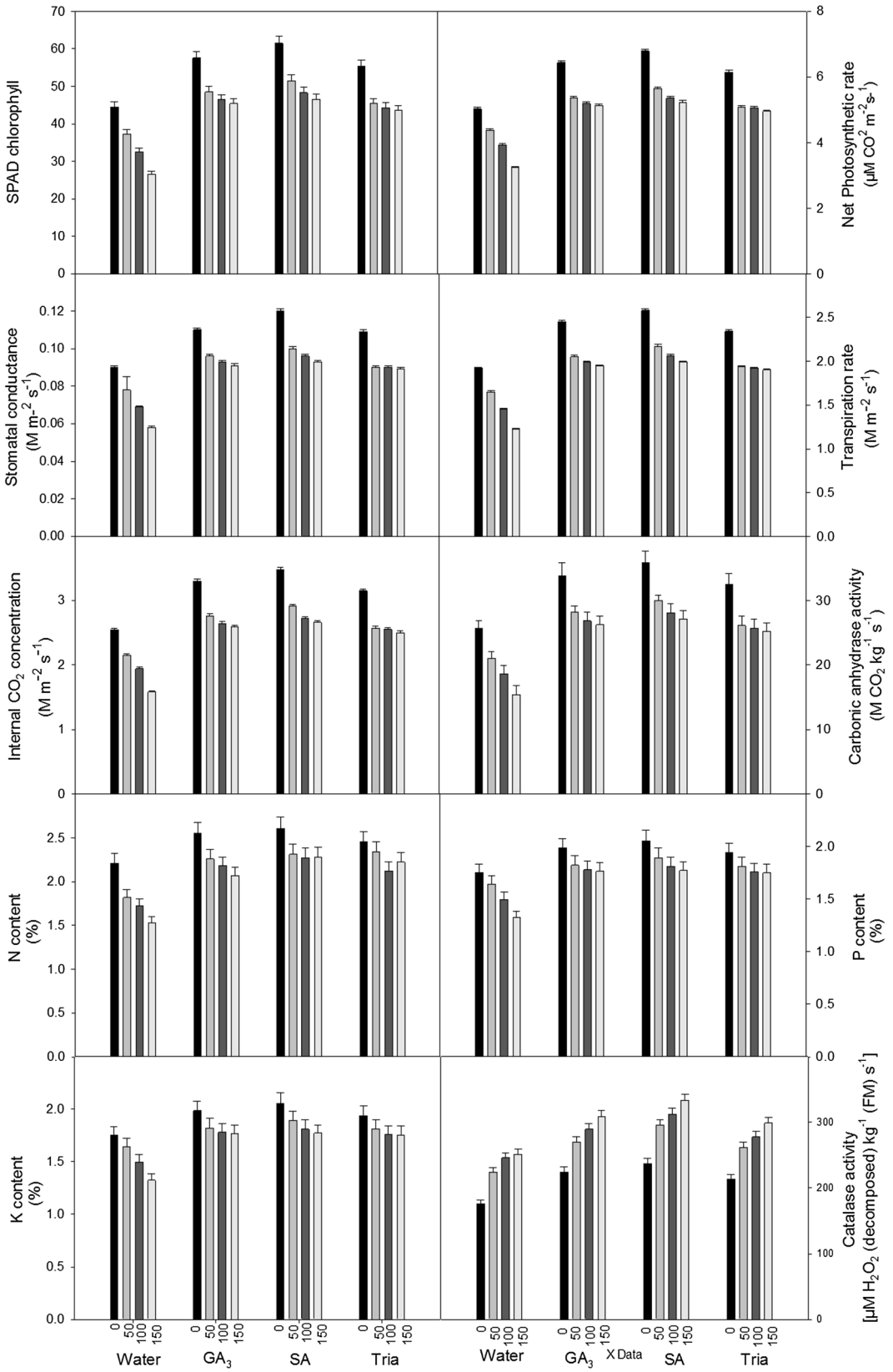

Fig. 2 Salt mediated effect of plant growth regulators on physio-biochemical parameters

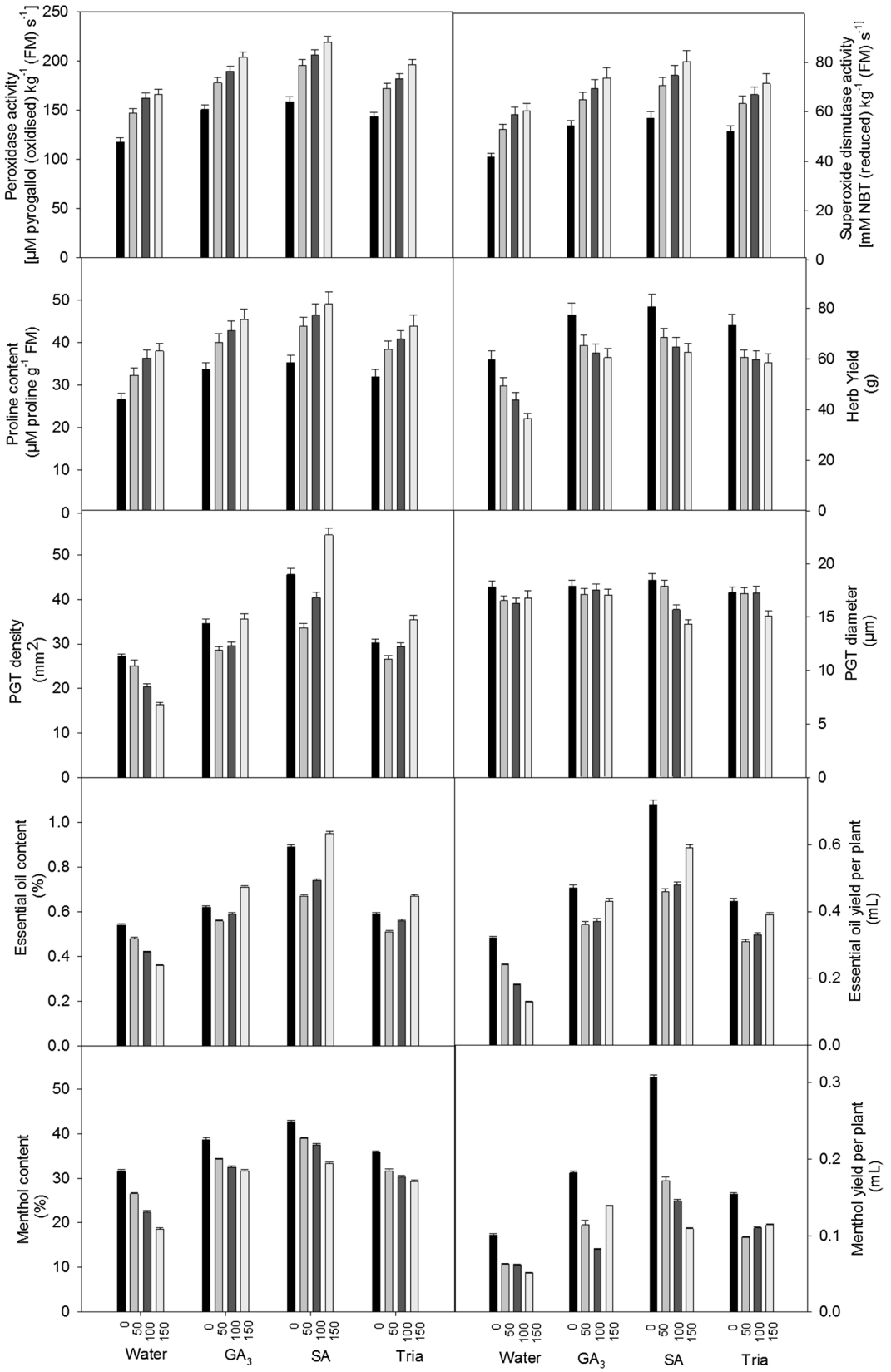

Fig. 3 Salt mediated effect of plant growth regulators on antioxidant enzyme activities, proline content and yield and quality attributes

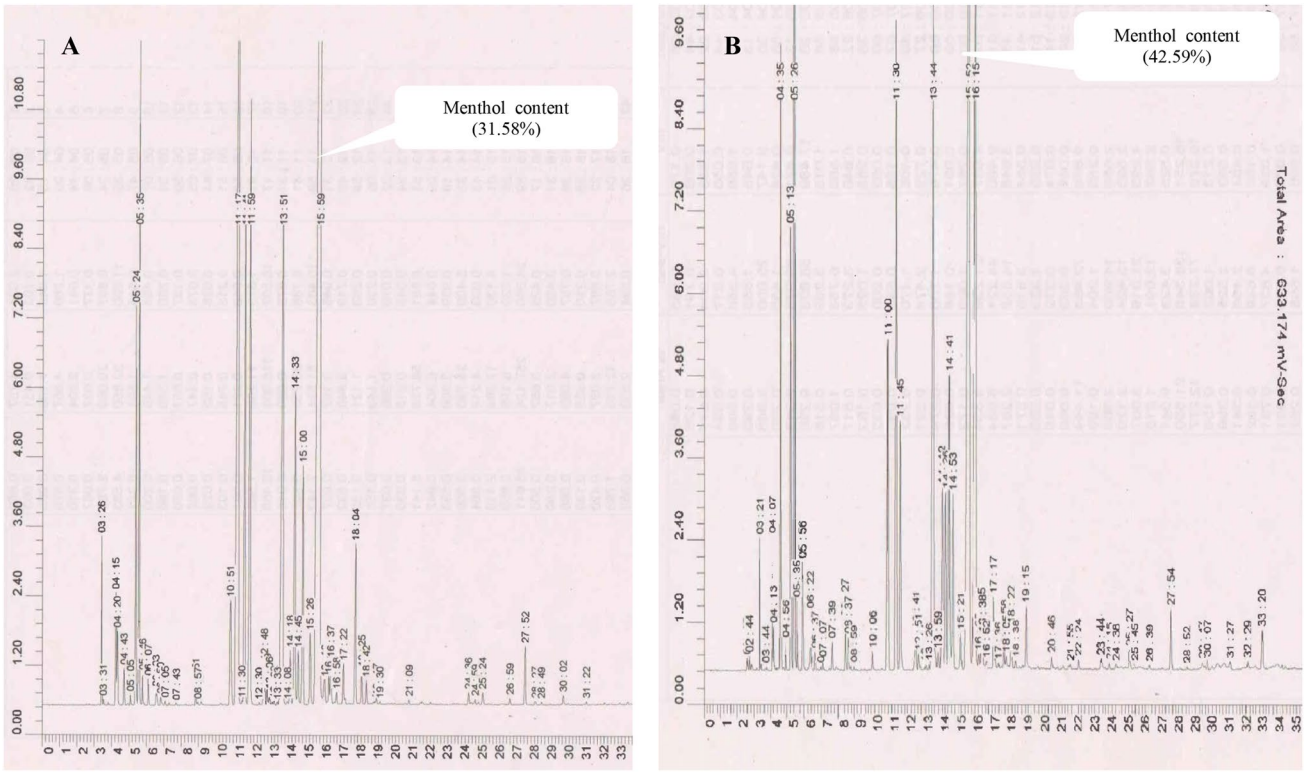

Fig. 4 Salt mediated effect of plant growth regulators on menthol content (GC graph). **a** Control and **b** 150 mM NaCl × 10^{-6} M salicylic acid

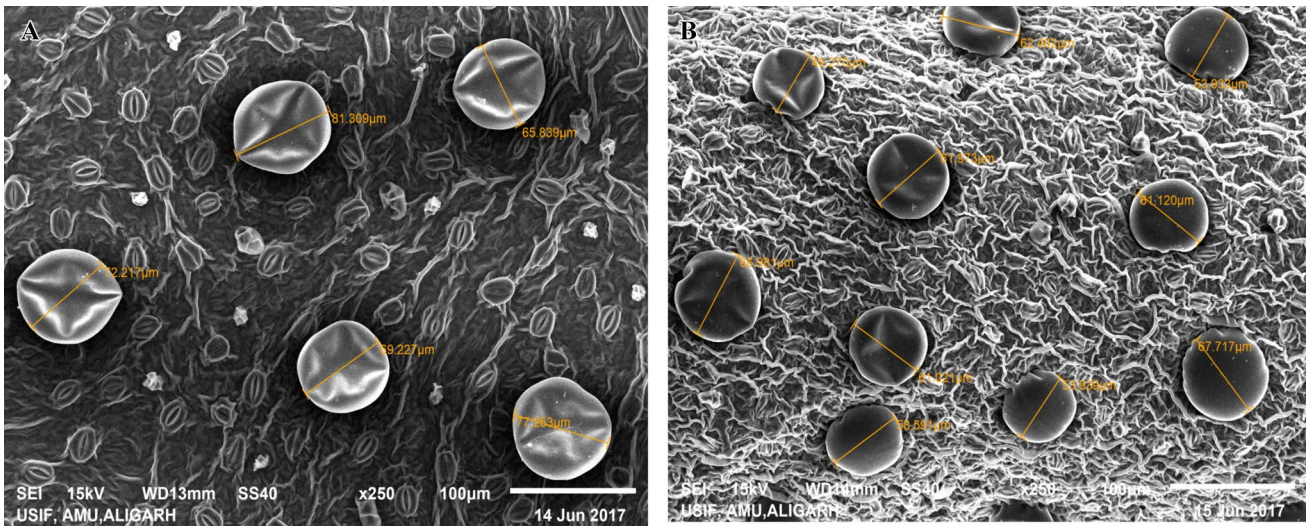

Fig. 5 Salt mediated effect of plant growth regulators on density and diameter of peltate glandular trichomes (SEM image). **a** Control, **b** 150 mM NaCl × 10^{-6} M salicylic acid

from salt stress. Application of SA was comparatively more pronounced. As a conclusion, foliar application of SA treatment may present new prospects for growing peppermint in soils even containing moderate and high amounts of NaCl.

Author contribution statement DK carried out the experimental work, analysed and prepared the manuscript. FM had supervised the work and was involved in the design of the experiment preparation and presentation of the manuscript.

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References

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry, 4th Edn. Allured Publishing Corporation, Carol Stream, USA

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Agarwal S, Pandey V (2004) Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol Plant 48:555–560

Aldesuquy HS, Mankarios AT, Awad HA (1998) Effect of some antitranspirants on growth, metabolism and productivity of salinetreated wheat plants. Induction of stomatal closure, inhibition of transpiration and improvement of leaf turgidity. Acta Bot Hung 41:1–10

Alonso-Ramírez A, Rodriguez D, Reyes D, Jimenez JA, Nicolas G, Lopez-Climent M, Gomez-Cadenas A, Nicolas C (2009) Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in arabidopsis seeds. Plant Physiol 150(3):1335–1344

Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53:1331–1341

Arzhe J, Izan T, Nasiri Y, Shekari F (2015) The effect of salicylic acid on some morphological features and yield of *Dracocephalum moldavica* in saline conditions. Bull Environ Pharmacol Life Sci 4:95–101

Ashraf M, Akram NA, Arteca RN, Foolad MR (2010) The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. Crit Rev Plant Sci 29:162–190

Aziz EE, Al-Amier H, Craker LE (2008) Influence of salt stress on growth and essential oil production in peppermint, pennyroyal, and apple mint. J Herbs Spices Med Plants 14:1–2

Babar S, Siddiqi EH, Hussain I, Bhatti KH, Rasheed R (2014) Mitigating the effects of salinity by foliar application of salicylic acid in fenugreek. Physiol J. https://doi.org/10.1155/2014/869058 **(article ID 869058)**

Barros AS, Morais SM, Ferreira PAT, Vieira IGP, Craveiro AA, Fontenelle ROS, Menezes JESA, Silva FWF, Sousa HA (2015) Chemical composition and functional properties of essential oils from *Mentha* species. Ind Crop Prod 76:557–564

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant soil 39:205–207

Bohnert HJ, Nelson DE, Jensen G (1995) Adaptations to environmental stresses. Plant Cell 7:1099–1111

Borsani O, Valpuesta V, Botella MA (2001) Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. Plant Physiol 126:1024–1030

Chance B, Maehly AC (1955) Assay of catalases and peroxidases. Methods Enzymol 2:764–775

Chen X, Yuan H, Chen R, Zhu L, Du B, Weng Q, He G (2002) Isolation and characterization of triacontanol-regulated genes in rice (*Oryza sativa* L.): possible role of triacontanol as a plant growth stimulator. Plant Cell Physiol 43(8):869–876

Dwivedi RS, Randhawa NS (1974) Evaluation of rapid test for the hidden hunger of zinc in plants. Plant Soil 40:445–451

Fiske CH, Subba Row Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66:375–400

Gengmao Z, Quanmei S, Yu H, Shihui L, Changhai W (2014) The physiological and biochemical responses of a medicinal plant (*Salvia miltiorrhiza* L.) to stress caused by various concentrations of NaCl. PLoS One 9:e89624

Guenther E (1972) The essential oils. History-origin in plants-production-analysis, vol 1. Kreiger Publishing Company, Malabar, Florida

Gunes A, Inal A, Alpaslan M, Cicek N, Guneri E, Eraslan F, Guzelordu T (2005) Effects of exogenously applied salicylic acid on the induction of multiple stress tolerance and mineral nutrition in maize (*Zea mays* L.). Arch Agron Soil Sci 51(6):687–695

Gunes A, Inal A, Alpaslan M, Eraslan F, Bagci EG, Cicek N (2007) Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. J Plant Physiol 164:728–736

Gupta PK (2004) Soil, plant, water and fertilizer analysis. Agrobios Agro House, Jodhpur

Hald PM (1946) The flame photometer for the measurement of sodium and potassium in biological materials. J Biol Chem 163:499–510

Horvath E, Szalai G, Janda T (2007) Induction of abiotic stress tolerance by salicylic acid signaling. J Plant Growth Regul 26:290–300

Jayakannan M, Bose J, Babourina O, Shabala S, Massart A, Poschenrieder C, Rengel Z (2015) The NPR1-dependent salicylic acid signalling pathway is pivotal for enhanced salt and oxidative stress tolerance in *Arabidopsis*. J Exp Bot 66:1865–1875

Khan NA (2003) NaCl inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. Biol Plant 47:437–440

Khorasaninejad S, Mousavi A, Soltanloo H, Hemmati K, Khalighi A (2010) The effect of salinity stress on growth parameters, essential oil yield and constituent of peppermint (*Mentha piperita* L.). World Appl Sci J 11:1403–1407

Kono Y (1978) Generation of superoxide radicle during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys 186:189–195

Lawrence BM (2006) Mint: the genus *Mentha*. CRC Press, Boca Raton

Li Z, Yang H, Wu X, Guo K, Li J (2014) Some aspects of salinity responses in peppermint (*Mentha* × *piperita* L.) to NaCl treatment. Protoplasma 252:885–899

Lindner RC (1944) Rapid analytical methods for some of the more common inorganic constituents of the plant tissues. Plant Physiol 19:76–89

Maffei M, Gallino M, Sacco T (1986) Glandular trichomes and essential oils of developing leaves in *Mentha viridis* lavanduliodora. Planta Med 52:187–193

Muthuchelian K, Murugan C, Harigovindan R, Nedunchezhian N, Kulandaivelu G (1996) Ameliorating effect of triacontanol on salt stressed *erythrina variegata* seedlings. Changes in growth, biomass, pigments and solute accumulation. Biol Plant 38:133–136

Naeem M, Khan MMA, Moinuddin, Idrees M, Aftab T (2011) Triacontanol mediated regulation of growth and other physiological attributes, active constituents and yield of *Mentha arvensis* L. Plant Growth Regul 65:195–206

Nazar R, Iqbal N, Syeed S, Khan NA (2011) Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. J Plant Physiol 168(8):807–815

Olfa B, Imen T, Maha Z, Mohamed C, Ben Nasri-Ayachi M (2016) Essential oil and trichome density from *Origanum majorana* L. shoots affected by leaf age and salinity. Biosci J Uberlândia 32:238–245

Ondrasek G, Rengel Z, Veres S (2011) Soil salinization and salt stress in crop production. In: Shanker AK, Venkateswarlu B (eds)

Abiotic stress in plants, mechanisms and adaptations. In Tech, Rijeka, pp 171–190
Özkan C, Baydar NG (2016) Brassinosteroid effects on some physical and biochemical properties and secondary metabolite accumulation in peppermint (*Mentha piperita* L.) under salt stress. Ind Crops Prods 86:251–258
Palma F, López-Gómez M, Tejera NA, Lluch C (2013) Salicylic acid improves the salinity tolerance of Medicago sativa in symbiosis with Sinorhizobium meliloti by preventing nitrogen fixation inhibition. Plant Sci 208:75–82
Pazoki A (2015) Influence of salicylic acid and jasmonic acid on chlorophyll, carotenes and xanthophyll contents of lemon balm (*Melissa officinalis* L.) under salt stress conditions. Biol Forum 7:287–292
Popova L, Pancheva T, Uzunova A (1997) Salicylic acid, properties, biosynthesis and physiological role. Bulg J Plant Physiol 23:85–93
Radhakrishnan R, Kumari BDR (2008) Effect of n-triacontanol on the growth of salt stressed soybean plants. J Biosci 19:53–62
Rajasekaran LR, Blake TJ (1999) New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. Plant Growth Regul 18:175–181
Raskin I (1992) Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol 43:439–463
Ries SK, Wert V, Sweeley CC, Leavitt RA (1977) Triacontanol: a new naturally occurring plant growth regulator. Science 195:1339–1341
Saharkhiz MJ, Goudarzi T (2014) Foliar application of salicylic acid changes essential oil content and chemical compositions of peppermint (*Mentha piperita* L.). J Essent Oil Bear Plants 17:435.–440
Sairam RK, Srivastava GC (2002) Changes in antioxidant activity in subcellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci 162:897–904
Santoro MV, Nievas F, Zygadlo J, Giordano W, Banchio E (2013) Effects of growth regulators on biomass and the production of secondary metabolites in peppermint (*Mentha piperita*) micropropagated in vitro. Am J Plant Sci 4:49–55
Senaratna T, Touchell D, Bunn E, Dixon K (2000) Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul 30:157–161
Shabrangi A, BeigiJazi EA (2014) Effect of salicylic acid on the amount of essential oil, phenolic compounds, flavonoids and antioxidant activity of *Mentha piperita* L. Int J Agric Crop Sci 7:499–502
Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA, Fatkhutdinova DR (2003) Changes in the hormonal status of wheat seedling induced by salicylic acid and salinity. Plant Sci 164:317–322
Singh HP, Tomar VKS, Kalra A, Tiwari R (2012) Aus Gyanya mint. CIMAP, Lucknow, p 4
Sperdouli I, Moustakas M (2012) Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of *Arabidopsis thaliana* to drought stress. J Plant Physiol 169:577–585
Srivastava MK, Dwivedi UN (2000) Delayed ripening of banana fruit by salicylic acid. Plant Sci 158(1–2):87–96
Stevens J, Senaratna T, Sivasithamparam K (2006) Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma), associated changes in gas exchange, water relations and membrane stabilisation. Plant Growth Regul 49:77–83
Sun T, Gubler F (2004) Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Biol 55(1):197–223
Tabatabaie SJ, Nazari J (2007) Influence of nutrient concentrations and NaCl salinity on the growth, photosynthesis, and essential oil content of peppermint and lemon verbena. Turk J Agric For 31:245–253
Timperio AM, Egidi MG, Zolla L (2008) Proteomics applied on plant abiotic stresses, role of heat shock proteins. J Proteomics 71:391–411
Tuna AL, Kayab C. Dikilitas M, Higgs D (2008) The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environ Exp Bot 62:1–9
Yildirim E, Turan M, Guvenc I (2008) Effect of foliar salicylic acid applications on growth, chlorophyll, and mineral content of cucumber grown under salt stress. J Plant Nutr 31:593–612
Zheljazkov VD (2010) Effect of plant hormones and distillation water on mints. Hortic Sci 45:1338–1340
Zohra ESF, Youssef M, Hassan E, Khalid AJ (2016) Effect of salicylic acid and salt stress on the growth and some biochemical parameters of *Mentha suaveolens*. Int J Sci Eng Res 7:54–62