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[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan
[2]Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia
[3]Department of Botany, GC University, Faisalabad, Pakistan

Regulation in some vital physiological attributes and antioxidative defense system in carrot (*Daucus carota* L.) under saline stress

[1]Saira Bano, [1,2]Muhammad Ashraf, [3*]Nudrat Aisha Akram, [2]F. Al-Qurainy

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Summary

Regulation of some key metabolic phenomena including anti-oxidative defense system involved in plant salt tolerance is of great concern. Changes in chlorophyll pigments, chlorophyll fluorescence and leaf gas exchange characteristics, glycinebetaine and proline contents, and enzymatic and non-enzymatic antioxidants was assessed in two carrot (*Daucus carota* L.) cultivars, DC-4 and T-29 under saline stress in a greenhouse study. Application of different saline regimes (0, 50, 100 and 150 mM NaCl) to the growth medium considerably reduced the shoot and root fresh and dry weights, shoot and root lengths, chlorophyll *b* contents, leaf water potential (Ψ_w), leaf osmotic potential (Ψ_s), photosynthetic rate (A), water-use efficiency, sub-stomatal CO_2 concentration (C_i), stomatal conductance (g_s), transpiration rate (E), C_i/C_a ratio, leaf and root K^+ and Ca^{2+} contents, leaf MDA, total phenolics, total soluble proteins, and activities of CAT, SOD and POD enzymes, while a marked increase was observed in leaf turgor potential (Ψ_p), leaf and root Na^+ and Cl^- contents, leaf proline, glycinebetaine (GB), ascorbic acid (AsA) and H_2O_2 contents in both cultivars. Of both carrot cultivars, cultivar T-29 was relatively higher in shoot and root fresh weights, root Na^+, leaf and root Ca^{2+}, leaf proline, MDA, total phenolics, soluble proteins and activity of SOD enzyme. In contrast, cultivar DC-4 was relatively higher in leaf Ψ_w and Ψ_s, leaf K^+, root Ca^{2+} and leaf GB as compared to those in the other cultivar. The relatively better growth of cultivar T-29 was found to be correlated with improved leaf water potential, leaf Ca^{2+}, proline, phenolics, and activity of SOD enzyme under saline conditions.

Introduction

Excessive accumulation of toxic ions such as Cl^- and Na^+ is one of the vital environmental factors, which reduce growth and yield in most salt-affected soils world-wide (Raathinasabapathi, 2000; Xue et al., 2004; Ashraf, 2004, 2009; Flowers et al., 2010; Kanwal et al., 2011). Most crop plants are salt sensitive and they are referred to as glycophytes (Xue et al., 2004; Parida and Das, 2005; Munns and Tester, 2008), because these ions cause impairment in many physiological and biochemical attributes including water relations, antioxidant defense system, photosynthesis, nutritional balance and metabolism of osmoprotectants (Shahbaz et al., 2008; Ashraf, 2009; Akram and Ashraf, 2011; Sabir et al., 2011).

A number of studies have been conducted to investigate salt tolerance potential in terms of better growth and yield production and the associated mechanisms including leaf fluorescence, gaseous exchange characteristics, antioxidant capacity, osmoprotectants as well as inorganic nutrient accumulation in various crops, e.g., *Panicum miliaceum* (Sabir et al., 2011), sunflower (Akram and Ashraf, 2011), safflower (Siddiqi et al., 2011), cucumber (Shi et al., 2008), sugar beet (Ghoulam et al., 2002), cotton (Ashraf, 2002), pea (Noreen et al., 2010), eggplant (Abbas et al., 2010), rice (Habib et al., 2010), maize (Ali and Ashraf, 2011), wheat (Perveen et al., 2010, 2011, 2012), and canola (Ashraf and Ali, 2008). Differential response has been observed in relatively salt tolerant cultivars as compared to that of sensitive ones within the same crop species. For example, recently while working with 10 cultivars of proso millet Sabir et al. (2011) and 10 cultivars of safflower, Siddiqi et al. (2011) have found a differential response under saline conditions in terms of high accumulation of proline and enhanced antioxidant capacity in relatively salt tolerant cultivars as compared to salt susceptible ones.

One of the potential adaptations to salt stress is the accumulation of osmoprotectants particularly proline and glycinebetaine in the cytoplasm (Kumar et al., 2004; Banu et al., 2010). As a consequence, inorganic ions such as Na^+ and Cl^- are sequestered into the vacuole leading to turgor maintenance and osmotic adjustment (Bohnert et al., 1995; Glenn et al., 1999; Ashraf, 2004; Banu et al., 2009). In plants, high production of reactive oxygen species (ROS) as a result of various abiotic stresses denature DNA, carbohydrates, proteins and lipids due to uncontrolled oxidative stress. However, plants possess a very efficient cascade of enzymatic as well as non-enzymatic antioxidant defense mechanism that shields plants against ROS (Mittler, 2002). Generally, to overcome the uncontrolled oxidation, plants accumulate superoxide dismutase (SOD), catalase (CAT), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), glutathione-*S*-transferase (GST), dehydroascorbate reductase (DHAR), guaicol peroxidase (GOPX), ascorbate peroxidase (APX), and glutathione peroxidase (GPX) as enzymatic antioxidants and apoprotein amino acids, glutathione (GSH), alkaloids, ascorbic acid (AsA), tocopherols and phenolics as non-enzymatic antioxidants for scavenging ROS (Yamaguchi and Blumwald, 2005; Ashraf, 2009; Gill and Tuteja, 2010; Siddiqi et al., 2011).

Vegetables generally show high antioxidant metabolism in response to salinity stress as already observed in a number of vegetables such as pea, radish, turnip, cauliflower and cucumber, etc. (Noreen and Ashraf, 2009 a,b; Volden et al., 2009; Colla et al., 2010; Neffatti et al., 2011; Shahbaz et al., 2012). Carrot (*Daucus carota*) is one of the most preferred vegetables worldwide for its edible tubers due to their enriched mineral composition, sugars, phytonutrients, carotene, vitamins A and C, dietary fibre and high culinary uses (Kumar et al., 2004). However, production of carrot is very low due to adaptation to a variety of abiotic stresses including salinity prevalent in many countries particularly falling under arid and semi-arid environments (Gonçalves et al., 2010). It is classified as a salt-sensitive plant as it grows best only at 20 mM salinity level. Furthermore, 7% growth reduction has been observed for increment of 1 dS/m electrical conductivity, which is mainly attributed to reduced photosynthetic capacity and gaseous exchange disorders (Gibberd et al., 2002). In view of the reports presented earlier, we hypothesized that a positive relationship exists between antioxidative defense system and regulation of some key metabolic phenomena and salt tolerance in carrot plants. Thus, the present study was conducted to determine the salinity tolerance potential in two carrot cultivars and the pattern of accumulation of chlorophyll regulation, chlorophyll fluorescence and leaf gas exchange characteristics, accumulation of GB, proline, and enzymatic and non-enzymatic antioxidants due to salt stress in carrot plants.

* Corresponding author

Materials and methods

A greenhouse experiment was carried-out during November-April, 2010 in the Botanical Garden, University of Agriculture, Faisalabad, Pakistan. During experimentation, average values of photoperiod, light intensity, relative humidity and temperature were recorded as 12 h, 890 µmol m^{-2} s^{-1}, 50%, and 26.7-37.9 °C, respectively. Seeds of two carrot cultivars (DC-4 and T-29) were supplied by the Ayub Agricultural Research Institute, Faisalabad, Pakistan. A completely randomized design was employed to set-up the experiment. After 10 days of germination, four seedlings were maintained at two-leaf stage per replicate and pots were separated in four sets supplied weekly with Hoagland's nutrient solution supplemented with four varied levels of salt (NaCl) [0, 50, 100 and 150 mM]. The salt concentrations increased step-wise per day with 50 mM NaCl. The plants were harvested four weeks after the commencement of the saline treatment. The plants were separated into shoots and roots, data recorded for fresh weights and after it, roots and shoots were oven-dried at 70 °C up till constant dry weights achieved. Before harvesting, fresh leaves were kept frozen using liquid nitrogen for the estimation of following attributes:

Chlorophyll contents

The fresh leaves (0.5 g) were extracted overnight using 80% acetone. The extract was centrifuged at 10, 000 x *g* for 5 min and the absorbance of the supernatant read at 645 and 663 nm using a spectrophotometer. Chlorophyll *a* and *b* were calculated using the formulae proposed by ARNON (1949).

Water relation attributes

Early in the morning (6.00-8.00 am), a fresh leaf from top was cut and used for the estimation of leaf water potential (Ψ_w) with the help of a Scholander type pressure chamber. The same leaf was frozen in a freezer at -80 °C for one week. Then cell sap was extracted from the frozen leaf material and directly used for the estimation of osmotic potential (Ψ_s) using an osmometer (Wescor, 5500). The difference between Ψ_w and Ψ_s was calculated as leaf turgor potential (Ψ_p).

Gas exchange characteristics

Gas exchange attributes including net CO_2 assimilation rate (*A*), sub-stomatal CO_2 concentration (C_i), stomatal conductance (g_s) and transpiration rate (*E*) were measured on a fresh leaf of each plant using infrared gas analyzer (LCA-4 ADC portable, Analytical Development Company, Hoddesdon, England).

Chlorophyll fluorescence

Maximal quantum yield of PSII (F_v/F_m), non-photochemical quenching (NPQ), photochemical quenching (qP) and co-efficient of non-photochemical quenching (qN) were examined using a OS5p Modulated Fluorometer (ADC BioScientific Ltd. Great Amwell Herts, UK) following STRASSER et al. (1995).

Determination of Na^+, K^+ and Ca^{2+}

Following ALLEN et al. (1985), dried ground leaf or root material (100 mg) was taken in a digestion flask and 1 mL digestion mixture (14 g $LiSO_4 \cdot 2H_2O$ + 0.42 g Se + 350 ml of hydrogen peroxide + 420 mL concentrated sulfuric acid) were added to the flask and placed on a hot plate. An increase in temperature was gradual from 50 °C to 200 °C until the mixture turned black. Then, perchloric acid (500 µL) was added to the flasks until the plant material became colorless. The mixture was cooled down, filtered and the solution was diluted up to 50 mL using distilled water with the help of volumetric flask. The filtrate was used for the determination of Na^+, K^+ and Ca^{2+} using a flame photometer (Model: Jenway, PFP-7). For the determination of Cl^-, the dried ground leaf or root sample (0.1 g) was extracted in 10 mL de-ionized water at 80 °C until the volume became half. Again the volume was maintained to 10 mL using deionized water. The Cl^- content was examined using a chloride analyzer (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

Leaf proline determination

Fresh leaf (500 mg) was extracted in freshly prepared 3% sulfo-salicylic acid (Mol. wt = 254.22) purchased from MP Biomedicals, Inc. Then, the filtrate (2.0 mL) was mixed with glacial acetic acid (2.0 mL) and 2.0 mL acid ninhydrin mixture, prepared by mixing ninhydrin (1.25 g), glacial acetic acid (30 mL) and 6M H_3PO_4 (20 mL) in a glass tube. The material was incubated at 100 °C for one h and cooled it. To it, 4.0 mL of toluene were added and mixed well. The optical density (OD) of the chromophore containing toluene was read at 520 nm. The proline concentration was estimated following the method described by BATES et al. (1973).

Glycinebetaine (GB)

Fresh leaf material (1.0 g) was shaken occasionally in 10 mL of 0.5% toluene solution and filtered. After filtration, 1 mL of the extract was blended with 1 mL of 2N H_2SO_4. Then 0.5 mL of this mixture was taken in a glass tube and to it potassium tri-iodide solution (0.2 mL) was added. The contents were shaken and ice-cooled for 90 min. Then 2.8 mL of distilled water and 6 mL of 1-2 dichloroethane were added to the mixture. The upper aqueous layer was discarded and OD of the organic layer determined at 365 nm.The GB concentration was calculated following GRIEVE and GRATTAN (1983).

Total phenolics

Total phenolics were analysed following JULKUNEN-TITTO (1985). Fresh leaf tissue (0.5 g) was homogenized with 80% acetone and centrifuged at 10,000 x *g* for 10 min. One hundred microliters of the supernatant were mixed with 2 mL of water and 1 mL of Folin-Ciocalteau's phenol reagent and shaken. Then 5 mL of 20% sodium carbonate (Na_2CO_3) were added and the volume was made up to 10 mL using distilled water. The contents were mixed and OD read at 750 nm.

H_2O_2 determination

Following VELIKOVA et al. (2000), fresh leaf (0.5 g) was extracted with 5 mL of 0.1% (w/v) TCA. The extract was centrifuged at 12,000 x *g* for 15 min. To 0.5 mL of the supernatant, 0.5 mL potassium phosphate buffer (pH 7.0) and 1 mL potassium iodide (KI) were added. The mixture was vortexed and its absorbance read at 390 nm.

Cell membrane injury (CMI)

The cell membrane injury was determined as described by YANG et al. (1996). The fully expanded youngest leaves of uniform size were excised and placed in test tubes each containing 10 mL deionized distilled water. Percent CMI was calculated as:

$$CMI\ (\%) = [EC_1\text{-}EC_0/EC_2\text{-}EC_0] \times 100$$

Activities of antioxidant enzymes

The fresh leaf (0.5 g) was ground well in 5 mL cooled phosphate buffer (50 mM; pH 7.8). Then the homogenate was mixed with a vortex and centrifuged at 15,000 x *g* for 15 min at 4 °C. The supernatant was separated and SOD activity determined by appraising the photoreduction of nitroblue tetrazolium (NBT) by the enzyme. For this purpose, the standard method as proposed by GIANNOPOLITIS and RIES (1977) was used. Three mL of the reaction solution contained 1.3 µM riboflavin, 50 µM NBT, 75 nM EDTA, 13 mM methionine, 20 mM phosphate buffer having 7.8 pH. 20 to 50 µL of the enzyme extract were homogenized in a test tube. This

solution was irradiated for 15 min under white fluorescent light. Then the OD of the solutions was read using a spectrophotometer at 560 nm. The amount of enzyme required to inhibit half of NBT photoreduction was considered equal to one unit of SOD activity.

Peroxidase (POD) and Catalase (CAT)

The protocol of CHANCE and MAEHLY (1955) was followed for the appraisal of peroxidase and catalase activities. Three mL of peroxidase reaction solution were mixed with 0.1 mL enzyme extract and then 40 mM H_2O_2, 20 mM guaiacol and 50 mM phosphate buffer (pH 5 .0) were added to it. The changes in absorbance of reaction solution were recorded at 470 nm after every 30 sec. A change in the absorbance of reaction mixture per min was considered equal to one unit of POD activity. Three mL reaction solution used for the determination of catalase activity contained 5.9 mM H_2O_2, 50 mM phosphate buffer having pH 7.8, and 0.1 mL enzyme extract. For the determination of catalase activity, the reaction was commenced by adding the enzyme extract and the changes in absorbance of the reaction solution after every 20 sec were measured at 240 nm. A change per min in absorbance was considered equal to one unit catalase activity. The activity of each enzyme was calculated and expressed on the basis of total protein measured as described by BRADFORD (1976).

Malondialdehyde (MDA)

MDA content was estimated with a spectrophotometer (U-2001 Hitachi Co., Tokyo) following CARMAK and HORST (1991). The concentration was calculated using coefficient of difference between OD at 600 and 532 nm.

Statistical analysis

ANOVA was obtained using JMP v.6.0 provided by SAS institute Inc., Cary, MC, USA. Data were presented as the mean ± S.E.; $n = 4$ for each cultivar and salt treatment. Significant differences between the salt levels were observed by a least significance difference test at 0.05% significance level.

Results

Analysis of variance of the data for shoot fresh and dry weights of two carrot (*Daucus carrota* L.) cultivars shows that imposition of salt (NaCl) stress caused a considerable decrease in shoot fresh and dry weights of both carrot cultivars (Tab. 1; Fig. 1). Of both carrot cultivars, cv. T-29 was relatively more tolerant to salt stress than cv. DC-4.

Salt stress had a marked reducing effect on root fresh and dry weights of both carrot cultivars. Of all salt regimes, 150 mM NaCl was highly inhibitory as compared to the other salt treatments. However, the response of both carrot cultivars to salt stress was non-significant in these attributes.

Imposition of NaCl stress induced a substantial decrease in shoot length of both carrot cultivars under test. Similarly, a slight reduction in root length was also observed under saline conditions. However, the response of both carrot cultivars to salt stress was inconsistent with respect to these attributes (Tab. 1; Fig. 1).

Root medium NaCl did not alter the chlorophyll *a* contents in both carrot cultivars (Tab. 1; Fig. 1). However, a marked reduction in chlorophyll *b* contents was observed in both carrot cultivars on exposure to varying salt treatments. In both carrot cultivars, the trend of increase or decrease in both pigments was almost constant (Fig. 1). Salt stress did not influence chlorophyll *a/b* ratio in both carrot cultivars. Both carrot cultivars were almost similar in chlorophyll *a/b* ratio (Fig. 2).

Application of varying levels of NaCl significantly affected all the three leaf water, osmotic and turgor potentials of both carrot cultivars under examination (Tab. 1; Fig. 2). Under saline conditions, a significant decrease (more negative) in leaf water potential as well as leaf osmotic potential (Ψ_s), while a considerable increase in leaf turgor potential of all carrot plants was observed. Of both carrot cultivars, cv. T-29 was lower in leaf water and osmotic potentials than cv. DC-4. However, both carrot cultivars did not differ in leaf turgor potential under saline conditions (Fig. 2).

Net CO_2 assimilation rate (A) in both carrot cultivars decreased on exposure to varying saline regimes. However, both carrot cultivars were similar in this gas exchange attribute (Tab. 1; Fig. 2). Under saline conditions, a considerable reduction in transpiration rate in carrot plants was observed. Both cultivars were similar in transpiration rate under saline and non-saline conditions (Tab. 1; Fig. 2). A significant decrease in stomatal conductance was found due to root growing medium salt stress. On the basis of analysis of variance, it is not possible to discriminate the carrot cultivars with respect to stomatal conductance. Sub-stomatal CO_2 concentration was significantly suppressed under saline regimes. Both carrot cultivars did not differ significantly under non-saline as well as saline regimes. Imposition of NaCl stress reduced the C_i/C_a ratio of both carrot cultivars. Of all salt regimes, lowest value of C_i/C_a was observed at 150 mM NaCl. Both carrot cultivars showed consistent values of C_i/C_a ratio under non-saline or saline regimes (Tab. 1; Fig. 3). Water-use-efficiency of both carrot cultivars decreased considerably at 150 mM NaCl, while in contrast, at 50 and 100 mM NaCl levels, a slight increase in WUE was observed (Tab. 1; Fig. 3). Both carrot cultivars were almost consistent in water-use-efficiency.

Leaf and root Na^+ concentrations increased significantly with increase in salt concentration. However, higher values of leaf and root Na^+ were observed in carrot plants at 150 mM NaCl and lower under normal as well as at 50 mM NaCl in both carrot cultivars. Both carrot cultivars were similar in leaf Na^+ at varying external saline regimes, while root Na^+ concentration was relatively higher in cv. T-29 than that in cv. DC-4 (Tab. 1; Fig. 4). Under saline conditions, leaf and root potassium (K^+) concentrations decreased markedly. Lowest values of leaf and root K^+ concentrations were observed at 150 mM NaCl. Both carrot cultivars significantly differed in leaf K^+ accumulation, and cv. DC-4 was reasonably higher in leaf K^+. A non-significant difference was observed between both carrot cultivars in root K^+ concentration (Tab. 1; Fig. 4). It was observed that leaf and root Ca^{2+} concentrations decreased significantly under saline conditions. Leaf Ca^{2+} concentration was almost consistent in both carrot cultivars, while root Ca^{2+} significantly varied and cv. DC-4 was relatively higher in root Ca^{2+} than cv. T-29 under saline regimes (Fig. 4). Rooting medium salt stress had a marked effect on leaf and root Cl^- accumulation in both carrot cultivars (Tab. 1; Fig. 4). Highest value of leaf and root Cl^- in carrot plants was observed at 150 mM NaCl. The difference between both cultivars was significant and of both carrot cultivars, cv. T-29 was relatively higher than cv. DC-4 in leaf and root Cl^- accumulation.

Leaf free proline accumulation increased significantly in both cultivars under salt stress treatments. The highest proline accumulation was observed at 150 mM NaCl (Tab. 1; Fig. 3). Of both carrot cultivars, cv. T-29 was relatively higher in proline content than cv. DC-4 under various salt regimes.

A marked increase in glycinebetaine concentration was examined in carrot plants when exposed to varying salt regimes. Cv. DC-4 accumulated relatively higher amount of glycinebetaine than cv. T-29 (Tab. 1; Fig. 3).

Leaf malondialdehyde (MDA) decreased considerably ($P \leq 0.001$) in both carrot cultivars under all salt regimes. Overall, cv. T-29 had markedly higher concentration of MDA than that in cv. DC-4, particularly under saline regimes (Tab. 1; Fig. 5).

Leaf hydrogen peroxide (H_2O_2) in both carrot cultivars increased consistently due to root growing medium salt regimes. Leaf H_2O_2

Tab. 1: Analyses of variance of data for growth, chlorophyll pigments, photosynthetic attributes, leaf fluorescence, proline, glycinebetaine, relative membrane permeability, mineral nutrients, enzymatic and non-enzymatic antioxidants of two cultivars of carrot (*Daucus carota* L.) grown for 30 days under varying NaCl levels.

Source of variation	df	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Shoot length	Root length	Chloro-phyll *a*	Chloro-phyll *b*
Cultivars (Cvs)	**1**	114.3*	0.018ns	0.368ns	0.123**	0.516ns	1.201ns	0.005***	0.012**
Salt stress (S)	**3**	777.5***	2.964***	3.116***	0.386***	157.9***	3.333**	0.0008*	0.005**
Cvs x S	**3**	9.91ns	0.045ns	0.227ns	0.068**	9.19ns	0.584ns	0.0004ns	0.0003ns
Error	**24**	19.98	0.089	0.295	0.01	7.242	0.465	0.0003	0.001
		Chloro-phyll *a/b* ratio	***A***	***E***	g_s	C_i	C_i/C_a	**WUE**	**Free proline**
Cultivars (Cvs)	**1**	0.329*	3.983ns	2.949***	1088.1ns	34871.7***	0.281***	11.15**	2048.0***
Salt stress (S)	**3**	0.164*	26.49***	0.218*	4338.3***	6127.5***	0.049***	9.88**	213.3***
Cvs x S	**3**	0.02ns	0.232ns	0.042ns	1715.4*	3635.4**	0.029**	1.648ns	0.00000008ns
Error	**24**	0.043	0.961	0.053	434.7	495.03	0.004	1.313	1.666
		Leaf GB	**Relative membrane permeability**	F_v/F_m	***NPQ***	***QP***	***NP***	**Leaf Na^+**	**Root Na^+**
Cultivars (Cvs)	**1**	0.04**	84.28ns	0.004*	0.011ns	0.0003ns	0.023**	0.781ns	2.53ns
Salt stress (S)	**3**	0.34***	971.0**	0.002ns	0.009ns	0.0033ns	0.003ns	97.66***	36.26***
Cvs x S	**3**	0.002ns	113.4ns	0.001ns	0.001ns	0.001ns	0.005ns	0.781ns	0.885ns
Error	**24**	0.007	146.9	0.001	0.004	0.001	0.003	7.614	2.395
		Leaf K^+	**Root K^+**	**Leaf Ca^{2+}**	**Root Ca^{2+}**	**Leaf Cl^-**	**Root Cl^-**	**MDA**	**H_2O_2**
Cultivars (Cvs)	**1**	73.5*	7.51ns	15.82**	136.1**	140.1ns	0.252ns	202.8***	2.33ns
Salt stress (S)	**3**	169.04***	34.34*	18.84***	23.93ns	1451.5***	72.33***	40.87*	6.335ns
Cvs x S	**3**	21.34ns	3.57ns	3.278ns	4.52ns	1.183ns	1.177ns	18.36ns	4.715ns
Error	**24**	15.25	9.75	1.757	13.33	68.32	2.935	9.598	2.178
		Total soluble proteins	**SOD**	**POD**	**CAT**				
Cultivars (Cvs)	**1**	18.49***	29.64***	0.531ns	18.16***				
Salt stress (S)	**3**	6.919***	1.448ns	1.703**	3.374***				
Cvs x S	**3**	1.851***	0.803ns	0.1ns	2.567***				
Error	**24**	0.195	0.623	0.305	0.265				

ns = non-significant; *, ** and *** = significant at 0.05, 0.01 and 0.001 levels, respectively.

contents were higher at 100 mM NaCl in cv. T-29, while in cv. DC-4 at 150 mM. However, both cultivars remained similar in leaf H_2O_2 contents under saline conditions (Tab. 1; Fig. 5).

Leaf total phenolic contents decreased in carrot cv. DC-4, while increased in cv. T-29 under all saline regimes (Fig. 5).

A marked reduction in leaf total soluble proteins of both carrot cultivars was observed due to root growing medium salt regimes. Salt-induced reduction in soluble protein content was significantly higher in cv. DC-4 than that in cv. T-29 (Tab. 1; Fig. 5).

In the present study, the activity of superoxide dismutase (SOD), a key antioxidant enzyme, decreased in both carrot cultivars under saline stress. Cultivar T-29 had significantly higher activity of SOD than that of cv. DC-4 under all salt levels (Tab. 1; Fig. 5). Leaf peroxidase (POD) enzyme activity in both carrot cultivars decreased significantly ($P \leq 0.05$) under saline conditions (Fig. 5). The salt-induced decrease in POD activity was inconsistent in both carrot cultivars under saline regimes. Salt stress had a considerable reducing effect on leaf CAT activity in cv. DC-4, whereas in cv. T-29, it remained similar to that in plants grown under non-saline conditions. Of both carrot cultivars, cv. T-29 had significantly higher activity of CAT than that of cv. DC-4 under all salt regimes (Tab. 1; Fig. 5).

Discussion

In the present study, there was a considerable decrease in shoot and root fresh and dry weights in both carrot cultivars (DC-4 and T-29) under salt stress. The salt-induced growth reduction in carrot is analogous to what has been earlier reported in different vegetable crops, e.g. turnip (Noreen et al., 2010), okra (Saleem et al., 2011), chilli (Maiti et al., 2010), cucumber (Navarro et al., 2006), eggplant (Abbas et al., 2010), radish (Noreen and Ashraf, 2009b), tomato (Frary et al., 2010), and pea (Noreen and Ashraf, 2009a). Reduction in plant growth takes place due to salt-induced alteration in various physio-biochemical characteristics (Mittler, 2002; Shi et al., 2008; Ashraf and Akram, 2009). Of various

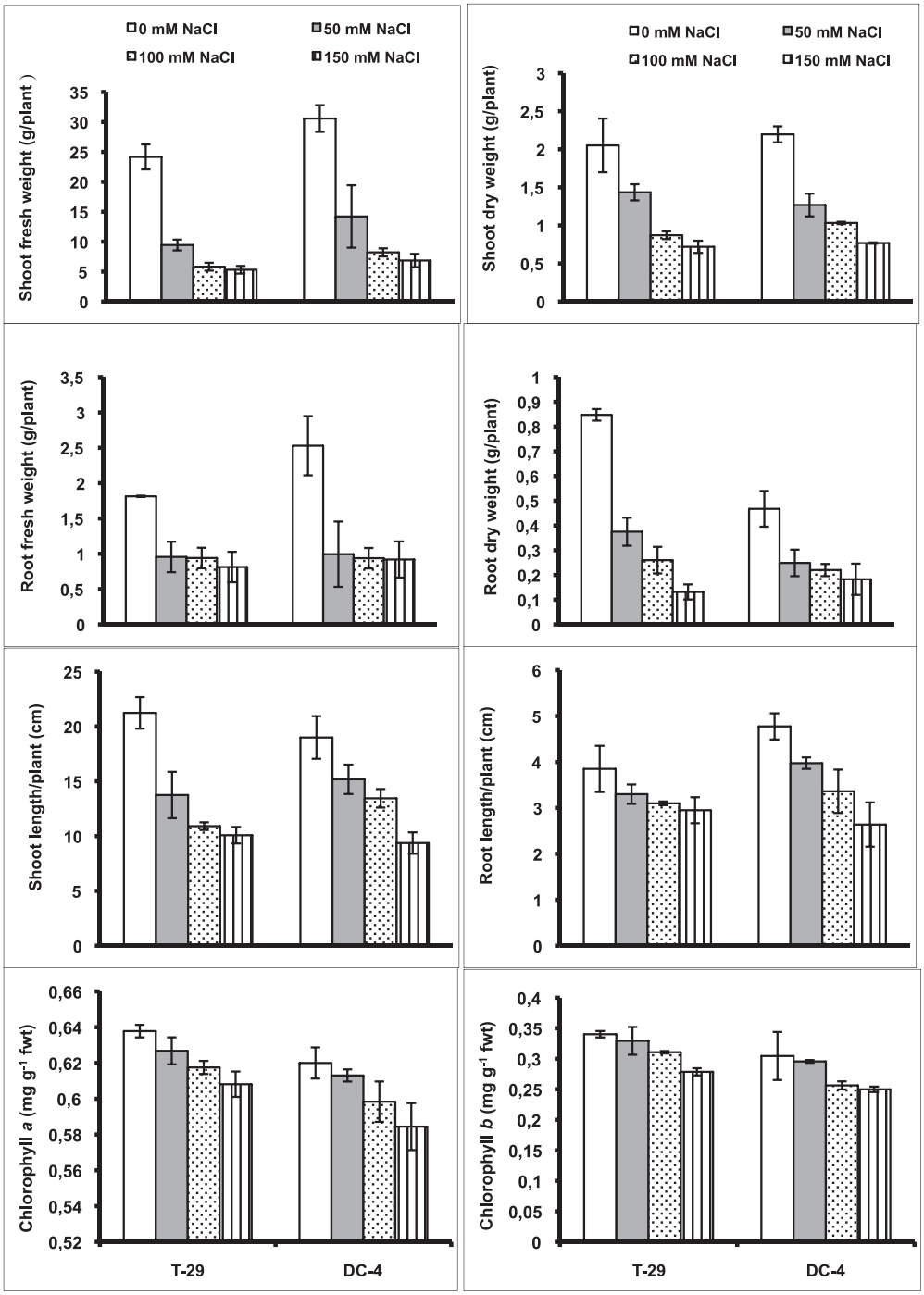

Fig. 1: Shoot fresh and dry weights, shoot and root lengths and chlorophyll *a* and *b* contents of two cultivars of carrot (*Daucus carota*) grown for 30 days under varying NaCl levels (Mean + S.E; *n*= 4).

physiological processes, photosynthesis is directly involved in plant growth and development as in this process light energy is converted into usable chemical energy, which is consumed in a variety of plant growth and developmental processes (Taiz and Zeiger, 2010). In the present study, exposure of carrot plants to saline stress showed considerable alteration in different gas exchange characteristics including photosynthetic rate (*A*), sub-stomatal CO_2 concentration (C_i), transpiration rate (*E*) and stomatal conductance (g_s). Generally, all these gas exchange characteristics decreased due to salt stress. Earlier studies reveal that salt-induced plant growth suppression is often correlated with decline in rate of photosynthesis (Noreen and Ashraf, 2008; Siddiqi et al., 2009; Akram and Ashraf, 2011; Saleem et al., 2011). For example, while screening 10 cultivars of safflower (*Carthamus tinctorius* L.) for salt tolerance, Siddiqi et al. (2009) observed a significant relationship of *A* with plant biomass under saline stress and suggested photosynthetic capacity as a potential indicator of salinity tolerance in this crop. In contrast, our results did not show such a strong relationship of photosynthetic rate with the growth of both carrot cultivars. So, it means that the differential salt tolerance of the two carrot cultivars is governed by factors other than photosynthesis.

Salinity impairs the ability of plant cells or tissues to take up water

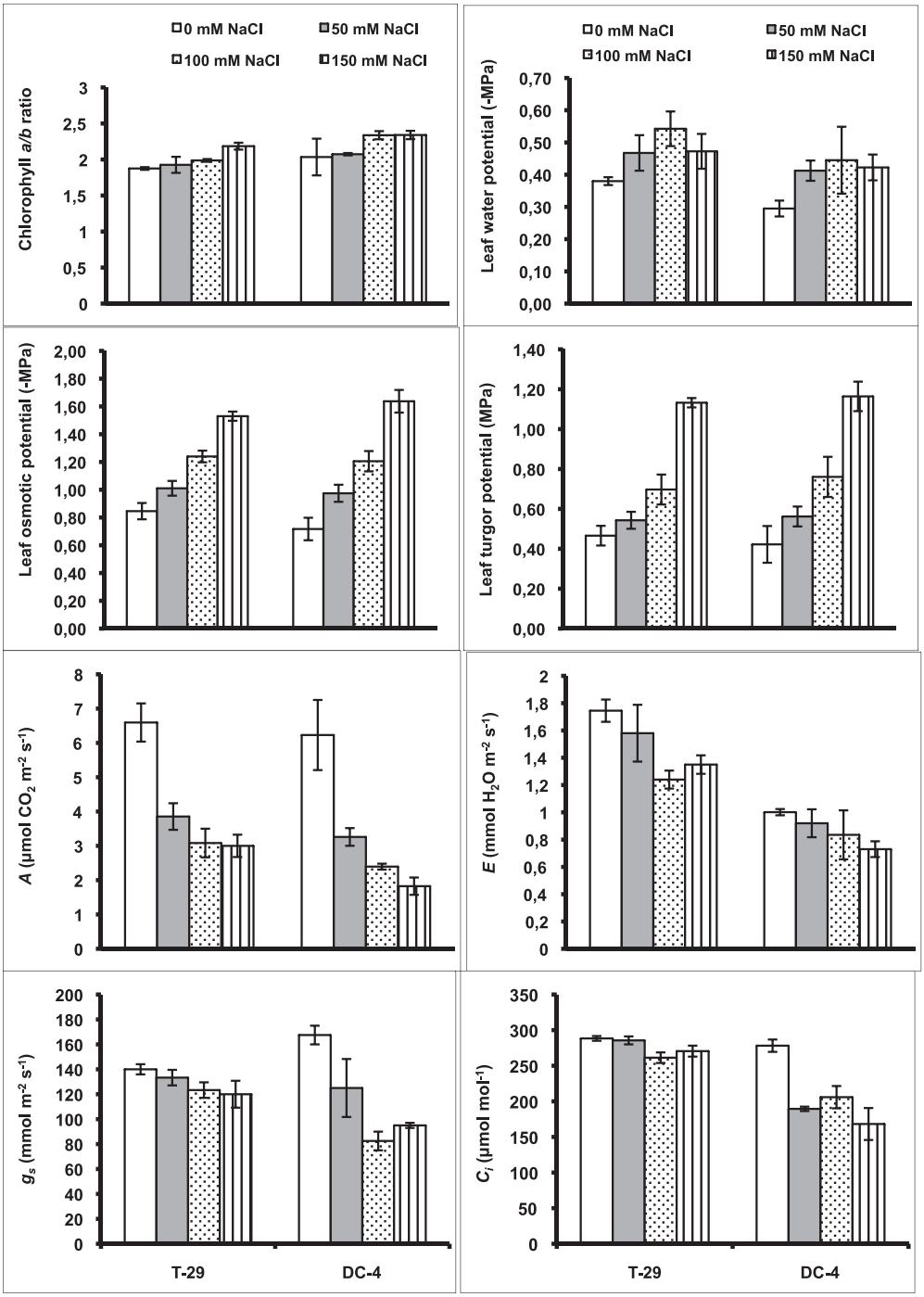

Fig. 2: Chlorophyll *a/b* ratio, water, osmotic and turgor potentials, photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i) of two cultivars of carrot (*Daucus carota*) grown for 30 days under varying NaCl levels (Mean + S.E; n= 4).

from the saline growing medium (MUNNS, 2002; ZHU, 2002). This leads to reduced tissue water potential, which in turn, adversely affects the plant growth metabolism (MELONI et al., 2001; SABIR et al., 2009). In the present study, a significant reduction in leaf water potential (Ψ_w), as well as leaf osmotic potential was observed, which could be one of the factors inducing biomass reduction in both carrot cultivars. Similarly, during a study with 18 accessions of proso millet, SABIR et al. (2009) found salt-induced reduction in leaf water potential, which ultimately suppressed the growth of proso millet plants. In another study, similar findings were reported by SIDDIQI and ASHRAF (2008) that salt stress caused reduction in shoot fresh biomass, which was attributed to suppression in leaf relative water content (RWC) and Ψ_w.

Plant cells/tissues under stress conditions exhibit different defense mechanisms to protect themselves from the adverse effects of oxidative stress caused due to over-accumulation of reactive oxygen species (ROS) (MITTLER, 2002; ASHRAF, 2009). To counteract the ROS, plants have generated a variety of non-enzymatic and enzymatic detoxification systems and protect cells from oxidative stress (YAMAGUCHI and BLUMWALD, 2005). Variation in the transcript and enzyme levels of major antioxidant enzymes during stress is well documented (AZOOZ et al., 2009). The major

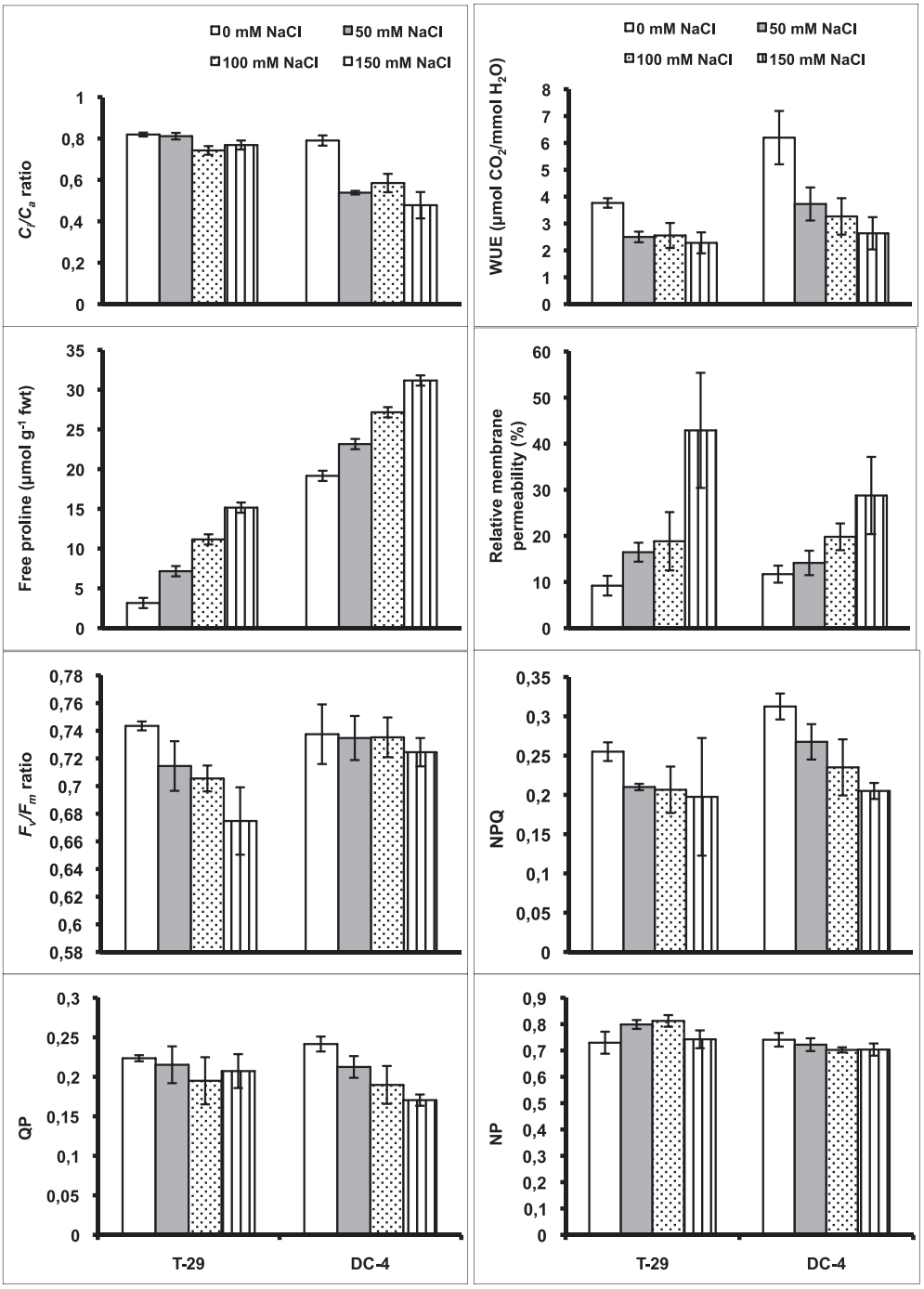

Fig. 3: C_i/C_a ratio, WUE, leaf proline, RMP, efficiency of photosystem-II (F_v/F_m), non-photochemical quenching (*NPQ*), photochemical quenching (*qP*) and co-efficient of non-photochemical quenching (*qN*) of two cultivars of carrot (*Daucus carota*) grown for 30 days under varying NaCl levels (Mean + S.E; *n*= 4).

antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (POX) and glutathione reductase (GR) can effectively detoxify ROS such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (Mittler, 2002). Recently, Azooz et al. (2009) examined the activities of antioxidant enzymes, APX, SOD, CAT and POD in maize plants and reported that salt-tolerant maize cultivars were higher in antioxidant enzyme activities as compared to those of salt sensitive ones under saline conditions. In addition, they observed a significant relationship between the activities of antioxidant enzymes and plant growth appraised in terms of dry biomass. However, contrarily, Noreen et al. (2010) found a non-significant relationship between growth and the activities of SOD, CAT and POD in six cultivars of turnip under saline conditions. Recently, Sabir et al. (2011) while screening 18 accessions of proso millet for salt tolerance found a significant enhancement in the activities of POD, CAT and SOD under saline conditions, but the relationship between antioxidant enzyme activities and yield of proso millet plants were found to be non-significant. However, in the present study, the activities of SOD, POD and CAT decreased in both carrot cultivars, but the high biomass producing cv. T-29 had significantly higher activities of CAT and SOD than those of the other cultivar under all salt regimes.

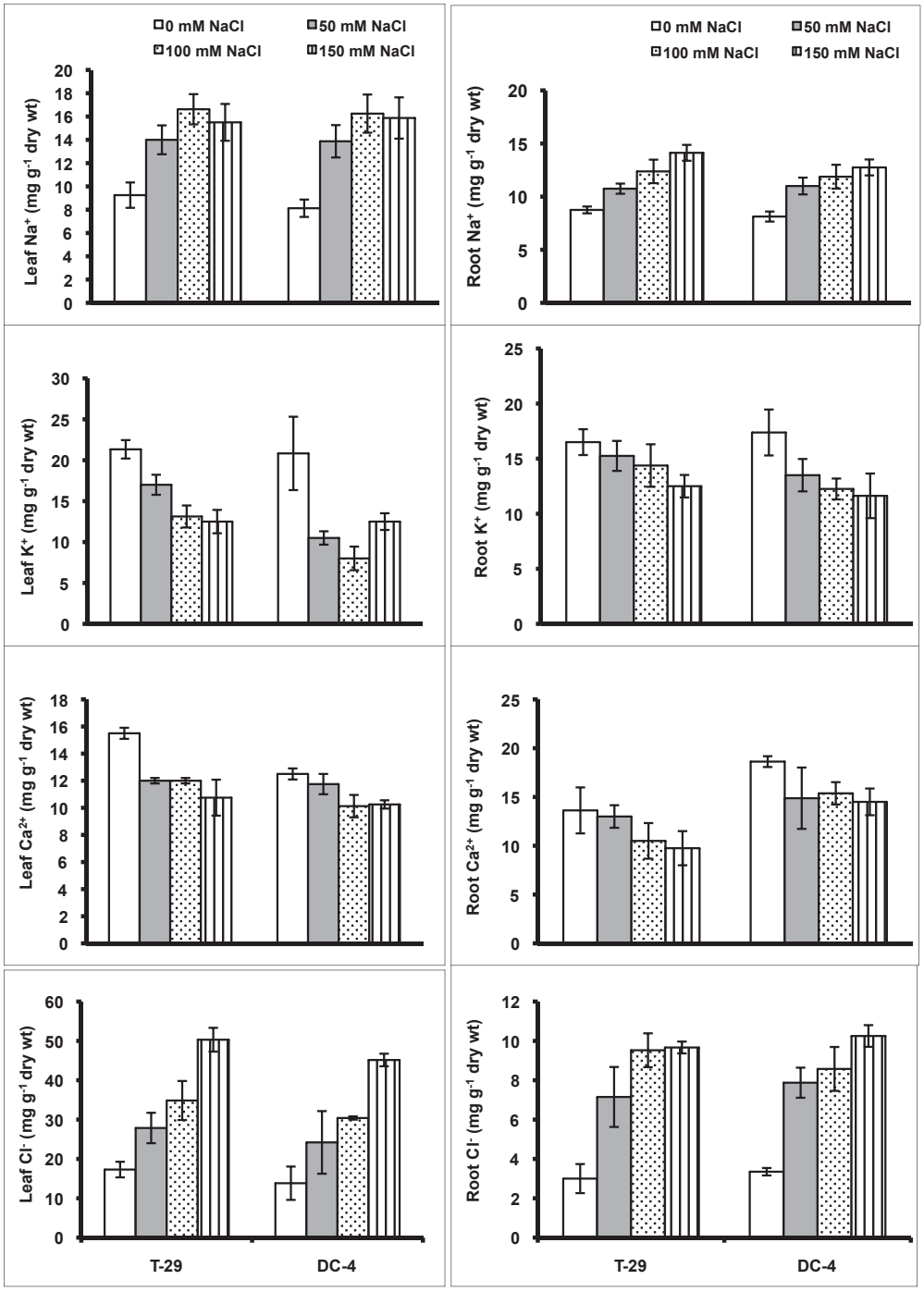

Fig. 4: Leaf and root Na^+, K^+, Ca^{2+} and Cl^- concentrations of two cultivars of carrot (*Daucus carota*) grown for 30 days under varying NaCl levels (Mean + S.E; n= 4).

In addition to enzymatic antioxidants, plants also use non-enzymatic antioxidants such as carotenoids, phenolics, tocopherols, ascorbic acid, and glutathione to scavenge ROS. Phenolics are antioxidant compounds which are highly soluble in water and have a key role in neutralizing ROS by transferring their hydrogen atoms (Sakihama et al., 2000; Frary et al., 2010). It is believed that plants with high antioxidant levels, whether induced or constitutive, have better resistance to ROS (Parida and Das, 2005; Ashraf, 2009). Plants show intricate antioxidant response when placed under saline stress, because antioxidant contents are regulated by different QTLs/genes under saline conditions (Frary et al., 2010). In this study, relatively salt tolerant carrot cultivar T-29 accumulated higher amount of total phenolics, while lower content of leaf MDA than the relatively low biomass producing carrot cv. DC-4. These findings are partially parallel to an earlier investigation on wheat in which salt-induced MDA content was more in salt sensitive wheat cv. MH-97 as compared to that in salt-tolerant S-24 under saline regimes.

Osmotic adjustment is one of the major physiological phenomena involved in stress tolerance, which involves high accumulation of organic and inorganic solutes in plant tissues/cells (Munns et al., 2006; Ashraf and Foolad, 2007; Abbas et al., 2010). In the present study, leaf proline and GB accumulation increased substantially in both carrot cultivars under saline regimes. These findings are supported by a number of reports which show a significant salt-

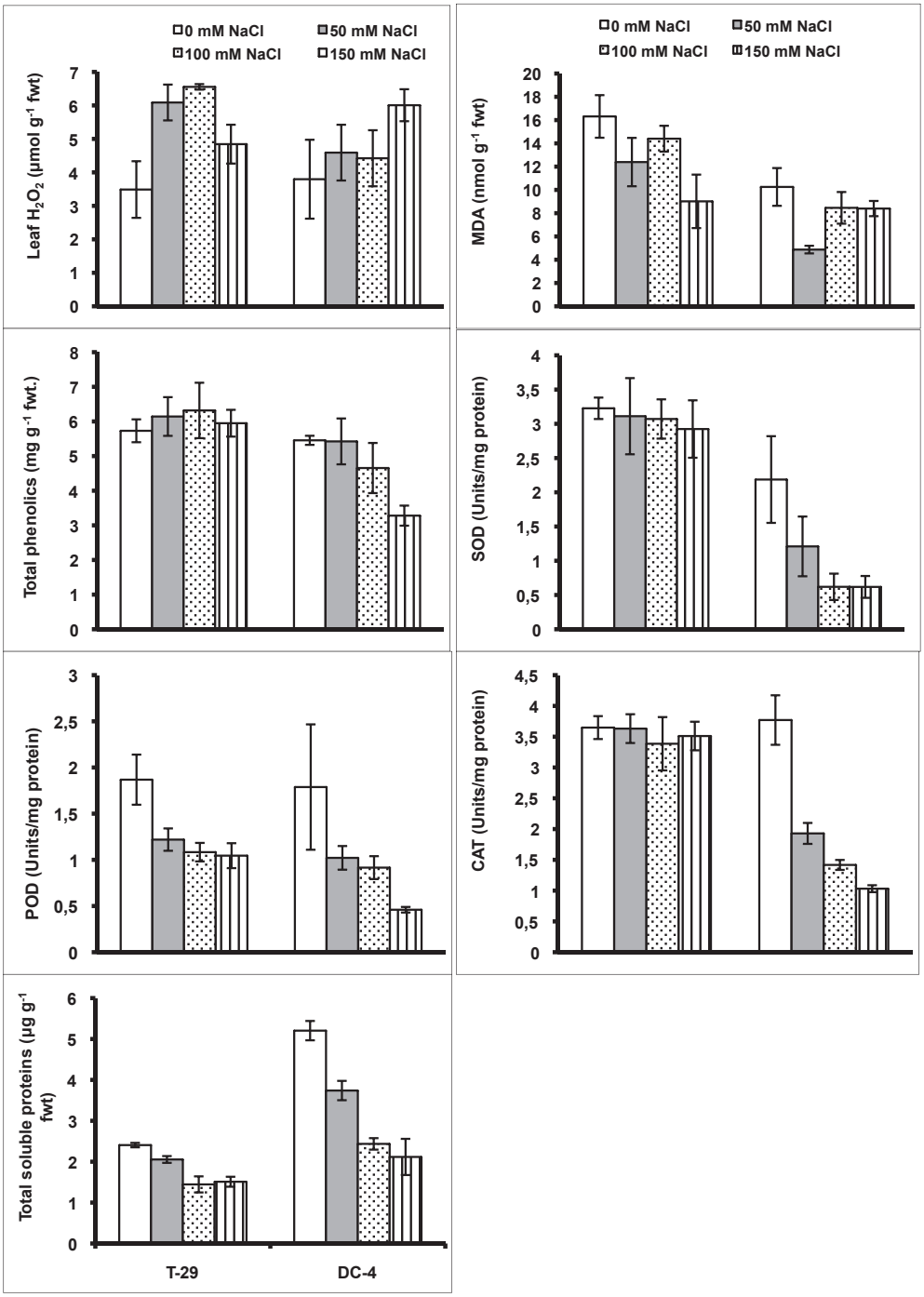

Fig. 5: Leaf H_2O_2, MDA, total phenolics, activities of SOD, POD and CAT enzymes and total soluble proteins of two cultivars of carrot (*Daucus carota*) grown for 30 days under varying NaCl levels (Mean + S.E; *n*= 4).

induced increase in both proline and GB accumulation in different plant species, e.g., proso millet (Sabir et al., 2011), okra (Saleem et al., 2011), eggplant (Abbas et al., 2010), pea (Noreen et al., 2010), and turnip (Noreen et al., 2010). In the present study, leaf and root K^+ levels decreased while Na^+ and Cl^- contents increased, a general trend of most glycophytes, under saline regimes (Munns and Tester, 2008). However, of both carrot cultivars, relatively tolerant cv. T-29 was higher in root Na^+, and leaf and root Cl^-, while, cv. DC-4 in leaf K^+ and root Ca^{2+} under saline conditions. Such type of differential inorganic ion accumulation has already been observed in a number of crop plants such as sunflower (Akram and Ashraf, 2011; Shahbaz et al., 2011), safflower (Siddiqi et al., 2011), and turnip (Noreen et al., 2010).

Overall, varying saline regimes considerably reduced the growth, chlorophyll *b* contents, leaf water potential (Ψ_w), leaf osmotic potential (Ψ_s), net CO_2 assimilation rate (A), water-use efficiency, stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), transpiration rate (E), C_i/C_a ratio, leaf and root K^+ and Ca^{2+} contents, leaf MDA, total phenolics, total soluble proteins, and activities of CAT, SOD and POD, while a considerable increase was observed in leaf turgor potential (Ψ_p), leaf and root Na^+ and Cl^- contents, leaf proline, GB, ascorbic acid (AsA), and H_2O_2 contents in both cultivars. Of both carrot cultivars, cv. T-29 was relatively higher in shoot and root fresh weights, leaf and root Ca^{2+}, leaf proline, MDA,

total phenolics, soluble proteins and activity of SOD, while cv. DC-4 was relatively higher in leaf Ψ_w and Ψ_s, leaf K^+, root Ca^{2+} and leaf GB as compared to those in the other cultivar. The relatively better growth of cv. T-29 was found to be associated with up-regulation of water relations and higher leaf Ca^{2+}, proline, phenolics and activity of SOD under saline conditions.

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Address of the corresponding author:
N.A. Akram, E-mail: nudrataauaf@yahoo.com, Tel.: +92-41-9201488