Acta Biologica Hungarica 68(1), pp. 73–87 (2017)
DOI: 10.1556/018.68.2017.1.7

PHYSICO-CHEMICAL CHANGES IN KARKADE (*HIBISCUS SABDARIFFA* L.) SEEDLINGS RESPONDING TO SALT STRESS

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(Received: August 15, 2016; accepted: October 26, 2016)

Salinity is one of the major abiotic stress factors affecting series of morphological, physiological, metabolic and molecular changes in plant growth. The effect of different concentrations (0, 25, 50, 100 and 150 mM) of NaCl on the vegetative growth and some physiological parameters of karkade (*Hibiscus sabdariffa* var. *sabdariffa*) seedling were investigated. NaCl affected the germination rate, delayed emergence and retarded vegetative growth of seedlings. The length of seedling as well as the leaf area was significantly reduced. The fresh weight remained lower in NaCl treated seedlings compared to control. NaCl at 100 and 150 mM concentrations had significant effect on the dry matter contents of the treated seedlings. The chloroplast pigments in the treated seedlings were affected, suggesting that the NaCl had a significant effect on the chlorophyll and carotenoid biosynthesis. The results showed that the salt treatments induced an increase in proline concentration of the seedlings. The osmotic potential (ψs) of NaCl treated seedlings decreased with increasing NaCl concentrations. Salt treatments resulted in dramatic quantitative reduction in the total sterol percent compared with control ones. Salt stress resulted in increase and decrease of Na^+ and K^+ ions, respectively. NaCl salinity increased lipid peroxidation. SDS-PAGE was used to evaluate protein pattern after applying salt stress. High molecular weight proteins were intensified, while low molecular weight proteins were faint. NaCl at 100 and 150 mM concentration distinguished with new protein bands. Salt stress induced a new peroxidase bands and increased the band intensity, indicating the protective role of peroxidase enzyme.

Keywords: Carotenoids – chlorophyll – karkade – peroxidase – pigments – salt stress – seedling emergence

INTRODUCTION

Plants have evolved complex mechanisms for adaptation to osmotic and ionic stresses caused by high salt. These mechanisms include osmotic adjustment by accumulation of compatible solutes, such as proline, glycine, betaine, polyols, sugar alcohols and soluble sugars, and lowering the toxic concentration of ions in the cytoplasm by restriction of Na^+ influx or its sequestration into the vacuole and/or its extrusion [44]. There are evidences showing that salinity changes photosynthetic parameters, includ-

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ing osmotic and leaf water potential, transpiration rate, leaf temperature, and relative leaf water content [47].

Salinity alters a wide array of metabolic processes in growing plants and induces changes in contents and activities of many enzymes [29, 49]. Salinity appears to affect two plant processes: water relations and ionic relations. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves [13, 24]. Salinity is expressed by a series of morphological, physiological, metabolic and molecular changes that cause delayed germination, poor stand establishment [5, 39], high seedling mortality, stunted growth and lower yields [4, 35].

Elevated NaCl causes an increase in Na^+ concentration and a decrease in K^+ and Ca^{2+} concentration [44]. In addition, accumulation of Na^+ ions changes ion balance such as Na/Ca and K/Na ratio in plant cells under saline condition. A high Na/Ca ratio results in increased cell permeability [32]. Ion disorders caused from salinity may also lead to changes in plant lipid metabolism [37]. When examining the effects of environmental stresses on plant membranes, researchers have also measured the products of lipid peroxidation, such as malondialdehyde (MDA) and/or ethane [48].

Roselle (*Hibiscus sabdariffa* L.) belongs to the family Malvaceae, locally called "karkade", is an important annual crop grown successfully in tropical and sub-tropical climates [33]. The commercially important part of the plant is the fleshy calyx (sepals) surrounding the fruit (capsules). The whole plant can be used as beverage, or the dried calyces can be soaked in water to prepare a colorful cold drink, or may be boiled in water and taken as a hot drink. It also has some medicinal properties [19]. The seeds contain 17.8–21% non-edible oil and 20% protein, and are sometimes used for animal feed [20]. The purpose of the present study was to determine some morphological, physiological and molecular changes during seed germination of Karkade (*Hibiscus sabdariffa* var. *sabdariffa*) under NaCl salinity stress.

MATERIALS AND METHODS

Plant material preparation

Germination test

Healthy and uniform seeds of Karkade (*Hibiscus sabdariffa* var. *sabdariffa*) were kindly provided by the Vegetable Crop Department, Agricultural Research Center, Ministry of Agriculture (Giza, Egypt). Surface sterilization was carried out with ethanol 70% followed by 10% commercial bleach then rinsed 3 times with sterile distilled water. Seeds were subsequently placed in sterile plates containing half-strength MS [35] medium supplemented with 2% sucrose, 0.4% agar and different concentrations (0, 50, 100 and 150 mM) of NaCl. Germination was performed in a growth chamber for two weeks under controlled environmental conditions of temperature (28 ± 2 °C),

photoperiod regime (16 h/day), irradiance intensity (50 µE m^{-2} S^{-1}) and humidity (70%). For each experiment 40 seeds were used and each experiment was made in triplicate. Daily observations were made on radicle emergence. Seed germination percentage was calculated using the following formula: Germination % = Number of germinated seeds/total number of seeds × 100. Speed of germination was calculated by the following formula given by Czabator [17]. Speed of germination = n1/d1 + n2/d2 + n3/d3 +---------. Where, n = number of germinated seeds, d = number of days.

Measurement of seedling height, fresh and dry weight and leaf area

Morphological parameters such as seedling height and fresh weight were measured in fresh samples. The fresh weight of each sample with its leaves was determined, then the samples were oven-dried at 80 °C and the dry weight was determined. Leaf area was measured by an area meter (Area Meter CI, 202).

Estimation of photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometrically (Spekol 11, Carl Zeiss, Jena, Germany) according to Metzner et al. [35].

Determination of proline

Proline was determined by the ninhydrin method described by Bates et al. [11]. In this method, proline was extracted from 0.5 g of fresh leaf tissue into 10 ml of 3% sulfosalicylic acid and filtered through Whatman No. 42 filter papers and determined in Shimadzu UV-1201 model spectrophotometer.

Estemation of sterol

Total lipids were fractionated according to the method of Farag et al. [21]. Estimation of total sterol was carried out using Perkin-Elmer 3920B gas chromatograph equipped with a flame ionization detector. A glass column (2 m × 2 mmID) packed with 3% (w/w) OV-I? of 80–100 mesh Gas-Chrom Q, was used isothermally at 240 °C. The carrier gas was helium at a flow rate of 40 ml/min. Injector and detector temperatures were 225 and 245 °C, respectively. For quantification, 5ct-cholestane was used as internal standard, and sterols were identified by cochromatography using authentic standards [1].

Determination of leaf water relations

Leaf samples were frozen in liquid nitrogen, and stored at –20 °C. Tissues were thawed and centrifuged at 1,200 × *g* for 25 min at 4 °C to extract the cell sap. Osmotic potential (ψs) of the cell sap was measured using a vapor pressure osmometer (model 5,500, Wescor, Logan, UT, USA). Osmotic adjustment (OA) was calculated as the differences in ψs between salinized and control plants.

Determination of ion content

For ions determination, fresh leaf samples were extracted in concentrated 0.1N nitric acid. N, K and Ca contents were determined by flame photometry in samples from leaves [5].

Lipid peroxide content (MDA)

Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid (TBA) reaction [27]. Frozen samples were homogenized in a pre-chilled mortar with two volumes of ice-cold 0.1% (w/v) tricloroacetic acid (TCA) and centrifuged for 15 min at 15,000 × *g*. Assay mixture containing 1 ml aliquot of the supernatant and 2 ml of 0.5% (w/v) thiobarbituric acid in 20% (w/v) tricloroacetic acid (TCA) was heated to 95 °C for 30 min and then rapidly cooled in an ice-bath. After centrifugation (10,000 × *g* for 10 min at 4 °C), the supernatant absorbance (532 nm) was read and values corresponding to non-specific absorption (600 nm) were subtracted. The MDA content was calculated according to the molar extinction coefficient of MDA (155 mM^{-1} cm^{-1}).

SDS-protein electrophoresis

Protein extraction was performed using two-week-old seedlings. SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total proteins according to the method described by Laemmli [30].

Isozyme analysis

Isozyme extraction was performed using two-week-old seedlings as well as leaf tissues from NaCl treated plants. Tissue (400 mg) was ground in 2 ml extraction buffer (0.1% (w/v) Tris-citric acid, pH 7.5; 1% (w/v) polyvinylpyrrolidone (PVP); 0.1% (w/v) ascorbic acid and 0.1% (w/v) cysteine) and centrifuged at 5333 × *g* (JS – 5.2

rotor), at 4 °C for 5 min. Twenty µl of extracted samples were used for electrophoresis on native (-PAGE) gel according to the method of Stigmann et al. [45], using a Pharmacia electrophoresis apparatus (GE-4).

Peroxidase detection

Peroxidase enzyme activity was detected by incubating the native (-PAGE) gel in darkness for one hour at 37 °C in a mixture of 15 ml of 10% benzidine (in 95% ethanol); 85 ml of 1 mM potassium acetate and 1 ml of 1% H_2O_2 (pH 4.7). After the incubation period, the gel was rinsed in distilled water and fixed in 50% glycerol for one hour.

Statistical analysis

Data were statistically analyzed by calculating the means, standard deviation (SD) and the least significant difference (LSD) using the statistical package of SAS program, version 9.1 [41].

RESULTS

Seed germination

Seed germination studies showed that NaCl stress had a significant effect on seed germination. The germination percent decreased as the result of salinity stress/ increasing NaCl concentration in medium. Seed germinated percent of the seeds treated with 25, 50, 100 and 150 mM were 83.6, 73.8, 24.9 and 11.7%, respectively. Delaying of germination was observed in the salt stress treatments. The 125 and 150 mM NaCl had a significant effect on the germination rate. Salt stress significantly affected the fresh weight and dry weight of seedlings. Elongation studies showed that NaCl had a significant effect on the lengths of seedlings (Table 1). Seedlings grown in media supplied with 25 or 50 mM NaCl were shorter, 12.63 and 7.35 cm, respectively. They could develop their secondary leaves. Seedlings grown in media supplied with 100 and 150 mM NaCl were much shorter, 3.77 and 1.54 cm, respectively. They could not even develop their secondary leaves. Seedlings could hardly growth at salinity higher than 150 mM (Table 1). The leaf area of seedlings subjected to NaCl salinity stress were significantly affected. The leaf areas of seedlings treated with 25, 50, 100 and 150 mM salt were, 27.65, 25.49, 13.08 and 7.13 mm^2, respectively (Table 1).

Table 1
Effects of NaCl on seed germination and growth of karkade seedling characteristics

NaCl treatment (mg/L)	Germination (%)	Speed of germination	Seedling fresh weight (g)	Seedling dry weight (g)	Seedling length (cm)	Leaf area (mm^2)
0.0	100	0.76^a	42.8^a	9.2^a	14.94^a	36.73^a
25	83.6	0.57^b	35.7^b	8.1^a	12.63^b	27.65^b
50	73.8	0.38^c	23.4^c	7.5^a	7.35^c	25.49^b
100	24.9	0.24^d	17.6^d	5.4^b	3.77^d	13.08^c
150	11.7	0.12^f	12.8^f	3.7^c	1.54^f	7.13^d
LSD ($\alpha < 0.05$)	11.3	0.09	2.58	1.3	1.17	3.43

The experiment was conducted twice with 3 replicates/treatment. Mean values indicated by the same latter in the same column are not significant different ($\alpha \leq 0.05$)

Table 2
Effects of NaCl on chlorophyll (a), chlorophyll (b) and carotenoid contents of karkade leaves subjected to different concentrations (0, 25, 50, 100 and 150 mM) of NaCl

Treatment of NaCl (mM)	Pigments content (mg/g fresh weight)			
	Chl a	Chl b	Total Chl	Carotenoids
Control	1.37±0.12	0.93±014	2.37±014	0.38±0.05
25	1.02±0.07	0.81±0.05	1.83±014	0.27±0.02
50	0.93±0.09	0.64±0.04	1.57±014	0.21±0.04
100	0.43±0.02	0.33±0.05	0.76±014	0.19±0.02
150	0.27±0.07	0.13±0.03	0.40±014	0.11±0.03
LSD	0.17	0.05	0.24	0.04

Data represented as means ± SD followed by LSD test at $\alpha < 0.01$.

Table 3
Effects of NaCl salinity on changes of Na^+, K^+ and Ca^{2+} ions contents and K^+/Na^+ and Na^+/Ca^{2+} ratios in karkade seedlings

NaCl (mM)	Na^+ (g/g f.w.)	K^+ (g/g f.w.)	K^+/Na^+	Cl^- (g/g f.w.)	Ca^{2+} (g/g f.w.)	Na^+/Ca^{2+}
0.0	0.430	1.453	3.38	0.570	1.643	0.261
25	2.556	0.967	0.378	2.564	1.030	2.482
50	4.346	0.845	0.194	3.432	0.920	4.724
100	5.673	0.689	0.121	4.436	0.870	6.521
150	6.123	0.545	0.089	5.053	0.630	9.719
LSD	0.050	0.030	0.030	0.010	0.030	0.020

Data represented as means followed by LSD test at $\alpha < 0.01$. f.w.: fresh weight.

NaCl effects on the photosynthetic pigments

NaCl affects on the photosynthetic pigments of leaves were examined (Table 2). Chlorophyll and carotenoid contents significantly decreased in NaCl treated seedlings. Chlorophyll b was more sensitive than chlorophyll a in the treated seedlings with 150 mM salt. Chlorophyll a and b decreased to 0.27 and 0.13 mg/g fresh weight, respectively. The total chlorophylls decreased from 2.37 mg/g fresh weight at control to 0.40 at the highest (150 mM) concentration of salinity (Table 2). Salinity had also a significant effect on carotenoid contents (Table 2).

NaCl effects on the total sterol profile

The results of this study showed dramatic quantitative reduction in the total sterol percent of the treated seedlings compared with control ones (Fig. 1). NaCl treatment significantly affected the total sterol percent in all concentrations (25, 50, 100 and 150 mM) tested (Fig. 1).

NaCl effects on ions contents

Under salinity conditions, NaCl concentrations showed a uniform increase in Na^+ ion and decrease in K^+ ion in stressed seedlings (Table 3). K^+/Na^+ ratios of karkade seedlings treated with salted solution were lower than those of controls. From the aspect of K^+/Na^+ ratios, differences among the treatments were significant. The lowest K^+/Na^+ ratio was found in 150 mM treated seedlings. The ratio of K^+/Na^+ decreased as the concentration of NaCl concentration increased. On the other hand, NaCl concentrations showed a uniform increase of Na^+ ion and decrease in Ca^{2+} ion contents in stressed seedlings. Na^+/Ca^{2+} ratios of seedlings treated with the salt containing solu-

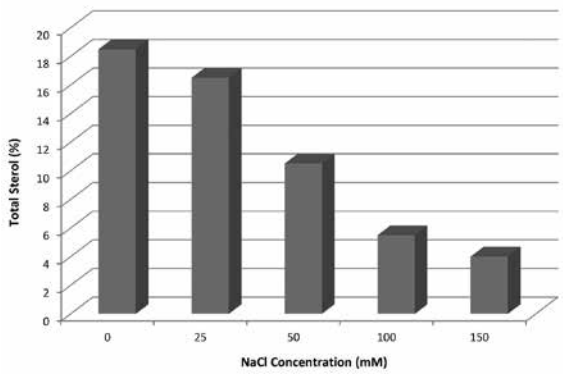

Fig. 1. Effect of different concentrations (0, 25, 50, 100 and 150 mM) of NaCl on total sterol content in karkade seedlings. Percentage was calculated relative to the total unsaponifiable matters

tion were higher than those of control ones. The ratio of Na^+/Ca^{2+} increased as the concentration of NaCl concentration increased (Table 3).

NaCl effects on the osmotic potential

The osmotic potential (ψs) of NaCl treated seedlings decreased with the increase of NaCl concentrations and the decrease was more pronounced at the highest concentration (150 mM NaCl) of salinity tested. Osmotic adjustment (OA) increased with the increase of NaCl concentration, and was more pronounced at the highest concentration (150 mM) of NaCl (Table 4).

NaCl effects on the lipid peroxide content (MDA)

Damage by NaCl to cellular membranes due to lipid peroxidation as indicated by the accumulation of the malondialdehyde (MDA) levels was observed. The results showed that MDA level was significantly increased with the increase of NaCl concentrations. NaCl treatment significantly affected the malondialdehyde (MDA) levels in all concentrations tested. MDA amounts increased as the concentration of NaCl concentration increased. These increases of MDA were very clear in the treated seedlings with 150 mM NaCl, while being the lowest in control one (Table 5).

NaCl effects on proline concentration

The results showed that the salt treatments induced an increase in proline concentration of the karkade seedlings (Table 6).

Table 4
Osmotic potential (ψs) and osmotic adjustment (O.A.) in karkade seedlings under different concentrations (0, 25, 50, 100 and 150 mM) of NaCl

	NaCl concentration (mM)				
	0.0	25	50	100	150
Ψs	2.97	–3.79	–5.21?	–6.45	–8.94
OA	–	1.42	2.66	5.15	6.43

Table 5
Effects of NaCl salinity on MDA contents in karkade seedlings

	NaCl concentration (mM)				
	0.0	25	50	100	150
MDA mol g FW	2.453^a	2.765^a	3.062^b	4.439^c	5.536^d

Mean values indicated by the same lower-case letters are not significantly different ($\alpha \leq 0.01$).

Table 6
Effects of NaCl on proline concentration of karkade seedlings treated with different concentrations (0, 25, 50, 100 and 150 mM) of NaCl

	NaCl concentration (mM)				
	0.0	25	50	100	150
Proline (μ mol g^{-1}) fresh weight	0.806^{a}	2.546^{b}	3.876^{c}	5.812^{d}	7.536^{af}

Mean values indicated by the same letter are not significantly different ($\alpha \leq 0.01$).

Protein profiles of the salt treated karkade seedlings

The protein profiles of the salt treated karkade seedlings are presented in Table 7. Comparing the protein profiles between control plants and those treated with different salt concentrations using SDS-PAGE showed that NaCl treatment induced changes in the protein patterns. It was found that the staining intensity of some protein bands was increased in salt-treated plants. Seedlings treated with 100 and 150 mM NaCl showed

Table 7
SDS-PAGE of total protein extracted from the leaves of salt treated karkade seedlings [Lane 1: Protein marker, lanes 2–6 are the treated seedlings with different concentrations (0, 25, 50, 100 and 150 mM) of NaCl]

Band No.	MW (KDa)	NaCl concentration				
		0	25	50	100	150
1	141.40	+	+	+	+	+
2	117.75	+	+	+	+	+
3	106.11	+	+	+	+	+
4	100.19	+	+	+	+	+
5	90.42	+	+	+	+	+
6	56.73	+	+	+	+	+
7	48.42	–	–	–	+	+
8	42.81	+	+	+	+	+
9	37.09	–	–	–	+	+
10	32.00	+	+	+	+	+
11	29.90	+	+	+	+	+
12	26.55	–	–	+	+	+
13	23.00	–	–	–	+	+
14	20.13	–	–	–	+	+
15	17.15	+	+	+	+	+
16	14.40	+	+	+	+	+

Table 8
RF values for peroxidase isozyme of the control (0 mM NaCl) and salt treated (25, 50, 100 and 150 mM NaCl) karkade seedlings as analyzed using gel documentation program software

Rf	NaCl concentration mM				
	0.0	25	50	100	150
0.37	+	+	+	+	+
0.42	+	+	+	+	+
0.48	–	–	+	+	+
0.55	–	–	+	+	+

RF = Relative mobility of bands, (+) Presence of bands and (–) absence of bands.

higher staining intensity of bands compared with those of control ones. The salt treated seedlings were distinguished with five new bands of about 20.13, 23, 26.55, 37.09 and 48.42, respectively.

Peroxidase profiles of the salt treated karkade seedlings

The peroxidase Rf values of the salt treated karkade seedlings (Table 8 and Fig. 2). Showed that salt stress increased the staining intensity of bands and induced some new bands. Treated seedlings with 100 and 150 mM NaCl showed higher staining intensity of bands compared with those of other ones. The salt treated seedlings with 50, 100 and 150 mM NaCl were distinguished with two bands of about 0.48 and 0.55 Rf values, respectively (Table 8 and Fig. 2).

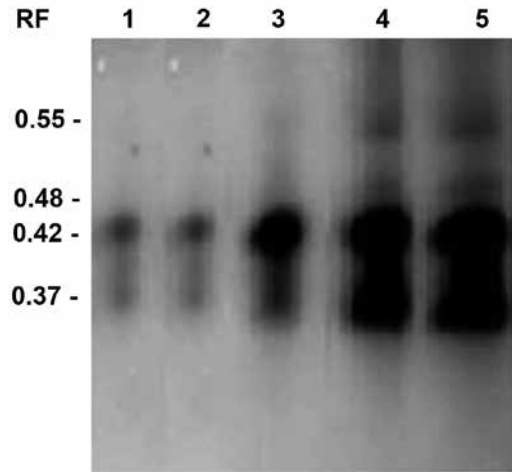

Fig. 2. Peroxidase isozyme profile of control (0 mM NaCl) and salt-treated (25, 50, 100 and 150 mM NaCl) karkade seedlings. Lane 1, Control and Lanes 2–5, salt-treated seedlings

DISCUSSION

Most of the cultivated plants are sensitive to salt-stress, in which NaCl salinity has harmful effects on growth and development of plants. The reduction in germination process relies on salinity may be related to its negative effect on the physiological processes influencing seed germination [8, 13]. Andriolo et al. [6] reported that salinity is the main cause of reduction the percentage and homogeneity of germination and dry weight of plants. Salinity delays plant growth by reducing photosynthesis effects. It includes closing stomata, reduction of water entrance into plants and a two-fold reduction of plant weight [2]. Comparison of seedling lengths under salinity at different levels showed that when the salinity level increases length of seedlings decreases. In this relation it was reported that salinity reduces the growth of seedlings and the rate of reduction increases with increasing the level of salinity [8]. In the present study it was observed that salinity suppressed growth of seedlings. The suppression of growth may be related to the osmotic pressure which resulted in the limitation of water absorption by germinating seeds, subsequently the cell division, differentiation and length of seedlings decreased. Similar result was obtained by Savvas and Lenz [42], who reported that the reduction in fresh weight duo to salt stress is a normal phenomenon and it is related to the limitation of water uptake by germinating seeds. Leaf area was decreased. This might partially be attributed to the lower leaf water potential and a reduction in relative leaf water content, resulted in loss of turgor, which in turn causes stomatal closure and limits CO_2 assimilation and reduced photosynthetic rate. This result agrees with the finding of Turner [47]. A marked growth reduction was also reported earlier to seedlings exposed to salt stress [36]. Some researcher have reported that vegetative growth of plants, i.e. shoot length, plant leaf area, fresh weight and dry weight were significantly reduced at salinities higher than 50 mM [2, 6, 36]. The results of this study demonstrate inhibition of the seed germination and reduction the growth of seedlings when expose to increase in salinity level. This study showed that photosynthetic pigment concentrations were reduced under the effect of salt stress. The decrease in Chl and tetrapyrroles content along with the increase of NaCl was also showed by Savvas and Lenz [42]. The results of this study are in agreement with those of Dagar et al. [18]. The decrease in chlorophyll concentration in salinized plants may be attributed due to activity increase in chlorophyll degrading enzyme chlorophyllase [3, 39]. Ion accumulation in the leaves also adversely affected chlorophyll concentration [3, 28]. The decrease in carotenoids under salt stress leads to degradation of β-carotene and formation of zeaxanthins, which are apparently involved in protection against photo inhibition [27, 43]. As salinity adversely influenced the photosynthetic process, photosynthetic production (e.g. sugar) was inhibited [18, 42]. It was found also that sugar content of leaves decreased in seedlings under NaCl stress [14]. One of the most important mechanisms by higher plants under salt-stress is the accumulation of compatible solutes such as proline. Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in the cells. Several reports show a significant role of proline in osmotic adjustment, protecting cell structure and its function in plants in

salt-tolerant and salt-sensitive cultivars of many crops [5, 9, 15]. On the other hand, a positive correlation was determined between proline and tissue Na concentrations under salt stress [9, 10]. Plants sterols are important components of the plant cell membrane regulating the permeability and modulating the activity of bond enzymes required for plant growth and development [23, 26]. Sterol regulates the activity of the Na^+/K^+-ATPase in plant cells [23]. The osmotic effect resulting from soil salinity may cause disturbances in water balance of plant and inhibiting growth as well provoking stomata closure and reducing photosynthesis [3, 13]. Plants respond by means of osmotic adjustment, normally by increasing the concentrations of Na^+, although such accumulation of inorganic ions may produce important toxic effects and cell damage and inactivate both photosynthetic and respiratory electron transport [43, 44]. This limited osmotic adjustment was not sufficient to avoid water stress in the treated plants [5, 28]. It was observed that high Na/Ca ratio results in increased cell permeability [31, 49]. Ion disorders caused by salinity was obtained when NaCl was applied in soil; the levels of K in plant were reduced in accordance with the antagonism between Na^+ and K^+ [7, 49]. It was shown that excess of NaCl leads to the loss of K due to membrane depolarization by Na ions [18, 44]. The increase in Na^+ ion content and decrease in K^+ ion uptake disturbs ionic imbalance as observed in most species exposed to salt stress [34]. The diminution of K^+ concentration in tissue may be related to the direct competition between K^+ and N^+ at plasma membrane. Inhibition of Na^+ on K^+ transport process in xylem tissues and/or Na^+ induced K^+ efflux from the roots [49]. High Na^+ accumulation in salt-sensitive foxtail millet cultivar and in lettuce roots have been reported [31], resulting in enhanced membrane damage, electrolyte leakage and oxidative damage. Wilson et al. [48] indicated that osmotic adjustment, accounted for decreases in the fresh weight and dry weight contents, increases in apoplastic water content and direct solute accumulation. MDA is produced when polyunsaturated fatty acids in the membrane undergo oxidation by the accumulation of free oxygen radicals. Lipid peroxidation is ascribed to oxidative damage and is often used as an indicator of increased damage [13, 24]. Comparing the protein profiles between control plants and those treated with different salt concentrations, using SDS-PAGE, showed that NaCl treatment induced changes in protein pattern. It was shown that the intensity of some protein bands was increased in salt-treated plants. The results obtained in this study are in agreement with those of Farida et al. [22], who reported that salt-stress induce alterations in protein profile and protease activity in mangrove (*Bruguiera parvi*). Peroxidases are enzymes related to polymer synthesis in the cell wall, as well playing an important role in prevention the oxidative damage caused by environmental stress to the membrane lipids [12]. Our results showed that salt stress elicits oxidative stress, measured as lipid peroxidation, as it has been seen in other species [32]. Salt stress increased peroxidase bands intensity and induced the appearence of some new bands. This may be associated with the potential role of this peroxidase in protecting lipid peroxidation. Salinity increases the content of H_2O_2 and induces oxidative stress in plant tissues [34]. Membrane injury under salt stress is related to increased production of highly toxic reactive oxygen species [28]. The results of our study are in agreement with those of Gaspar et al. [25], who

reported that the increase in peroxidase due to salt, could be responsible for the ability to adapt to external stimulus. There are reports that salinity disrupts membrane permeability by peroxidation of the lipid membrane [12, 14, 27]. Salinity also increases the content of H_2O_2 and induces oxidative stress in plant tissues [40, 49]. Membrane injury under salt stress is related to increased production of highly toxic reactive oxygen species [28]. Our study proved that salt stress has a harmful effect on some morphological, physiological and molecular processes of seed germination in karkade (*Hibiscus sabdariffa* var. *sabdariffa*), injuring and/or influencing germination rate, vegetative growth, and photosynthetic pigments, sterol contents, the osmotic potential and protein profile of the plant.

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