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Influence of NaCl treatments on growth and biochemical parameters of castor bean (*Ricinus communis* L.)

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ABSTRACT

Castor bean (*Ricinus communis* L.) is widely cultivated throughout the world for use as a medicinal plant and oil consumption purposes; however its salt tolerance has not been clarified. To investigate the effect of salt stress on its growth and on activity of antioxidative enzymes in different organs, castor bean plants at the 4-leaf stage were subjected to 50, 100 and 200 mM NaCl admixed to Hoagland's solution for 10 days under greenhouse conditions. The results showed that salt stress inhibited plant growth (root and shoot length, fresh root and shoot weight) but root growth was more affected than shoot. Relative water content of leaves and the membrane stability of the leaves were decreased with increasing NaCl concentration. The activity of guaiacol peroxidase (GPX) and catalase (CAT) was sharply decreased by escalation of salt stress. However activity of ascorbate peroxidase (APX) was enhanced under moderate salt stress (100 mM NaCl) in both root and shoot but then decreased with increased NaCl concentration. The activity of superoxide dismutase (SOD) increased with the increase of the concentration of NaCl in shoots and root. However alternation in enzymatic antioxidant activity was noticed in shoot compared to root. Increased H₂O₂, total soluble protein, proline content and malondialdehyde (MDA) concentration in both plant's organs was linearly and positively correlated with increasing NaCl concentration. The results of this study suggest that the salt sensitivity of Castor bean plant under salt stress conditions is probably due to a lack of efficient activity of CAT and GPX probably lead to imperfect H₂O₂ scavenging.

Key words: Castor bean, salt stress, oxidative stress, APX, CAT, H₂O₂, lipid peroxidation, MDA

IZVLEČEK

VPLIV TRETMAJA Z NaCl NA RAST IN BIOKEMIČNE PARAMETRE NAVADNEGA KLOŠČEVCA (*Ricinus communis* L.)

Navadni kloščevca (*Ricinus communis* L.) se v svetovnem merilu pogosto goji kot zdravilna rastlina ali zaradi uporabnega olja, njegova toleranca na solni stres pa še ni bila raziskana. Za preučevanje vpliva solnega stresa na rast in aktivnost antioksidacijskih encimov smo navadni kloščevca izpostavili v razvojni fazi 4 listov koncentracijam NaCl 50, 100 in 200 mM, raztopljenih v Hoaglandovi raztopini in rastline gojili deset dni v rastlinjaku. Rezultati so pokazali, da je solni stres inhibiral rast rastlin (dolžino korenin in poganjkov in njihovo svežo težo), vendar je bila rast korenin bolj inhibirana. Relativna vsebnost vode in integriteta membran listnega tkiva sta upadali z naraščajočo koncentracijo NaCl. Aktivnost gvajakol peroksidaze (GPX) in katalaze (CAT) je močno upadla po povečanju solnega stresa. Aktivnost askorbat peroksidaze (APX) se je povečala v razmerah zmernega solnega stresa (100 mM NaCl) v koreninah in poganjku, vendar je s povečevanjem koncentracije NaCl potem upadla. Aktivnost superoksid dismutaze (SOD) se je s povečevanjem koncentracije NaCl povečevala v koreninah in poganjku. Kljub temu je bilo v poganjku opaziti večje spremembe v encimski antioksidacijski aktivnosti v primerjavi s koreninami. Vsebnost H₂O₂, celokupnih topnih proteinov, prolina in malondialdehida (MDA) se je v obeh organih linearno povečevala z naraščajočo koncentracijo NaCl. Rezultati te raziskave kažejo, da je občutljivost navadnega kloščevca na solni stres posledica nezadostne aktivnosti encimov CAT in GPX, kar verjetno vodi do nepopolne presnove H₂O₂.

Ključne besede: navadni kloščevca, solni stres, oksidacijski stres, APX, CAT, H₂O₂, peroksidacija lipidov, MDA

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1 INTRODUCTION

Salty soils extensively exist in arid and semi-arid climate regions of the world and cause salt stress in plants (Khan et al., 2001). Salinity is an important abiotic stress factor seriously affecting plant productivity and survival. The ability of vegetation to survive under higher salinity conditions is important for the distribution of plants and agriculture around the world. Enhancing the salt tolerance of plants is an important breeding objective in areas, which are affected by soil salinity (Flowers and Flowers, 2005). A plant's ability to acclimate to salt stress includes alterations at the leaf level, associated with morphological, physiological and biochemical characteristics whereby many plants adjust to high salinity and the consequent low soil water availability (Munns, 2002). Morphologically the most typical symptom of saline injury to plant is reduction of growth (Azooz et al., 2004), which is a consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, photosynthetic efficiency, carbon allocation and utilization (Ismail 2003; Taylor et al., 2004).

From physiological and metabolic aspects salt stress can also stimulate formation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals. ROS injure the cellular components of proteins, membrane lipids and nucleic acids (Foyer et al., 1994). Evidence suggests that membranes are the primary sites of salinity injury to cells and organelles (Candan and Tarhan, 2003) because ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasma membrane or in the membranes of organelles (Karabal et al., 2003). Malondialdehyde (MDA), a product of lipid peroxidation indicates well the oxidative stress and can be used as a tool for determining salt tolerance in plants (Yildirim et al., 2008). To scavenge ROS, plants possess specific mechanisms, which include activation of antioxidant enzymes and non-enzymatic antioxidants such as, carotenoids and ascorbic acid (Mittler, 2002).

The enzymatic antioxidant system is including superoxide dismutase (SOD: EC 1.15.1.1), which can be found in various cell compartments and catalyses the disproportion of two O_2^- radicals to H_2O_2 and O_2 (Scandalios, 1993). H_2O_2 is eliminated by various antioxidant enzymes such as catalases (CAT: EC 1.11.1.6) (Scandalios 1993) and guaiacol peroxidase (GPX: EC 1.11.1.7) (Gara et al., 2003) which convert H_2O_2 to water. Ascorbate peroxidase (APX: EC 1.11.1.11) also play a key role by reducing H_2O_2 to water through the Halliwell–Asada pathway (Noctor and Foyer, 1998). However, under salt stress, the mechanisms developed by Castor bean plants to scavenge ROS are still poorly understood.

Castor bean (*Ricinus communis* L) is a crop plant of commercial relevance since its oil is used for manufacturing surfactants, coatings, greases, fungistats, pharmaceuticals, cosmetics, and many other products. Most part of castor bean is grown in semiarid regions where salinity stress may affect germination and plant growth intensively (Pinheiro et al., 2008). Plant with high constitutive and induced antioxidant levels has better resistance to damage (Parida and Das, 2005). The degree of damage by ROS depends on the balance between the product of ROS and its removal by these antioxidant scavenging systems (Khan and Panda, 2008). However, to the best of our knowledge, the antioxidant responses of Castor bean to salt stress have not been reported.

The aim of this work was to study the effects of different concentrations of salt on the growth, lipid peroxidation and antioxidant enzyme activities (e.g., CAT, POX, GPX & APX) of Castor bean plant and to analyze the trends of these parameters under different levels of salinity stress. This may be helpful in developing a better understanding of tolerance threshold and provide additional information on the mechanisms of salt tolerance.

2 MATERIAL AND METHODS

2.1 Trials protocol

Seeds of castor bean (*Ricinus communis* L.) were obtained from local seed mass, Isfahan, Iran. Homogenous seeds of were surface sterilized using 5% sodium hypochlorite solution for 5 min and then rinsed 3 times with sterile distilled water. Seeds were germinated in 120 mm covered Petri dishes on two layers of filter paper moistened with 15 ml of distilled water and then approximately 20-25 seedlings were planted onto plastic trays covered with cheesecloth containing half-strength Hoagland's solution. Nutrient solution was permanently aerated and renewed 2-3 times a week to minimize a pH shifts and nutrient depletion. Hydroponics were kept in a growth room (approximately 14h light/10 h dark) providing white fluorescent light and natural light with an photosynthetically

active radiation of $250 \mu\text{mol m}^{-2}\text{s}^{-1}$, day/night temperature of $25 \pm 2 \text{ }^\circ\text{C}$ / $15 \pm 2 \text{ }^\circ\text{C}$ and $60 \pm 5 \%$ relative humidity. The plants were grown in normal growth conditions until 4-5 leaf stage. Then, twenty-three-day old plants were treated with Hoagland's solution containing 50, 100 and 200 mM NaCl and maintained for 10 days in these conditions. Control plants were kept in Hoagland solution without NaCl. After treatment for 12 days, the castor bean plants were sampled (leaf and root). The plants were first washed with tap water, and then with distilled water. Roots and shoots were separated and the fresh weight was determined for each plant. Portion of the fresh samples were taken to measure the physiological indices and some of them transferred to liquid nitrogen and maintained at -80°C for future extraction.

2.2 Measurement of plant water status

Five fresh leaves of same size and same age of five plants from each treatment were collected and weighted (Fw). Leaf segments were kept immersed in distilled water for 24 h at room temperature in the dark. The turgid weight (Tw) of leaves were measured and then oven-dried at 80°C for 72 h until constant weight and reweighing (Dw). The fresh weights, turgidity and dry weights of the leaf segments were used to determine the hydration and relative water content according to Sangakkara et al. (1996). Hydration was determined as $H (\%) = 100 - 100 (Dw / Fw)$. The relative water content (RWC) was determined as $RWC (\%) = [(Fw - Dw) / (Tw - Dw)] \times 100$.

Leaf Membrane Stability Index (MSI) was measured as ion leakage, the washed leaves were cut into 1 cm pieces and placed in a glass beaker containing 10 mL deionised water. The beakers were kept at 30°C for 3 h and the conductivity of solution was measured by a conductivity meter. The same samples were boiled for 2 min and then their conductivity was measured again, when the solution was cooled to room temperature. The percentage of Membrane Stability was calculated as follows, $MSI (\%) = \{1 - (C1/C2)\} \times 100$. Where C1 and C2 are the electrolyte conductivities measured before and after boiling, respectively.

2.3 Total soluble protein and proline content assay

Total soluble protein content was measured according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard. Fresh leaf samples (1 g) were homogenized with 4 mL Na-Phosphate buffer (pH 7.2) and then centrifuged at 4°C. Supernatants and dye were pipetting in spectrophotometer cuvettes and absorbance was measured using a UV-vis spectrophotometer (PG instruments T80) at 595 nm. Proline was extracted and its concentration determined by the method of Bates et al. (1973). Leaf tissue was homogenized in sulfosalicylic acid, and the homogenate was centrifuged at $3000 \times g$ for 20 min. The supernatant was treated with acetic acid and acid-ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined.

2.4 Enzyme extraction

For SOD, CAT and GR extraction, leaf and root samples (0.5 g) were homogenized in ice cold 0.1 M phosphate buffer (pH=7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at $15000 \times g$. The supernatant was used for enzyme activity assay (Esfandiari et al., 2007).

2.5 Enzyme activity assay

SOD activity was estimated by recording the decrease in absorbance of superoxidenitro blue tetrazolium complex by the enzyme (Sen-Gupta et al., 1993). About 3 ml of reaction mixture, containing 0.1 ml of 200 mM methionine, 0.01 ml of 2.25 mM nitro-blue tetrazolium (NBT), 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1 ml

distilled water and 0.05 ml of enzyme extraction, were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 ml riboflavin (60 µM) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed maximal color. A non-irradiated complete reaction mixture which did not develop color served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

CAT activity was measured according to Aebi (1984). About 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH=7), 0.5 ml of 75 mM H₂O₂, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. Reaction started by adding H₂O₂ and decrease in absorbance recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of H₂O₂ decomposed. APX activity was measured according to Yoshimura et al. (2000) by monitoring the rate of ascorbate oxidation at 290 nm ($E=2.8\text{mM}^{-1}\text{cm}^{-1}$). The reaction mixture contained 25 mM phosphate buffer (pH=7), 0.1 mM EDTA, 1 mM H₂O₂, 0.25 mM AsA and the enzyme sample. No change in absorption found in the absence of AsA in the test medium.

Guaiacol peroxidase was determined by measuring the oxidation of guaiacol. The assay mixture contained 10 mM/L potassium phosphate (pH 6.4), 8 mM/L guaiacol, and 2.75 mM/L H₂O₂. The increase in absorbance was recorded at 470 nm within 2 min (linear phase) after the addition of H₂O₂ (Huang et al., 2006). Malondialdehyde (MDA) was measured by colorimetric method (Stewart and Bewley, 1980). 0.5 g of leaf samples were homogenized in 5ml of distilled water. An equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid solution was added and the sample incubated at 95°C for 30 min. The reaction stopped by putting the reaction tubes in the ice bath. The samples then centrifuged at $10000 \times g$ for 30 min. The supernatant removed, absorption read at 532 nm, and the amount of nonspecific absorption at 600 nm read and subtracted from this value. The amount of MDA present calculated from the extinction coefficient of $155 \text{mM}^{-1}\text{cm}^{-1}$. Enzyme activity and MDA content of samples were recorded with duplication.

2.6 Statistical analysis

Primary statistical analyses such as frequency distribution and normality tests were conducted. Normality test of the data was assessed using the Anderson-Darling normality test (Minitab, 14.0) and homogeneity test of variances with Levene test. Data were determined by analysis of variance and differences between treatment means were separated by the least significant difference (LSD) at a 0.05 probability level. All the statistical analyses were carried out using version 14 (SPSS Institute, 2004) and MINITAB version 14 (2005) software.

3 RESULTS

Relative to control plants, growth of castor bean plant was drastically reduced due to NaCl at all tested concentrations (Table 1). The dry matter production of the different organs (root and shoot) differed in their response to salinity stress. The reduction percentage in dry weight was averagely higher in root (about 78%) than of shoot (56%) as compared with control plants displayed a highly significant reduction in dry matter of different organs at the most salinization levels as compared with the control. Leaf area was also significantly smaller than control at higher NaCl concentration by 81%. In addition, the relative water content (RWC) of leaf was decreased from 96.4% in

control to 78.6, 74.3 and 66.5% at 50, 100 and 200 mM NaCl treatment, respectively (Table 1). In this connection at the end of the experimental period, the leaves of NaCl treated plant showed a visual symptoms (chlorosis and necrotic). To examine the biochemical responses to salt stress, we first determined the effect of NaCl on soluble protein content in castor bean plants. As a result to the exposure to NaCl, soluble protein content showed a significant increase trend with the increase of the concentration of NaCl (Fig. 1). Soluble protein increased by 23.4, 54.6 and 65% in shoots, 14.53, 45.15 and 73% in roots, at 50, 100 and 200 mM NaCl, respectively.

Table 1.: Dry weight (Dw) of root and shoot (g plant^{-1}), percentage water content (Wc%), leaf area (cm^{-2}), percentage relative water content (RWC%) and membrane stability index of castor bean (*Ricinus communis* L) in response to different NaCl concentrations

NaCl concentration	Root		Shoot		Leaf		
	Dw	WC%	Dw	WC%	Area	RWC	MSI
Control	0.60±0.045	93.0±5.25	2.75±0.254	92.8±5.24	22.57±1.41	96.4±7.71	73±7.42
50	0.39±0.050	88.1±6.85	2.43±0.243	87.6±8.10	13.41±1.25	78.6±7.09	59±8.07
100	0.35±0.045	73.5±10.68	1.64±0.237	79.4±5.74	8.77±1.29	74.3±6.04	41±8.54
200	0.13±0.037	69.8±6.45	1.22±0.177	76.2±8.63	4.29±1.03	66.5±7.96	39±7.69
LSD at 5%	0.154	19.56	0.691	13.24	4.38	8.22	27.41

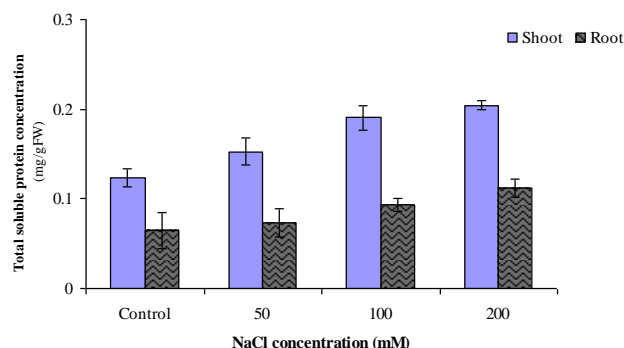


Figure 1.: Effect of NaCl stress on total protein concentration in root and shoot of castor bean (*Ricinus communis* L.) plant. The values and standards errors (vertical bars) of three replications are shown.

For better understanding the interrelationships among the mentioned traits, simple correlation coefficients were computed (Table 2). The Dw had significant positive correlation with the other traits. Also, similar results were seen for all of studied traits which were

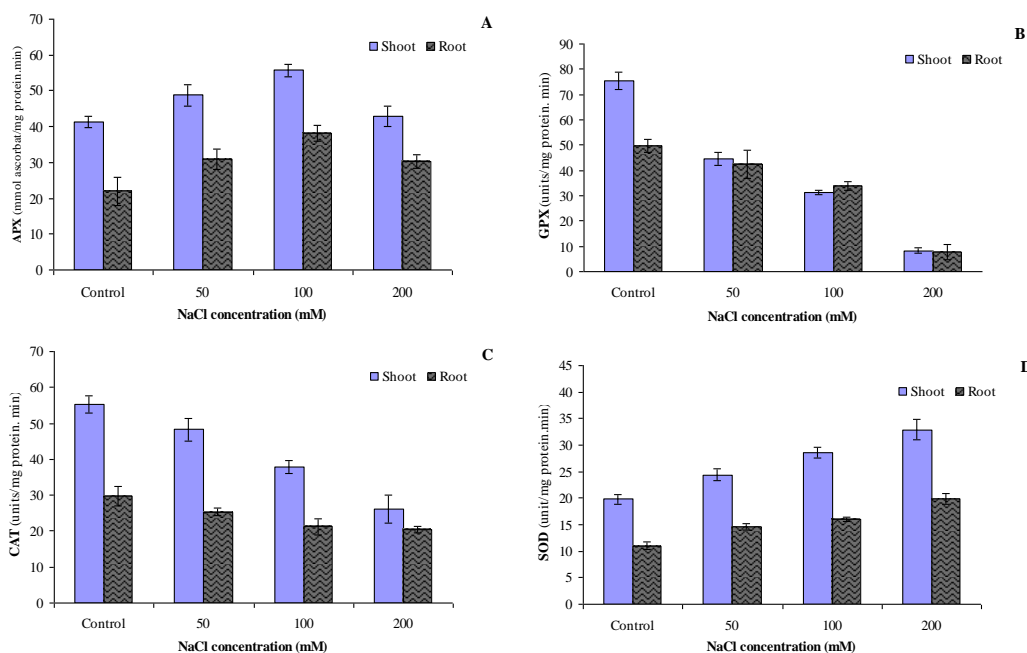
correlated with each other. Due to low numbers of dataset, some of these high correlations were not significant. However it seems that there were good associations among stress tolerance indices in salt stress conditions.

Table 2.: Dry weight (Dw) of root and shoot (g plant⁻¹), percentage water content (Wc%), leaf area (cm²), percentage relative water content (RWC%) and membrane stability index of castor bean (*Ricinus communis* L.) in response to different NaCl concentrations

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The activity of antioxidant enzymes such as APX, GPX, CAT and SOD was differentially affected by salinity. The activity of APX in shoots and roots of castor bean showed increase at low and medium NaCl concentrations but decreased at higher concentration. It considerably increased in 100 mM NaCl treated plants, with 35.14% (roots) and 74% (shoots) increase compared to control ones. The trends in APX activity were similar in root and shoot tissues. The increase of NaCl concentration up to 200 mM caused and 24.14% and 21% decrease in APX activity compared with 100 mM NaCl treated plants, respectively in root and shoot (Fig. 2A). The GPX activity in roots and shoots decreased significantly with rising NaCl concentration. Results showed similar level if GPX activity in shoots

and roots under stress condition, but under control condition GPX activity in shoots was significantly higher than in roots (Fig. 2B). In case of CAT, a significant decrease was noticed in castor bean cells under salt stress. The activity of CAT nearly halved in shoots at 200 mM of NaCl (Fig. 2C). By contrast, in root tissues the decline in CAT activity was not so sharp; e.g., it was 31% at 200 mM. SOD activity in shoot increased significantly with increasing NaCl concentration compared to the control, reaching increase of 66% at 200 mM NaCl. Similarly, the highest SOD activity in the roots increased by 81% at NaCl concentration of 200 mM compared to control (Fig. 2D).

**Figure 2.:** Effect of NaCl stress on antioxidant enzymes (A) ascorbate peroxidase (APX); (B) guaiacol peroxidase (GPX); (C) catalase (CAT); and (D) Superoxide dismutases (SOD) activities in root and shoot of castor bean (*Ricinus communis* L.) plant. The values and standards errors (vertical bars) of three replications are shown

According to Table 2, the APX activity in shoot had significant positive correlation with the APX activity in root. In contrast the APX activity in shoot did not show any significant positive or negative correlations with the other enzymes. The GPX activity in root indicated relatively high negative correlation with CAT activity in root, and relatively moderate negative correlation with CAT activity in shoot while showed relatively moderate positive correlation with SOD activity in shoot. It is mentioned that these correlations were not significant due to low numbers of observations. The GPX activity in shoot indicated significant positive correlation with GPX activity in root, CAT activity in shoot and CAT activity in root while showed significant negative correlation with SOD activity in root and shoot (Table 2). The GPX activity in root indicated significant positive correlation with CAT activity in shoot while showed significant negative correlation with SOD activity in root and shoot. The CAT activity in shoot and root had significant negative correlation with SOD activity in root and shoot while

both of SOD activity in root and shoot were associated with each other (Table 2).

The result revealed that increased salt concentration caused a significantly higher MDA production in shoot compared to root. Changes in lipid peroxidation in shoots and roots of castor bean at different treatments were shown in Fig. 3. For different concentrations of NaCl in shoots it increased by 1.2-, 5.1- and 5.6-fold and in roots it increased by 3.3-, 5.2- and 6.2-fold, respectively. MDA response of shoot and root at 50 mM were not significantly different. The amount of hydrogen peroxide (a product of SOD and GPX reactions) showed a significant increase in shoots and roots of castor bean plant treated with NaCl particularly at higher concentrations compared to untreated plants and were more pronounced in shoots than in roots (Fig. 4). The level of H₂O₂ in roots of NaCl treated plants drastically increased by 110, 236.4 and 319% compared to the controls. Similar trend also occurred in shoot with a significantly lower increase that observed in roots.

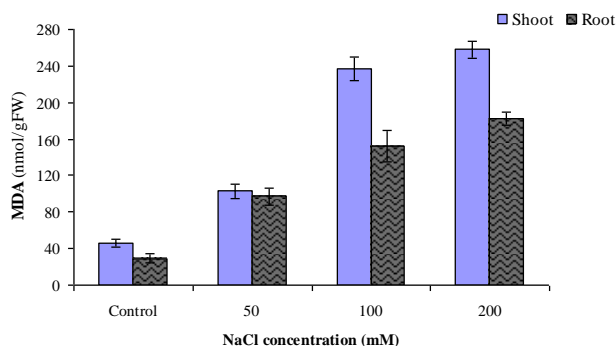


Figure 3.: Effect of NaCl stress on malondialdehyde (MDA) concentration in root and shoot of castor bean (*Ricinus communis* L.) plant. The values and standards errors (vertical bars) of three replications are shown.

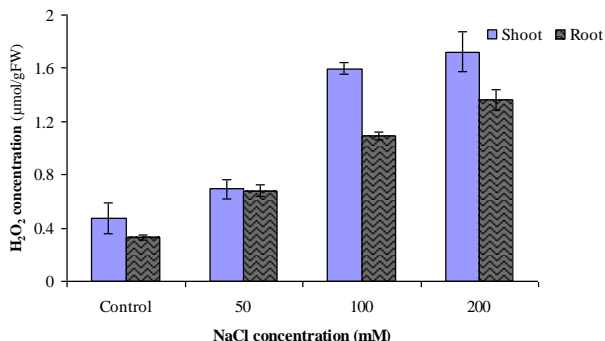


Figure 4.: Effect of NaCl stress on H₂O₂ concentration in root and shoot of *Ricinus communis* L. plant. The values and standards errors (vertical bars) of three replications are shown.

Proline content of the shoots was significantly enhanced with NaCl at all tested concentrations; by contrast, the roots appeared less sensitive to salt exposure and only at 100 mM an increase of 26% was compared to control

(Fig. 5). The increase of proline concentration in the shoots is dose dependent, and it increases by 19.6, 69.3 and 137% in the shoots treated with 50, 100 and 200 mM of NaCl, respectively, compared to the controls.

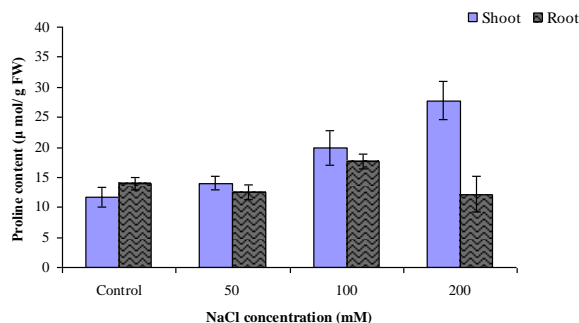


Figure 5: Effect of NaCl stress on proline content in root and shoot of castor bean (*Ricinus communis* L.) plant. The values and standards errors (vertical bars) of three replications are shown.

4 DISCUSSION

The effect of salinity on plant growth is due to an osmotic effect and/or ion toxicity. However, variation of adaptive mechanisms exists in different species (Rehman et al., 1996). In present study, 50 mM NaCl treatment resulted in a significant decrease in the root dry weight and leaf area. In a 200 mM NaCl concentration, growth reduction of roots was much higher than that of shoots. Our finding suggested that different changes are related to effects of different NaCl concentrations on different organs of plants. The results suggested that the roots were more susceptible to salt stress; perhaps it is result of roots vicinity to Na^+ and Cl^- ions. Differences between shoots and roots response to same level of salinity stress might result from pattern of sodium partitioning between them. Investigation of castor bean plants during late vegetative growth under salt stress revealed that high selectivity exist for Na^+ , so that sodium cations noticeably retained in the root (Jeschke and Pate, 1991). Furthermore, lateral uptake of Na^+ from xylem by hypocotyl, stem internodes and petioles can result in low intake by young leaf laminae and substantial cycling from older leaves back to the root may causing more inhibition of root growth.

Plants according to their sensitivity to different levels of salt content are divided into four groups which including halophytes, halophilic crop species, Salt tolerant crop species and Salt sensitive crop species. Although In the literature the coaster bean plant is considered moderately salt tolerant (Jeschke and Pate, 1991; Pinheiro et al., 2008), the result obtained here

showed that even high level of applied salinity (200 mM) was not lethal. However, with increasing of the salinity level growth was strongly inhibited. Comparison of current result with previous experiments suggests there is a high diversity of salt tolerance in different varieties or local masses of coaster bean.

Like to other stresses, salt stress is also expected to alter activities of antioxidative enzyme and thereby creating an oxidative stress situation. Superoxide dismutase, CAT, GPX and APX are important antioxidative enzymes that function in the cells to prevent the build-up of ROS (Elster 1982; Halliwell and Gutteridge, 1988). Because of NaCl induction to generation of oxidative stress, this study showed that membrane stability index decreased in leaves tissue as a result of oxidative damage induced by NaCl treatments (Table 1).

Under salt stress the protection against H_2O_2 becomes weakened due to the decline in activities of GPX and CAT which may favor the elevated steady state level of H_2O_2 . This ROS can react in presence of transition metal ions to produce the hydroxyl radical (Elster, 1982). Result indicated that in the case of castor bean, even though there was some protection against superoxide, the H_2O_2 detoxification mechanism was insufficient. In aerobic cells, hydroxyl radicals the most potentially toxic species - are known to be formed from H_2O_2 in presence of transition metal ions (Halliwell and Gutteridge, 1988).

Generally, peroxidases catalyse the oxidoreduction between hydrogen peroxide and reductants. As reported by Lobarzewski et al. (1991) peroxidases are involved in auxin and ethylene metabolism, redox reactions in plasma membranes, cell wall modifications (lignification and suberization) as well as in developmental and defense processes. In present study GPX and APX differently responded to NaCl stress, the activity of GPX sharply decreased while APX showed dose dependent increase in shoot and roots of castor bean. Plant peroxidase isozymes are differentially expressed during plant cell development (Ros-Barcelo and Sabater, 1986) thus observed result might be due to different sensitivity of GPX to salt stress or its expression in other growth stages.

Among the antioxidant enzymes SOD was the one which showed highest activity increase. At organ level shoots showed highest activity when compared to roots. In this study, salt-induced increase in SOD activity suggests that due to imposition of NaCl stress, *de novo* synthesis of enzymatic protein may have occurred which would be more pronounced in shoot tissues. A decreased superoxide concentration is thus to be expected, but parallel with an increased production of H₂O₂. A positive correlation between salt stress and the abundance of SOD formerly reported by Cavalcanti et al., (2007) in cowpea cells. In our case the lack of efficient activity of CAT and GPX probably lead to imperfect H₂O₂ scavenging. Accumulation of peroxide is a general stress response, which has been observed in plants exposed to various biotic and abiotic stresses. A similar increase in H₂O₂ level was marked under salinity stress as seen for other plants (Esfandiari et al., 2007; Cavalcanti et al., 2007).

Lipid peroxidation is a process by which the functionality and integrity of the membrane is affected and can produce irreversible damage to cell function. Lipid peroxidation gets initiated by ROS or by lipoxygenases. Salt stress enhanced the MDA level in castor bean, which is an index of lipid peroxidation and

oxidative stress and represent a balance of oxidative stress that induced production of MDA in relation to NaCl treatments. Thus the increased MDA indicates the prevalence of oxidative stress and perhaps this may be one the possible mechanisms by which toxicity due to NaCl stress could be manifested in plant tissues. Probably this oxidative stress situation might have occurred due to alternation in activity of antioxidative enzymes.

Proline concentration showed a remarkable increase at shoots with the increase of NaCl, also its increase in root was in salt concentration dependent manner. Higher concentrations of proline in shoot might be the reason of higher salt tolerance or prevention of NaCl effects when compared to roots. Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important protective roles. Environmental stress may lead to proline accumulation. Proline accumulation in plant tissues has been suggested to result from (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d) hydrolysis of proteins (Charest and Phan, 1990). In Arabidopsis, the gene of A'-pyrroline-5-carboxylate synthetase, as a key enzyme in proline biosynthesis, is induced by salt stress and other forms of osmotic stress (Yoshida et al., 1995).

In conclusion, growth parameters and cell membranes of castor bean plants were drastically affected under salinity stress. Despite of the significant increase in activity of some antioxidant enzyme under salt stress conditions, it might not be enough for ROS scavenging and no recovery of root and shoot growth as well as membrane integrity was observed, suggesting the tested castor bean is sensitive to long-term salt stress at least during the investigated stage. But since all seedlings survived even under the highest stressful conditions at the end of experiment it seems that salt tolerance in local masses (especially from arid and semi arid regions) still remain poorly understood.

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