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Full Length Research Paper

Salinity induced changes in cell membrane stability, protein and RNA contents

Muhammad Jamil[1]*, Muhammad Ashraf[2], Shafiq ur Rehman[4], Mushtaq Ahmad[5] and Eui Shik Rha[3]*

[1]Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat 26000, Pakistan.
[2]Department of Botany, University of Agriculture, Faisalabad, Pakistan.
[3]College of Agriculture and Life Sciences, Sunchon National University, Suncheon 540-742, Republic of Korea.
[4]Department of Plant Science, Kohat University of Science and Technology, Kohat 26000, Pakistan
[5]Department of Plant Science, Quid-i-Azam University, Islamabad, Pakistan

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Seedlings of sugar beet (*Beta vulgarus* L.) were used at salinity levels of 0 (control), 4.7, 9.4 and 14.1 dS m^{-1} to determine the effect of salt on vegetative growth, relative water content, cell membrane stability, protein and RNA contents in sand culture experiment. Fresh and dry weights of plants, shoots and roots decreased significantly with increasing salt concentration. Salinity significantly reduced leaf area and relative water content while cell membrane injury increased with increasing salt concentration. Leaf protein content decreased significantly and sodium dodecyl sulphate-polyacrylamide gel electrophresis (SDS-PAGE) analysis showed significant change in protein profiles in salt treated samples, which suggests that NaCl altered protein pattern. Salinity induced RNA degradation with increasing salt level. Cell membrane stability exhibited negative correlation with fresh and dry weight, leaf area, leaf water content and total protein content. There was also a significant positive correlation between cell membrane injury and RNA degradation.

Key words: Salt stress, membrane injury, growth, RWC, protein activity, RNA, *Beta vulgarus* L.

INTRODUCTION

Abiotic stress is a main factor in limiting plant growth and food production in many regions of the world (Osakabe et al., 2011; Jamil et al., 2010). In general, salinity limits plant growth and productivity (Munns, 2002; Ashraf and Foolad, 2007). Salt tolerance in plants is a complex mechanism involving morphological, physiological and biochemical processes. Water deficiency is one of the most common example of salt stress (Tabaei-Aghdaei et al., 2000) that results in malfunctioning of the cellular membranes by increasing their ion leakage. Plasma membrane may be the primary site of salt injury (Maas and Nieman, 1978; Mansour, 1997) the harmful effect of salinity on the plasma membrane is basically due to the action of salt ions (Mansour, 1997).

*Corresponding author. E-mail: m.jamil@kohat.edu.pk, euishik@sunchon.ac.kr

It has well known fact that salinity decreased performance of RNA machinery. Na^+ initiated RNA degradation *in vitro* but *in vivo* RNA stability depended on the relative Amount of the ion accumulated (Rauser and Hanson, 1966; Aspinall, 1986; Munns and Termaat, 1986). Therefore, the reduction in RNA content will ultimately reduce the protein content, since RNA is required for the process of protein synthesis through transferring the amino acids into protein synthesis centers (Udovenko et al., 1971).Sugar beet is a member of the Cheno-podiaceae. Sugar beet is a salt-tolerant crop that is common grown in saline soil. Sugar beet is well established in the saline soil to tolerate at high salt level (Bernstein, 1964) and soil water stress (Hills et al., 1990). It has been reported that sugar yield of sugar beet was not affected by salt stress up to an electrical conductivity value of f 7dSm^{-1} (Roades and Loveday, 1990).The objective of this study was to determine the effect of salinity on growth, cell membrane stability,

protein synthesis and RNA of sugar beet and whether cell membrane injury was attributable to biological (fresh and dry weights, leaf area) and biochemical changes such as total protein content and RNA.

MATERIALS AND METHODS

Plant material

Sugar beet seeds (*Beta vulgaris* L. cv Tianjin qing pielan) used in this experiment were obtained from Institute of Soils and Fertilizers, Beijing, China.

Green-house experiment

A green-house experiment was initiated to investigate the effect of salt stress on vegetative growth, relative water content (RWC), cell membrane stability (CMS), and protein and RNA contents. Seedlings of sugar beet were grown in plastic pots in sand culture irrigated with full strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Plants were irrigated for 2 weeks with full strength Hoagland's nutrient solution. Salt treatments in Hoagland's nutrient solution were applied two week days after the start of the experiment. The NaCl treatments used were 0 (control), 4.7, 9.4 and 14.1 dS m^{-1} in full strength Hoagland's nutrient solution. The experiment was designed in three replicates with average temperature of 25/15°C for day/night and photoperiod for the day/night cycle was 16/8 h. The plants were uprooted carefully after 30 days of treatments, washed with distilled water, and then the fresh weight of plants, shoots and roots was recorded. Dry weights were determined by drying plant samples in an oven at 80°C.

Leaf area and relative water contents (RWC)

Area meter (AM-200, ADC Bio Scientific Ltd., England) was used for the measurement of individual plant leaves area. Relative water content was determined by detaching fresh leaves from each replication and weighed instantly to note fresh weight (FW), followed by dipping in DW for 12 h. The leaves were blotted dry to remove surplus water and weighed to note fully turgid weight (TW). The leaves were then dried in the oven at 80°C for 24 h to note the dry weight (DW). The RWC were calculated by using the equation developed by Turner (1986) that is RWC = [FW−DW]×100/[TW–DW].

Measurement of cell membrane stability or cellular injury

Cell membrane stability (CMS) was investigated from each treatment and replication by using fully expanded young plant leaves. Twenty pieces (1 cm diameter) were cut from leaves and sunken in DW contained in test tubes. The tubes were kept in cooled chamber for 24 h at 10°C, followed by warming at 25°C and measuring the electrical conductivity (C_1) of the samples. The leaves samples were then autoclave for 15 min at 121°C and the electrical conductivity (C_2) of the samples was measured again. CMS was calculated by using the formula: (C_1/C_2)*100 where C represents conductivity one and two.

Isolation of total cell RNA

RNA was extracted by the method of TRIzol Reagent (MRC, USA). The material was ground to a fine powder with liquid nitrogen and homogenized in 1 ml (1 ml/50 - 100 mg tissue) of TRIzol solution. The homogenized samples were incubated for 5 min at 15 to 30°C. The material was extracted with 0.2 ml chloroform per 1 ml of TRIzol. The two phases was separated by centrifugation for 15 min at 12000´g at 2 to 8°C. RNA was precipitated with 500 µl isopropanol overnight at 20°C and centrifuged for 10 min at 12000´g. The pellet was washed with 75% ethanol, resedimented for 5 min at 10000 g and dried carefully. The RNA was re-dissolved with diethylpyrocarbonate (DEPC) water to remove the supernatant. Samples (10 µl) were subjected to electrophoresis on 1.1% agarose gel in TAE buffer. RNA was stained with 0.5 µg ml^{-1} ethidium bromide for 30 min.

Total soluble protein

Total leaf protein was determined by using the procedure described by Santoni et al. (1994). The protein of the supernatant was determined with SmartSpec™ 3000 Spectrophotometer according to Bradford (1976) method using bovine gamma globulin as standards.

SDS-PAGE analysis of protein

SDS polyacrylamide gel electrophoresis (PAGE) was used for protein synthesis of control and NaCl treated plants by following the procedure of Laemmli (1970). Fifty microgram (50 µg) protein with sample buffer [62.5 mM Tris–HCl, pH 6.8, 20% (w/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 0.01% (w/v) bromophenol blue] was loaded in each lane of 12.5% polyacrylamide gel. Electrophoresis was done at 500 V for 30 min by using Bio-Rad, Mini Protein II electrophoresis system. The gel was stained with 0.25% Coomassie Brilliant Blue R-250 (Sigma) for 2 h and destained with 50% methanol and 10% acetic acid. Densitometer (GS-710, Bio-Rad, USA) was used for photographs and scanning. Standard proteins were as follows: phosphorylase b (97.4 kDa); bovine serum albumin (66.0 kDa); ovalbumin (43.0 kDa); carbonic anhydrase (29.0 kDa); soyabean trypsin inhibitor (20.1 kDa); lysozyme (14.3 kDa).

Statistical analysis

Analysis of variance was calculated by using the Microsoft Excel version 5.0. Means values for different plant characteristics were compared through least significance difference (LSD) test (Li, 1964). Correlation between morphological parameters and biochemical attributes were developed by using Minitab statistical software package.

RESULTS

Increased salt concentration caused a significant reduction in the vegetative growth of sugar beet (Figure 1). Fresh and dry weights of sugar beet decreased significantly with increasing salt concentration in the growth medium (Figure 1). However, significant reduction was observed at high salinity levels. Furthermore, root was found to be more sensitive towards salt stress than that of shoot as decrease in fresh weight was more pronounced as compared to that of shoot at 9.4 and 14.1 dS m^{-1} (Figure 1A). A significant reduction in leaf area was also observed with increasing salt concentration but

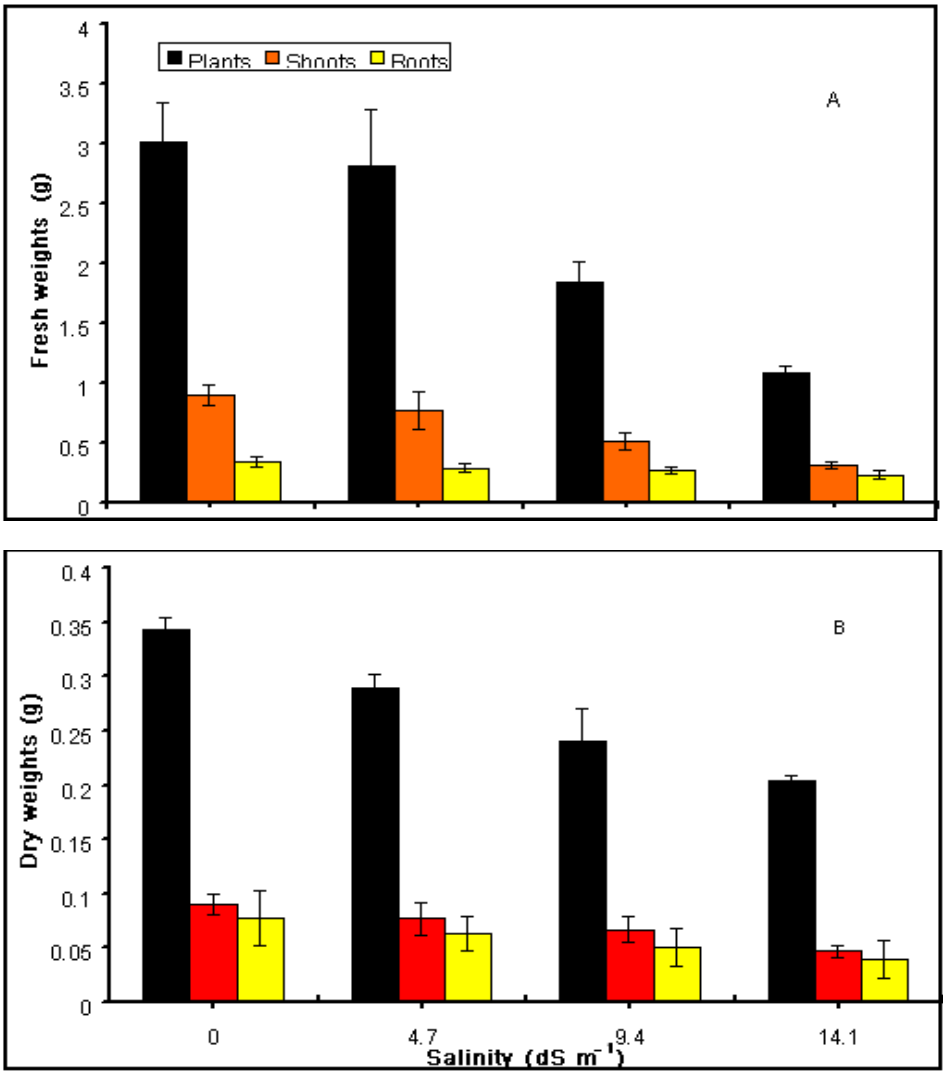

Figure 1. Effect of various concentrations of salinity on fresh (A) and dry (B) weights of sugar beet.

the magnitude of decrease was more pronounced at 9.4 and 14.1 dS m^{-1}(Figure 2A).

Considerable differences were observed for RWC and cell membrane injury (Figures 2B and 3). Salinity induced significant decrease in RWC, but the pattern of decrease varies at different salinity levels. Maximum reduction was observed at high salinity levels as compared to the control (Figure 2B). Increased salt concentration caused an increased in cell membrane injury (Figure 3). Cellular injury increased significantly at high salt concentrations, but the enormity of increase was more intense at 9.4 and 14.1 dS m^{-1} as compared to the control (Figure 3).

There was a decrease in the amount of total soluble protein content with the consequent raise in salinity level (Figure 4). Decrease in total protein content was more at high salt concentration (Figure 4). SDS-PAGE analysis showed significant changes in protein profiles of in all treated samples. It was observed that molecular weight markedly decreased with increasing salt stress (Figure 5).

Biochemical analysis revealed that nuclear RNA commenced disintegration after salt stress (Figure 6). Fragmentation of RNA was clearly detected at higher NaCl stress, but not in the control. With the increase of NaCl concentration, more nuclear RNA degraded and the degraded genomic RNA formed a smear in the gel and the genomic RNA band became undefined. The RNA band intensity also increased with the increased in salt concentration (Figure 6).

There was a significant negative correlation between cellular injury and growth parameters (Table 1). Correlation also revealed a strong (R^2 = 0.99, P = 0.001) significant negative relationship between cellular injury and leaf area. Table 1 also shows a weak (R^2 = 0.95, P = 0.047) significant negative relationship between cellular

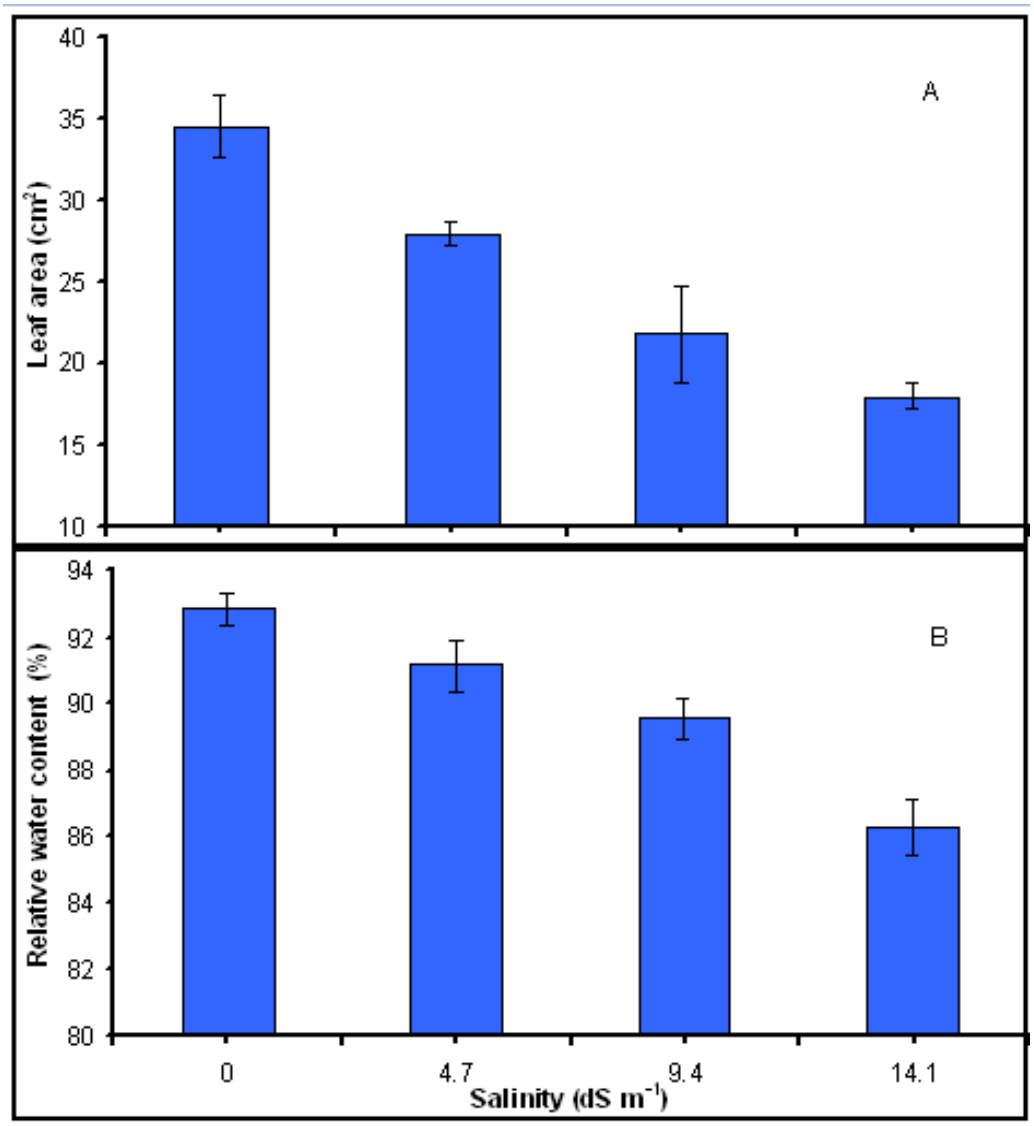

Figure 2. Effect of various concentrations of salinity on leaf area (A) and relative water content (RWC)(B) of sugar beet.

injury and fresh plant weight. Cellular injury was also significantly (P=0.05) and negatively correlated with RWC (R^2 = 0.95) and with total protein content (R^2 = 0.95). Cellular injury and RNA also exhibited positive (R^2 = 0.95) correlation and the value was also significant (P=0.05).

DISCUSSION

Decrease in plant growth under different salinity concentration has been reported in various plants by many scientists (Alpaslan and Gunes, 2001; Greenway and Munns, 1980). Salinity significantly reduced fresh and dry weights of plants, shoots and roots and leaf area (Figures 1 and 2A). It has been documented that the effect of salt stress on leaf area was more pronounced than on dry weight because salt accumulation in the shoot occurs through transpiration stream, which is highest in old leaves (Greenway and Munns, 1980). Decrease in plant growth under saline soil condition is a common process in mesophytes (Ashraf and Harris, 2004), but such decrease occurs differently in different plant parts. For example in the present study, fresh root weight was affected more than fresh shoot weight (Figure 2). Our results are similar in line with the results earlier reported by Jamil et al. (2005). They investigated that salinity inhibited the growth of shoot more than root in *Brassica* species.

Our results indicate continue increase in cellular membrane injury and decrease in RWC with increasing salt concentration (Figures 3 and 2B). Cellular membrane injury exhibited a negative correlation with fresh weight of

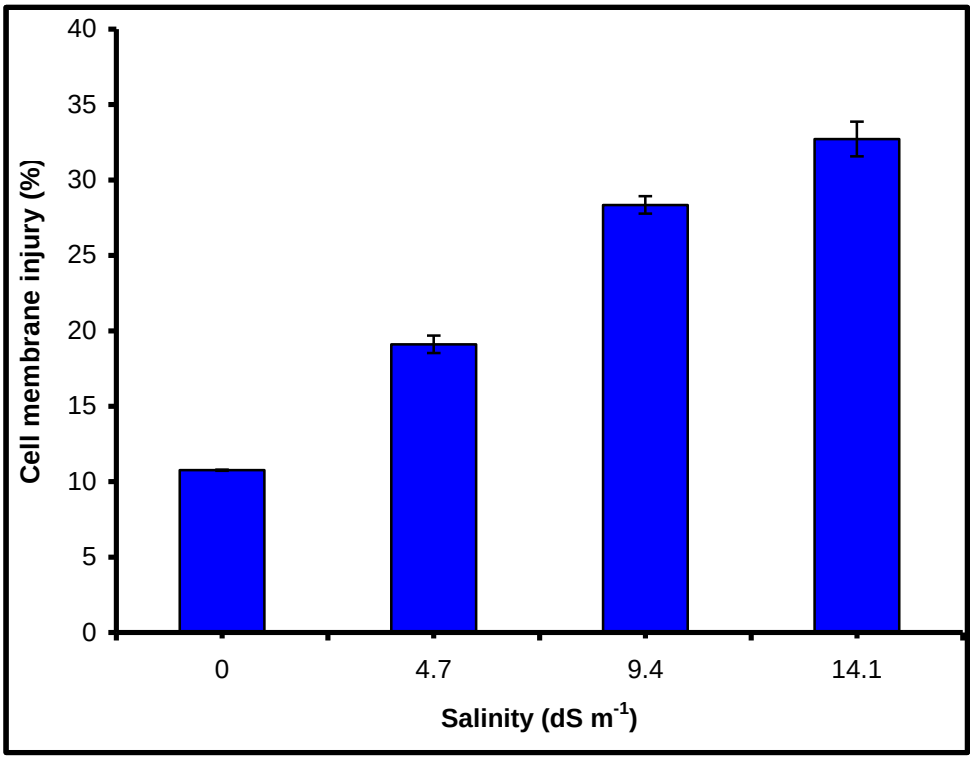

Figure 3. Effect of various concentrations of salinity on cell membrane stability (CMS) of sugar beet.

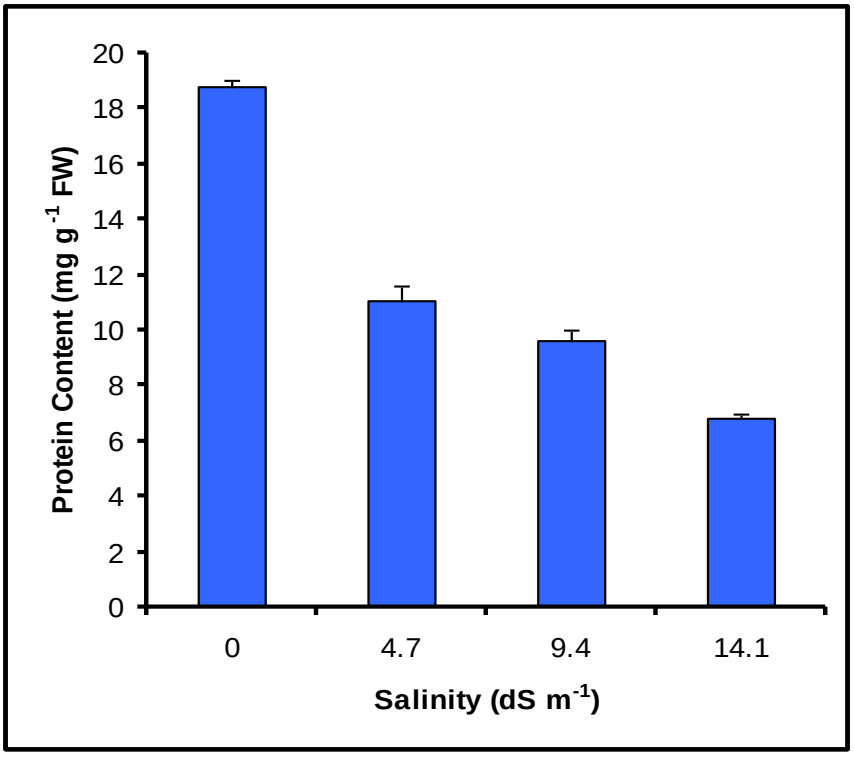

Figure 4. Effect of various concentrations of salinity on total protein content of sugar beet.

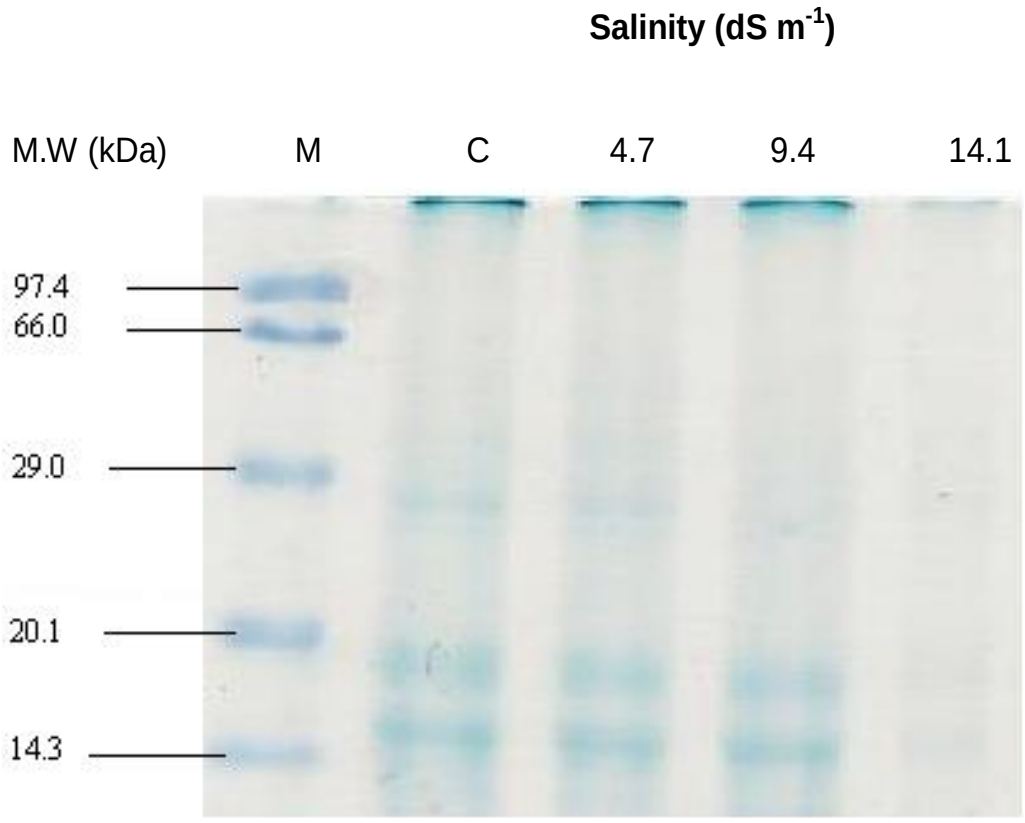

Figure 5. Effect of various concentrations of salinity on polypeptide patterns of total protein from leaf as analyzed by SDS-PAGE. Lanes C and 4.7, 9.4 and 14.1 represent proteins extracted from the control and NaCl treated plants after 30 days of treatment and lane M represents molecular weight marker.

plants, roots, shoots, leaf area, and relative water contents (Table 1): the parameters that are equally affected by salt stress (Munns, 2002). However, water deficiency one of the most common examples of salt stress (Tabaei-Aghdaei et al., 2000) results in the mal-functioning of the cellular membranes by increasing their ion leakage. The deleterious effect of salinity on the plasma membrane is essentially due to the action of salt ions (Mansour, 1997). It has been also reported that salinity induced decrease in RWC (Gadallah, 1999) and cell membrane stability (Bhattacharjee and Mukherjee, 1996).

Specific expression of stress proteins is an important adaptive manifestation in maintaining the integrity, native configuration and topology of cellular membranes components to ensure their normal functioning under salinity stress (Wahid et al., 2007). The decrease in protein content and protein molecular weights in sugar beet suggests that salinity exposure affect protein activities in this plant (Figures 4 and 5). A significant negative relation was found between cell membrane stability and total protein content (Table 1). Changes in the expression of proteins occur due to stress, yet it is probable that only some of these proteins are directly involved in stress tolerance. It is possible that in some cases the synthesis of a protein indicates sensitivity to a stressor rather than being part of a tolerance mechanism. Ashraf and Waheed (1993) reported that leaf soluble proteins decreased due to salt stress in all lentil lines, irrespective of their salt tolerance. It has been well documented that salinity decreased protein contents of leaves in glycophytes (Alamgir and Ali, 1999; Gadallah, 1999; Wang and Nil, 2000). In case of Rhizobium, certain outer membrane proteins of molecular weight 22, 38, 40, 42, 62 and 68 kDa noticeably decreases with increasing NaCl levels (Unni and Rao, 2001).

Salinity induced RNA degradation with increasing salt stress (Figure 6). Na^+ initiated RNA degradation *in vitro* but *in vivo*, RNA stability depended on the relative amount of the ion accumulated (Rauser and Hanson, 1966; Aspinall, 1986; Munns and Termaat, 1986). RNA exhibited a negative relationship with total protein content and positive relation with cell membrane stability (Table 1). The reduction in RNA content will ultimately reduce the protein content, since RNA is required for the process of protein synthesis through transferring the amino acids into protein synthesis centers (Udovenko et al., 1971). Structural changes of nuclei caused by salt stress have

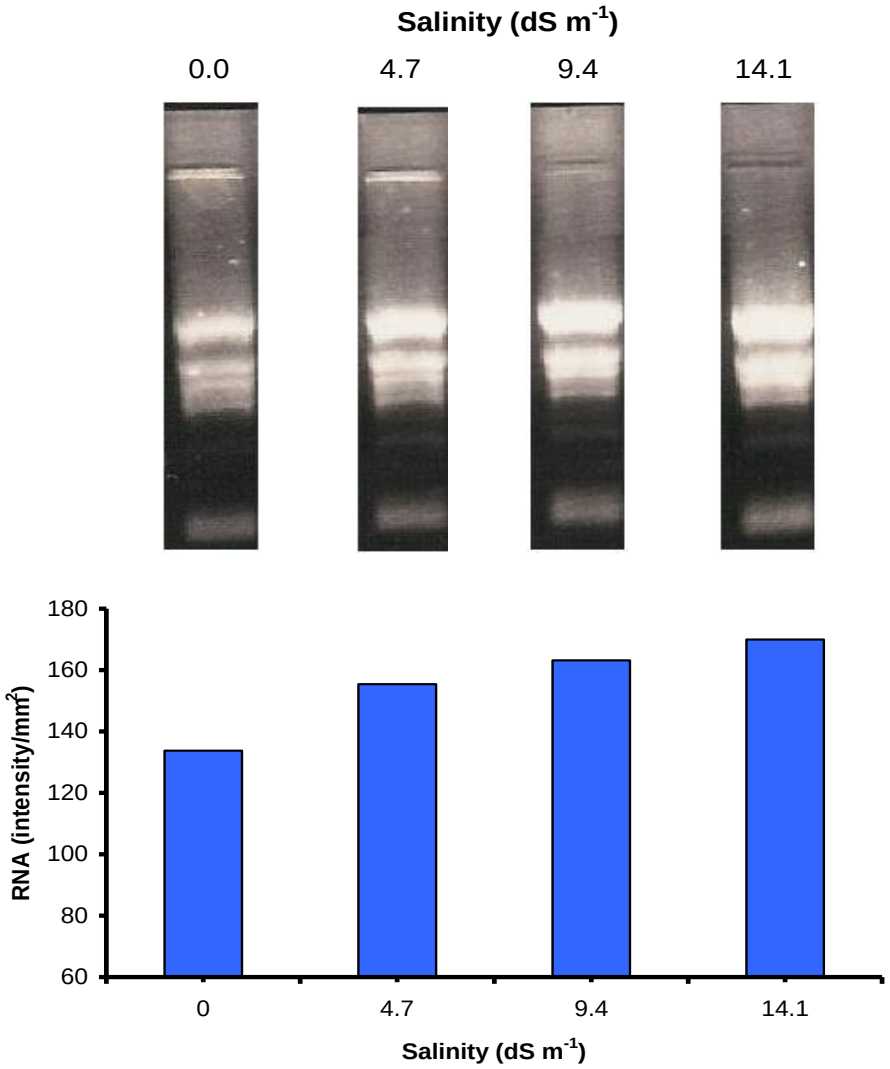

Figure 6. Effect of various concentration of salinity on RNA of sugar beet. Histograms represent the relative intensity/mm^2 of the RNA bands.

Table 1. Relationships among cell membrane injury and growth, RWC, total protein content (TPC) and RNA (Band intensity/mm^2) of sugar beet under various level of salt concentration.

Parameter	FPW	FRW	FSW	DPW	DRW	DSW	LA	CMS	RWC	TPC
FRW	**0.925** ^{ns}									
FSW	0.993**	0.959*								
DPW	0.952*	0.993**	0.981*							
DRW	0.963*	0.986*	0.988*	0.999**						
DSW	0.969*	0.981*	0.980*	0.980*	0.979*					
LA	0.951*	0.985*	0.981*	0.998**	0.999**	0.969*				
CMS	-0.953*	-0.977*	-0.982*	-0.995**	-0.997**	-0.961*	-0.999**			
RWC	0.975*	0.968*	0.980*	0.969*	0.970*	0.998**	0.957*	-0.949*		
TPC	0.838 ^{ns}	0.979*	0.894 ^{ns}	0.965*	0.951*	0.921*	0.959*	-0.950*	0.897 ^{ns}	
RNA -	0.864 ^{ns}	-0.982*	-0.917 ^{ns}	-0.977*	-0.967*	-0.927 ^{ns}	-0.976*	0.970*	-0.905 ^{ns}	0.996**

**, * Significant at P = 0.01 and P = 0.05, respectively; ns = Non-significant. FPW; Fresh plant weight, FRW; fresh root weight, FSW; fresh shoot weight, DPW; dry plant weight, DRW; dry root weight, DSW; dry root weight, LA; leaf area, CMS; cell membrane stability, RWC; relative water content, TPC; total protein content.

also been reported by Werker et al. (1983) and Katsuhara and Kawasaki (1996).

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