E3 Journal of Environmental Research and Management Vol. 4(1). pp. 0163-0170, January, 2013
Available online http://www.e3journals.org
ISSN 2141-7466 © E3 Journals 2012

Full length research paper

Effect of salinity on growth, chlorophyll, carbohydrate and protein contents of transgenic *Nicotiana Plumbaginifolia* over expressing P5CS gene

Riahy Mahboobeh[1], Ehsanpour Ali Akbar[2]

[1,2]Department of Biology, University of Isfahan, Isfahan, Iran

Accepted 13 January, 2013

Transgenic tobacco plant (*Nicotiana Plumbaginifolia*) over expressing P5CS (Δ-pyrroline-5-carboxylate synthetase) and non transgenic plant as control were grown on MS medium supplemented with 0, 100, 150, 200 and 250 mM NaCl for 28 days. The effect of NaCl concentrations on fresh and dry weight, the chlorophyll (chl. a and b and total), carbohydrate and protein content were measured. Results showed a significant increase of chlorophyll, fresh weight, dry weight and carbohydrate in transgenic compared to the non transgenic plants under salt treatments. The total protein was decreased with increasing of salt concentration.

Keywords: Salt stress, P5CS, *Nicotiana plumbaginifolia*, Proline

INTRODUCTION

Salinity is one of the major widespread environmental stresses that can limit growth and development of plants (Munns, 1993). Salt tolerance of crops may vary with their growth stage (Mass et al., 1994). Despite the essentiality of chloride as micro nutrient for all higher plants and of sodium as mineral nutrient for many halophytes and some C_4 species, salt accumulation many convert agricultural areas in unfavorable environments reduce local biodiversity, limit growth and reproduction of plants, and may lead to toxicity in non salt tolerant plants, known as glycophytes (Ashraf and Harris, 2004). Species of halophytes are able to avoid ion toxicity and maintain water uptake in the presence of high salt concentrations (Flowers et al., 1977; Munns, 2002). Two mechanisms of cellular adaptation to salinity are the accumulation of osmo protectants, such as glycine betaine or proline (especially present in glycophytes), and the control of ion movements allowing high inorganic ion concentrations (especially present in halophytes) (Bohnert et al., 1995).
Δ-pyrroline-5-carboxylate synthetase (P5CS) is key enzyme in proline synthesis and proline has been correlated with tolerance to salinity stresses in plant. In addition to various known roles of proline, it was also involved in the synthesis of key proteins that are necessary for stress responses (Iyer and Caplan, 1998). In response to the stress conditions, plants increase the osmotic potential within their cells by synthesizing and accumulating compatible osmolytes (Hanson and Hitz, 1980). Most of the plants are sensitive to salt stress, in with high concentration of salinity causes reduction in carbohydrates that are needed for cell growth. Carbohydrates are supplied mainly through the process of photosynthesis and photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl (Ashraf and Harris, 2004; Parida and Das, 2005). It has been reported that over expression of P5CS increased the salt tolerance and drought tolerance of some plant species. The aim of the present study is to understand to what extend over expression of P5Cs gene affects on some biochemical and physiological parameters of tobacco plant as a model system

MATERIAL AND METHODS

2.1. Determination Of Fresh And Dry Weights

After 10 day of stress treatment seedlings were removed

*Corresponding Author Email: ehsanpou@sci.ui.ac.ir

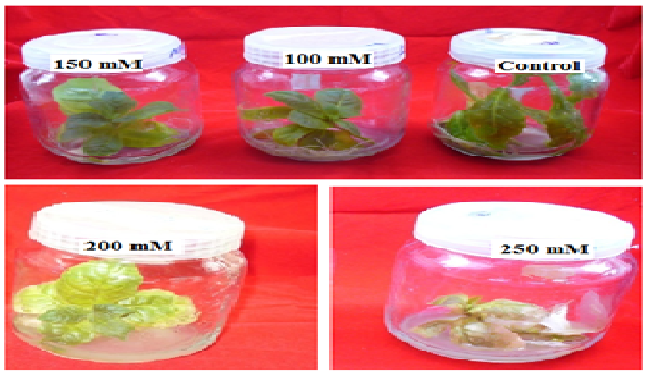

Figure 1: Effect of different concentrations of NaCl on the growth and the general appearance of tobacco plants.

from the culture medium, washed were briefly in distilled water and dried between two layers of filter paper. Three seedlings from each treatment were measured. The fresh weight was then recorded. Seedings were incubated in a plant growth chamber at 70°C until used for further analysis. The dry weights were then recorded.

2.2. Determination of Total Chlorophyll Concentration

Leaf segments of plants (100 mg) were harvested and used for determination of chlorophyll a, b and total based on method of Arnon (Arnon, 1949). The absorbance of the extract was recorded at 663, 645 and 480 nm.

2.3. Determination Of Reduced Carbohydrate Content

Determination of reduced carbohydrate was carried out according to dinitrosalicyclic assay (DNSA) (1% of 3,5-dinitrosalicylic acid (DNSA), 30% of Sodium potassium tartrate and 0.4 M NaOH) (Jeffries et al., 1998). Equal volumes of the leaf extract sample and DNSA reagent were mixed and heated in a boiling water bath for 10 min. Solution then was diluted with 10 volumes of distilled water, and finally the absorbance was recorded at 570 nm. Sucrose was used as a reference for the standard curve.

2.4. Determination Of Total Carbohydrate Content

Determination of total carbohydrate was carried out according to modified anthrone reagent (Fales, 1951). Ethyl alcohol was also incorporated, into the reagent, since it was found that the colored product was thereby stabilized. About 400mg of anthrone was dissolved in 200 ml of mixed solution %85 Sulfuric acid and %95 ethyl alcohol. The solution was then cooled and mixed. 10 ml of the modified anthrone reagent were added to 1 ml of the extract solution. The color was developed in a boiling water bath by the method of Seifter and Dayton (1950). The absorption was reported at 620 nm.

2.5. Protein Extraction

Protein was determined by method described by Bradford (1976) and Bollag and Edelstein, (2001), using bovine serum albumin as standard. Leaf samples (100 mg) were homogenized with 3ml extraction buffer (50mM Tris-HCl (pH:7.5), 2mM EDTA, 1mM 2-Mercaptoethanol , 1mM DTT). Samples then were centrifuged at 14000 rpm for 25 min at 4◦˚c. and supernatants were isolated and used for protein assay.

2.6. SDS-PAGE

The polyacrylamide gels (stacking gel, 5% and separating gel, 12%) were prepared. Leaf protein extracts were heated to 95◦C for 4 min then cooled at room temperature. To avoid variability in loading, equal amount of protein in each extract were applied to the gel wells. The protein was separated at 120 V. Finally gel was stained using method of Bohnert (1995).

Statistical Analysis

All experiments were carried out in a completely randomized design and data statistically analyzed by ANOVA and Tukey test.

RESULTS

3.1. Growth Parameters

The effect of salinity on the general pattern of growth of transgenic plants are summarized in Figure 1. The

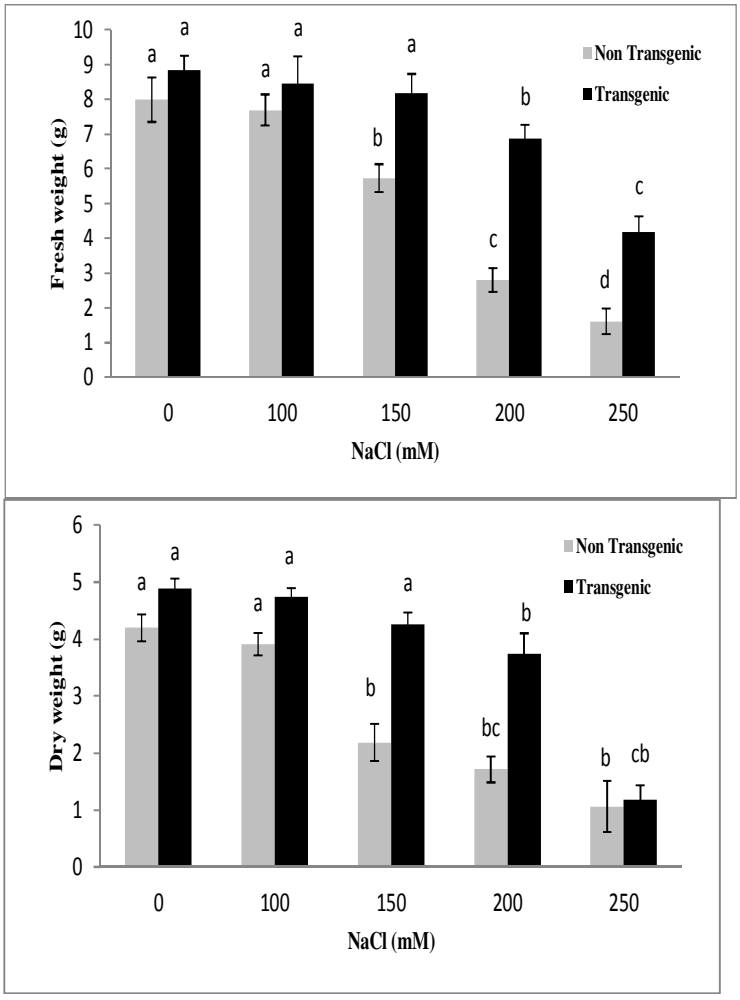

Figure 2: Effect of NaCl concentration on the dry and fresh weight of leaves of N. plumbaginifolia plants. Similar letters are not significant (P<0.05) based on Tukey test.

growth appearance of transgenic and nontransgenic tobacco plant decreased by increasing of NaCl in particular at higher than 150 mM NaCl. The leaf color in 200 and 250 mM became yellow. While non transgenic plants did not grow very well in the medium containing higher than 100 mM NaCl.

3.2. The Fresh And Dry Weight

The effect of salinity on the fresh and dry weight are illustrated in Figure 2. The fresh weight of tobacco plants after NaCl treatment decreased in transgenic plants at salinity higher than 150mM but in non transgenic plants the fresh and dry weight decreased at concentration more than 100 mM significantly. Whereas 250 mM of NaCl showed more negative effects on the plants. The pattern of dry weight was more and less similar to fresh weight. Tobacco plants (transgenic and non transgenic) grown at the low levels of NaCl exhibited relatively higher dry weights and did not imply toxicity symptoms in high concentration of NaCl. Dry weight in transgenic and non transgenic plants under salt stress was similar to the fresh weight. Between transgenic and non transgenic plants decreasing of dry weight was significant at 200 and 250 mM of NaCl.

3.3. Changes In Chlorophyll Content

The chlorophyll content of plants in response to NaCl showed a similar pattern in transgenic as well as non

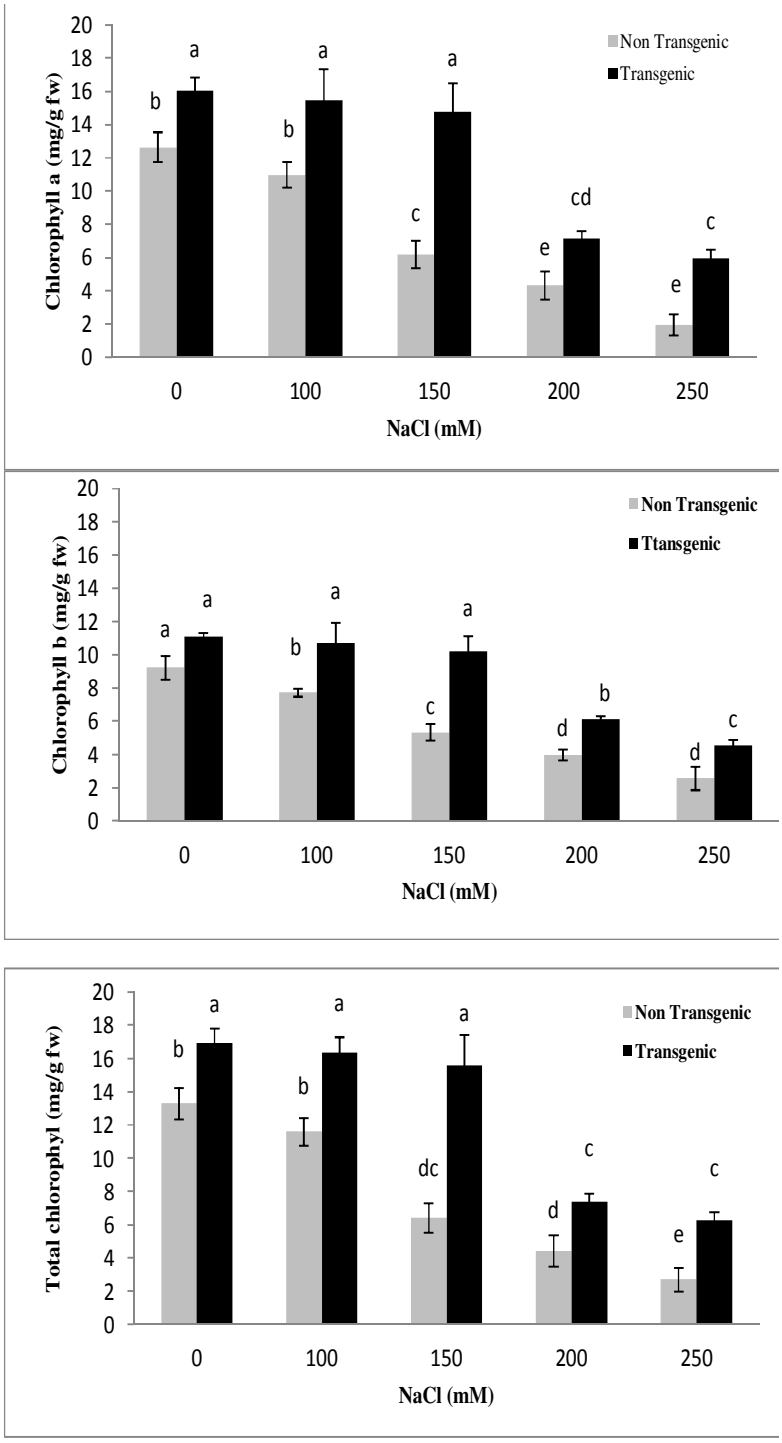

Figure 3: Effect of NaCl on Chlorophyll a, b and total Chlorophyll contents of leaves in tobacco plants. Similar letters are not significant ($P<0.05$) based on Tukey test

transgenic plants (Figure 3). It was observed that the high level of salinity, '150, 200 and 250 mM NaCl' in transgenic and 100,150, 200 and 250 mM NaCl in non transgenic tobacco induced insignificant decrease in the chlorophyll content compare with the control plants. Chlorophyll a, b and the total chlorophyll content in

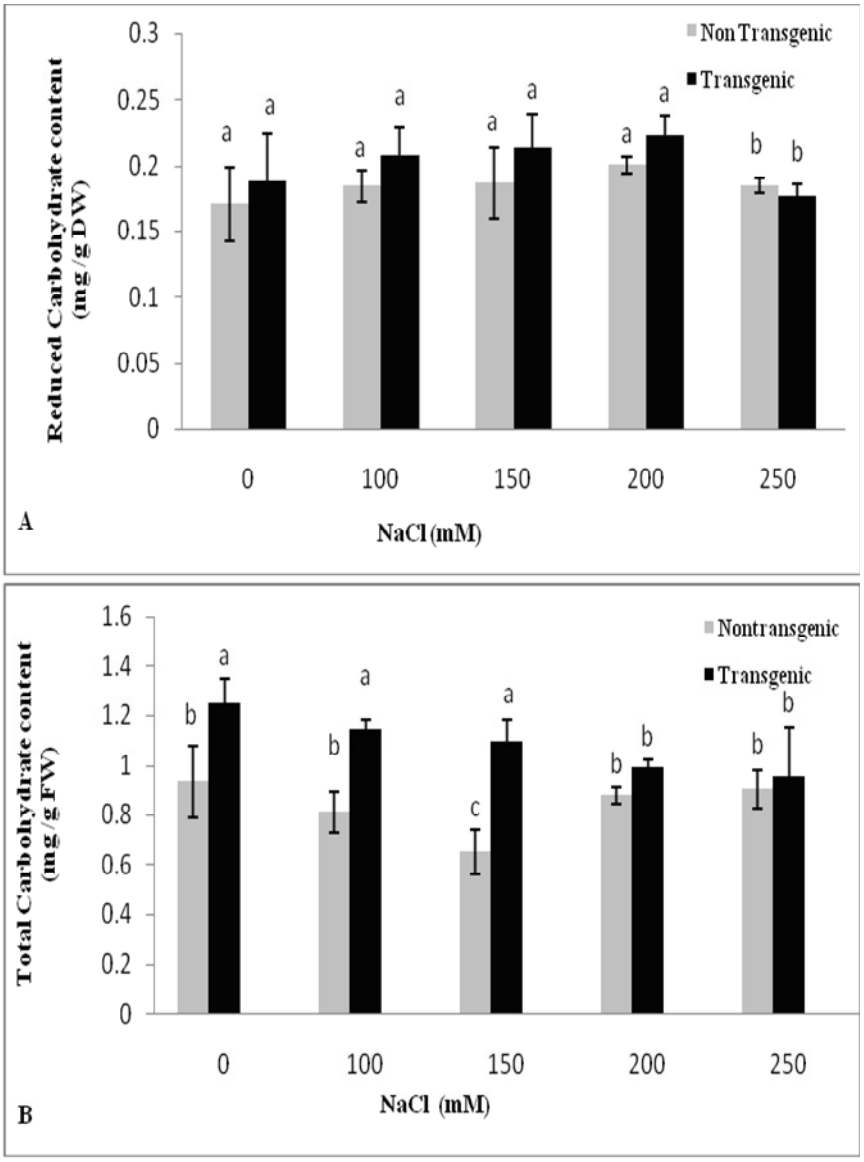

Figure 4: Effect of NaCl on reduced (A) and total carbohydrate (B) of tobacco leaves. Similar letters are not significant (P<0.05) based on Tukey test

transgenic plants was much higher than non transgenic plants.

3.4. Changes In Total Carbohydrate And Reduced Carbohydrate

In transgenic and non transgenic plants the reduced carbohydrates content did not show significant differences in any of NaCl concentration except for 250mM, however, this kind of sugar in transgenic plants was higher than non transgenic (Figure 4A). Total carbohydrate content decreased in 150 mM NaCl in non transgenic plant but it was increased in 200 and 250 mM NaCl. Similar changes did happen in transgenic plant but in 200 and 250 mM NaCl treatment (Figure 4B).

3.5. Protein content and SDS PAGE pattern

The influence of salinity levels on protein content of tobacco plant is shown in Figure 5. Protein content of the non transgenic plants decreased when salinity was increased but, in 200 and 250mM were much lower than the other salt concentrations. In contrast, the protein content of transgenic plants did not change up to 200mM NaCl. Despite of protein reduction, protein content in transgenic plant was much higher than non transgenic

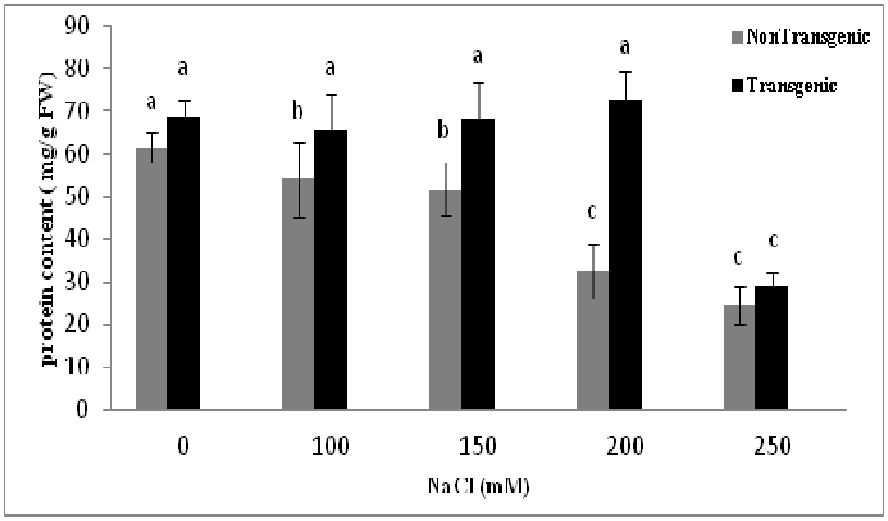

Figure 5: Effect of NaCl on protein content of tobacco leaves. Similar letters are not significant (P<0.05) based on Tukey test.

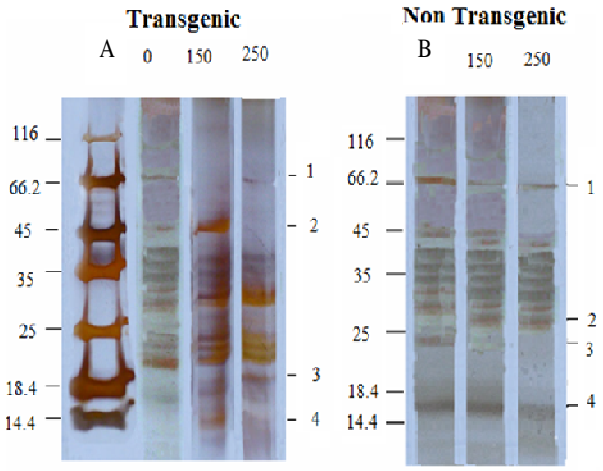

Figure 6: SDS PAGE pattern of protein in transgenic and non transgenic tobacco plant after salt treatments (0, 150, 200mM NaCl).

plant significantly. However, in 250 mM salt, protein content decreased.SDS PAGE pattern of proteins in transgenic as well as non transgenic plants showed some variations in protein bands. Four bands named 1, 2, 3 and 4 in both plants showed some changes either in respect to presence or absence or the intensity of protein bands. (Figure.6). When we analyzed the protein pattern four bands were identical in both transgenic as well as non transgenic plants. In transgenic plant band No 1 in 150 mM, Band 2 in 250 and Band No 3 and 4 in control were down regulated. In non transgenic plant the pattern was completely different. The intensity of protein bands are illustrated in Figure 7.

DISCUSSION

Salinity is a major factor in reducing the growth and the productivity of plants (Ehsapour and Fatahian, (2003). To cop with this problem, one of the most important

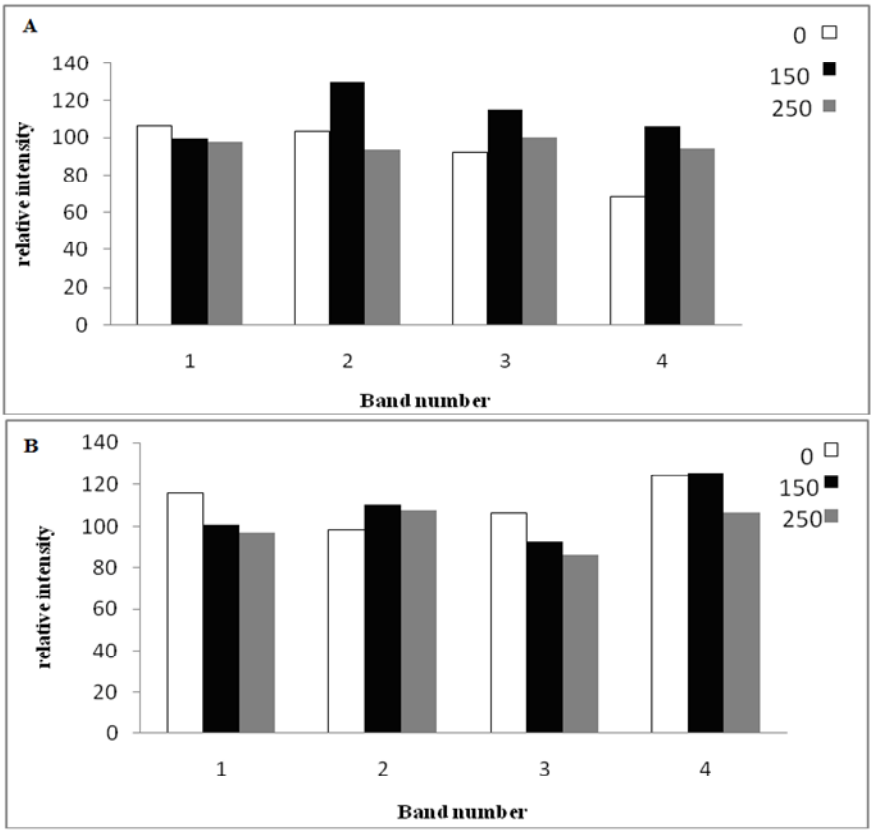

Figure 7: The intensity of protein bands A: transgenic plant, B: non transgenic plant

mechanisms by higher plants under salt- stress is the accumulation of compatible solutes such as proline. Osmotic adjustment, protecting cell structure and antioxidant activity are its function in plants many plants (Desingh and Kanagaraj, 2007). On the other hand, a positive correlation was determined between proline and tissue-Na concentrations under salt stress (Bajji, et al., 2001). The present study showed that the salt treatments induced and increased the proline concentration due to over expression of P5CS in transgenic plant (data are not shown). We measured dry weight and fresh weight in both transgenic and non transgenic plants under normal and salt stressed conditions. We observed significant differences between non transgenic and transgenic plants when grown under salt stress condition. Some studies have reported that biomass accumulation decreased during stress conditions while others have found that it increased (Hanson and Hitz, 1982). Since, dry and fresh weight in transgenic plant was higher than non transgenic plant, thus increasing of dry and fresh weight is associated with cell division and new material synthesis ([Sunderland, 1960). Our results indicated that over expression of P5CS gene in transgenic plant improved the salinity tolerance of tobacco plant than non transgenic plant. However the dry and fresh weight was decreased in 250 mM NaCl which might be due to proline content. The total chlorophyll content of *Niccotiana plumbaginifolia* leaves was reduced by increasing of NaCl concentration. Similar observation was reported in two maize varieties (Cha-um and Kirdmanee, 2009). The low level of salt treated tobacco plant in this experiment might be interpreted as the sensitivity of Photosynthesis machinery. Chlorophyll content is one of the major component in the plant cell which is sensitive to salt stress (Ahmad et al., 1978; Hajar and Heikal, 1993). In the present study, chlorophyll content in transgenic plants was much higher than non transgenic plants when salt concentration of the medium was increased. Looking at the carbohydrate content in both transgenic as well as non transgenic plants, we can concluded, that it was increased after salt treatment. The pattern of chlorophyll and carbohydrate content changes in transgenic plants might be due to over expression of P5CS gene and the function of this gene in producing of proline and stabilizing of the key enzymes related to photosynthesis and carbohydrate synthesis. Moreover, carbohydrate is a source of energy and osmolyte. Higher level of carbohydrate in transgenic plants associated with proline

and chlorophyll might be a good strategy for transgenic plant over expressing P5CS gene to increase salt tolerance.

Decreasing of protein and changing of protein pattern is another phenomenon which is common in salt stress condition for many plant species (Merril, 1990).

ACKNOWLEDGMENT

Author wish to thank University of Isfahan and Plant Stress Center of Excellence (PSCE) for their support.

REFERENCES

Ahmad AM, Heikal MM and Shaddad MA (1978). Photosynthetic activity, pigment content and growth of Helianthus annus and *Linum usitatissiumm* plants as influenced by salinization treatments," Assiut. Univ. 7: 49-56.

Arnon DT (1949) Copper enzymes in isolated Chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiology 24:1-15.

Ashraf M and Harris PJC (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Science 166: 3-16.

Bajji M, Lutts S and Kient JM (2001). Water deficit effects on solute contribution to osmotic adjustment as a function of leaf aging in three durum wheat (*Triticum Durum Defs*) cultivars performing differently in arid conditions. Plant Science 160: 669-681.

Bohnert HJ. Nelson DE. and Jensen RG (1995). Adaptation to environmental stresses. Plant Cell 11: 1099-1111.

Bollag DM and Edelstein SJ (2001). Protein methods. Wiley-Less, New York. pp. 221-230.

Bosshard HF and Datyner A (1977). The use of a new reactive dye for quantitation of pre-stained proteins on polyacrylamide gels," Analytical Biochemistry 82: 327-333.

Bradford M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Cha-um S.and Kirdmanee C (2009). Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars," Journal of Botany 42: 78-98.

Desingh R and Kanagaraj G (2007). Influence of salinity stress on phtosynthesis and antioxidative systems in two cotton varieties. Plant Physiology 33: 221-234.

Ehsapour AA and Fatahian N (2003). Effect of salt and proline on *Medicago sativa* callus. Plant Cell Tissu and Organ Culture. 73: 73-56.

Fales FW (1951). The assimilation and degradation of carbohydrates of yeast cells. Journal of Biological Chemistry 193: 113-116.

Flowers TJ, Troke PF and Yeo AR (1977).The mechanism of salt tolerance in halophytes. Annual Review of Plant Physiology 28: 89-121.

Hajar AS, Heikal MM, Maghrabi YM and Abuzinadah RA (1993) Responses of *Arachis hypogaea* (Peanut) to salinity stress," Journal of King AbdulAziz University Science 5: 5-13.

Hanson AD and Hitz WD (1980). Metabolic response of mesophytic plants to water deficits. Annual Review of Plant physiology Assiut. University 33: 163-203.

Hanson AD and Hitz WD (1982). Metabolic responses of plant water deficit. Plant Physiology 33: 163-203.

Iyer S and Caplan A (1998). Products of proline catabolism can induce osmotically regulated genes. Plant Physiology 116: 203-211.

Jeffries TW, Yang VW and Davis MW (1998).Comparative study of xylanase kinetics using dinitrosalisylic, arsenomolibdate and ion chromatographic assays. Applied Biochemistry and Biotechnology 257: 70-72.

Mass EV and Grieve CM (1994). Tiller development in salt stressed wheat. Crop Science 34: 1594-1603.

Merril CR (1990). In Methods in Enzymology," Academic Press 182: 477-488.

Munns R (1993). Physiological processes limiting plant growth in saline soils:some dogmas and hypothesis," Plant Cell Environ 16:15-24.

Munns R (2002). Comparative Physiology of salt and water stress. Plant Cell Environment 25: 239-250.

Parida AK and Das AB (2005). Salt tolerance and salinity effects on plants. A Review Ecotoxicology of Environment 60: 324-349.

Seifter S and Dayton S (1950). The estimated of glycogen with the anthrone reagent. Arch Biochem 25: 191-200.

Sunderland N (1960). Cell division and expansion in the growth of the leaf. Journal of Experimental Botany 11: 68-80.