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Differential accumulation of physio-biochemical parameters in nitere bush (*Nitraria schoberi L.*) plants against salinity

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Abstract

The present investigation was made to study the effect of different levels of soil salinity on biochemical constituents and photosynthetic pigments of the seedlings of *Nitraria schobery*. Pigment components (Chl *a*, Crt) and Chl (*a*+*b*) content decreased up to MOS level. Beyond this level the contents decreased marginally. Organic compounds such as soluble sugars, amino acids and proline content increased with the increasing of soil salinity. Highest amount of pigment components, total soluble sugars and protein contents in nonsaline condition (control) indicates that *N. schobery* plants can grow well even in nonsaline soils, but the metabolic pathways of proline, total soluble sugars and free amino acids appeared that the species can be considered as a miohalophyte species.

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Introduction

The genus *Nitraria* (Zygophyllaceae), comprising 15 species, is a dominant vegetation component of the sandy and clay deserts across Central Asia (Zhao *et al.* 2002). The genus is very broadly distributed in Middle Asia, the Middle East, Iran, North-West China and Near East deserts (Vladimir *et al.*, 1999). Its special physiological characteristics of drought resistance and salt-resistance make it an ideal plant with remarkable ecological values (Li *et al.*, 2006). *Niraria schobery* is a strong vegetation of hot sandy deserts; the species also dominates in clay and saline arid regions (Netchaeva *et al.* 1973).

In the past, the actual plains of the central of Iran were big and small lakes, which then, gradually turned to dessert and barren salt lands (Mojiri *et al.*, 2011). Iran is the classic country of great salines and Kavirs; saline and alkaline soils are expanding in arid and semiarid regions. Desertification prone lands in the country occupy two third of its terrestrial land (Mehrabian *et al.* 2009; Amiraslani and Dragovich, 2011). One way to prevent the spread of blowing sand in a desert area is through biological fixation using compatible plant species (Honarjoo *et al.*, 2010) such as *N. schoberi*. *Nitraria schoberi* is a drought-resistant shrub with numerous ramifications which is halophyte and suffered to high salt concentrations (Khajeddini *et al.* 2012). Furthermore, some authors distinguished that some halophytes genus (such as *Nitraria* genus species) often used as ruminant feeding systems (Ben Salem *et al.*, 2010) or used as drought reserve to fill annual feed shortages within grazing systems (Osman *et al.*, 2006).

Salinity in soil or water is one of the major stresses and, especially in arid and semi-arid regions, can severely limit plant production (Shanon, 1998; Zhu, 2002). The antagonistic effects of salinity on plant growth can be attributed low osmotic potential of soil solution, nutritional imbalance, specific ion effect, or a combination of the mentioned factors (Qasim and Ashraf. 2006).

Under stress conditions, plant appears ability to prevent water loss and to maintain the continuous growth. Plants commonly react to these stresses by accumulation of compatible solutes in cells which results in the improvement of environmental stress tolerance (Ashraf and Foolad, 2007). Salinity induces oxidative stress through the generation of reactive oxygen species within the plant cells (Talukdar 2011b). Salinity affects numerous physiological or biochemical processes, imposing ionic, osmotic and secondary stress such as nutritional disorders and oxidative stress leading to membrane disorganization, metabolic toxicity and inhibition of photosynthesis, many of which are seen at the cellular level (Madhuri *et al.* 2010).

The main objectives of the present study were to evaluate the photosynthetic pigments, proteins, free amino acids, proline and sugars content in *N. schobery* plants as influenced by salinity stress.

Material and methods

Niter bush seeds were collected in November 2011 from typical habitat of the Maranjab in Kashan County, Isfahan Province, Iran (34°00 –34°10 N, 51°27–51°35 E, 800–950 m a.s.l.). Seeds were sown on wet tissue paper in Petri dishes. After germination, seedlings with uniform size were planted into 6-L plastic pots filled with soil mix (soil: farm yard manure, 10:1 [w/w]). After 40 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. Seedlings were grown under natural conditions (maximum PAR 1800-2000 µmol m^{-2} s^{-1}on a clear day and daily maximum minimum temperatures 48 - 25 °C, respectively) for three months.

The mixed salts used to obtain the required salinity were NaCl, $MgCl_2$ and $CaCl_2$, and they were throughly mixed with the pot materials. The experiment was arranged in a complete randomized design (CRD) with four replicates in pots. The soil salinity content treatment was divided into four levels: control (untreated soil), MIS (mild salinity, 70 mM kg^{-1} dry

soil), MOS (moderate salinity, 140 mM kg^{-1} dry soil) and HS (high salinity, 200 mM kg^{-1} dry soil) (Asish and Bhavanath, 2010). In order to prevent water deficiency, soil water content in all the pots was kept at field capacity.

Chlorophyll and carotenoid

were extracted from the leaves and estimated by the method of Arnon (1949). Half of a gram fresh leaf material was ground with 10 ml of 80 per cent acetone at 4°C and centrifuged at 2500xg for 10 minutes at 4°C. This procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube and made up to 10 ml with 80 per cent acetone and assayed immediately.

Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with a spectrophotometer (U-2001-Hitachi) against 80 per cent acetone as blank. Chlorophyll content was calculated using the formula of Arnon and expressed in milligram per gram fresh weight (mg g^{-1} FW). Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in mg g^{-1} FW.

Total soluble sugar

was estimated by the method of Nelson (1944). Leaf samples were treated with 80 percent boiling ethanol for taking extractions (5 ml extract representing 1 g of tissue). Five readings for each sample were taken.

One ml of ethanol extract taken in the test tubes was evaporated in a water bath. To the residue, 1 ml of distilled water and 1 ml of 1 N sulphuric acid were added and incubated at 49 °C for 30 min. The solution was neutralised with 1 N sodium hydroxide using methyl red indicator. One ml of Nelson's reagent was added to each test tube prepared by mixing reagent A and reagent B in 25:1 ratio (Reagent A: 25 g sodium carbonate, 25 g sodium potassium tartarate, 20 g sodium bicarbonate and 200 g anhydrous sodium sulphate in 1000 ml; Reagent B: 15 g cupric sulphate in 100 ml of distilled water with 2 drops of concentrated sulphuric acid). The test tubes were heated for 20 min in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent (25 g ammonium molybdate, 21 ml concentrated sulphuric acid, 5 g sodium arsenate dissolved in 475 ml of distilled water and incubated at 37 °C in a water bath for 48 h) was added. The solution was thoroughly mixed and diluted to 25 ml and measured at 495 nm in a spectrophotometer. The reducing sugar contents of unknown samples were calculated from glucose standard.

Free amino acids content

was determined according to Moore and Stein (1948). One ml ethanol extract was taken in 25 ml test tubes and neutralized with 0.1 N sodium hydroxide using methyl red indicator. One ml of ninhydrin reagent was added (800 mg stannous chloride in 500 ml citrate buffer, pH 5.0, 20 g ninhydrin in 500 ml methyl cellosolve; both solutions were mixed). The contents were boiled in a water bath for 20 min, 5 ml of diluent solution (distilled water and n-propanol mixed in equal volume) was added, cooled and diluted to 25 ml with distilled water. The absorbance was measured at 570 nm in a spectrophotometer. The standard graph was prepared using leucine.

Free proline content

was determined according to Gilmour *et al.*, (2000). Seedling samples from each variety was homogenized in 3% (w/v) Sulphosalicylic acid 1 mL at room temperature and then stored at 4°C over night. The supernatant was added with acid ninhydrin and glacial acetic acid. The mixture was heated at 100°C for 45 min in a water bath. Reaction was then stopped by using an ice bath. The mixtures were extracted with toluene and measured at wavelength 519 nm. Proline concentration was determined using calibration curve and expressed as mg g^{-1}FW.

Protein content

was determined according to Lowry *et al.* (1948). Fresh tissue weighing 0.5 g was macerated in 20 per cent trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 600 rpm for 30 min and the supernatant was discarded. Five ml of

0.1 N NaOH was added to the pellet and it was centrifuged for 30 min. The supernatant was saved for the estimation of protein. To 0.5 ml of the extract, 5 ml of copper reagent 'C' was added (Reagent C: mixture of reagents A and B in the 50:1 ratio; Reagent A: 2 per cent Na2CO in 0.1 N NaOH; Reagent B: equal volume of 1 per cent CuSO3 and 2 per cent sodium potassium tartrate). The tubes were shaken well and allowed to stand in dark for 10 min at room temperature, 0.5 ml of properly diluted Folin-Ciocalteau reagent was added to the solution and mixed thoroughly. The absorbance was read at 500 nm in a spectrophotometer against an appropriate blank. Bovin serum albumin was used as the standard.

Results

The results on the effect of salinity on the pigment parameters in leaves of *N. schoberi* are presented in (Table 1). Chlorophyll *a* content decreased progressively with increase in soil salt content. The maximum Chl *a* (0.96 mg g^{-1} FW) content was observed at control and it reduced deeply when salinity level reached at HS (0.70 mg g^{-1} FW). Significant reduction in Chl *b* initiated at MIS and continued to the lowest at HS (0.22 µg g^{-1} FW). A decreasing trend in the main photosynthetic pigments content [Chl (*a* + *b*)] was observed with increasing soil salt content, as well as was obtained a reduction 32% at HS, when compared to control plants. An increase in salinity stress level provoked significant decrease in carotenoid concentration, and the values shown were 31.80 and 16.10 µg g^{-1} FW, in control and HS respectively. A steady significant increase in Chl *a* to Chl *b* ratio [Chl (*a*/*b*)] was observed with increasing soil salt content. Maximum increase in Chl. (a/b) ratio was observed at HS (135% compared to control). Drastic effects of soil salt content on the ratio of Chl (*a*+*b*) to carotenoid pigments (Table 1).

Total soluble sugars (TSS) exhibited a decreasing trend at salinity treatments. The minimum significant reduction in this organic substance (30%) was evident at MOS followed by 49.5% at HS. Free amino acids content increased significantly with increasing of soil salt content. This organic material increased by 136 and 147% in MOS and HS treatments, respectively, compared to controls (Table 2). A similar pattern was observed for increased proline (Prl). On the contrary, increasing soil salinity led to significant decrease in the total protein (Prt) content. Maximum control compared decrease in Prt content was observed in HS treatment (40.7%).

Table 1. Effects of different levels of salinity stress on pigment components (PGC) in leaves of *N. schoberi* (values are mean ± S.E., n =4).

PGC	Chl. *A* (mg g^{-1})	Chl. *B* (mg g^{-1})	Car (µg g^{-1})	Chl. (*a*+*b*) (mg g^{-1})	Chl. (*a*/*b*)
SSC (mM salt kg^{-1} DS)					
Ctrl	0.96±0.08^{a}	0.40±0.05^{a}	31.80±5.4^{a}	1.63±0.11^{a}	2.39±0.23^{a}
MIS	0.86±0.07^{b}	0.34±0.03^{b}	25.67±4.3^{b}	1.20±0.10^{b}	25.67±4.3^{b}
MOS	0.74±0.06^{c}	0.28±0.04^{b}	25.67±4.3^{b}	1.03±0.07^{c}	25.67±4.3^{b}
HS	0.70±0.03^{c}	0.22±0.03^{c}	16.10±3.2^{c}	0.92±0.05^{c}	16.10±3.2^{c}

Different letters in each column show significant difference at $P < 0.05$ by Duncan's Multiple Range Test (DMRT). SCC: soil salinity content; DS: dry soil; Chl. *a*: chlorophyll *a*; Chl. *b*: chlorophyll *b*; Chl. (*a*+*b*): sum of chlorophyll *a* and *b*; Car: carotenoid.

Different letters in each column show significant difference at $P < 0.05$ by Duncan's Multiple Range Test (DMRT). SCC: soil salinity content; DS: dry soil; TSS: total soluble sugars; Prl: proline; FAA: free amino acid and Prt: protein content.

Discussion

Salinity represents one of the most important

environmental stresses since it limits plant production disturbing the normal physiology and entire metabolic balance (Moore and Wolcott 2001). Our results showed that there was clear effect of soil salination on the leaf pigment contents. The reduced level of total chlorophyll content under salt stress condition can be attributed to chloroplastid membrane deterioration, leading toward lesser accumulation of chlorophyll (Bo-Guan *et al.*, 2011) and decrease in photosynthetic efficiency as reported earlier by several researchers (Singh and Dubey 1995; Turan *et al.*, 2009). It was observed that Chl (*a*/*b*) increased along with increasing soil salt content (Table 1), this is parallel to the results of Ramani *et al.* (2011) on *Sesuvium portulacastrum* plants and it appears that the light harvesting complex (LHCs) of thylakoid membranes are altered by salt exposure (Mitra and Banerjee, 2010).

Table 2. Effects of different levels of salinity stress on some phytochemical constituents (PCC) in leaves of *N. schoberi* (values are mean ± S.E., n =4).

PCC	TSS (mg g-1)	Prl (µg g-1)	FAA (mg g-1)	Prt (mg g-1)
SSC (mM salt kg^{-1} DS)				
Ctrl	23.93±3.60^{a}	3.26±0.50^{a}	9.17±0.93^{a}	1.67±0.34^{a}
MIS	21.68±4.26^{a}	3.50±0.30^{a}	9.34±0.63^{a}	1.38±0.13^{b}
MOS	16.66±1.27^{b}	4.70±0.26^{b}	12.43±1.40^{b}	1.20±0.10^{c}
HS	12.25±1.56^{c}	6.65±1.20^{c}	13.45±1.20^{c}	0.99±0.17^{d}

Our study clearly demonstrated that an increase in soil salt content decreases Cr content of *N. schobery* leaves (Table 1). Sharma and Hall (1991) highlighted that salinity stress induces degradation of β-carotene, which causes a decrease in the content of carotenoids that are integrated constituents of thylakoid membranes and act in absorption and light transfer to chlorophyll; besides, they protect chlorophyll from photooxidation (Thaiz and Zeiger, 2009). Thus, degradation in Cr synthesis may imply degradation of chlorophylls (Maria *et al.*, 2011).

With respect to the results obtained from the present study, it is obvious that salt stress caused a reduction in the TSS of *N. schobery* plants (Table 2). The change in TSS contents under salt stress has already been reported for a number of plant species (Khattab 2007; Rady *et al.*, 2011). This reduction concluded that salt stress may inhibit the photosynthetic activity and/or increased partial utilization of carbohydrates into other metabolic pathways (Hassenein *et al.*, 2009).

Proline accumulation in stressed plants is a primary defense response to maintain osmotic pressure in a cell. Our study showed a significant increase in Prl concentration in stressed plants as compared to the control (Table 1). Proline is known to accrue widely in higher plants in response to salinity, playing an adaptive role in mediating osmotic adjustment and protecting the sub-cellular structures. In several studies, a positive correlation between the accumulation of Prl and stress tolerance in plants has been noted (Kumar *et al.*, 2003; Desingh and Kanagaraj 2007; Madhuri 2010).

Amino acids acts as a putative osmoprotective solute leading to lowering osmotic potential in several tissues exposed to stress. The exposure of the niter bush (*N. schobery*) plants to salt stress induced an accumulation of FAA (Table 1). These results are in agreement with those observed by Azooz (2004), Khattab (2007), and Rady *et al.* (2011). These results can be attributed to the decrease in Prt synthesis and/or to the increase in its degradation (proteolysis). The different changes in phytochemical constituents in *N. schoberi* response to soil salinity, enabled to distinguish the metabolic events caused by ionic or

osmotic components of salinity. The metabolic pathways of proline, total soluble sugares and free amino acids appeared that niter bush can be considered as a miohalophyte species.

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