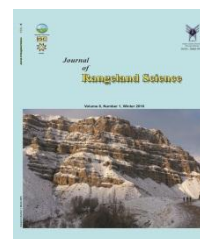


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Research and Full Length Article:

Impact of Salinity Stress on Photochemical Efficiency of Photosystem II, Chlorophyll Content and Nutrient Elements of Nitre Bush (*Nitraria schoberi* L.) Plants

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Abstract. Salinity is one of major stresses which can severely limit plant production, especially in the arid and semi-arid regions. The present study was carried out to evaluate the impact of salinity stress on some physio-biochemical parameters in nitre bush plants (*Nitraria schoberi*). Thus, an experiment was carried out under natural conditions and salinity stress was induced by a combination of different salts (NaCl, MgCl₂ and CaCl₂) at four levels. The salinity treatments were: Control (Ctrl), Low Salinity (LS), Medium Salinity (MS) and High Salinity (HS) of the combined salts. In this study, photosynthetic apparatus of *N. schoberi* was damaged to a certain extent as it has been observed from leaf chlorophyll fluorescence parameters (Chl. FPs) such as minimal fluorescence (F₀), maximal fluorescence (F_m) and maximal photochemical efficiency (F_v / F_m). A significant alteration in chlorophyll content of leaf was not noticed with the increased soil salt content up MS and thereafter, it significantly declined at HS. The reduced level of total chlorophyll content under salt stress conditions can be attributed to chloroplastid membrane deterioration leading to lesser accumulation of chlorophyll. The amount of inorganic ions in nitre bush plant leaves altered with an increase in salinity stress. The concentration of Na⁺ and Cl⁻ steadily increased and on the contrary, the concentrations of K⁺, Ca²⁺ and Mg⁺ showed significant decreases only at HS. This phenomenon is explainable by the inhibition of K⁺ uptake by high Na⁺ levels because these cations are transported by the same proteins. In our experiments, we did not observe significant differences between control plants and those grown in presence of 300 mMol salt kg⁻¹ dry soil (DS). Thus, nitre bush is considered to be a salt tolerant species.

Key words: Photosynthesis, Photochemical, Fluorescence, Pigment, Ions, Salinity

Introduction

The genus *Nitraria* (Zygophyllaceae) comprising 15 species is a dominant vegetation component of 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-Western China and Near East deserts (Vladimir *et al.*, 1999; Li *et al.*, 2006). Niter bush (*N. schoberia*) constitutes the 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 central of Iran were big and small lakes which gradually turned to dessert and barren salt lands (Mojiri *et al.*, 2011).

In this country, the areas with saline and alkaline soils are expanding, especially in the arid and semi-arid regions. Some authors distinguished that some tolerant genus such as *Nitraria* genus species have been often used as ruminant feeding systems (Ben-Salem *et al.*, 2010) or drought reserves to fill the annual feed shortages within grazing systems (Osman *et al.*, 2006).

Salinity in soil or water is one of the major stresses which especially in the arid and semi-arid regions can severely limit plant production (Shanon, 1998; Zhu, 2002). Environmental conditions that provide the concentration of intracellular Na^+ ions, K^+ , and Cl^- lead to irreversible inactivation of photosystem1(PSI) and photosystem2 (PSII). This inactivation may also occur in the electron transport respiratory chain (Allakhverdiev *et al.*, 2000). Abiotic stresses such as salinity which may affect plant growth have been investigated using the measurements of quantum efficiency of PSII (Baker, 2008). Chlorophyll fluorescence kinetics is an informative tool for studying the effects of different environmental stresses on photosynthesis (Stirbet and Govindjee, 2011).

Chlorophyll is the main color agent responsible for photosynthesis. In the

adverse circumstances, the chlorophyll level is a good indicator of photosynthesis function. It has been found that the chlorophyll level decreases with the aggravated salt stress (Furdi *et al.*, 2013) due to enzymatic chlorophyll degradation (Xu *et al.*, 2000; Khan, 2003) and decreases in the content of carotenoids that are the integrated constituents of thylakoid membranes and act in absorption and light transfer to chlorophyll; besides, they protect chlorophyll from photo oxidation (Thaiz and Zeiger, 2009). Thus, the degradation in carotenoid synthesis may imply the degradation of chlorophylls (Maria *et al.*, 2011).

There is little information about the influence of salinity stress on photosynthesis in niter bush, and there is even less knowledge regarding the effects of salinity stress on the inhibition of PSII function. The objectives of this study are: (a) to evaluate the effects of salinity on functionality of *N. schoberia* photosynthetic apparatus measured by chlorophyll fluorescence parameters, (b) changes in the content of pigments (chlorophylls and carotenoid) and (c) examine the changes in the ion accumulation in plant leaves.

Materials and Methods

Plant materials and treatments

Niter bush seeds were collected in November 2011 from typical habitat of 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 the mixed soil (soil: farm yard manure, 10:1 [w/w]). After 45 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. Seedlings were grown under natural conditions [maximum Photosynthetically Active Radiation (PAR) 1800-2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on a clear day and daily maximum

and minimum temperatures of 48 and 25 °C, respectively) for two months.

The mixed salts used to obtain the required salinity were NaCl, MgCl₂ and CaCl₂ (78, 20 and 2%, respectively), and they were applied to the plants as millimoles per kg dry soil (mmol kg⁻¹ DS). The following concentrations of salt were applied: control (untreated soil, Ctrl), 150 mmol (low salinity, LS), 300 mmol (medium salinity, MS) and 450 mmol kg⁻¹ (high salinity, HS) (Mojiri and Jalalian, 2011). In order to prevent from water deficiency, soil water content in all the pots were kept at field capacity using tap water (EC= 0.3 dS m⁻¹). The experiment was arranged in a Completely Randomized Design (CRD) with four replicates in pots.

Analysis of chlorophyll fluorescence

Chlorophyll fluorescence yields were measured using a portable fluorometer PAM-2500 (*H. walz*, Effeltrich, Germany). Before measuring chlorophyll fluorescence yields (Chl FYs), leaves were put in a dark adapted state for 30 min (Genty *et al.*, 1989) using light exclusion clips. During dark adapted state, all the reaction centers and electron carriers of PSII were re-oxidized; this situation is essential for rapid fluorescence induction kinetics and recording chlorophyll fluorescence parameters (Chl FPs).

The following Chl FYs were measured: minimal fluorescence yield of dark-adapted state (F₀), minimal fluorescence yield of light-adapted state (F'₀), maximal fluorescence yield of dark-adapted state (F_m), maximal fluorescence yield of light-adapted state (F'_m), and steady-state fluorescence yield (F_s). Some basic mutually independent Chl FPs such as maximal photosystem 2 (PSII), photochemical efficiency (F_v/F_m), effective quantum yield of photochemical energy conversion in PSII (ΦPSII), photochemical dissipation of absorbed energy (q_p) and non-photochemical dissipation of absorbed energy (NPQ) can

be calculated with respect to these five essential Chl FYs that give insight into the photosynthetic processes in chloroplasts and can be used effectively in photosynthesis research (Ranjbarfordoei *et al.*, 2006).

Chlorophyll content

At the end of greenhouse experiment, fully expanded non-senescent and undamaged leaves were collected from each plant. Leaves were immediately wrapped in aluminum foil to avoid the degradation of pigments by light. Soon afterwards, 0.5-g samples were taken from the collected leaves. These samples were then pulverized with liquid nitrogen. Subsequently, 0.25 g of each sample was extracted by 80 % acetone and put in the freezer at -5°C for 24 h. Pigments were determined according to Lichtenthaler (1987) using a spectrophotometer (Hitachi U-2001, Hitachi Ltd, Japan). Amounts of chlorophyll *a* (Chl. *a*) and Chl. *b* [mg g⁻¹ fresh mass (FM)] were calculated according to Wellburn (1994). Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in μg g⁻¹ FM.

Ion content

Dried ground leaf material (1g) was digested with sulfuric acid and hydrogen peroxide according to the method of Wolf (1982). The digested material was filtered and used for the determination of cations. K⁺, Na⁺ and Ca²⁺ were determined by a flame photometer (*Jenway, UK*) and Mg²⁺ was determined by a micro flame photometer (Varian, Austria). Chloride analysis was performed with a titrimetric method using a flame photometer (Johnson and Ulrich, 1959).

Results

Exposure of niter bush plants to the selected salinity stress levels induced the alterations in chlorophyll fluorescence parameters (Table 1). F₀ was not changed significantly with the increased salinity

stress levels from Ctrl to MS, but a further increase in salinity led to a significant increase in the value of this parameter at HS. A gradual reduction in F_m was observed with increasing salinity stress and significantly lower F_m at the HS. A decreasing trend in F_v/F_m was observed with increasing soil salt content, but a significant difference occurred

between the plants subjected to Ctrl and HS treatments. Photochemical dissipation of absorbed energy (q_p) and $\Phi PSII$ were the highest at control and lowest at SH. A steadily ascending trend was observed in NPQ from control to MS. A drastic increase in NPQ was emerged at MS and decreased slightly at HS.

Table 1. Effects of different levels of Soil Salinity (SS) on chlorophyll fluorescence parameters (Chl. FPs) in *N. schoberi* (values are mean \pm S.E., n =4)

Chl. FPs	F_0	F_m	F_v/F_m	$\Phi PSII$	q_p	NPQ
SS						
Ctrl	290 \pm 28 ^a	1915 \pm 47 ^a	0.85 ^a	0.54 \pm 0.09 ^a	0.67 \pm 0.12 ^a	0.76 \pm 0.11 ^a
LS	317 \pm 23 ^a	1890 \pm 59 ^a	0.83 ^a	0.51 \pm 0.11 ^a	0.64 \pm 0.16 ^a	0.72 \pm 0.14 ^a
MS	331 \pm 34 ^a	1853 \pm 43 ^a	0.82 ^a	0.48 \pm 0.07 ^a	0.59 \pm 0.10 ^a	1.06 \pm 0.09 ^b
HS	464 \pm 37 ^b	1586 \pm 62 ^b	0.71 ^b	0.38 \pm 0.10 ^b	0.43 \pm 0.11 ^b	0.98 \pm 0.16 ^b

Different letters in each column show significant difference at $P \leq 0.01$ by Duncan's Multiple Range Test

The results on the effects of soil salinity on the pigment parameters in the leaves of *N. schoberi* are presented in Table 2. A significant alteration in chlorophyll content of the leaf was not noticed with increasing soil salt content up to 300 mmol kg⁻¹ (MS) and thereafter, it significantly declined at SH. The Chl. *a* was always higher than that of Chl. *b* at all the concentrations. A decreasing trend in Chl. (*a* + *b*) content was observed with

increasing soil salt content, but the significant difference occurred between the plants subjected to control and HS treatments. A similar trend was also observed with carotenoid (*Car*) concentration. An increasing trend in Chl. (*a/b*) was observed with increasing soil salt content, but the significant difference occurred between the plants subjected to Ctrl and HS treatments.

Table 2. Effects of different levels of Soil Salinity (SS) on Pigment Components (PC) in leaves of *N. schoberi* (values are mean \pm S.E., n =4)

PC	Chl. <i>a</i> (mg g ⁻¹)	Chl. <i>b</i> (mg g ⁻¹)	Car (μ g g ⁻¹)	Chl. (<i>a+b</i>) (mg g ⁻¹)	Chl. (<i>a/b</i>)
SS					
Ctrl	0.58 \pm 0.09 ^a	0.33 \pm 0.06 ^a	44.52 \pm 7.5 ^a	0.91 ^a	1.76 ^a
LS	0.56 \pm 0.10 ^a	0.31 \pm 0.05 ^a	41.94 \pm 8.8 ^a	0.87 ^a	1.80 ^a
MS	0.51 \pm 0.07 ^a	0.28 \pm 0.08 ^a	39.66 \pm 6.3 ^a	0.79 ^a	1.82 ^a
HS	0.41 \pm 0.10 ^b	0.20 \pm 0.07 ^b	26.54 \pm 9.2 ^b	0.61 ^b	2.05 ^b

Different letters in each column show significant difference at $P \leq 0.01$ by Duncan's Multiple Range Test

The results presented in Table 3 show that an increase in soil salt content steadily increased Na⁺ and Cl⁻ ions. On the contrary, continual decreases were found in the concentrations of K⁺, Mg⁺ and Ca²⁺ in the leaves of *N. schoberi* plants subjected to salinity stress. A significant decrease in the contents of

mentioned cations was only observed at HS. As compared with control, K⁺ content was decreased as 2.9, 6.8 and 35.7% at LS, MS and HS, respectively, but a significant decrease was observed between MS and LS. In parallel with the Na⁺ accumulation and decline in K⁺ content, the K⁺/ Na⁺ ratio was decreased

significantly. As a consequence, the parameter (K^+/Na^+) was sharply decreased at all the levels of salinity. Ca^{2+} content was decreased as 1.18, 9.31 and 38.8% at LS, MD and HS, respectively from control. Maximum reduction given as 45.94% of control was observed for Mg^{2+} at HS. Salinity had a

significant effect on Cl^- content in *N. schoberi* plants. This anion was significantly increased with the increase in salinity level. It was 25.78 mg g^{-1} dry matter (DM) in plants at control level while in HS, it rose to 60.51 mg g^{-1} DM (Table 3).

Table 3. Effects of different levels of Soil Salinity (SS) on Nutrient Elements (NE) in leaves of *N. schoberi* (values are mean \pm S.E., n =4)

NE	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	K^+/Na^+
SS						
Ctrl	34.80 \pm 6 ^a	12.70 \pm 2.0 ^a	17.71 \pm 4.5 ^a	24.38 \pm 5 ^a	25.78 \pm 5.5 ^a	0.36 ^a
LS	51.90 \pm 5 ^b	12.33 \pm 4.0 ^a	17.50 \pm 3.2 ^a	23.33 \pm 7 ^a	35.17 \pm 3.8 ^b	0.24 ^b
MS	80.91 \pm 9 ^c	11.84 \pm 0.7 ^a	16.06 \pm 4.0 ^a	21.67 \pm 3.7 ^a	47.80 \pm 10.6 ^c	0.15 ^c
HS	95.57 \pm 11 ^d	8.17 \pm 1.0 ^b	10.84 \pm 3.3 ^b	13.18 \pm 4.0 ^b	60.51 \pm 8.4 ^d	0.09 ^d

Different letters in each column show significant difference at $P \leq 0.05$ by Duncan's Multiple Range Test

Discussion

In this study, photosynthetic apparatus of *N. schoberi* was damaged to a certain extent as it was observed from leaf chlorophyll fluorescence parameters such as F_0 , F_m , F_v/F_m , qP and NPQ (Table 1). The observed significant increase in F_0 with the corresponding decrease in F_m at HS level indicates the impairment of light harvesting complex of PSII (Naumann *et al.*, 2007). In higher plants in the unstressed conditions, F_v/F_m is close to 0.83 (Björkman and Demmig, 1987). Some researchers have found the reduced F_v/F_m due to salinity and drought stresses (Hao *et al.*, 2011; Zlatev, 2009). The F_v/F_m value in *N. schoberi* plants grown at MS was 0.82; this indicates that in the plants at MS, reaction centers are photochemically active and electron transport rate in PSII has not changed (Hazem *et al.*, 2011). A significant increase in NPQ at MS suggests an enhancement in thermal dissipation in PSII in such a way to match the decrease in photosynthesis in order to avoid the photodamage induced by the mentioned salinity levels (Li *et al.*, 2010; Qiu *et al.*, 2003). Our results can be related to some

earlier findings in which it has been observed that salt stress has significant effects on PSII photochemical activity, e.g. in *Rumex patientia* (Hua-Xin *et al.*, 2004) and *Suaeda salsa* (Hao *et al.*, 2011). Our results showed that there was a clear effect of soil salinization on the leaf pigment contents at HS (Table 2). The reduced chlorophyll contents of leaves can fulfill a similar protecting function as photoinhibition at higher salinities. On one hand, it reduces the assimilation rate, but on the other hand, it decreases the light absorption of leaves (Christian, 2005). Our study also revealed a decrease in Mg^{2+} at SH which belongs to the central structure of Chl. *a.* molecule. Thus, the decrease in Mg^{2+} content can also be attributed to the decrease in photosynthetic pigment content (Maria *et al.*, 2011).

In addition, the reduced level of total chlorophyll content under high salinity conditions can be attributed to chloroplastid membrane deterioration leading to lesser accumulation of chlorophyll (Bo-Guan *et al.*, 2011) and a 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) was affected at high saline conditions (SH) in the selected species which was increased along with the increased soil salt content (Table 1); this is parallel to the results of Ramani *et al.* (2006) on *Sesuvium portulacastrum* plants and it appears that the light harvesting complex (LHCs) of thylakoid membranes may be relatively altered by salt exposure (Mitra and Banerjee, 2010). Salt-induced decreases in photosynthetic pigments have been reported previously in various species (e.g. Meloni *et al.*, 2003; Aghaleh *et al.*, 2009; Rahdary *et al.*, 2012).

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

The amount of inorganic ions in niter bush leaves altered with an increase in salinity stress. Our study revealed that an increase in soil salinity steadily increased Na^+ and Cl^- in plant leaves. Higher Na^+ and Cl^- levels in the leaves of control and treated plants (Table 3) indicate that in *N. schobery*, an ion inclusion mechanism operates. Accumulation of Na^+ and Cl^- has been reported to account for salt tolerance in plants (Boursier and Lauchli, 1990) and this capacity has been proposed as a trait of salt tolerance (Naidoo and Raghunathan, 1990). The concentrations of K^+ , Ca^{2+} and Mg^{2+} were significantly decreased only at SH. This phenomenon is explainable by the inhibition of K^+ uptake by high Na^+

levels because these cations are transported by the same proteins (Hatamnia *et al.*, 2013). Thus, maintenance of low ratios of K^+/Na^+ will be suitable for metabolic processes occurring within the plants and essential for the plants to survive salt stress (Turkyilmaz *et al.*, 2011). The decrease in the K^+/Na^+ ratios with the decreased K^+ and Ca^{2+} content under salinity stress conditions is a common result of many studies. Our current findings are in agreement with other reports proposing that salt stress reduces K^+ , Ca^{2+} and the K^+/Na^+ ratio of *Atriplex prostrata* (Wang *et al.*, 1997), *Salicornia persica* (Aghaleh *et al.*, 2009), *Chenopodium quinoa* (Esia *et al.*, 2012) and *Atriplex halimus* (Belkheiry and Mulas, 2013).

Conclusion

In our experiments, we did not observe significant differences (for the measured parameters) between control plants and those grown in presence of 300 mmol salt kg^{-1} dry soil (MS). Thus, an important conclusion can be drawn from the results achieved through this study. Niter bush is considered to be a salt tolerant species. Our results indicate its ability to maintain high physiological activities when subjected to relatively high levels of salinity.

Acknowledgements

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اثر تنش شوری بر کارائی فتوشیمیایی فتوسیستم ۲، محتوی کلروفیل و عناصر غذایی گیاه قره‌داغ (*Nitraria sccoberi* L.)

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چکیده. شوری یکی از تنش‌های بزرگ است و بویژه در نواحی خشک و نیمه خشک، می‌تواند تولید گیاه را محدود کند. گونه‌های متعلق به جنس قره‌داغ (*Nitraria*) اغلب بعنوان اندوخته غذا بمنظور جبران کمبود علوفه در سیستم‌های چرای دام استفاده شده‌اند. مطالعه حاضر برای ارزیابی اثر تنش شوری بر برخی پارامترهای فیزیو-بیوشیمیایی گیاه قره‌داغ (*Nitraria schoberi*) انجام شد. بنابراین، آزمایشی تحت شرایط طبیعی و تنش شوری ایجاد شده با ترکیبی از نمک‌های گوناگون (کلریدهای سدیم، منیزیم و کلسیم) در چهار سطح اجرا شد. در این مطالعه، همچنانکه از پارامترهای فلورسنس کلروفیل مانند فلورسنس مبنا (کمینه) (F_0)، فلورسنس بیشینه (F_m)، و حداکثر کارایی فتوسیستم دو (F_v/F_m) مشاهده شد، دستگاه فتوسنتزکننده گیاه قره‌داغ (*N. schoberi*) تا حدی آسیب دید. با افزایش محتوی نمک خاک تا سطح شوری متوسط (MS)، تغییر در محتوی کلروفیل برگ قابل توجه نبود و از آن پس، بطور معنی‌دار در سطح شوری بالا (HS) کاهش یافت. کاهش محتوی کلروفیل کل تحت شرایط شوری را می‌توان به تخریب غشاء کلروپلاستید که منجر به تجمع کمتر کلروفیل می‌شود نسبت داد. مقدار یون‌های غیر آلی در برگ‌های گیاه با افزایش تنش شوری تغییر کرد. تراکم یون‌های سدیم (Na^+) و کلر (Cl^-) بطور پیوسته و معنی‌دار افزایش یافت و برعکس تراکم یون‌های پتاسیم (K^+)، کلسیم (Ca^{2+}) و منیزیم (Mg^{2+}) بطور معنی‌دار در سطح شوری بالا (HS) کاهش یافت. این پدیده با ممانعت جذب یون پتاسیم (K^+) توسط سطوح بالای یون سدیم (Na^+) قابل توضیح است، زیرا این کاتیون‌ها توسط پروتئین یکسان حمل می‌شوند. در آزمایش حاضر، اختلاف معنی‌دار بین گیاهان تیمار شاهد و آنهایی که در حضور ۳۰۰ میلی مول نمک بر کیلوگرم وزن خاک خشک (تیمار MS) رشد کردند یافت نشد. بنابراین، گیاه قره‌داغ به عنوان یک گونه مقاوم به شوری تلقی می‌شود.

کلمات کلیدی: فتوسنتز، فتوشیمیایی، فلورسنس، رنگدانه، یون‌ها، خشک