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Photosynthetic Responses in Reed (*Phragmites australis* (CAV.) TRIN. ex Steud.) Seedlings Induced by Different Salinity-Alkalinity and Nitrogen levels

Ch. Deng[1,2], G. Zhang[1]*, and X. Pan[3]

ABSTRACT

Many *Phragmites*-dominated wetlands have been markedly salinized and alkalinized in the Songnen Plain, northeastern China. Agricultural wastewater with high nitrogen content has been discharged into these alkalinized-salinized wetlands. To understand the effect of salinity-alkalinity on reed (*Phragmites australis*) seedlings at various nitrogen levels, we examined photosynthesis rate, chlorophyll fluorescence characteristics, and chlorophyll content of reed seedlings using gas exchange and chlorophyll *a* fluorescence tests. The greatest decreases (by 82%, 15%, 82% and 98%) of net photosynthesis rate (P_n), maximal efficiency of photosystem II (PSII) photochemistry (F_V/F_M), comprehensive photosynthesis performance index (PI_{ABS}) and plant height growth rate were observed at high salinity-alkalinity (mixed with 150 mM NaCl and 100 mM $NaHCO_3$). Stomatal limitation was the main reason for decreased photosynthesis rate at low salinity-alkalinity (mixed with 50 mM NaCl and 25 mM $NaHCO_3$). The activity of PSII was significantly inhibited at high salinity-alkalinity. Both donor and acceptor sides of PSII are the target sites of high salinity-alkalinity. High N (30 mM) at low salinity-alkalinity and moderate N (15 mM) at high salinity-alkalinity mitigated the toxicity of salinity-alkalinity on reeds and promoted plant height growth, chlorophyll synthesis, and PSII activity. Proper levels of N partly reduced the toxicity of salinity-alkalinity on the donor and acceptor sides of PSII. This suggests that agricultural wastewater containing high level of nitrogen may be helpful in restoration of *Phragmites*-dominated salinized wetland, though the N level needed for salinity-alkalinity stressed reed varies with the salinity-alkalinity level.

Keywords: Alkalinized-salinized wetlands, Chlorophyll fluorescence, Gas exchange, Nitrogen, *Phragmites australis*.

INTRODUCTION

Salinization of wetlands is increasingly getting serious in arid and semi-arid areas. Excess salinity induces adverse effects on plants photosynthesis (Long and Baker, 1986) including reduction of CO_2 assimilation and stomatal conductance and decelerating activity of photosystem II (PSII) (Everard *et al.*, 1994). The reduction of photosynthesis is often due to both stomatal and non-stomatal limitations (Loreto *et al.*, 2003; Jeranyama and DeMoranville, 2008). Excess alkalinity also inhibits photosynthesis and retards plant growth (Shi and Wang, 2005; Yang *et al.*, 2009). When soil salinization and alkalization co-occur,, their effect on plants is much more complex. To our best knowledge, only a few reports are

[1] Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, Jilin, 130012, People Republic of China.
[2] School of Tourism and Geographic Science, Yunnan Normal University, Kunming, Yunnan, 650092, People Republic of China.
[3] Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, Xinjiang, 830011, People Republic of China.
* Corresponding author, e-mail: zhanggxcc@yahoo.cn

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available about the effect of combined salinity-alkalinity stress on photosynthesis rate of plants (Anjum *et al.*, 2005; Zhang and Yin, 2009). Previous limited studies showed that salinity-alkalinity mixed stress led to decrease in chlorophyll concentration (Kaya *et al.*, 2002), reduction of stomatal conductance and transpiration rate (Khan and Abdullah, 2003), and inhibited the plant growth. However, the underlying knowledge about the inhibitory effects of combined salinity-alkalinity stress on photosynthetic activity is yet not fully understood.

The metabolic process of plants is largely dependent upon an adequate supply of nitrogen. There is a strong correlation between the nitrogen supply and plant photosynthetic capacity. Nitrogen limitation to plant production is believed to be widespread, especially in salinized soil (van Wijnen and Bakker, 1999). Plants growing under nitrogen-limited conditions exhibit decreases in photosynthetic capacity and leaf chlorophyll concentrations (Terashima and Evans, 1988; Lu and Zhang, 2000). Nitrogen, as a crucial macronutrient for plants, and Cl, as a micronutrient and a major salinity agent, are known to be antagonistic to each other in the nutrient uptake system of plants (Tisdale *et al.*, 1993). Increasing salinity usually decreases nitrogen availability and affects nitrogen movement to plants. Increasing nitrogen supply decreases the toxicity of salinity to the plants (Tisdale *et al.*, 1993). Apparent increases in salt tolerance (Papadopoulos and Rendig, 1983) and photosynthetic rate have been found when excess N is supplied under saline conditions (Kao *et al.*, 2001), implying that excess N may overcome some of the inhibitory effects of salinity. Although many researchers have studied the interaction of nitrogen and salinity (Papadopoulos and Rendig, 1983; Kao *et al.*, 2001; Tabatabaei, 2006), there is little information available on the effect of nitrogen on plant physiology under combined salinity-alkalinity stress.

Chlorophyll *a* fluorescence is a sensitive indicator of photosynthetic efficiency in plants and has been proved as a rapid, non-invasive, and reliable method to assess photosynthetic performance under various environmental stresses (Krause and Weis, 1991; Schreiber *et al.*, 1994). The O-J-I-P fast chlorophyll *a* fluorescence transient has been found to be very sensitive to various environmental stresses (Strasser *et al.*, 2000). The JIP-test is thus a useful tool for the *in vivo* investigation of the behavior of the photosynthetic apparatus. In the present study, we employed this approach to get a more thorough insight into the responses of PSII to salinity-alkalinity stress under various N conditions.

Phragmites australis is one of the most widespread and productive wetland plant species. In the Songnen Plain, northeastern China, a number of *Phragmites*-dominated wetlands have been markedly salinized and alkalinized due to overexploitation or irrational development of water resources and climate change. Agricultural wastewater containing high nitrogen has become a source of water for alkalinized-salinized wetlands; while it has been purified by wetlands. Knowledge about the effects of nitrogen level on plant stressed by salinity-alkalinity is important for understanding nutrient source control in agricultural wastewater discharged into alkalinized-salinized wetlands. The objective of this study was to investigate the photosynthetic responses of *P. australis* to combined salinity-alkalinity stress at various nitrogen levels by gas exchange measurement and *in vivo* chlorophyll *a* fluorescence test.

MATERIALS AND METHODS

Materials and Treatments

Rhizomes of reed (*P. australis*) were taken from Momoge wetland in the Songnen Plain, northeastern China. Rhizomes of about 20cm were cultivated in outdoor sand tanks and watered with pond water for 3 weeks until seedlings emerged. *P. australis* seedlings, about 15 cm long, were transplanted into 8-L plastic pots with sands under a photosynthetic photon flux density

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(PPFD) of approximately 600 µmol m^{-2} s^{-1} provided by metal halide bulbs, 60% relative humidity, a photoperiod of 14h, and a day/night temperature of 25/16°C. Seedlings were acclimated in the greenhouse for at least one week prior to experimental treatment. Each pot contained 5 seedlings and there were three pots for each treatment.

NaCl and $NaHCO_3$ were selected based on the main salt components in saline-alkaline soils of Momoge wetland. The salts and N supply of various concentrations were mixed as shown in Table 1. Nitrogen, in the form of NH_4NO_3,was added into the salt solution at four levels (0, 7.5, 15 and 30 mM). Some seedlings watered with Hoagland nutrient solution (including 15 mM N at recommended level) were used as the control. Plants under various stress conditions were watered every three days with Hoagland nutrient solution containing various salinity-alkalinity and nitrogen levels.

Growth Measurement

Plant heights were recorded at the beginning and the end of the 15-day treatment. Height was measured as the distance between the stem base and the shoot tip. The plant growth rate was estimated as follows: Height rate $=H_2 - H_1$. H_1= plant height at the start of treatment; H_2 = plant height at the end of treatment.

Chlorophyll Fluorescence Measurement

The fourth fully expanded leaf was used for fast Chlorophyll *a* fluorescence measurements with a portable fluorometer (FP100, PSI, Brno, Czech Republic). The plant leaf was dark-adapted for 10 min using leaf-clip before fluorescence measurement. This fluorometer instrument is devised for measurements of fast fluorescence transients (O-J-I-P rise) according to the commonly accepted illumination protocol. Chlorophyll fluorescence is excited by a visible light band peaking at 475 nm (half bandwidth 25 nm). Fast fluorescence transients induced by actinic light were recorded at sampling intervals varying from 10 µs to 10 ms. The polyphasic fast fluorescence induction curve provides valuable information about the function of PSII (Strasser *et al.*, 1995). Upon the triggering of strong actinic light, the increase in Chlorophyll *a* fluorescence of dark-adapted photosynthetic materials will follow a triphasic kinetic from its initial level (F_O), two intermediate levels (F_J and F_I) to its maximal level (F_M or F_P). The initial Chl fluorescence F_O (fluorescence intensity at 50 µs) indicates all molecules of Q_A are in the oxidized state. F_J (fluorescence intensity at around 2 ms) reflects the accumulation of Q_A^-. F_I (fluorescence intensity at around 30 ms) demonstrates an accumulation of $Q_A^-Q_B^-$, whereas F_M (maximal fluorescence intensity, usually reached at 200-500 ms) indicates all molecules of Q_A are in the reduced state with

Table 1. Concentrations of various compounds of salinity-alkalinity and N levels in different treatments.

Treatment	NaCl (mM)	$NaHCO_3$ (mM)	N level (mM)
control	0	0	15
SA_1	50	25	0
SA_2	150	100	0
SA_1+N_1	50	25	7.5
SA_1+N_2	50	25	15
SA_1+N_3	50	25	30
SA_2+N_1	150	100	7.5
SA_2+N_2	150	100	15
SA_2+N_3	150	100	30

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the accumulation of $Q_A^-Q_B^{2-}$ (Strasser and Govindjee, 1992; Strasser *et al.*, 1995). It can be used to analyze changes in electron transfer reaction on both donor (Delosme and Joliot, 2002) and acceptor side of PSII (Strasser and Govindjee, 1992). The JIP-test analysis (Strasser and Strasser, 1995) was employed to analyze each chlorophyll *a* fluorescence transient. The JIP-test represents translation of the original data to biophysical parameters that quantify the energy flow through PSII. F_O, $F_{300\mu s}$, F_J and F_M from the original measurements were used. Other JIP-test parameters were derived from these original parameters (Table 2) (Strasser *et al.*, 2000).

Gas Exchange Measurement

After Chlorophyll *a* fluorescence measurement, the same leaves were used for measurement of net photosynthesis (P_n), stomatal conductance (g_s), internal CO_2 concentration (C_i) at a PPFD level of 1500 µmol m $^{-2}$ s^{-1} at ambient CO_2 concentration (350 µmol mol $^{-1}$) with a portable photosynthesis system (LI-6400, LI-COR Biosciences Inc., Lincoln, NE, USA).

Determination of Chlorophyll Content

The chlorophyll content indices (CCI) of the same leaves used for chlorophyll fluorescence and gas exchange measurement were measured with a CCM-200 chlorophyll content meter (Opti-Sciences, Tyngsboro, MA, USA). The relative change in chlorophyll content ratio was used to characterize the degree of stress-induced inhibition of plant chlorophyll synthesis, which was expressed as: The change in chlorophyll content (%) = $(C_2 - C_1) / C_1 \times 100\%$, where $C_1$is the chlorophyll content at the start of treatment and C_2 is the chlorophyll content at the end of treatment.

Data were presented as the means ± standard errors for each treatment (n = 5). Analysis of variance was performed using the SPSS software (ver.13.0).

RESULTS AND DISCUSSION

Growth Measurement

The plant height growth rates were significantly inhibited after SA_1 and SA_2 treatments (Figure1), being 40% and 2% of the

Table 2. Equations and terms used in the JIP-test analysis (Strasser *et al.*, 2000)

Formulae and terms	Illustrations
$V_J = (F_{2ms} - F_O)/(F_M - F_O)$	Relative variable fluorescence intensity at the J-step
$F_V = F_M - F_O$	Variable fluorescence
$M_O = 4\ (F_{300\ \mu s} - F_O)/(F_M - F_O)$	Approximated initial slope of the fluorescence transient
Quantum efficiencies or flux ratios	
$\varphi_{Po} = TR_O/ABS = [1 - (F_O/F_M)] = F_V/F_M$	Maximum quantum yield for primary photochemistry (at $t = 0$)
$\varphi_{Eo} = ET_O/ABS = [1 - (F_O/F_M)]\ \Psi_O$	Quantum yield for electron transport (at $t = 0$)
$\varphi_{Do} = 1 - \varphi_{Po} = F_O/F_M$	Quantum yield for energy dissipation (at $t = 0$)
$\Psi_O = ET_O/TR_O = (1 - V_J)$	Probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (at $t = 0$)
Specific fluxes or specific activities	
$ABS/RC = M_O\ (1/V_J)\ (1/\varphi_{Po})$	Absorption flux per reaction center
$TR_O/RC = M_O\ (1/V_J)$	Trapped energy flux per reaction center (at $t = 0$)
$ET_O/RC = M_O\ (1/V_J)\ \Psi_O$	Electron transport flux per reaction center (at $t = 0$)
$DI_O/RC = (ABS/RC) - (TR_O/RC)$	Dissipated energy flux per reaction center (at $t = 0$)
Performance indexes	
$PI_{ABS} = (RC/ABS)\ [\varphi_{Po}/(1 - \varphi_{Po})]\ [\Psi_O/(1 - \Psi_O)]$	Performance index on absorption basis

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control, respectively. The plant height growth was markedly reduced with increasing salinity-alkalinity level. Restriction on plant height and shoot biomass is one of the obvious symptoms of salinity-alkalinity stress (Guan *et al.*, 2009), which mainly resulted from the inhibition of expansion growth of new leaves and shoot elongation rate. The restriction on leaf expansion under saline-alkaline conditions minimizes water losses, which happens to many species under osmotic stress (Tabatabaei, 2006). Low N level (7.5 mM) had no improvement on plant height growth under salinity-alkalinity stress. The height rate of plants under SA_1+N_2, SA_1+N_3 and SA_2+N_2 treatments were less stressed, being 67%, 79%, and 11% of the control, respectively. It was demonstrated that the high level of N (30mM) partially mitigated the adverse effects of low salinity-alkalinity on plant height growth (Figure 1), while moderate N level (15mM) showed the greatest mitigation effect at high salinity-alkalinity. One reason for the mitigation effect is that ammonium nitrate might convert $NaHCO_3$ into NH_4HCO_3, resulting in decreased alkalinity. Besides, enough fertilization enhanced the plant resistance to stress. Under the high salinity-alkalinity and N supply condition, salinity-alkalinity was the dominant factor governing the reed growth and N uptake, and over-fertilization did not improve N uptake. Thus, moderate N level mitigated the adverse effect induced by high salinity-alkalinity. Abdelgadir *et al.* (2005) reported that N supply increased rice growth under salinity stress. Tabatabaei (2006) found that plants growth were improved at 200 mg l^{-1} N in two olive cultivars (cvs Manzanillo and Zard), but it was reduced when the N concentration increased to 300 mg l^{-1}. However, the growth of another olive cultivar (Mission) progressively increased with the increase in both salinity and N level. These findings are similar to our results, in which the optimum N concentration for mitigation varies between species and the levels of salinity-alkalinity.

Photosynthesis Rate Analysis

The net photosynthesis rate (P_n), stomotal conductance (g_s), and intercellular CO_2 concentration (C_i) are shown in Table 3. Compared with the control, plants P_n decreased by 41% and 82% under SA_1 and

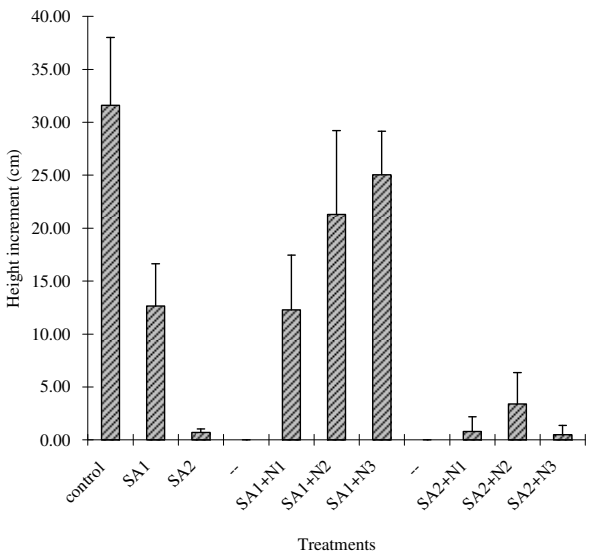

Figure 1. The height increment of the control and 15-day old salinity-alkalinity stressed reed seedlings under various N conditions. Vertical lines are standard error of the means (n = 5)

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Table 3. The leaf net photosynthesis (P_n), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of the control and 15-d salinity-alkalinity stressed reed seedlings under various N conditions.

Treatment	P_n (μmol CO_2 m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})	C_i (μmol mol^{-1})
control	24.23 ± 2.36	0.693 ± 0.012	291 ± 22
SA_1	14.20 ± 1.25	0.286 ± 0.005	279 ± 17
SA_2	4.38 ± 0.31	0.099 ± 0.002	359 ± 10
SA_1+N_1	12.97 ± 0.88	0.180 ± 0.002	241 ± 8
SA_1+N_2	20.47 ± 1.55	0.284 ± 0.007	233 ± 11
SA_1+N_3	19.90 ± 2.23	0.297 ± 0.010	243 ± 14
SA_2+N_1	14.67 ± 2.15	0.217 ± 0.012	266 ± 20
SA_2+N_2	9.80 ± 0.92	0.075 ± 0.004	229 ± 6
SA_2+N_3	9.58 ± 1.05	0.059 ± 0.006	174 ± 15

SA_2 treatments, respectively. The sensitivity of P_n shows that it is an ideal indicator of salinity-alkalinity stress, as also shown in a pervious study (Xu *et al.*, 2008). Reduction in net photosynthesis rate strongly correlated with stunted growth, implying that inhibition of plant growth can be at least partially attributed to the reduction of carbon assimilation under stress. Stomatal conductance (g_s) decreased significantly in plants under SA_2 treatment at various N levels. Intercellular CO_2 concentration (C_i) decreased by 4% in plants under SA_1 treatment, but increased by 23% under SA_2 treatment. According to Farquhar *et al.* (1989), lower P_n accompanied by lower g_s and C_i at low salinity-alkalinity (SA_1) might be mainly ascribed to stomatal closure, which restricts entry of CO_2 into leaves. However,, lower P_n accompanied by lower g_s and higher C_i at high salinity-alkalinity (SA_2) may be attributed to non-stomatal limitation, including changes in leaf biochemistry that results in inhibition or down-regulation of photosynthesis and the reduction of the chlorophyll content. Previous studies demonstrated that stomatal limitation is more significant at medium salinities and non-stomatal restriction is more pronounced at high salinity levels (Everard *et al.*, 1994; Netondo *et al.*, 2004). A similar change pattern in response to salinity-alkalinity stress was found in the present study. Our study suggests that the stomtal closure is likely the first defense mechanism of plant against salinity-alkalinity stress. Non-stomatal limitation increases progressively under high salinity-alkalinity stress. This means that non-stomatal limitation may play a major role through inhibiting biochemical metabolism and adversely affecting chlorophyll *a* fluorescence.

Similar to the observation of Kao *et al.* (2001), who reported that increasing nitrogen availability increased CO_2 assimilation of the mangrove species, N supply enhanced the photosynthetic capacity of plants under salinity-alkalinity stress in the present study. There was a clear recovering tendency of photosynthesis rate for plants treated with low salinity-alkalinity at moderate and high N levels. As shown in Table 3, P_n for SA_1+N_2 and SA_2+N_1 treated plants were up-regulated to 84% and 61% of the control, respectively. The stomotal conductance (g_s) changed little at moderate and high N and low/high salinity-alkalinity levels, but showed an increase at low N and high salinity-alkalinity, suggesting that low N supply decreased stomatal closure at high salinity-alkalinity. Intercellular CO_2 concentration (C_i) decreased slightly for plants at various N levels at low/high salinity-alkalinity. A higher P_n accompanied by lower C_i suggests that optimum N supply may increase photobiochemical activity. These results revealed that N supply increased the photosynthesis rate of reed stressed by low/high salinity-alkalinity; the decrease of non-stomotal limitation being one of the important reasons. This was further confirmed by the results of chlorophyll fluorescence measurements (Table 4).

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Table 4. The selected JIP-test parameters of the control, salinity-alkalinity stressed plants under various N conditions. All parameters were normalized to the values of the control.

Treatment	F_M	F_V	V_J	F_M/F_O	F_V/F_O	F_V/F_M	M_o	Ψ_O	φ_{Eo}	φ_{Do}	PI_{ABS}	ABS/RC	TR/RC	ET/RC	DI/RC
control	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SA_1	0.93	0.91	1.05	0.89	0.86	0.97	1.18	0.95	0.92	1.12	0.65	1.16	1.13	1.08	1.3
SA_2	0.68	0.6	1.45	0.64	0.56	0.85	1.58	0.57	0.49	1.63	0.18	1.29	1.09	0.63	2.15
SA_1+N_1	0.96	0.95	1.06	0.97	0.96	0.99	1.06	0.95	0.94	1.03	0.82	1.02	1.01	0.96	1.05
SA_1+N_2	0.96	0.95	0.93	0.96	0.95	0.99	0.87	1.07	1.06	1.04	1.1	0.95	0.94	1.01	1
SA_1+N_3	0.96	0.95	0.84	0.96	0.95	0.99	0.8	1.15	1.13	1.04	1.3	0.96	0.95	1.1	1
SA_2+N_1	0.99	0.94	1.29	0.82	0.78	0.95	1.43	0.73	0.69	1.22	0.4	1.18	1.11	0.82	1.46
SA_2+N_2	0.88	0.85	1.04	0.89	0.86	0.97	1.01	0.97	0.94	1.12	0.77	1.01	0.99	0.96	1.14
SA_2+N_3	0.88	0.84	1.16	0.84	0.8	0.95	1.25	0.85	0.81	1.19	0.51	1.13	1.08	0.92	1.36

Chlorophyll Fluorescence

To investigate the effects of salinity-alkalinity and N level on the electron transport of PSII of plants, the poly-phases fast fluorescence induction test was performed. Examples of the fast kinetic induction curves of plants treated with various salinity-alkalinity and various concentrations of nitrogen are shown in Figure 2. No significant change of F_O was found under salinity-alkalinity stress. F_M decreased drastically and the J-P phase gradually leveled off with high salinity-alkalinity concentration (Figure 2 and Table 4), suggesting that electron transport was hindered after Q_A (Haldimann *et al.*, 1995; Strasser *et al.*, 1995; Toth *et al.*, 2005), and OEC failed to provide electrons for PSII to reduce the quinone acceptors (Falk and Palmqvist, 1992); that is, the electron transport on the donor side of PSII was inhibited, and $P680^+$, a strong fluorescence quencher, accumulated at higher salinity-alkalinity (Govindjee, 1995), indicating that high salinity-alkalinity stress had inhibitory effects on the donor side of PSII. The depression of F_M was also found in higher plants and algae stressed by pollutants (Appenroth *et al.*, 2001; Pan *et al.*, 2008). The decrease of F_M led to a decrease of the variable fluorescence (F_V), and finally led to a decline of F_V/F_M. The values of F_V/F_M were significantly reduced under SA_2 treatment with respect to the control (Table 4). This was in accordance with many other studies (Larcher *et al.*, 1990; Xu *et al.*, 2008; Kafi, 2009). According to Kafi (2009), the non-stomatal limitation such as reduction in F_V/F_M, show that plant might experience some degree of photoinhibition at high salinity, indicating that the RuBP regeneration, which needs adequate electron translocation from PSII to electron acceptors, might be disturbed by high salinity. Usually, under moderate stress, F_V/F_M of plants changes little and its decline under severe stress reflects weakened ability of PSII to reduce the primary acceptor Q_A (Calatayud and Barreno, 2001).

Since the O-J-I-P fluorescence transient reflects the state of Q_A, Q_B, and PQ pool (Strasser and Govindjee, 1992), more information was obtained from parameters extracted from fluorescence rises (Table 4). Results of the JIP-test (Table 4) demonstrated that high salinity-alkalinity stress resulted in an increase in the effective antenna size per reaction center (ABS/RC,

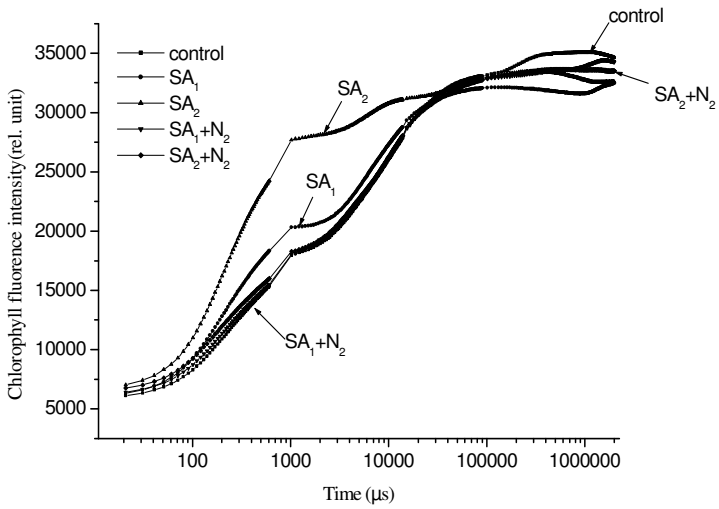

Figure 2. Examples of fast chlorophyll fluorescence transient of the control and salinity-alkalinity stressed reed seedlings under various N conditions.

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29%) and a decrease in electron transport flux (ET_o/RC, φ_{Eo}, Ψ_o). Under SA_2 stress, lower φ_{Eo} (49% of the control) and Ψ_o (57% of the control) revealed that the activity of electron transport beyond Q_A, i.e. the acceptor side of PSII, was considerably hindered at high salinity-alkalinity. The increasing trend of fluorescence intensity at J step at high salinity-alkalinity (SA_2, Figure2) is usually interpreted as an evidence of accumulation of reduced Q_A^- pool (Strasser *et al.*, 1995), possibly due to a decrease of electron transport beyond Q_A^-. The value of φ_{Do} under SA_2 stress increased to 163% of the control, resulting in a decrease of the performance index (PI_{ABS}) by 82%. Parameters such as PI_{ABS}, φ_{Do}, and ABS/RC showed higher sensitivity than F_V/F_M to salinity-alkalinity stress, which is in agreement with many other studies (Christen *et al.*, 2007). This is because these parameters provide information on the heterogeneity of PSII reactive centers while F_V/F_M only reflects the efficiency of all the PSII units including both activated and inactivated reactive centers (Wen *et al.*, 2005).

Nitrogen addition at high salinity-alkalinity caused F_V/F_M to recover to 95-97%, suggesting N supply promoted the ability of PSII to reduce the primary acceptor Q_A. The greatest improvement of electron transfer (ET_o/RC, φ_{Eo} and Ψ_o), dissipated energy (DI/RC,φ_{Do}), and performance index (PI_{ABS}) occurred at moderate N with high salinity-alkalinity, indicating that the activity of electron transport beyond Q_A was enhanced. Plants growing under N-limited conditions exhibit decreases not only in photosynthetic capacity but also in leaf chlorophyll concentrations (Demmig-Adams and Adams, 2003), suggesting that photo-damage may occur under N deficiency. Increased thermal energy dissipation under N deficiency has been found in maize (Lu and Zhang, 2000). Therefore, nitrogen supplement counteracts these adverse effects on the PSII, i.e., nitrogen supplement promotes electron transfer and decreases the energy dissipation, thus, enhances the PSII activity.

Chlorophyll Content

To further understand the damage of salinity-alkalinity stress to photosynthetic apparatus, chlorophyll content change was calculated. Fifteen days after the onset of the treatments, the chlorophyll content of reed seedlings decreased by 15% and 28% under SA_1 and SA_2 treatments, respectively (Figure 3). A decrease in leaf chlorophyll content has been described in plants irrigated with water containing high concentrations of NaCl (Demir and Kocacaliskan, 2008). This decrease may be attributed to the formation of proteolytic enzymes, such as chlorophyllase, which degrade chlorophyll and damage the photosynthetic apparatus (Yasseen, 1983). Chlorophyll content of the reeds receiving high N (30 mM) with low salinity-alkalinity and moderate N (15 mM) with high salinity-alkalinity showed the greatest increase, i.e. 94% and 22%, respectively. Increase in chlorophyll content after addition of nitrogen might be explained by the fact that the synthesis of chlorophylls and related proteins, as well as precursor molecules such as amino acids, is dependent on N availability (Terashima and Evans, 1988). Under high salinity-alkalinity stress, N uptake of reed seedling will decrease, thus, high nitrogen may cause more inhibitory effect on it. Therefore, chlorophyll content reduction in SA_2+N_3 decreased significantly. The changes of the chlorophyll content in various conditions were in accordance with the change of chlorophyll fluorescence and plant height growth.

CONCLUSION

The growth of reed seedling was retarded by salinity-alkalinity stress through down-regulation of chlorophyll content and inhibition of photosynthesis. Decreased

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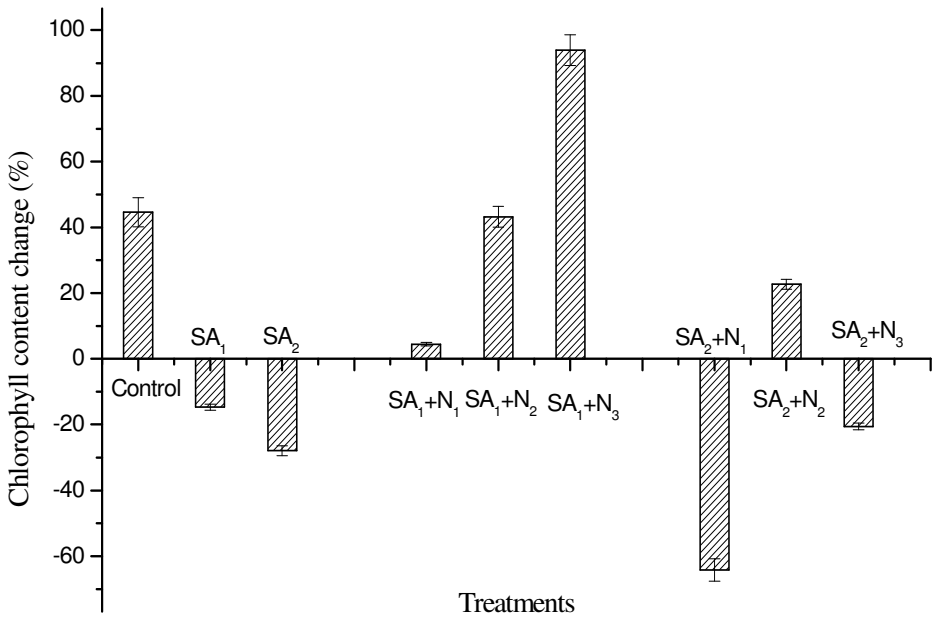

Figure 3. Chlorophyll content change (%) of the control and salinity-alkalinity stressed plants under various N conditions. Vertical lines are standard error of the means. (n = 5).

photosynthesis rate under low salinity-alkalinity condition is associated with the increase in stomatal closure, while under high salinity-alkalinity stress it may mainly be attributed to non-stomatal limitation, i.e. down-regulation of photochemical activity and damage of photosynthetic apparatus. Both donor and acceptor sides of PSII are the target sites under high salinity-alkalinity. At high salinity-alkalinity, the electron transfer was markedly inhibited and energy dissipation was enhanced, eventually, leading to decrease in the comprehensive photosynthesis performance index and photosynthesis rate. The highest N supply in low salinity-alkalinity treatment, or moderate N supply in high salinity-alkalinity treatment, partially mitigated the toxicity of salinity-alkalinity to plants. The mitigation effect included promotion of the plant height growth and chlorophyll synthesis and decreasing the inhibitory effects of salinity-alkalinity on PSII activity. Nitrogen supply could enhance the electron transfer on the donor side and the acceptor side of PSII, decrease the energy dissipation, and increase the comprehensive photosynthesis performance and photosynthesis rate. These results suggested that agricultural wastewater with high nitrogen content may be used for the recovery of *Phragmites*-dominated alkalinized-salinized wetland.

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REFERENCES

1. Abdelgadir, E. M., Oka, M. and Fujiyama, H. 2005. Nitrogen Nutrition of Rice Plants under Salinity. *Biologia Plantarum*, **49 (1):** 99-104.
2. Anjum, R., Ahmed, A., Ullah, R., Jahangir, M. and Yousaf, M. 2005. Effect of Soil

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[DOR: 20.1001.1.16807073.2011.13.5.4.5]

Salinity/ Sodicity on the Growth and Yield of Different Varieties of Cotton. *Int. J. Agri. Biol.*, **7(4):** 606-608.

3. Appenroth, K.J., Stockel, J., Srivastava, A. and Strasser, R.J., 2001. Multiple Effects of Chromate on the Photosynthetic Apparatus of *Spirodela polyrhiza* as Probed by OJIP Chlorophyll a Fuorescence Measurements. *Environ. Pollut.*, **115(1):** 49–64.
4. Calatayud, A. and Barreno, E. 2001. Chlorophyll a Fluorescence, Antioxidant Enzymes and Lipid Peroxidation in Tomato in Response to Ozone and Benomyl. *Environ. Pollut.*, **115 (2):** 283-289.
5. Christen, D., Schonmann, S., Jermini, M., Strasser, R. J. and Defago, G. 2007. Characterization and Early Detection of Grapevine (*Vitis vinifera*) stress responses to Esca Disease by in situ Chlorophyll Fluorescence and Comparison with Drought Stress. *Environ. Exp. Bot.*, **60 (3):** 504-514.
6. Delosme, R. and Joliot, P. 2002. Period Four Oscillations in Chlorophyll *a* Fluorescence. *Photosynth. Res.*, **73 (1-3):** 165-168.
7. Demir, Y. and Kocacaliskan, I. 2008. Effects of Proline on Maize Embryos Cultured in Salt Stress. Fresenius Environ. Bull., **17 (5):** 536-542.
8. Demmig-Adams, B. and Adams III, W. W. 2003. Photoinhibition. In: Thomas, B., Murphy, D. and Murray, B. (eds.) *"Encyclopedia of Applied Plant Science"*. Academic Press, New York. pp 707–714.
9. Everard, J. D., Gucci, R., Kann, S. C., Flore, J. A. and Loescher, W. H. 1994. Gas-Exchange and Carbon Partitioning in the Leaves of Celery (*Apium-Graveolens* L) at Various Levels of Root-Zone Salinity. *Plant Physiol.*, **106 (1):** 281-292.
10. Falk, S. and Palmqvist, K. 1992. Photosynthetic Light Utilization Efficiency, Photosystem-II Heterogeneity, and Fluorescence Quenching in *Chlamydomonas-Reinhardtii* during the Induction of the CO_2-Concentrating Mechanism. *Plant Physiol.*, **100 (2):** 685-691.
11. Farquhar, G. D., Ehleringer, J. R. and Hubick, K. T. 1989. Carbon Isotope Discrimination and Photosynthesis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.,* **40:** 503-537.
12. Govindjee, 1995. Sixty-three Years since Kautsky: Chlorophyll *a* Fuorescence. *Aust. J. Plant Physiol.* **22:**131–60.
13. Guan, F. C., Yan, C. and Liang, Z. W. 2009. Differences of the Soil and Vegetation in *Phragmites australis* Community between Enclosed and Grazed Alkali-salinity Wetlands. *Wetland science*, **7(1):** 47-52.
14. Haldimann, P., Srivastava, A. and Strasser, R. J. 1995.In: P. Mathis (Editor), *"Photosynthesis: From Light to Biosphere"*, vol. II, Kluwer, Dordrecht. 911-914.
15. Jeranyama, P. and DeMoranville, C. J. 2009. Gas Exchange and Chlorophyll Content of Cranberry under Salt Stress. *Acta Hort. (ISHS),* **810:** 753-758.
16. Kafi, M. 2009. The Effects of Salinity and Light on Photosynthesis, Respiration and Chlorophyll Fluorescence in Salt-tolerant and Salt-sensitive Wheat (*Triticum aetivum* L.) cultivars. *J. Agr. Sci. Tech.,* **11:** 535-547.
17. Kao, W. Y., Tsai, H. C. and Tsai, T. T. 2001. Effect of NaCl and Nitrogen Availability on Growth and Photosynthesis of Seedlings of a Mangrove Species, *Kandelia candel* (L.) Druce. *J. Plant Physiol.,* **158 (7):** 841-846.
18. Kaya, C., Higgs, D. and Ikinci, A. 2002. An Experiment to Investigate Ameliorative Effects of Potassium Sulphate on Salt and Alkalinity Stressed Vegetable Crops. *J. Plant Nutr.,* **25 (11):** 2545-2558.
19. Khan, M. A. and Abdullah, Z. 2003. Salinity-sodicity Induced Changes in Reproductive Physiology of Rice (*Oryza sativa*) under dense soil conditions. *Environ. Exp. Bot.,* **49 (2):** 145-157.
20. Krause, G. H. and Weis, E. 1991. Chlorophyll Fluorescence and Photosynthesis - the Basics. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **42:** 313-349.
21. Larcher, W., Wagner, J. and Thammathaworn, A. 1990. Effects of Superimposed Temperature Stress on Invivo Chlorophyll Fluorescence of *Vigna-Unguiculata* under Saline Stress. *J. Plant Physiol.*, **136 (1):** 92-102.
22. Long, S. P. and Baker, N. R. 1986. Saline Terrestrial Environments. In: Baker, N. R and Long, S. P. (eds.) *"Photosynthesis in Contrasting Environments"*. Elsevier, New York. pp. 63–102.
23. Loreto, F., Centritto, M. and Chartzoulakis, K. 2003. Photosynthetic Limitations in Olive Cultivars with Different Sensitivity to Salt Stress. *Plant Cell Environ.* **26 (4):** 595-601.
24. Lu, C. M. and Zhang, J. H. 1998. Effects of Water Stress on Photosynthesis, Chlorophyll

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[DOR: 20.1001.1.16807073.2011.13.5.4.5]

Fuorescence and Photoinhibition in Wheat Plants. *Aust. J. Plant Physiol.,* **25:** 883–892.

25. Lu, C. M. and Zhang, J. H. 2000. Photosystem II Photochemistry and Its sSensitivity to Heat Stress in Maize Plants as Affected by Nitrogen Deficiency. *J. Plant Physiol.*, **157:** 124-13.
26. Netondo, G. W., Onyango, J. C. and Beck, E. 2004. Sorghum and Salinity: II. Gas Exchange and Chlorophyll Fuorescence of Sorghum under Salt Stress. *Crop Sci.*, **44(3):** 806-811.
27. Pan, X., Deng, Ch., Zhang, D., Wang, J., Mu, G. and Chen Y. 2008. Toxic Effects of Amoxicillin on the Photosystem II of *Synechocystis* sp. Characterized by a Variety of in vivo Chlorophyll Fluorescence Tests. *Aquat. Toxicol.* **89(4):** 207-213.
28. Papadopoulos, I. and Rendig, V.V. 1983. Interactive Effects of Salinity and Nitrogen on Growth and Yield of Tomato Plants. *Plant Soil.* **73:** 47–57.
29. Schreiber, U., Bilger, W.and Neubauer, C. 1994. Chlorophyll Fuorescence as a Non-invasive Indicator for Rapid Assessment of in vivo Photosynthesis. In: Schulze, E. D.and Calswell, M. M. (eds.), *"Ecophysiology of Photosynthesis"*. Springer, Berlin. pp. 49-70.
30. Shi, D. C. and Wang, D. L. 2005. Effects of Various Salt-alkaline Mixed Stresses on *Aneurolepidium chinense* (Trin.) Kitag. *Plant and Soil*, **271 (1-2):** 15-26.
31. Strasser, B. J. and Strasser, R. J. 1995. Measuring Fast Fuorescence Transients to Address Environmental Questions: the JIP-test. In: Mathis, P. (ed), *"Photosynthesis: from light to biosphere"*. Kluwer Academic Publishers, Dordrecht. pp 977-980
32. Strasser, R. and Govindjee 1992. On the O-J-I-P Fluorescence Transient in Leaves and D1 Mutants of *Chlamydomonas-Reinhardtii*. *Photosynth. Res.*, **34 (1):** 135-135.
33. Strasser, R. J., Srivastava, A. and Tsimilli-Michael, M. 2000. The Fluorescence Transient as a Tool to Characterize and Screen Photosynthetic Samples. In: Yunus M., Pathre U. and Mohanty P. (eds.), *"Probing Photosynthesis: Mechanism, Regulation and Adaptation"*. Taylor & Francis, London. pp. 443-480.
34. Strasser, R. J., Srivastava, A. and Govindjee 1995. Polyphasic Chlorophyll-Alpha Fluorescence Transient in Plants and Cyanobacteria. *Photochem. Photobiol.,* **61 (1):** 32-42.
35. Tabatabaei, S. J. 2006. Effects of Salinity and N on the Growth, Photosynthesis and N Status of Olive (*Olea europaea* L.) trees. *Sci. Hortic.,* **108 (4):** 432-438.
36. Terashima, I. and Evans, J. R. 1988. Effects of Light and Nitrogen Nutrition on the Organization of the Photosynthetic Apparatus in Spinach. *Plant Cell Physiol.,* **29 (1):** 143-155.
37. Tisdale, S. L., Nelson, W. L., Beaton, J. D. and Havlin, J. L. 1993. Soil Fertility and Fertilizers. Macmillan Publishing Company, NewYork.
38. Toth, S. Z., Schansker, G. and Strasser, R. J. 2005. Intact Leaves, the Maximum Fluorescence level (F_M) is Independent of the Redox State of the Plastoquinone Pool: A DCMU-inhibition study. *Biochim. Biophys. Acta,* **1708 (2):** 275-282.
39. van Wijen, H.J., and Bakker J.P. 1999. Nitrogen and Phosphorus Limitation in a Coastal Barrier Salt Marsh: The Implications for Vegetation Succession. *Journal of Ecology*, **87:**265-272.
40. Wen, X. G., Qiu, N. W., Lu, Q. T. and Lu, C. M. 2005. Enhanced Thermotolerance of Photosystem II in Salt-adapted Plants of the Halophyte *Artemisia anethifolia*. *Planta*, **220 (3):** 486-497.
41. Xu, Z. Z., Zhou, G. S., Wang, Y. L., Han, G. X. and Li, Y. J. 2008. Changes in Chlorophyll Fluorescence in Maize Plants with Imposed Rapid Dehydration at Different Leaf Ages. *J. Plant Growth Regul.,* **27 (1):** 83-92.
42. Yang, C. W., Xu, H. H., Wang, L. L., Liu, J., Shi, D. C.and Wang, D. L. 2009. Comparative Effects of Salt-stress and Alkali-stress on the Growth, Photosynthesis, Solute Accumulation, and Ion Balance of Barley Plants. *Photosynthetica*, **47 (1):** 79-86.
43. Yasseen, B.T. 1983. An Analysis of the Effects of Salinity on Leaf Growth in Mexican Wheats. Ph.D. Thesis, University of Leeds.
44. Zhang, Y. and Yin, B., 2009. Influences of Salt and Alkali Mixed Stresses on Antioxidative Activity and MDA Content of Medicago sativa at Seedling Stage. *Acta Prataculturae Sinica,* **18(1):** 46-50.

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واکنش فتوسنتزی گیاهچه نی(*Phragmites australis* (CNV.) TRIN. ex Steud.) درشرایط مختلف شوری-قلیائی وسطوح نیتروژن

چ. دنگ، ج. ژنگ، و ی. پان

چکیده

بسیاری ازمانداب های دشت Songen درشمال شرقی چین که درآن ها گیاه نی غالب است، به علت تخلیه پساب های کشاورزی با محتوای بالای نیتروژن شدیداً شور وقلیائی شده اند. . به منظورشناخت اثر شوری وقلیائی روی گیاهچه نی (*Phragmites australis*) درمقادیرمختلف نیتروژن، میزان فتوسنتز، ویژگی های فلورسانس کلروفیل ومحتوای کلروفیل گیاهچه های نی بااستفاده ازسیستم های تست تبادل گازی وفلورسانس کلروفیل a ارزیابی شد. درشوری-قلیائی بالا(مخلوط ۵۰ میلی مولارNaCl و۲۵ میلی مولار $NaHCO_3$) بیشترین کاهش ها (درحد ۸۲٪، ۱۵٪، ۸۲٪ و۹۸٪) درمیزان فتوسنتزخالص (*P*n)، ماگزیمم کارائی فتوشیمی (F_V/F_M)فتوسیستم II (PSII)، شاخص کارائی فتوسنتزکل (PI_{ABS}) ومیزان افزایش ارتفاع بوته مشاهده شد. درشوری قلیائی بالافعالیت PSII به طور معنی داربازداشته شد. هردو سمت دهنده وگیرنده PSII نقطه هدف برای شوری-قلیائی بود. نیتروژن بالا(۳۰ میلی مولار) درشوری-قلیائی کم و نیتروژن متوسط (۱۵ میلی مولار) درشوری-قلیائی زیاد سمیت شوری-قلیائی روی بوته نی راکاهش داده وافزایش ارتفاع بوته، سنتز کلروفیل فعالیت PSII را موجب شد. بر پایه این یافته ها، کار برد پس آب های کشاورزی حاوی مقدارزیاد نیتروژن ممکن است برای احیای مجدد مانداب های شوری که درآن ها *Phragmites* غالب است قابل استفاده باشد، ولی باید توجه داشت که مقدار N مورد نیاز برای گیاه نی تحت تنش شوری با شدت شوری-قلیائی تغییر می کند.

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