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BRIEF COMMUNICATION

Photosynthesis, photosystem II efficiency, amino acid metabolism and ion distribution in rice (*Oryza sativa* L.) in response to alkaline stress

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Abstract

Alkalies are important agricultural contaminants complexly affecting plant metabolism. In this study, rice seedlings were subjected to alkaline stress ($NaHCO_3$:Na_2CO_3 = 9:1; pH 8.9) for 30 days. The results showed that stress mightily reduced net photosynthetic rate (P_N), but slightly decreased transpiration rate and stomatal conductance. This indicated that decline of P_N might be a result of nonstomatal factors. Alkaline stress caused a large accumulation of Na^+ in leaves up to toxic concentration, which possibly affected chloroplast ultrastructure and photosynthesis. We found that alkaline stress reduced chlorophyll fluorescence parameters, such as ratios of F_v'/F_m', F_v/F_m, photosystem (PS) II efficiency, and electron transport rates in rice plants, *i.e.* it influenced the efficiencies of photon capture and electron transport by PSII. This might be a main reason for the decrease of P_N under such conditions. Deficiency of minerals could be another reason for the decline of P_N. Alkaline stress lowered contents of N, K, Cu, Zn, P, and Fe in rice plants. In addition, the stress strongly affected metabolism of amino acids. This might be caused by imbalance in carbon metabolism as a result of photosynthesis reduction.

Additional key words: alkaline stress; rice; photosynthesis; photosystem II efficiency; amino acid metabolism.

Alkaline stress has complex effects on plant metabolism, specifically on root physiology. Some reports demonstrated clearly the existence of this type of stress and showed it as being more serious than salt stress (Kawanabe and Zhu 1991, Läuchli and Lüttge 2002, Wang *et al.* 2008). In previous studies, it was suggested that salt stress can be defined as the stress of neutral salts; alkaline stress is the stress of alkaline salts (Shi and Sheng 2005, Shi and Wang 2005). In some areas, alkalization of soil due to $NaHCO_3$ and Na_2CO_3 might be a more serious problem than soil salinization caused by neutral salts, such as NaCl and Na_2SO_4. In northeast China, more than 70% of land area is an alkaline grassland (Kawanabe and Zhu 1991) with soil pH > 10, where only a few alkali-tolerant plant species can survive (Zheng and Li 1999). However, to date, salt stress–research emphasizes NaCl as the main subject, with lesser attention to alkaline stress.

Salt stress generally involves osmotic stress and ion injury (Munns 2002, Munns and Tester 2008). Alkaline stress exerts the same stress factors but with the additional influence of high-pH stress. The high-pH environment surrounding roots can cause directly precipitation of Fe^{2+}, Ca^{2+}, Mg^{2+}, and $H_2PO_4^-$ and it may inhibit ion uptake and disrupt the ion homeostasis of plant cells (Yang *et al.* 2007). As a result, growth and photosynthesis are negatively affected (Yang *et al.* 2008a,b; 2009). Rice (*Oryza sativa* L.) is one of the most important cereal crops in tropical and temperate regions of the world. In many agricultural areas of Asia, especially in north

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Abbreviations: C_i – internal CO_2 concentration; Chl – chlorophyll; E – transpiration rate; ETR – apparent rate of electron transport at the PSII level; F_v/F_m – maximum quantum yield of photosystem II; F_v'/F_m' – efficiency of excitation capture by open PSII centres; g_s – stomatal conductance; GDH – glutamate dehydrogenase; GS – glutamate synthetase; P_N – net photosynthetic rate; PSII – photosystem II; Φ_{PSII} – PSII efficiency; q_N – nonphotochemical quenching; q_P – photochemical quenching.
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China, soil alkalinity (high pH) is an important, limiting factor of rice productivity. Several studies, focused on rice alkali tolerance (Wang *et al*. 2011, 2012a,b), showed that alkaline stress strongly affects growth and nitrogen metabolism. In this study, the rice seedlings were subjected to alkaline stress ($NaHCO_3$:Na_2CO_3 = 9:1; pH 8.9) for 30 d. Photosynthesis, chlorophyll (Chl) fluorescence, free amino acids, and a content of mineral elements were determinated to investigate rice alkali tolerance.

ChangBai-9, a major rice cultivar in north China, was used in this experiment. As the alkali-tolerant cultivar, it is widely grown in the moderately alkalinized field of northeast China. ChangBai-9 exhibits stronger resistance to the blast disease and a higher seed yield. The seeds were provided by Institute of Rice Science, Jilin Agricultural University, Changchun, China. The seeds were germinated and grown in petri dishes for 7 d in a growth cabinet (28°C during the day and 25°C during the night, 16/8 h photoperiod at 300 μmol m^{-2} s^{-1}). Seedlings were transferred to buckets containing 2 L of aerated, sterile, nutrient solution for hydroponic cultivation. The nutrient solution was replaced daily. The buckets were placed in a growth chamber maintained at 27°C during the day and 22°C during the night, under a 16/8 h photoperiod at 250 μmol m^{-2} s^{-1}. The nutrient solution contained components described by the International Rice Research Institute (Yoshida *et al.* 1976).

In northeast China, alkali-tolerant rice cultivars are widely grown on alkaline land of pH 8.8–9.2. Two alkaline salts ($NaHCO_3$ and Na_2CO_3) were selected as the main salt components and pH affecting factors in the majority of salt–alkaline soils. Both salts were mixed in a 9:1 molar ratio ($NaHCO_3$:Na_2CO_3, pH 8.9) to reach the alkaline stress environment. The total salt concentration was 35 mM. After 18 d in the hydroponic medium, rice plants were transferred to another bucket containing 2,000 mL of the alkaline solution amended with the above nutrients and salts for the stress treatment. A bucket including 10 seedlings represented one replicate; there were five replicates per treatment. Ten buckets of seedlings were randomly divided into two sets (control *vs*. alkaline treated), five buckets per set. Each bucket was considered as one replicate with five replicates per set, one set was used as a control, and another set was alkali-treated . The nutrient solution without the salts was used as a control. The stress treatment continued for 30 d.

Photosynthesis and Chl fluorescence parameters were determined with a portable open-flow gas-exchange system *LI-6400x* (*LICOR*, USA). The apparent rate of electron transport at the PSII level (ETR), PSII efficiency (Φ_{PSII}), photochemical quenching (q_P), nonphotochemical quenching (q_N), efficiency of excitation capture by open PSII centres (F_v'/F_m'), and maximum quantum yield of PSII (F_v/F_m) were determined according to method of Qiu *et al.* (2003).

P_N, stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) of leaves were determined at 08:30–10:30 h in fully expanded blades. The photosynthetically active radiation (PAR) was 1 200 μmol m^{-2} s^{-1} (*i.e*., saturation level). Free amino acids were separated and detected by an automated amino acid analyzer (*L-8900*, *Hitachi Ltd.*, Japan) as described in detail by Dluzniewska *et al.* (2006). Dry samples of 200 mg were digested with mixed acids (HNO_3:HCl:HF = 5:15:3) in a microwave digestion instrument (*HG08C-4*, *Huagang*, Beijing, China), and the extract was used to determine the contents of mineral elements. An inductively coupled plasma atomic emission spectrometer (*ProdigyXP, Prodigy*, USA) was used to determine the contents of Na, K, Ca, Mg, Fe, Mn, Cu, Zn, and P.

Statistical analysis of the data was performed using the statistical program *SPSS 13.0* (*SPSS*, Chicago, USA). All data were represented by an average of the five replicates and the standard errors (SE). The treatment mean values in the same organ were compared by *t*-test ($P<0.05$) .

Alkaline stress reduced substantially P_N, but only slightly E and g_s (Table 1), indicating that the decrease of P_N might result from nonstomatal factors. The greater reduction of P_N could be due to high-pH injury. High pH caused by alkalies might seriously affect chloroplast structure and photosynthetic process. We found that alkaline stress lowered ratios of F_v'/F_m' and F_v/F_m, Φ_{PSII}, and ETR of rice plants (Table 1). This indicated that the stress affected the efficiency of photosynthetic electron transport and the capture of light energy by PSII, which might be the main reason for the decrease of P_N under conditions of alkaline stress.

Na^+ is the main toxic ion in salinized soils. Na^+ enters plant cells through the K^+ transporter pathways and nonselective cation channels (Zhu 2003, Munns and Tester 2008). Our results showed that alkaline stress remarkably enhanced Na^+ content, but reduced K^+ content (Table 1). It is well known that many plant species posses a Na^+ exclusion mechanism that is dependent on a Na^+/H^+ antiport, such as SOS1, which exchanges cytoplasmic Na^+ with external H^+ (Zhu 2003, Munns and Tester 2008). This exchange activity relies on the transmembrane proton gradient achieved by H^+-ATPase (Zhu 2003, Munns and Tester 2008). Under alkaline stress, the lack of external protons might weaken the exchange activity of the Na^+/H^+ antiport on the root plasma membrane, possibly reducing the exclusion of Na^+ into the rhizosphere and enhancing *in vivo* accumulation of Na^+, even up to toxic concentrations. The excess of Na^+ could damage chloroplasts and thus reduce P_N and photosynthetic electron transport (Table 1) under alkaline stress. This could be the main basis of alkaline injury. Another reason for the decrease of P_N was deficiency of minerals under alkaline stress. We observed that the contents of N, K, Cu, Zn, P, and Fe decreased in alkaline-treated rice plants (Table 1). It is well known that mineral ion uptake relies on the transmembrane H^+ gradient. Under alkaline stress, the lack of external H^+ reduces the

Table 1. Effects of alkaline stress on photosynthesis, chlorophyll fluorescence, and contents of mineral elements in rice plants. The values are means (± SE) of five replicates. Statistically significant differences among alkali stress and control treatments at same organ were determined by *t*-test, and marked as $*P < 0.05$. C_i – internal CO_2 concentration; E – transpiration rate; ETR – apparent rate of electron transport at the PSII level; F_v/F_m – maximum quantum yield of PSII; F_v'/F_m' – efficiency of excitation capture by open PSII centres; g_s – stomatal conductance; P_N – net photosynthetic rate; Φ_{PSII} – PSII efficiency; q_N – nonphotochemical quenching; q_P – photochemical quenching.

Parameter	Leaf		Leaf sheath		Root	
	Control	Alkaline stress	Control	Alkaline stress	Control	Alkaline stress
N [%]	3.90 ± 0.30	2.63 ± 0.22*	1.92 ± 0.17	1.71 ± 0.11	1.84 ± 0.3	1.68 ± 0.14
P [%]	0.46 ± 0.05	0.25 ± 0.03*	0.60 ± 0.05	0.26 ± 0.02*	0.46 ± 0.04	0.14 ± 0.01*
K [%]	2.19 ± 0.17	1.94 ± 0.19	1.66 ± 0.17	2.17 ± 0.17*	1.33 ± 0.14	0.23 ± 0.02*
Na [%]	0.04 ± 0.002	1.14 ± 0.20*	0.14 ± 0.01	1.18 ± 0.09*	0.21 ± 0.02	1.45 ± 0.15*
Ca [%]	0.18 ± 0.01	0.39 ± 0.03*	0.07 ± 0.01	0.04 ± 0.003*	0.11 ± 0.01	0.19 ± 0.02*
Mg [%]	0.43 ± 0.02	0.76 ± 0.06*	0.40 ± 0.05	0.21 ± 0.01*	0.23 ± 0.04	0.15 ± 0.01*
Cu [μg g^{-1}(DM)]	16.95 ± 1.35	11.97 ± 1.51	11.99 ± 1.09	6.58 ± 0.46*	40.2 ± 3.65	13.7 ± 2.15*
Zn [μg g^{-1}(DM)]	23.9 ± 1.84	19.6 ± 1.64	24.1 ± 2.19	10.6 ± 0.86*	40 ± 3.63	17.9 ± 1.40*
Mn [μg g^{-1}(DM)]	51.5 ± 3.86	82.2 ± 6.85*	42.6 ± 3.87	23.6 ± 1.77*	9.3 ± 0.84	15.9 ± 1.62*
Fe [μg g^{-1}(DM)]	147 ± 12.27	199 ± 17.61	128 ± 12.66	49 ± 3.34*	362 ± 32.92	232 ± 19.29*
P_N [μmol(CO_2) m^{-2} s^{-1}]	16.93 ± 1.72	6.92 ± 0.118*				
g_s [μmol(H_2O) m^{-2} s^{-1}]	0.43 ± 0.02	0.31 ± 0.02*				
C_i (μmol mol^{-1})	449 ± 1	442 ± 8				
E [mmol(H_2O) m^{-2} s^{-1}]	4.71 ± 0.14	3.48 ± 0.14*				
F_v/F_m	0.82 ± 0	0.77 ± 0.01*				
F_v'/F_m'	0.58 ± 0.01	0.49 ± 0.01*				
Φ_{PSII}	0.39 ± 0.02	0.27 ± 0.02*				
q_P	0.67 ± 0.02	0.53 ± 0.04*				
q_N	2.41 ± 0.08	1.98 ± 0.05*				
ETR	86 ± 5	58 ± 5*				

Table 2. Effects of alkaline stress on the contents of free amino acids in rice plants. The values are means (± SE) of five replicates. Statistically significant differences among alkaline stress and control treatments of the same organ were determined by *t*-test and marked as * at $P<0.05$. The contents of free amino acids are expressed as % of dry mass (DM).

Amino acid	Root [% DM]		Leaf [% DM]		Leaf sheath [% DM]	
	Control	Alkaline stress	Control	Alkaline stress	Control	Alkaline stress
Asp	0.21 ± 0.01	0.58 ± 0.05*	1.33 ± 0.07	1.57 ± 0.10	1.23 ± 0.10	0.90 ± 0.09
Thr	0.26 ± 0.01	0.10 ± 0.01*	0.70 ± 0.05	0.66 ± 0.04	0.24 ± 0.02	0.27 ± 0.03
Ser	0.45 ± 0.03	0.18 ± 0.01*	1.02 ± 0.07	1.53 ± 0.09*	0.96 ± 0.06	1.44 ± 0.12*
Glu	0.09 ± 0.01	0.52 ± 0.03*	0.87 ± 0.06	1.36 ± 0.09*	0.22 ± 0.02	0.93 ± 0.08*
Gly	0.04 ± 0.00	0.03 ± 0.00	0.09 ± 0.00	0.14 ± 0.01*	0.08 ± 0.01	0.09 ± 0.01
Ala	0.27 ± 0.02	0.46 ± 0.03*	1.68 ± 0.14	0.93 ± 0.05*	0.77 ± 0.06	0.89 ± 0.06
Val	0.11 ± 0.00	0.10 ± 0.00	0.57 ± 0.04	0.49 ± 0.02	0.25 ± 0.02	0.27 ± 0.03
Met	0.004 ± 0.000	0.003 ± 0.000	0.017 ± 0.001	0.010 ± 0.001*	0.004 ± 0.000	0.006 ± 0.000*
Lle	0.07 ± 0.00	0.05 ± 0.00*	0.39 ± 0.02	0.26 ± 0.02	0.15 ± 0.01	0.15 ± 0.01
Leu	0.10 ± 0.01	0.08 ± 0.00	0.91 ± 0.06	0.57 ± 0.04*	0.21 ± 0.02	0.15 ± 0.01*
Tyr	0.05 ± 0.004	0.02 ± 0.00*	0.34 ± 0.02	0.16 ± 0.01*	0.13 ± 0.01	0.07 ± 0.01*
Phe	0.12 ± 0.01	0.08 ± 0.00*	0.93 ± 0.07	0.64 ± 0.04*	0.58 ± 0.04	0.33 ± 0.04*
His	0.09 ± 0.00	0.05 ± 0.00*	0.30 ± 0.02	0.27 ± 0.02	0.22 ± 0.02	0.25 ± 0.02
Lys	0.09 ± 0.01	0.04 ± 0.00*	0.32 ± 0.02	0.21 ± 0.01*	0.10 ± 0.01	0.08 ± 0.01
Arg	0.12 ± 0.01	0.06 ± 0.00*	0.40 ± 0.03	0.18 ± 0.01*	0.18 ± 0.02	0.18 ± 0.02
Pro	0.07 ± 0.00	0.08 ± 0.00	0.55 ± 0.04	0.43 ± 0.02	0.22 ± 0.02	0.20 ± 0.02
Total	2.13 ± 0.11	2.44 ± 0.13	10.41 ± 0.64	9.40 ± 0.59	5.53 ± 0.44	6.21 ± 0.54

transmembrane H^+ gradient in roots, possibly limiting the uptake of mineral ions. Thus, alkaline stress should more negatively affect the roots than other organs concerning the accumulation of mineral ions (Table 1).

Plant survival and growth in saline environments is a result of adaptive processes, such as ion transport and compartmentation, osmotic compound synthesis and their accumulation. Many of these protective osmolytes are

N-containing compounds, such as amino acids, amides or betaines, thus, the nitrogen metabolism is of central importance under salinity stress (Läuchli and Lüttge 2002). However, interference between salinity and nitrogen nutrition is a very complex network affecting almost all processes in plant metabolism and development (Läuchli and Lüttge 2002). Our study showed that the effect of alkaline stress on nitrogen metabolism might be more complex than that of salt stress. The alkaline stress strongly affected the metabolism of amino acids in rice (Table 2). It had unlike effects on different amino acids, *e.g.*, it reduced contents of tyrosine and phenylalanine but it enhanced contents of aspartic and glutamic acids (Table 2). The altered metabolism of amino acids might be a result of imbalance in carbon metabolism. It has been well recognized that NO_3^- is reduced to NO_2^- by nitrate reductase and then to NH_4^+ by nitrite reductase. NH_4^+ from both nitrate reduction and photorespiration are incorporated into amino acids by glutamine synthetase (GS) and glutamate synthase (Fd-GOGAT and NADH-GOGAT) or alternative glutamate dehydrogenase (GDH) pathway (Wang *et al.* 2012b). Photosynthesis provides ammonium assimilation with reducing force (electrons) and carbon, and photorespiration provides amino acid synthesis with 80–90% of NH_4^+. It is well known that NO_3^- uptake is mediated by a H^+/NO_3^- symport mechanism, which relies on the transmembrane proton gradient. Under alkaline stress, the lack of external protons limits uptake of NO_3^- by roots and strongly affects nitrogen metabolism of the roots and leaves. It had been reported that alkaline stress mightily altered NH_4^+ assimilation pathway of rice plants, *i.e.*, weakened the NH_4^+ assimilation by the GS2/GOGAT pathway in chloroplast and elevated the frequency of NH_4^+ assimilation by the GDH pathway (Wang *et al.* 2012a,b). The lowered NH_4^+ assimilation influences a removal of toxic NH_4^+ from chloroplasts and reduces the photosynthesis of stressed plants. Our results showed that alkaline stress caused the large accumulation of Na^+ in leaves up to toxic concentrations, which decreased P_N; as a result, reducing force, carbon, and NH_4^+ were reduced, which might strongly influence the metabolism of amino acids.

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