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Full Length Article

Biomass, Gas Exchange and Chlorophyll Fluorescence in Wheat Seedlings under Salt and Alkali Stress

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Abstract

Gas exchange and chlorophyll fluorescence are two very important physiological processes affecting plant growth. The experiment was simulated by salt and alkali stresses. Shoots biomass, gas exchange and chlorophyll fluorescence parameters of wheat were measured after 9 d of treatments. The shoot biomass and gas exchange indices decreased significantly with the increasing salt concentration, and more for alkaline stress at the same concentration, except for *C*i concentration. Compared to saline and alkaline stresses with controls, the F_v/F_m was not changed under both stresses, but *qp* increased; PSII maximum efficiency (F_v'/F_m') was not affected by salinity, but decreased under alkalinity; PSII efficiency (φ_{PSII}) and electron transport rates (ETR) only increased significantly at lower concentration (40 mmol/L) under salt stress, and didn't change at other concentration, but ETR decreased at higher concentration (120 mmol/L) under alkali stress; q_N did not change at salinity, but enhanced significantly at 120 mmol/L concentration under alkalinity. Therefore, photosynthetic performance response of wheat seedling to salt and alkali stresses was very different. P_N was affected by both stomatal and non-stomatal factors under salt and alkali stresses. Photo-protection caused by photo-inhibition happened at the highest level of alkali stress, depending on high pH and salinity. The wheat variety Jimai 3 in present study has tolerance to moderate salinity and alkalinity. © 2020 Friends Science Publishers

Keywords: Chlorophyll fluorescence; Gas exchange characteristics; Wheat; Salt stress; Alkali stress

Introduction

Salt stress is one of the most serious abiotic stresses, which limit plant production in arid-semiarid region (Zhao *et al*., 2007). Saline-alkaline inland is a globally rare ecosystem, the soil of which is saline with the existence of CO_3^{2-} and HCO_3^- simultaneously. This kind of soil distributed widely in China and occupies one-tenth (9910.3×10^4 hm^2) of total saline-alkaline land areas in the world (Deng *et al*., 2006). West of Songnen Plain is the main distribution area of saline-alkaline inland. In the past two decades, scientists have paid more attention on alkaline salt effects on plants and reported that alkaline stress were more destructive to plants, and the plants' responses are varied (Zhang and Mu, 2009).

Phyotosynthesis (CO_2 assimilation) is the key process for which plants survived and gained high productivity in normal or stressed environments. In general, photosynthetic capacity decreases in plants under salt stress (Brugnoli and Bjorkman, 1992; Dionisio-Sese and Tobita, 2000). The CO_2 assimilation was related to PSII operating efficiency, which could be estimated by Chl fluorescence measurements (Krall and Edwards, 1991; Siebke *et al*., 1997) that can provide information on photosynthesis of plant at stress (Méthy *et al*., 1997). And maximum quantum efficiency decrease in *Fv/Fm*, such as in early salt-stressed mango trees (De-Lucena *et al*., 2012) have been reported. But some reports showed no significant change of *Fv/Fm* in other plants which respond to NaCl (Netondo *et al*., 2004). Other traits of Chl fluorescence, such as Φ_{PSII}, *Fv'/Fm'*, *qP*, *qN* and ETR can be determined and calculated under salt stress (Zribi *et al*., 2009). However, the change in traits was related to plants species and their tolerance to salinity.

Wheat is an important crop world-wide and many studies have reported its responses to NaCl stress, such as ion changes (Ruan *et al*., 2007; Ehsanzadeh *et al*., 2009; Li *et al*., 2014) and antioxidant enzyme activities and osmotic solutes changes (Heidari and Mesri, 2008). Comparison of salt-alkali stress on wheat gas exchange characteristics (Guo *et al*., 2009) and ions balance (Li *et al*., 2009) had been

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studied. However, the photosynthesis capacity and its related PSII efficiency under alkali stresses are still unclear. Therefore, pot experiments with simulated saline and alkaline conditions were conducted to measure the PSII efficiency, Chl fluorescence attributes of wheat seedlings, biomass and gas exchange characteristics, and analyze the tolerance of wheat variety to salt-alkali stress condition. These results will provide supplement as the theory basis for utilizing saline-saline soil.

Materials and Methods

Pot Experiments

Wheat (cv. Jimai 3) was used as the experimental materials. Fifteen wheat seeds were sowed in plastic pots. Hoagland's nutrient solution was added every day after seedlings emerged.

NaCl, Na_2SO_4 and $NaHCO_3$, Na_2CO_3 were mixed in 9:1 (molar ratio), added to Hoagland's nutrition solution for salt-alkali stress, respectively (Li *et al*., 2009). Three concentrations were applied: 40 (S1 and A1), 80 (S2 and A2) and 120 (S3 and A3) mmol/L. The pH ranges were 6.27–6.45 and 9.10–9.17, respectively in salt and alkali stresses. The pots with only Hoagland's nutrition solution were used as controls.

Twenty-one pots were divided into 7 sets when seedlings were 10 d. One pot was a replicate and there were three replications in one treatment. Two hundred and fifty mL of stress solution were used to treat per pot daily at 16:30–17:30 h. All pots were put in a greenhouse to protect against rain after treatments. The experiments were last for 9 days until the seedlings seemed died at the highest salinity under alkali stress.

Gas Exchange Characteristics

Before harvest of seedlings, P_N, E rates, g_s and Ci of leaves were measured on a fully expanded youngest leaf at 9:00, using a 1200 μmol m^{-2} s^{-1} light illumination by a portable open flow gas exchange system LI-6400. The experiment was repeated for 5 times with 2 blades per pot and 6 leaves per treatment and the averages were calculated.

Chlorophyll Fluorescence

The portable open gas exchange system LI-6400 with an integrated fluorescence chamber head (LI-6400-40 Leaf Chamber fluorometer) was used to measure leaf Chl fluorescence attributes. Seedlings were kept in darkness environment for at least 30 min before measuring. The F_0 value was measured by a modulated light (< 1 μmol m^{-2} s^{-1}). The F_m value was measured at 4200 μmol m^{-2} s^{-1} light intensity for 0.8 s on dark-adapted leaves. Then F_v/F_m was recorded. The F_m' value in light-adapted leaves and Φ_{PSII} were determined by a 0.8 s saturation pulses at 6000 μmol m^{-2} s^{-1}, whereas the actinic light was 200 μmol m^{-2} s^{-1} light intensity (Liu and Shi, 2010). All descriptions of Chl fluorescence parameters and calculated formulas of Φ_{PSII}, ETR, qP and q_N were showed in Table 1.

Harvest

After measuring the gas exchange characteristics and chlorophyll fluorescence parameters, all plants shoots were harvested and washed three times, then were oven-dried at 105°C for 10 min. The biomass were recorded after oven-dried at 70°C for 48 h.

Statistical Analyses

The experimental parameters were analyzed by one-way analysis of variance (ANOVA) using SPSS 17.0 and plotted in a histogram using SigmaPlot 10.0. Means and standard errors were reported and compared by the least significant difference ($LSD_{0.05}$) test if ANOVA tests were significant (P < 0.05).

Results

Shoot biomass of wheat seedlings decreased significantly with the increasing salinity under both stresses (P < 0.05, Fig. 1). At the highest stress concentration (120 mmol/L), the decrements were about 31% and 47% respectively at salinity and alkalinity, comparing to controls. Alkali stress showed more decrease than salt stress.

P_N, g_s and E (P < 0.05, Fig. 2) decreased significantly under both stresses, amounting to 82%, 50%, 71% under salt stress and 92%, 84%, 83% under alkali stresses, respectively at the highest concentration. More reductions were found in alkali stress than in salt stress. However, C_i increased markedly only at the highest level under salt stress. The changes of C_i were significant at all alkaline stress levels as compared to controls (P < 0.05, Fig. 2).

Chl fluorescence parameters were affected distinctly under salt and alkali stresses except of F_v/F_m (Table 2), the values of which were around 0.83 in all treatments. F_0 and F_m of wheat leaves decreased significantly under both stresses. There was a decrease tendency in F_0' and F_m' with increasing salinity, but the significance was found only at higher concentration of salt (120 mmol/L) and alkali stresses (80-120 mmol/L) (Table 3). The change tendency of F_s was similar to F_0' and F_m'. Then F_v'/F_m' ratio didn't change under salt stress, but decreased markedly at the highest salinity under alkali stress (120 mmol/L).

φ_{PSII} and ETR were higher significantly at 40 mmol/L than controls and unchanged at other salinity under salt stress, but lower markedly at 120 mmol/L than controls and unchanged at other salinity under alkali stress (Fig. 3). q_N reduced significantly only at 40 mmol/L and kept a similar value with controls under salt stress, but increased significantly at 120 mmol/L under alkali stress. Photochemical quenching (qP) increased markedly at 40 mmol/L then kept unchanged under salt stress, but increased markedly when salinity was equal or greater than 80 mmol under alkali stress.

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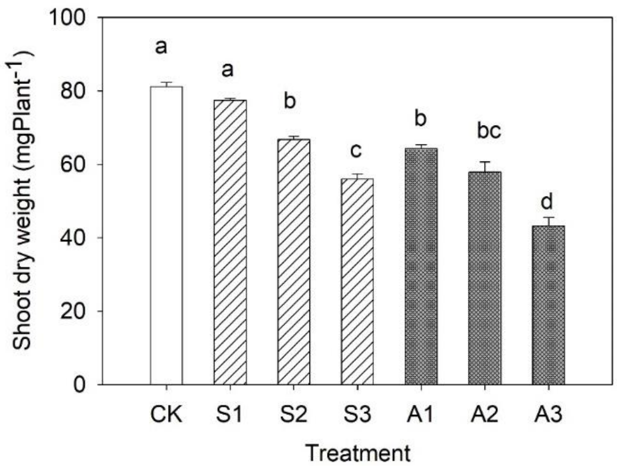

Fig. 1: Shoot dry weight of wheat seedlings under salt (NaCl: Na_2SO_4) and alkali stresses ($NaHCO_3$: Na_2CO_3), presented with means ± standard error (n=3). CK is the control plants without treatments, S1-S3 are the salt-treated plants, A1-A3 are the alkali-stressed plants. Different letters showed the significant variances among treatments using the least significant difference (LSD) test (*P* < 5%)

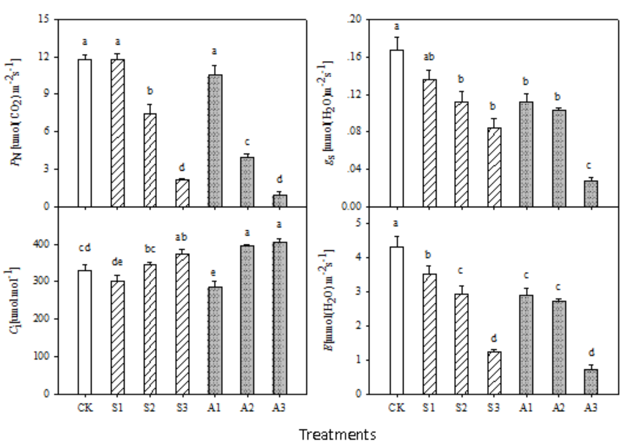

Fig. 2: Photosynthetic parameters of wheat seedlings under salt (NaCl: Na_2SO_4) and alkali stresses ($NaHCO_3$: Na_2CO_3), presented with means ± standard error (n=4). CK is the control plants without treatments, S1-S3 are the salt-treated plants, A1-A3 are the alkali-stressed plants. Different letters showed the significant variances among treatments using the least significant difference (LSD) test (*P* < 5%)

Na^+/K^+ in wheat seedlings under alkali stress were much higher than those under salt stress at the same stress concentration, and the Na^+/K^+ in the control group showed lowest (Fig. 4).

Discussion

Soil salination and alkalization is becoming an increasing problem in world environments, which affected the production of crops seriously. Alkali stress inhibited the growth of wheat seedlings more significant than salt stress

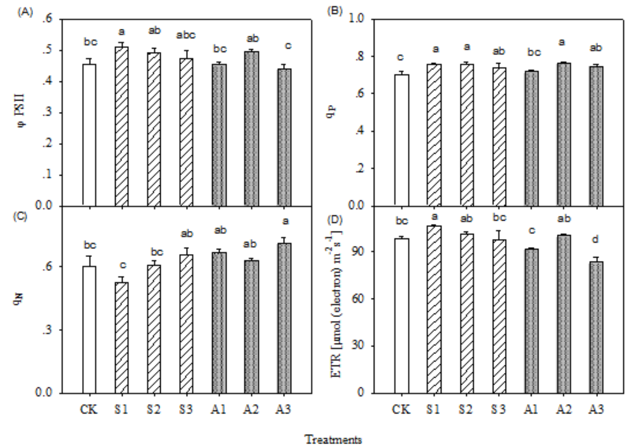

Fig. 3: Effect of salt and alkali stresses on actual PSII efficiency (φPSII), non-photochemical quenching coefficient (qN), photochemical quenching coefficient (qp) and photosynthetic electron transport efficiency (ETR), presented with means ± standard error (n=4). CK is the control plants without treatments, S1-S3 are the salt-treated plants, A1-A3 are the alkali-stressed plants. Different letters showed the significant variances among treatments using the least significant difference (LSD) test (*P* < 5%)

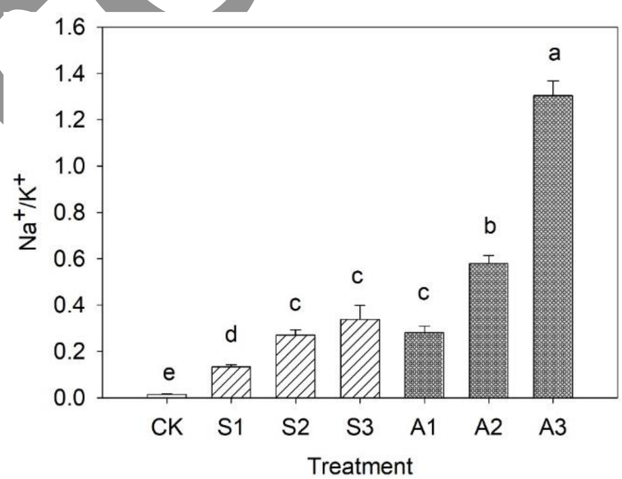

Fig. 4: Na^+/K^+ in the shoot of wheat seedlings under salt (NaCl: Na_2SO_4) and alkali stresses ($NaHCO_3$: Na_2CO_3), presented with means ± standard error (n=3). CK is the control plants without treatments, S1-S3 are the salt-treated plants, A1-A3 are the alkali-stressed plants. Different letters showed the significant variances among treatments using the least significant difference (LSD) test (*P* < 5%)

(Li *et al*., 2009). Plants usually maintain photosynthetic carbon gain and lessen transpiration under salt stress by decreasing stomatal conductance (Läuchli and Lüttge, 2002; Benlloch-González *et al*., 2008). Clark *et al*. (1999) indicated that the reductions of stomatal and transpiration represented the physiological responses to cope with salt condition. The reductions of g_s and *E* increased with increasing salinity, indicating the photosynthetic adaptation of wheat seedlings to both salt and alkali stresses.

Table 1: Summary of Chl fluorescence outputs parameters with general descriptions and where applicable, mathematical expressions

Parameter	Description	Mathematical expression
F_0	minimal Chl a fluorescence in dark-adapted	
F_0'	minimal Chl a fluorescence in light-adapted	
F_m	maximal Chl a fluorescence in dark-adapted	
F_m'	maximal Chl a fluorescence in light-adapted	
F_s	steady-state Chl fluorescence	
F_v/F_m	maximum quantum efficiency of PSII photochemistry	
Fv'/Fm'	PSII maximum efficiency	
Φ_{PSII}	quantum yield of PSII (actual PSII efficiency)	$\Phi_{PSII} = (F_m' - F_s)/F_m'$
q_P	photochemical quenching	$q_p = (F_m' - F_s)/(F_m' - F_0')$
q_N	non-photochemical quenching	$q_N = (F_m - F_m')/(F_m - F_0)$
ETR	electron transport rate	$ETR = \Phi_{PSII} \times PFDa \times 0.5$

Table 2: ANOVA results of salt (NaCl: Na_2SO_4) and alkali ($NaHCO_3$: Na_2CO_3) treatments on chlorophyll fluorescence parameters

Parameters	F_0	F_m	F_v/F_m	F_0'	F_m'	F_s	F_v'/F_m'	Φ_{PSII}	ETR	qP	qN
Treatments	*	*	ns	*	**	**	**	*	***	**	**

Note: the meanings of chlorophyll fluorescence parameters refer to Table 1

Table 3: The major fluorescence parameters of wheat seedlings under salt (NaCl: Na_2SO_4) and alkali stresses ($NaHCO_3$: Na_2CO_3)

Concentration (mmol/L)		F_0	F_m	F_v/F_m	F_0'	F_m'	F_s	F_v'/F_m'
control	0	73.38a	428.98a	0.83a	75.50a	213.98a	116.13a	0.64a
Salt	40	62.25b	367.35b	0.83a	68.85ab	210.20ab	103.05ab	0.67a
Stress	80	64.25ab	376.58b	0.83a	65.48ab	187.45abc	94.80bc	0.65a
	120	60.43b	363.95b	0.83a	58.65b	163.20c	85.08c	0.64a
Alkali	40	72.58a	429.60a	0.83a	69.33ab	188.60abc	103.15ab	0.63a
Stress	80	62.38b	367.30b	0.83a	61.95b	174.93bc	88.50bc	0.65a
	120	64.90ab	374.68b	0.83a	62.65b	152.35c	85.10c	0.59b

Notes: Difference letters showed the significant difference among different treatments ($P < 5\%$)

It has been reported that decreased photosynthetic rate exposure to salt for long-term might be due to reduced g_s (Ouerghi *et al*., 2000). Stomatal closure (Abbruzzese *et al*., 2009; Shahbaz and Zia, 2011; Ashraf and Ashraf, 2012) and non-stomatal factors (Ai-Abdoulhadi *et al*., 2012) could result in a lower C_i and then led to the reduction of P_N under stress conditions, thereby causing reduction in growth. However, C_i of wheat seedlings increased when gs and P_N decreased with increasing salinity, especially P_N remarkable decreased only when salinity over 80 mmol/L in the present study (Fig. 2). The decreases of g_s and P_N values with increasing of C_i, suggesting that non-stomatal factor was dominant for inhibiting of photosynthesis (Yan *et al*., 2012). Thus, P_N were affected by both stomatal non-stomatal factors in salt and alkali stressed wheat seedlings.

PSII was the importance stage to fix CO_2 in the photosynthetic process, which could be estimated by Chl fluorescence measurements. Chl fluorescence attributes were affected by salt stress in salt-sensitive genotypes (Atlassi *et al*., 2009; Baker and Rosenqvist, 2004). Ashraf and Ashraf (2012) reported that salt stress declined the activity of PSII of wheat during all the growth stages. However, Perveen *et al*. (2013) hold a contrary opinion, which indicated most Chl fluorescence attributes of wheat remained unaffected under salt stress. In present study, both stress types affected Chl fluorescence attributes significantly except of F_v/F_m (Table 2). Usually, Chl fluorescence was negatively correlated to photosynthesis. The concentration of CO_2 in leaves will increase going with the decreasing fluorescence intensity. Although F_m and F_m' decreased and C_i increased at salt-alkali stress, there was no significant correlation between C_i and F_m (R^2=0.34, P > 0.05), C_i and F_m' (R^2=0.59, P > 0.05).

Some reports showed that F_v/F_m wasn't affected in tolerant plant cultivars, such as wheat (Zair *et al*., 2003), rice (Dionisio-Sese and Tobita, 2000), sorghum varieties (Netondo *et al*., 2004) and maize (Shabala *et al*., 1998). When compared NaCl and $NaHCO_3$ effects on tomato, the ratio of F_v/F_m declined with increasing levels and the reduction was more significant in later (Gong *et al*., 2013). There, no photo-inhibition happening in present wheat seedlings under salt stress. Our experimental material (Jimai 3) was a salt-tolerant line of wheat varieties. Higher Fv'/Fm' was beneficial to improve the transformed efficacy of light energy in plants, accelerating the carbon assimilation and organic solutes accumulation (Baker and Rosenqvist, 2004). Wheat seedlings under both stresses could keep the similar Fv'/Fm' value with controls in most stress concentration except of the highest alkalinity, proved the maintain mechanisms of wheat seedlings under stresses and higher tolerance.

Plants had developed certain photo-protective mechanisms to dissipate excess excitation energy which protected the photosynthetic apparatus to avoid photo-damaging PSII (Qiu *et al*., 2003). q_P in leaves are the most sensitive photosynthetic characteristics for measuring salinity tolerance in maize (Shabala *et al*., 1998). In wheat seedlings, q_p didn't decreased even increased significantly at some salinity. Both φPSII and q_p increased at lower concentrations of saline stress and P_N maintain a stable

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value as the same as control. This result proved that higher φPSII and q_p value could accelerate the photosynthetic activity, which may be the adaptation of wheat to salt stress.

Stepien and Johnson (2009) concluded that increasing salinity resulted in a substantial increase in non-photochemical quenching (NPQ) in *Arabidopsis thaliana*. Moradi and Ismail (2007) reported no significant difference in quantum yields of PSII (Φ_{PSII}) were observed with increasing salinity levels at vegetative stages in rice, but NPQ increased significantly. The NPQ increase is suggested to occur of photo-protection to dissipate excess excitation energy (Demmig-Adams and Adams, 1992; Yan *et al*., 2012), in which a higher proportion of absorbed photons are lost as thermal energy instead of being used to drive photosynthesis (Shangguan *et al*., 2000). In the wheat seedlings of present research, Φ_{PSII} was not affected but non-photochemical quenching (q_N, similar like NPQ) increased significantly at the highest salinity under alkali stress (Fig. 3). High pH from alkali stress may have caused a series of harmful effects including destruction of photosynthetic machinery and primary electron acceptors, weakening PSII activity, and a reduction in the photochemical reaction. This resulted in plants being exposed to photo-inhibition, which then activated photo-protection by increasing NPQ (Liu and Shi, 2010). It was concluded that the photo-protection caused by photo-inhibition would happen depending on high pH and salinity. Under such conditions, increasing q_N could play a key role in excess energy dissipation to keep photosynthetic machinery from being destroyed.

Conclusion

Although shoot biomass of wheat seedling decreased significantly under both salt and alkali stresses, the photosynthetic performance response mechanisms were quite different between them. The inhibition of P_N was related to stomatal and non-stomatal factors under both stresses. According to the Chl fluorescence parameters, Jimai 3 was a kind of tolerant line wheat for some extent of salinity and alkalinity. There was no photo-inhibition observed under salt stress. Photo-protection caused by photo-inhibition happened at the highest level of alkali stress, depending on high pH and salinity. Based on Chl fluorescence parameters, Photosystem II response of wheat seedlings to salt and alkali stresses was different.

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