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Significance of mesophyll conductance for photosynthetic capacity and water-use efficiency in response to alkaline stress in *Populus cathayana* seedlings

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Abstract

Cuttings of *Populus cathayana* were exposed to three different alkaline regimes (0, 75, and 150 mM Na_2CO_3) in a semicontrolled environment. The net photosynthesis rate (P_N), mesophyll conductance (g_m), the relative limitations posed by stomatal conductance (L_s) and by mesophyll conductance (L_m), photosynthetic nitrogen-use efficiency (PNUE), carbon isotope composition ($\delta^{13}C$), as well as specific leaf area (SLA) were measured. P_N decreased due to alkaline stress by an average of 25% and g_m decreased by an average of 57%. Alkaline stress caused an increase of L_m but not L_s, with average L_s of 26%, and L_m average of 38% under stress conditions. Our results suggested reduced assimilation rate under alkaline stress through decreased mesophyll conductance in *P. cathayana*. Moreover, alkaline stress increased significantly $\delta^{13}C$ and it drew down CO_2 concentration from the substomatal cavities to the sites of carboxylation (C_i-C_c), but decreased PNUE. Furthermore, a relationship was found between PNUE and C_i-C_c. Meanwhile, no correlation was found between $\delta^{13}C$ and C_i/C_a, but a strong correlation was proved between $\delta^{13}C$ and C_c/C_a, indicating that mesophyll conductance was also influencing the $^{13}C/^{12}C$ ratio of leaf under alkaline stress.

Additional key words: carbon isotope discrimination; chloroplast CO_2 concentration; curve-fitting method; gas exchange; intercellular CO_2 concentration; leaf nitrogen; specific leaf area; stomatal conductance.

Introduction

Land salinization or alkalization belong to the most significant abiotic stresses that affect the growth and production of plants in many areas worldwide. Meanwhile, the warming climate, land clearing, and irrigation exacerbate the situation (Munns and Tester 2008). Photosynthesis is influenced by numerous environmental and physiological

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Abbreviations: C_a – concentration of atmospheric CO_2; C_c – CO_2 concentration in chloroplasts; C_i – intercellular CO_2 concentration; C_c/C_a – chloroplastic to ambient CO_2 concentration; C_i/C_a – substomatal to ambient CO_2 concentration; C_a-C_i – drawing down CO_2 concentration from ambient CO_2 concentration to substomatal CO_2 concentrations; C_i-C_c – drawing down CO_2 concentration from the substomatal cavities to the sites of carboxylation; g_m – mesophyll conductance; g_s – stomatal conductance; $J_{max\text{-}Cc}$ – maximal rate of electron transport driving regeneration of RuBP determined on the basis of C_c; $J_{max\text{-}Ci}$ – maximal rate of electron transport driving regeneration of RuBP determined on the basis of C_i; K_c – Michaelis-Menten constants for RuBP carboxylation; K_o – Michaelis-Menten constants for RuBP oxygenation; LED – light emitting diode; L_m – relative limitations posed by mesophyll conductance; L_s – relative limitations posed by stomatal conductance; N_a – leaf nitrogen content on area basis in g m^{-2}; O – oxygen concentration; P_N – net photosynthesis rate; PNUE – photosynthetic nitrogen-use efficiency; PPFD – photosynthetic photon flux density; R_d – mitochondrial respiration in the light; RSD – relative standard deviations; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP – ribulose-1,5-bisphosphate; SLA – specific leaf area; $V_{cmax\text{-}Cc}$ – maximum rate of carboxylation determined on the basis of C_c; $V_{cmax\text{-}Ci}$ – maximum rate of carboxylation determined on the basis of C_i; WUE – water-use efficiency; WUE_i – intrinsic water-use efficiency (P_N/g_s); Γ^* – CO_2 compensation point in the absence of mitochondrial respiration in the light; $\delta^{13}C$ – carbon isotope composition.

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regulators. Changes in mesophyll conductance (g_m) that reduces the CO_2 concentration in chloroplasts (C_c) relative to that in substomatal cavities (C_i) can limit photosynthesis (Warren 2008). Photosynthetic response to environmental stresses has been extensively studied. Nevertheless, there is still large uncertainty concerning the role played by environment-related changes in g_m, especially under alkaline stress. On the other hand, g_m has been recognized to reduce photosynthetic nitrogen-use efficiency (PNUE) (*e.g.* Flexas *et al.* 2008). However, the relative contribution of g_m to variation in PNUE has been quantified in several species (Flexas *et al.* 2008). It is clear that the role of g_m in determining PNUE warrants further research. Furthermore, it has been recently reported that g_m limits carbon assimilation and influences carbon isotope discrimination (Bickord *et al.* 2010), which can be used as a surrogate of water-use efficiency (WUE) (Farquhar *et al.* 1989). However, studies testing the role of g_m in $\delta^{13}C$ predictions are limited, and the degree to which g_m influences water-use efficiency as indicated by $\delta^{13}C$ is still neglected in most studies (Soolanayakanahally *et al.* 2009).

In the present study, *Populus cathayana* Rehd., a fast growing tree species, which is widely distributed in southwestern regions of China, was employed as a model species to assess plant responses to alkaline stress. The occurrence and performance of *P. cathayana* are affected by increased areas of saline soil (Yang *et al.* 2009). Meanwhile, soil salinization and alkalization frequently co-occur in nature. Although ecophysiological response to environmental change in *P. cathayana* has been widely measured (Chen *et al.* 2010, Xu *et al.* 2010, Zhang *et al.* 2011), no measurements of g_m for *P. cathayana* have been conducted to our knowledge under alkaline stress. In the present investigation, photosynthesis, g_m, PNUE, as well as $\delta^{13}C$ of *P. cathayana* were assessed under alkaline stress. Specifically, the following questions should be answered: (*1*) how does g_m respond to alkaline stress in *P. cathayana*?, and (*2*) what is the importance of g_m in regulating PNUE and $\delta^{13}C$ during alkaline stress periods?

Materials and methods

Plant materials and experimental design: Cuttings of *P. cathayana* were collected in their natural habitat (35°56′N, 101°35′E) in the Qinghai Province, China. The cuttings were planted in March 2011. After sprouting and growing for about 2 months, 60 cuttings of similar crown size and of an equal height were chosen and replanted (one cutting per pot) in 10-l plastic pots filled with 8 kg of a homogenized soil. The cuttings were grown in a naturally lit greenhouse under ambient conditions with a daytime temperature of 19–28°C, a night-time temperature of 12–18°C, and an average daytime and night-time relative humidity of 40% and 85%, respectively, at the Chengdu Institute of Biology. The alkaline-treated plants were irrigated with 1 l of Na_2CO_3 solution, including low alkaline (75 mM Na_2CO_3) and moderate alkaline (150 mM Na_2CO_3) solutions, whereas control plants were irrigated with 1 l of the tap water once a week. In each treatment, there were 20 seedlings arranged in five blocks (four seedlings per treatment in each block). Moreover, the locations of five blocks in the greenhouse were randomized every week to eliminate block effects. The treatment lasted for 6 weeks, when leaves of stressed plants fell off after a gentle touch.

Gas-exchange measurements and estimation of g_m by a curve-fitting method: The photosynthetic gas-exchange measurements were conducted on the fourth fully expanded, intact leaf from five randomly chosen individuals in each treatment using the portable photosynthesis system (*LI-6400, LI-COR Inc.*, Lincoln, NE, USA) between 08:00 and 11:30 h (local time) on July 2011 for a period of one week. Prior to measurements, the samples were illuminated with saturated photosynthetic photon flux density (PPFD) provided by the light emitting diode (LED) light source of the equipment for 10–30 min to achieve full photosynthetic induction. After inducing steady-state photosynthesis, the photosynthetic responses to varying C_i were measured. The concentration of atmospheric CO_2 (C_a) was lowered stepwise from 360 to 50 μmol mol^{-1} and then returned to 360 μmol mol^{-1} to reestablish the initial steady-state value of photosynthesis (Galmés *et al.* 2007). C_a was then increased stepwise from 360 to 1,800 μmol mol^{-1}. The air in the leaf chamber (80 ml) was maintained at 25°C with 36–55% of relative humidity inside the leaf chamber. The ratio of net photosynthetic rate (P_N) to leaf nitrogen content on area basis (N_a), measured for the leaves at 360 μmol mol^{-1}, was regarded as PNUE and the ratio of P_N to g_s was taken as the instantaneous water-use efficiency (WUE$_i$). The molar flow rate of air through the chamber varied between 200 and 250 μmol s^{-1}. Preliminary experiments had established that with a molar flow rate of 200 μmol s^{-1}, there was never more than 1.5 μmol mol^{-1} diffusion of CO_2 into an empty chamber maintained at 50 μmol(CO_2) mol^{-1}, and the correction for leaks obtained with a dead leaf suggested that leakage resulted in errors of only ± 4% in the estimations of P_N, depending on C_i (Warren *et al.* 2004). The recalculation of data assuming that photosynthesis changed ± 4% made no consequence for g_m. Thus, we made no correction for CO_2 diffusion.

A curve-fitting method was used to estimate g_m (Ethier and Livingston 2004, Ethier *et al.* 2006). Following Ethier and Livingston (2004), photosynthetic curves were fitted with a nonrectangular hyperbola version of the biochemical model of C_3 leaf photosynthesis by Farquhar *et al.* (1980) that accounts for g_m and, whereby, P_N was given as (1), (2), and (3):

$$P_N = \min\{P_c, P_j\} \quad (1)$$

$$P_c = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (2)$$

$a = -1/g_m$
$b = (V_{cmax} - R_d/g_m + C_i + K_c\,(1 + O/K_o)$
$c = R_d\,[C_i + K_c\,(1 + O/K_o)] - V_{cmax}\,(C_i - \Gamma^*)$

$$P_j = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (3)$$

$a = -1/g_m$
$b = (J/4 - R_d)/g_m + C_i + 2\Gamma^*$
$c = R_d\,(C_i + 2\Gamma^*) - J/4\,(C_i - \Gamma^*)$

where P_c and P_j were the ribulose-1,5-bisphosphate (RuBP)-saturated and RuBP-limited net CO_2 assimilation rates, respectively, V_{cmax} was the maximal CO_2 carboxylation rate, J was the photochemical electron transport rate under RuBP-limited conditions, R_d was the mitochondrial respiration in the light, Γ^* was the CO_2 compensation point in the absence of mitochondrial respiration in the light, and K_c and K_o were Michaelis-Menten constants for RuBP carboxylation and oxygenation, respectively, and O was the oxygen concentration. For $[K_c\,(1+O/K_o)]$, a value of 736 μmol mol^{-1} was used (von Caemmerer *et al.* 1994), while Γ^* was calculated by using the Laisk method (Laisk 1977). The accuracy of the photosynthesis model depends on proper representation of the kinetic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Pons *et al.* 2009). Consequently, we selected a general set of kinetic parameters (Tcherkez *et al.* 2006). This approach was justified by the belief that the kinetic properties of Rubisco among C_3 plants have been shown to be relatively conserved. The C_i cut-off point was determined based on the method proposed by Ethier *et al.* (2006). Detailed derivations of Eqs. 2 and 3, as well as a thorough evaluation of errors resulting from assuming an infinite g_m, when fitting the Farquhar *et al.* (1980) model equations to P_N-C_i curves are given in Ethier and Livingston (2004). P_N-C_i curves that had convergence problems during the fit were discarded, and the rest of them was kept, when the standard error of the g_m estimate was < 50% of the estimated value. The precision of the present estimates with relative standard deviations (RSD) were between 11–17%, similar to commonly reported values (10–15% RSD, *e.g.* Warren and Adams 2006). Using estimated g_m and measured P_N and C_i, C_c was calculated as: $C_c = C_i - (P_N/g_m)$.

Relative limitation on photosynthesis imposed by g_m and g_s: The limitation of photosynthesis imposed by finite g_m and g_s was based on the estimates of the potential rate of photosynthesis assuming that the conductances were either infinite or as measured (Farquhar and Sharkey 1982, Warren *et al.* 2003). The estimates of photosynthesis were based on CO_2-response curves, and measured as g_m and g_s. The rates of net photosynthesis were estimated assuming that g_m and g_s were as measured (P_N, the light-saturated rate of photosynthesis at C_a = 360 μmol mol^{-1}), assuming g_m being infinite and g_s as measured (P_{il}, the light-saturated rate of photosynthesis at $C_c = C_i$), or assuming g_m as measured and g_s being infinite (P_{sl}, the light-saturated rate of photosynthesis at C_i = 360 μmol mol^{-1}). The relative limitations due to g_m resistances (L_m) and stomatal resistances (L_s) were estimated as:

$L_m = (P_{il} - P_N)/P_{il}$
$L_s = (P_{sl} - P_N)/P_{sl}$

SLA and leaf nitrogen (N): At harvest, the leaf area was determined with a portable laser area meter (*CI-203, CID Inc.*, Camas, USA). Leaf biomass components were dried separately at 70°C to constant mass and weighed. Specific leaf area (SLA, leaf area divided by dry mass, DM) was calculated. Leaf samples were ground and passed through a 20-μm mesh screen after being dried at 70ºC for 36 h. Leaf N was determined by the semi-micro Kjeldahl method (Helrich 1990). Digests were performed with concentrated sulphuric acid on block digesters. Initial block temperature was 160ºC, then it was ramped up to 390ºC in 60 min and held at that temperature for 2 h. Quantitative analyses were performed by flow injection analysis (*Lachat Instruments QuikChem* method *13-107-06-2B*, *HACH Company*, USA).

Carbon isotope composition: Mature leaves, which became fully expanded during the imposition of the alkaline stress, were randomly selected from each replication per treatment for carbon isotope analysis. Samples of 100 mg of plant material, oven-dried at 70°C for 24 h, were homogenized by grinding in a ball mill. The stable carbon isotope abundance in the combusted samples was measured with a mass spectrometer (*Finnegan MAT Delta-E*, San Jose, CA) as described by Li *et al.* (2004). The overall precision of the δ-values was better than 0.1‰, as determined from repeated samples.

Statistical analyses: The data were subjected to one-way analysis of variance (*ANOVA*). The *Duncan*'s test was employed to detect possible differences between the alkaline regimes. Statistical analysis was performed using the *SPSS 11.5* for *Windows* statistical software package. *Pearson's* correlation coefficients were calculated to determine the relationships between variables.

Results

Photosynthetic CO_2-response characteristics and $\delta^{13}C$: Alkaline stress significantly decreased P_N, PNUE, g_s, g_m, $J_{max\text{-}Ci}$, and $J_{max\text{-}Cc}$ (Table 1). Moreover, alkaline stress resulted in significantly higher L_m, C_i-C_c, and $\delta^{13}C$. The results also showed that L_m (0.50) under moderate alkaline stress was greater than L_s (0.24). However, alkaline stress had no significant effects on N_a, L_s, R_d, WUE_i, and $V_{cmax\text{-}Cc}$ (Table 1). Mean values of V_{cmax} and J_{max} calculated on a C_c basis were not greater than those calculated on a C_i basis.

Table 1. The effects of alkaline stress on parameters tested in *Populus cathayana* cuttings. P_N – net photosynthetics rate; PNUE – photosynthetic nitrogen-use efficiency; N_a – leaf nitrogen content on area basis; g_s – stomatal conductance; g_m – mesophyll conductance; C_i-C_c – draw-down in the CO_2 concentration from the substomatal cavities to the sites of carboxylation; L_s – relative limitation to net photosynthesis posed by stomatal conductance; L_m – relative limitation to net photosynthesis posed by mesophyll conductance; R_d – rate of mitochondrial respiration in the light; $V_{cmax\text{-}Ci}$ – maximum rate of carboxylation determined on the basis of C_i; $V_{cmax\text{-}Cc}$ – maximum rate of carboxylation determined on the basis of C_c; $J_{max\text{-}Ci}$ – maximal rate of electron transport driving regeneration of RuBP determined on the basis of C_i; $J_{max\text{-}Cc}$ – maximal rate of electron transport driving regeneration of RuBP determined on the basis of C_c; WUE_i – intrinsic water-use efficiency; $\delta^{13}C$ – carbon isotope composition. The values are means ± SE, $n = 5$. *Letters* refer to differences within traits between the alkaline regimes. Values followed by *different letters* are significantly different from each other at the $P<0.05$ level.

Parameter	Control	Low alkaline stress	Moderate alkaline stress	*P*
P_N [µmol(CO_2) m^{-2} s^{-1}]	13.84 ± 0.13^a	11.92 ± 0.23^b	8.63 ± 0.29^c	0.000
PNUE [µmol g^{-1} s^{-1}]	7.53 ± 0.26^a	6.37 ± 0.21^b	5.03 ± 0.27^c	0.000
N_a [g m^{-2}]	1.84 ± 0.06^a	1.87 ± 0.03^a	1.72 ± 0.05^a	0.111
g_s [mol(H_2O) m^{-2} s^{-1}]	0.65 ± 0.01^a	0.60 ± 0.02^a	0.42 ± 0.03^b	0.010
g_m [mol m^{-2} s^{-1}]	0.24 ± 0.01^a	0.15 ± 0.01^b	0.05 ± 0.00^c	0.000
C_i-C_c [µmol mol^{-1}]	58 ± 3^a	83 ± 4^b	168 ± 8^c	0.000
L_s	0.25 ± 0.01^a	0.26 ± 0.01^a	0.24 ± 0.01^a	0.060
L_m	0.18 ± 0.01^a	0.26 ± 0.01^b	0.50 ± 0.02^c	0.000
R_d [µmol m^{-2} s^{-1}]	0.76 ± 0.03^{ab}	0.87 ± 0.03^a	0.72 ± 0.02^b	0.044
$V_{cmax\text{-}Ci}$ [µmol m^{-2} s^{-1}]	54.8 ± 0.6^a	51.3 ± 0.7^b	52.2 ± 1.3^{ab}	0.045
$V_{cmax\text{-}Cc}$ [µmol m^{-2} s^{-1}]	57.3 ± 0.6^a	53.6 ± 0.7^a	54.9 ± 1.5^a	0.060
$J_{max\text{-}Ci}$ [µmol m^{-2} s^{-1}]	82.5 ± 0.6^a	74.2 ± 1.2^b	63.5 ± 1.3^c	0.000
$J_{max\text{-}Cc}$ [µmol m^{-2} s^{-1}]	79.3 ± 0.7^a	71.4 ± 1.1^b	60.7 ± 1.1^c	0.000
WUE_i [µmol(CO_2) mol^{-1}(H_2O)]	21.3 ± 1.3^a	19.9 ± 1.5^a	20.7 ± 3.1^a	0.594
$\delta^{13}C$ [‰]	-26.04 ± 0.03^a	-25.00 ± 0.30^b	-23.30 ± 0.10^c	0.000
SLA [cm^2 g^{-1}]	153.8 ± 6.5^a	122.8 ± 3.5^b	89.4 ± 4.2^c	0.000

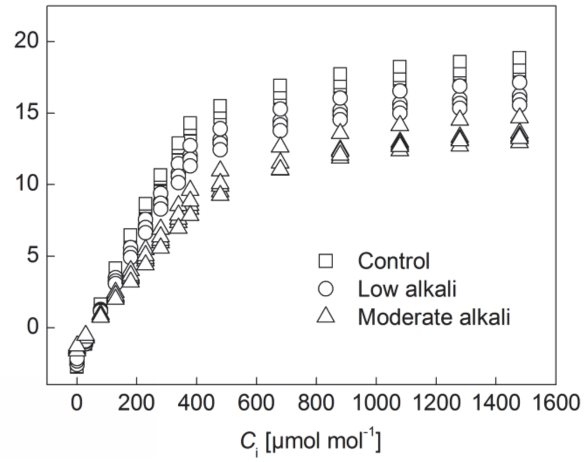

Fig. 1. The relationship between net photosynthetic rate (P_N) and intercellular CO_2 concentration (C_i) in *Populus cathayana* as affected by alkaline stress. □ – control, ○ – low alkaline stres, △ – moderate alkaline stress.

Relationships between variables: The shape of the P_N-C_i curves was substantially affected by alkaline stress (Fig. 1). PNUE was not correlated with C_a-C_i ($r = 0.45$, $P=0.08$) (Fig. 2*A*), but it was found the negative correlation with C_i-C_c ($r = 0.87$, $P=0.000$) (Fig. 2*B*). A strong correlation between $\delta^{13}C$ and C_c/C_a was observed ($r = 0.90$, $P=0.000$) (Fig. 3*B*), but $\delta^{13}C$ was not correlated with C_i/C_a ($r = 0.35$, $P=0.201$) (Fig. 3*A*). Moreover, $\delta^{13}C$ was not correlated with WUE_i ($r = 0.12$, $P=0.667$) (Fig. 4).

Discussion

The g_m mean value across all treatments was 0.15 mol m^{-2} s^{-1}, close to those reported for *Quercus* spp., *Eucalyptus* spp., *Fagus sylvatica*, and *Castanea sativa* (0.10–0.19 mol m^{-2} s^{-1}, von Caemmerer and Evans 1991, Loreto *et al.* 1992, Epron *et al.* 1995). In alkali-stressed plants, g_m was reduced. There was a general trend of

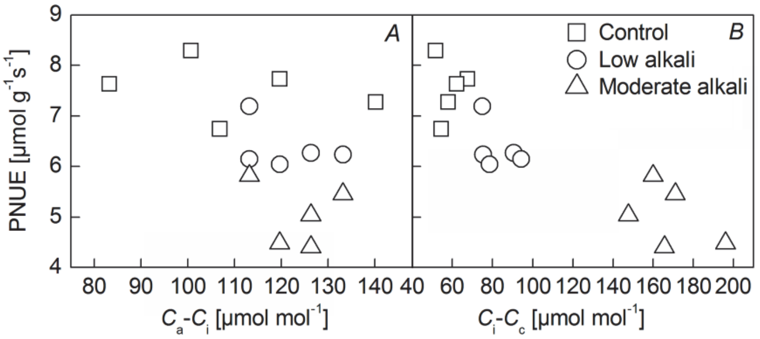

Fig. 2. Relationship between photosynthetic nitrogen-use efficiency (PNUE) and the draw-down in the CO_2 concentration from ambient CO_2 concentration to substomatal CO_2 concentrations (C_a-C_i) (r = 0.45, P=0.08 for all treatment data pooled together) (*A*), and relationship between photosynthetic nitrogen utilization efficiency and the draw-down in the CO_2 concentration from the substomatal cavities to the sites of carboxylation (C_i-C_c) (r = 0.87, P=0.000 for all treatment data pooled together) (*B*) in *Populus cathayana* as affected by alkaline stress. The values are shown for individual replicates. □ – control, ○ – low alkaline stres, △ – moderate alkaline stress.

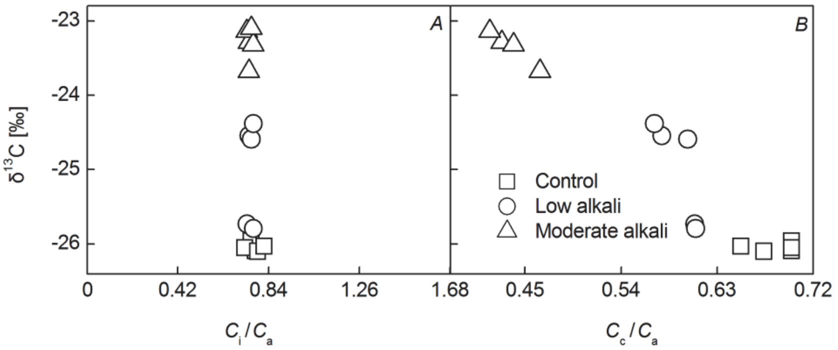

Fig. 3. Relationship between carbon isotope composition ($\delta^{13}C$) ratio of substomatal to ambient CO_2 concentration (C_i/C_a) (r=0.35, P=0.201 for all treatment data pooled together) (*A*) and $\delta^{13}C$ ratio of chloroplastic to ambient CO_2 concentration (C_c/C_a) (r=0.90, P=0.000 for all treatment data pooled together) (*B*) in *Populus cathayana* as affected by alkaline stress. The values are shown for individual replicates. □ – control, ○ – low alkaline stres, △ – moderate alkaline stress.

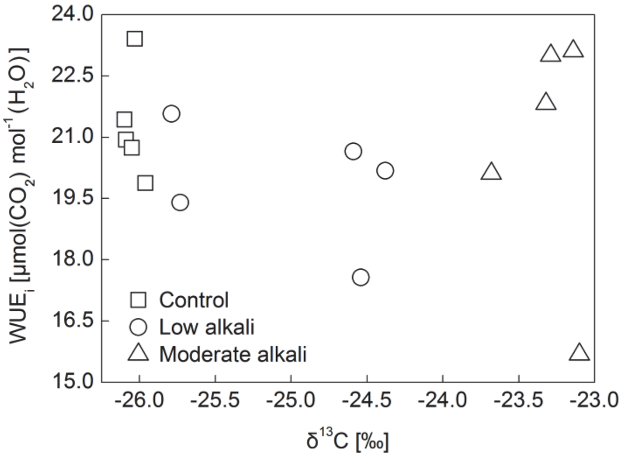

Fig. 4. Relationship between carbon isotope composition ($\delta^{13}C$) and intrinsic water-use efficiency (WUE_i) (r = 0.12, P=0.667 for all treatment data pooled together) in *Populus cathayana* as affected by alkaline stress. The values are shown for individual replicates. □ – control, ○ – low alkaline stres, △ – moderate alkaline stress.

increasing mesophyll limitations under alkaline stress, while the stomatal limitation remained unchanged. In fact, g_s decreased by 35%, while g_m decreased by 79% (Table 1) under moderate alkaline stress. Thus, it might be that g_s did not decrease enough (when compared with g_m) to be reflected in calculating their relative limitations to photosynthesis. The relative limitations reported here showed that g_m posed a large limitation on CO_2 fixation. In all cases, the relative limitation due to g_m was 0.18–0.50, confirming the importance of including this component into detailed study of gas-exchange response to environment stress such as alkaline stress (Flexas *et al*. 2008). The reduced g_m resulted in a large decrease in CO_2 concentrations from C_i to C_c of approx. 83–168 μmol mol^{-1} (Table 1), which was very similar to the mean value derived from the review of Warren (2008). We do not know what accounted for the observed decrease in g_m under alkaline-stress treatments. We suggest that the alkali-stressed leaves might invest more in tougher leaves (reflected as lower SLA) with thicker cell walls or in other structural components that might increase path lengths or otherwise limited diffusion of CO_2 into chloroplasts (Warren 2008, Soolanayakanahally *et al.* 2009).

It has been reported that neglecting the decline from C_i to C_c resulted in estimates of V_{cmax} that were 60% of the 'true' V_{cmax} (Ethier and Livingston 2004, Warren *et al.* 2004). However, in our study, failure to account for finite g_m had little effects on the estimates of V_{cmax}, which was similar to the results reported by Flexas *et al.* (2007) in *Arabidopsis thaliana* young plants. The choice of kinetic parameters involved in the equations (K_c, K_o) might be the largest possible source of error in this study. The problem with estimating V_{cmax} from apparent kinetic constants is that these constants are not universally applicable (Piel *et al.* 2002). Hence, while there might be some uncertainty concerning the absolute values of V_{cmax} and J_{max} (Flexas *et al.* 2007), these data suggest that low g_m to CO_2 combined with a low carboxylation capacity limits photosynthesis in *P. cathayana.*

Nevertheless, the low PNUE under alkaline stress was not a consequence of changes in N contents, as leaf N did not change during the experiment. Rather, the treatment-related PNUE decline was related to the limitation due to greater g_m, and it was accompanied by a greater draw down of C_i-C_c. g_m has been also recognized as an important factor influencing the $^{13}C/^{12}C$ ratio of a leaf material (Flexas *et al.* 2008), which has implications for interpreting WUE and terrestrial carbon exchange. In our study, WUE_i (P_N/g_s) decreased slightly (not significantly) in response to alkaline stress. These data seem to be contradictory with those of isotopic C^{13}/C^{12} that showed significant increases (less negative values) under low and moderate alkaline stress, which weakens the correlation between $\delta^{13}C$ and WUE_i (Fig. 4). Similar results were observed by Hanba *et al.* (2003) in *Polygonum* plants, which showed that substantial differences (3‰) were found in leaf $\delta^{13}C$ between highland and lowland *Polygonum* plants, with no changes in P_N/g_s. Furthermore, $\delta^{13}C$ of control and stressed plants was not strongly associated with the C_i/C_a (Fig. 2*A*). However, a strong inverse correlation between $\delta^{13}C$ and C_c/C_a (Fig. 2*B*) was found when taking into account g_m. Thus, our results suggest a profound effect of g_m on $\delta^{13}C$, as proposed by Barbour *et al.* (2010). Similarly, Duan *et al.* (2009) also reported the tight correlation between leaf WUE and g_m.

Conclusion: We found a strong reduction of photosynthesis in *P. cathayana* trees by alkaline stress. Alkaline stress also caused a significant reduction in g_s, g_m, and the enhancement in C_i-C_c, and L_m in *P. cathayana* seedlings, which might mean P_N and g_m varied nonproportionally between leaves in control and under alkaline stress. Moreover, our results showed that PNUE decreased through decreased photosynthetic capacity and g_m in *P. cathayana* under alkaline stress. Furthermore, the results also showed qualitatively that high $\delta^{13}C$ values under alkaline stress could be explained more by carbon in the chloroplast than by carbon in the intercellular spaces. Our results contributed to understanding the response of g_m to environmental change.

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