

The chlorophyll fluorescence CO<sub>2</sub> response curve of plants were measured by the LI-6400XT photosynthesis analyzer and fluorescence leaf chamber (LI-COR, United State) (set PPFD to 400  $\mu\text{Mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The CO<sub>2</sub> response curve of plants were modified by Tissue et al. (2005) and it was performed using Photosynthetic Assistant software (Version 1.1, Dundee Scientific, Dundee, Scotland). The maximum Rubisco carboxylation rate ( $V_c$ ), maximum electron transfer rate ( $J_{\text{max}}$ ), maximum photosynthetic rate under saturated light intensity and CO<sub>2</sub> ( $A_{\text{max}}$ ), and respiration rate under light ( $R_d$ ) are obtained from the CO<sub>2</sub> response curve.

The mesophyll conductance ( $g_m$ ) is calculated using the "variable electron rate method" (Harley et al., 1992), as follows:

$$g_m = A_N / (C_i) (\Gamma^* (J_{\text{flu}} + 8(A_N + R_d)) / (J_{\text{flu}}) 4(A_N + R_d))$$

Where,  $A_N$  and  $C_i$  was a CO<sub>2</sub> response curve at a saturation light intensity and initial external CO<sub>2</sub> concentration ( $C_a$ ) of 380  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , where  $\Gamma^*$  was referring to Bernacchi et al. (2002);  $J_{\text{flu}}$  was the electron transfer rate and is also measured by the chlorophyll fluorescence CO<sub>2</sub> response curve at an external  $C_a$  of 380  $\mu\text{mol}\cdot\text{mol}^{-1}$ . The value of  $K_C$  ( $1+O/K_O$ ) was based on von Camemmer et al. (1994), with a value of 736  $\mu\text{mol}\cdot\text{mol}^{-1}$ .

Referring to Grassi and Magnani (2005) and Farquhar et al. (1980), different functional components divide relative photosynthetic limitation into several different factors, namely CO<sub>2</sub> mesophyll conductance limitation ( $M_{CL}$ ), stomatal conductance limitation ( $S_L$ ), and biochemical limitation ( $B_L$ ). The specific calculation process is as follows:

$$A_N = \frac{V_{c \text{ max}}}{C_c + K_C \cdot (1 + O/K_O)} \cdot (1 - \frac{\Gamma^*}{C_c}) - R_d \quad (1)$$

Ignoring slight changes in respiratory function, formula (1) can be rewritten as:

$$dA_N = \frac{\partial A_N}{\partial C_c} \cdot dC_c + \frac{\partial A_N}{\partial V_{c \text{ max}}} \cdot dV_{c \text{ max}} \quad (2)$$

The CO<sub>2</sub> concentration reaching the chloroplast can be represented by the stomatal conductivity of CO<sub>2</sub> ( $g_{sc}$ ;  $g_{sc}=g_{sw}/1.6$ ), the mesophyll conductivity of CO<sub>2</sub>, and photophosphorylation:

$$dC_c = \frac{A_N}{g_{sc}} \cdot \frac{dg_{sc}}{g_{sc}} + \frac{A_N}{g_m} \cdot \frac{dg_m}{g_m} - \left( \frac{1}{g_{sc}} + \frac{1}{g_m} \right) \cdot dA_N \quad (3)$$

Meanwhile,  $A_N$  can be expressed using formula (4):

$$\frac{\partial A_N}{\partial A_{c \text{ max}}} = \frac{A_N}{V_{c \text{ max}}} \quad (4)$$

Substitute formulas (3) and (4) into formula (2), and the photosynthesis under light saturation can be represented by stomata, mesophyll, and biochemical capacity, resulting in formula (5):

$$\frac{dA_N}{A_N} = S_L + MC_L + B_L = l_s \cdot \frac{dg_{sc}}{g_{sc}} + l_{mc} \cdot \frac{dg_m}{g_m} + l_b \cdot \frac{dV_{c \max}}{V_{c \max}} \quad (5)$$

$$l_s = \frac{g_{\text{tot}}/g_{sc} \cdot \partial A_N / \partial C_c}{g_{\text{tot}} + \partial A_N / \partial C_c} \quad (6)$$

$$l_{mc} = \frac{g_{\text{tot}}/g_{mc} \cdot \partial A_N / \partial C_c}{g_{\text{tot}} + \partial A_N / \partial C_c} \quad (7)$$

$$l_b = \frac{g_{\text{tot}}}{g_{\text{tot}} + \partial A_N / \partial C_c} \quad (8)$$

where,  $g_{\text{tot}}$  is the total conductivity of  $\text{CO}_2$  ( $1/g_{\text{tot}} = 1/g_{sc} + 1/g_m$ ),  $S_L$ ,  $MC_L$ ,  $B_L$  were the contributions of stomatal and mesophyll conductivity, as well as were maximum carboxylation efficiency to  $dA_N/A_N$ .  $l_s$ ,  $l_{mc}$ , and  $l_b$  are corresponding relative limits, with values between 0 and 1.

We define that the relative change in assimilation rate under light saturation is the ratio of the actual value of each treatment to the maximum of all treatments, as shown in formula (9):

$$\frac{dA_N}{A_N} \approx \frac{A_{\max}^{\text{ref}} - A_{\max}}{A_{\max}^{\text{ref}}} \quad (9)$$

Similarly, the relative changes in stomatal conductance, mesophyll conductance, and carboxylation potential were also obtained based on formulas similar to those in Equation (9). Based on the growth and photosynthetic parameters among different treatments, we set 5  $\mu\text{M}$  Cd treatment was used as a control.

Finally, non stomatal limitation is defined as the sum of mesophyll conductance and biochemical limitation ( $NS_L = MC_L + B_L$ ), diffusion limitation is the sum of stomatal conductance limitation and mesophyll conductance limitation ( $D_L = S_L + MC_L$ ), and total limitation value is the sum of stomatal limitation and non stomatal limitation ( $T_L = S_L + NS_L$ ).

## References:

1. Harley, P.C.; Loreto, F.; Di Marco, G.; Sharkey, T.D. Theoretical Considerations when Estimating the Mesophyll Conductance to  $\text{CO}_2$  Flux by Analysis of the Response of Photosynthesis to  $\text{CO}_2$ . *Plant Physiol.* **1992**, *98*, 1429–1436.
2. Bernacchi, C.J.; Portis, A.R.; Nakano, H.; Von Caemmerer, S.; Long, S.P. Temperature Response of Mesophyll Conductance. Implications for the Determination of Rubisco Enzyme Kinetics and for Limitations to Photosynthesis in Vivo. *Plant Physiol.* **2002**, *130*, 1992–1998.
3. Grassi, G.; Magnani, F. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ.* **2005**, *28*, 834–849.
4. Farquhar, G.D.; Firth, P.M.; Wetselaar, R.; Weir, B. On the Gaseous Exchange of Ammonia between Leaves and the Environment: Determination of the Ammonia Compensation Point. *Plant Physiol.* **1980**, *66*, 710–714.