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Photosynthetic performance and acclimation of *Incarvillea delavayi* to water stress

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Abstract

The photosynthetic performance and related leaf traits of *Incarvillea delavayi* Bur. *et* Franch were studied at different water regimes to assess its capacity for photosynthetic acclimation to water stress. The initial response of *I. delavayi* to water stress was the closure of stomata, which resulted in down-regulation of photosynthesis. The stomatal limitation (S_L) represented the main component to photosynthetic limitations but non-stomatal limitation (NS_L) increased quickly with the increasing water stress, and had similar magnitude to S_L under severe water stress (soil moisture 25 - 30 % of field capacity). Chlorophyll (Chl) *a* fluorescence parameters characterizing photosystem (PS) 2 photochemical efficiency (Φ_{PS2}), electron transport rate (J) and photochemical quenching (qP) decreased with the increasing water stress, indicating impaired photosynthetic apparatus. However, the water-stressed plants had a increased mesophyll CO_2 diffusional conductance, Chl *a*/*b* ratio, leaf nitrogen partitioning in RuBPCO and bioenergetics in later grown parts, indicating that *I. delavay* had a substantial physiological plasticity and showed a good tolerance to water stress.

Additional key words: chlorophyll, fluorescence, net photosynthetic rate, nitrogen, photosynthetic limitation, RuBPCO, stomatal conductance, water stress.

Introduction

Water availability is considered as one of the most important environmental factors affecting plant growth and productivity (Boyer 1982). At high altitude, plant life is commonly less constrained by moisture shortage than at low altitudes, but seasonal and regional water shortage does occur frequently, and contributes to the overall impact of the physical environment on mountain plant (Körner 1999). The ability of species to acclimate and adapt to different environments is directly or indirectly associated with their ability to acclimate at the level of photosynthesis (Pearcy 1977), which in turn affects biochemical and physiological processes of the leaf and, consequently, the physiology and growth of the whole plant (Chandra 2003). Therefore, photosynthesis is widely used as a tool for indicating environmental stress and selection of growth conditions suitable for plant (Lin and Hsu 2004).

The response of photosynthesis to water stress has received much attention in the past, but most researches concentrated on economic crops, such as soybean, wheat, sunflower and coffee while there are limited studies on the photosynthetic adaptation of alpine plant to water

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Abbreviations: Chl - chlorophyll; c_i - intercellular CO_2 concentration; E - transpiration rate; F_m - maximum fluorescence of dark-adapted leaves; F_0 - minimum fluorescence of dark-adapted leaves; F_v/F_m - variable to maximum fluorescence ratio (maximum photochemical efficiency of PS 2); g_s - stomatal conductance; g_m - mesophyll conductance; J_{max} - light saturated rate of electron transport driving regeneration of RuBP; J_{mc} - the capacity for photosynthetic electron transport per unit cytochrome *f*; LMA - leaf mass per unit area; LNC - leaf nitrogen content per unit area; N_m - mass-based leaf nitrogen content; NPQ - non-photochemical quenching; NS_L - non-stomatal limitation; NUE - photosynthetic nitrogen use efficiency; PAR - photosynthetically active radiation; P_B - partitioning coefficients for leaf nitrogen in bioenergetics; P_{max} - light saturated photosynthetic rate at ambient CO_2 concentration; P_N - net photosynthetic rate; P_R - partitioning coefficients for leaf nitrogen in RuBPCO; qP - photochemical quenching; R_d mitochondrial respiration rate in the light; RGR - relative growth rate; R/S - root/shoot ratio; RuBp - ribulose-1,5-bisphosphate; S_B - biochemical limitation; S_L - stomatal limitation; S_M - mesophyll limitation; V_{cmax} - maximum rate of RuBPCO carboxylation; V_{cr} - the maximum rate of RuBP carboxylation per unit RuBPCO protein; Φ_2 - photochemical efficiency of PS 2; Γ^* - CO_2 compensation concentration.

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availability (Kao and Tsai 1998, DaMatta *et al.* 2002). In previous studies, the understanding of the biochemistry and physiology of water stress relies largely on short-term experiments or growth under conditions, which are far different from their natural environment (Yeo 1998). García *et al.* (2007) suggest that long-term water stress is needed to simulate more realistic responses to drought, since the way in which water stress is imposed might be important in understanding the response to drought, and also in evaluating the plant capacity to acclimate to stress.

The strong correlation between photosynthetic capacity and water relation has been widely accepted. However, photosynthetic constraint imposed by water stress on photosynthesis is still open to debate. Photosynthetic limitation has been traditionally analysed in terms of stomatal limitation (S_L) and non-stomatal limitation (NS_L) (Lawlor 2002, Galmés *et al.* 2007). This view suggests that the decreasing CO_2 assimilation in response to water stress is ascribed to either stomatal closure, restricting CO_2 entry into leaves (S_L), or to changes in leaf biochemistry resulting in the inhibition or down-regulation of photosynthesis (NS_L). It can also result from both of them, stomata closure being an immediate response and the change of biochemistry becoming increasingly important with increasing water stress (Chaves *et al.* 2002). However, NS_L traditionally interpreted as biochemical limitation is erroneous, because it includes both a diffusive (*i.e.* mesophyll conductance) and a truly biochemical component. Therefore it is possible to partition NS_L into mesophyll conductance limitation (S_M) and biochemical limitation (S_B).

Incarvillea delavayi is a perennial herb, and can be found in grasslands and slopes at altitudes of 2400 - 3900 m in western Szechwan and northwestern Yunnan, China. This species usually bears 2 - 6 red flowers from June to July, and the fruits mature from August to September. It is not only known as an ornament plant but also used as medicine. Up to now, the research of *I. delavayi* is concentrated on taxonomy (Chen *et al.* 2004) and extraction of useful pharmaceutical components (Nakamura *et al.* 2000). We compared the component of soil in natural habitat and nursery garden, and found that water supply limitation could be one of dominant limitations for the growth of *I. delavayi*, but the details about physiological responses to water supply is unclear. The aims of this study were to evaluate the response of photosynthesis to water stress, and explore the dominant limitation for photosynthesis to assess the capacity for photosynthetic acclimation of *I. delavayi* to water stress.

Materials and methods

Plants and experimental treatments: The experiment was carried out in the nursery of Shangri Ge-Sang Flower Company, northwest Yunnan, China (alt. 3200 m, 99°38'E, 27°46'N). Yearly mean precipitation and temperature is 625 mm and 5.4 °C, respectively.

The seedlings of *Incarvillea delavayi* Bur. *et* Franch were grown three years in the nursery of Ge-Sang Flower Company. Similar-sized plants with dormant buds were selected (26 May 2007) and grown in plastic pot containing 18 kg natural habitat soil. Soil available nitrogen was 139.10, phosphorus 105.37 and K 92.82 mg kg^{-1}, and pH was 7.02.

The plants were sufficiently watered until budding, and then separated randomly into five groups of 30 plants. Four water regimes (w1: 80 - 85, w2: 60 - 65, w3: 40 - 45 and w4: 25 - 30 % of field soil capacity) were maintained by weighing the pots before irrigation and applying the required amount of water. During the following period, plants received half strength Hoagland solution twice in one month in order to ensure non-limiting nutrient supply. All plants were grown for a total period of 50 d under these conditions before measurements. In order to understand the tolerance of *I. delavayi* to progressive water stress, one group of plants was irrigated every 3 d until their leaves were fully expanded, and then irrigation was withheld. Measurements were made on the 6, 9, 12, 16 and 20 d after the last irrigation.

Water potential was determined in fully expanded healthy leaves using a thermocouple psychrometer *PSYPRO* (*Wescor* , Logan, UT, USA). The predawn water potential (ψ_{pd}) was taken before sunrise, and the midday water potential (ψ_{mid}) was taken between 12:00 and 12:30 on July 13, 2007.

Gas exchange and chlorophyll *a* fluorescence were measured simultaneously on the fully expanded leaves with a combined open gas exchange system and chlorophyll *a* fluorescence system (*LI-6400- 40*, *Li-Cor*, Lincoln, NE, USA) on 13 - 15 July and 14 - 16 August 2007. Before measurement, the leaf was adapted in the dark for more than 2 h. After the minimum fluorescence (F_0) was determined at a weak irradiance, a saturating irradiance of 6000 μmol m^{-2} s^{-1} for 0.8 s was used to determine the maximum fluorescence (F_m). Then the leaf was irradiated by an actinic radiation of 1200 μmol $m^{-2}s^{-1}$ (10 % blue, 90 % red) for 10 - 15 min until stable photosynthesis occurred. The following fluorescence parameters were calculated: the maximum quantum efficiency of PS 2 $F_v/F_m = (F_m - F_0)/F_m$, PS 2 photochemical efficiency $\Phi_2 = (F_m' - F_s)/F_m'$ (Genty *et al.* 1989), electron transport rate $J = \Phi_2 \times PAR \times 0.5 \times 0.84$ (Krall and Edwards 1992), photochemical quenching $qP = (F_m' - F_s)/(F_m' - F_o')$, and non-photochemical quenching $NPQ = F_m/F_m' - 1$, where F_m', F_0', and F_s are maximum, minimum and steady-state fluorescence in light-adapted leaves, respectively.

Response of net photosynthetic rate (P_N) to irradiance (P_N-PAR) and internal CO_2 concentration (P_N-c_i) curves were measured at the same leaf under controlled leaf temperature (20 °C) and relative humidity (45 - 60 %). These curves were analysed by a curve fitting software (*Sigmaplot for Windows 8.0*) with a three-component exponential function (Watling *et al.* 2000):

$P_N = a(1 - e^{-bx}) + C$,

where P_N is photosynthetic rate, x is c_i or PAR, a, b and C are constants. Using P_N-PAR curves, light-saturated photosynthesis (P_{max}) was estimated by *Photosyn assistant* software (Ver *1.1*, *Dundee Scientific*, Scotland, UK). P_N-c_i response curves were used to calculate maximum carboxylation rate (V_{cmax}) and light-saturated electron transport (J_{max}) by *Photosyn assistant* software that applied biochemical model described by Von Caemmerer and Farquhar (1981).

Mesophyll conductance (g_m) was calculated using 'variable' method as described by Harley *et al.* (1992):

$g_m = PN/ci - [\Gamma^*\{J + 8(P_N + R_d)\}/J - 4(P_N + R_d)]$,

where P_N and R_d were measured from P_N-c_i curve and J was estimated from chlorophyll fluorescence on the same leaf, Γ* was 33.06 μmol mol^{-1} at 20 °C according to Bernacchi (2002). The values of g_m were calculated for measurements of net assimilation rate at c_i of 100 - 300 μmol mol^{-1}, and the average value of g_m was determined for each leaf.

Jones (1985) proposed to take g_m into account of photosynthesis limitation. This method, implemented by Grassi and Magnani (2005), allowed to partition photosynthetic limitation into components related to stomatal conductance (S_L), mesophyll conductance (S_M) and leaf biochemical characteristics (S_B). The plant with maximum value of CO_2 assimilation rate should be assumed as a reference in this method, so W_1 plants in July and W_2 plants in August were used as the references.

Leaf traits and plant production: After measuring P_N response curves, six plants of each treatment were harvested and dry mass was obtained after drying at 80 °C for 48 h. Relative growth rate (RGR) was estimated. Leaf area was measured with a leaf area meter *LI-3000A* (*LI-COR*, Inc., Nebraska, USA). Leaves were extracted by dimethylformamide and chlorophyll content in leaf extract was determined using a spectrophotometer *UV-2550* (*Shimadzu*, Tokyo, Japan) and calculated according to Inskeep and Bloom (1985). Leaf N content was analysed using an element analyser system (*GmbH VarioEL*, Hanau, Germany). The partitioning coefficients for leaf nitrogen in RuBPCO (P_R) and bioenergetics (P_B) were estimated according to the method of Niinemets and Tenhunen (1997):

$P_R = V_{cmax}/6.25 \times N_m \times V_{cr} \times LMA$ and

$P_B = J_{max}/8.06 \times N_m \times J_{mc} \times LMA$,

where from the temperature dependency described by Niinemets and Tenhunen (1997), the values of V_{cr} and J_{mc} at 20 °C were equal to 12.6 μmol(CO_2) g^{-1}(RuBPCO) s^{-1} and 131.9 mol(electron) mol^{-1} (cyt *f*) s^{-1}, respectively, and N_m was mass-based leaf nitrogen content [%]. Nitrogen use efficiency (NUE) was calculated as P_{max}/LNC, where LCN was leaf nitrogen content per unit area.

Statistical analysis: Statistical analysis was performed with *SPSS 10.0* software (*SPSS Inc.*, Chicago, USA). Differences between means were tested by one-way *ANOVA* and LSD multiple comparisons test. Difference was considered significant at $P \leq 0.05$. All graphs were carried using *SigmaPlot* for windows version *9.0*.

Results and discussion

The effect of water stress on photosynthesis: The increasing water stress slightly decreased Ψ_{pd} from w1 to w3 treatment, but the Ψ_{pd} of w4 treatment was dramatically decreased (Fig. 1*A*) as expected. However, we also found that well-watered plants (w1) had the lowest Ψ_{mid} (Fig. 1*A*) accompanied with relatively high stomatal conductance (g_s, Table 1). This was similar to the result of Pollock (1979), who found that alpine plant *Chionochloa* in New Zealand had relatively high and stable g_s under low leaf water potential. Some studies suggest that the stomata of alpine plants are relatively insensitive to changes in leaf water potential (Enquist and Ebersole 1994, Bowman 1995). Low Ψ_{mid} not only results from insufficient uptake (low soil moisture, cold soil) or impaired transport (frozen stems, xylem cavitation) but also from the high transpiration rate due to fully open stomata at high soil moisture (Körner 1999).

When the plants experienced water stress, the leaf size and RGR decreased significantly (Fig. 1*B*,*C*). The reduction of leaf size can effectively control transpiration water loss and prevent dehydration of leaf tissues. The increase in R/S is considered as one of adaptive mechanisms of plants to drought. However, *I. delavayi* grown under different water regimes had similar R/S (Fig. 1*D*). This result was in agreement with the finding of Sobrado and Turner (1986).

The increasing water stress significantly reduced g_s and g_m, therefore limited CO_2 entry into photosynthetic apparatus, and resulted in lower P_{max}, V_{cmax} and J_{max} (Table 1). Under the condition of water stress, the reduction in g_s was considered as one of the important strategies to diminish transpiration rate and maintain cell pressure potential. The increase in LMA reduced g_m, since g_m varies significantly with leaf structure and often decrease with the increasing LMA (Terashima *et al.* 2005). The increase in LMA could partially explain the decrease in g_m and P_N, as LMA was sensitive to drought (Flexas *et al.* 2004, García *et al.* 2007, Melgar *et al.* 2008).

The quantitative limitation analysis according to Farquhar and Sharkey (1982) was used to determine the relative contributions of decreased CO_2 diffusion and biochemical efficiency to decrease in photosynthesis under the water stress. Our study indicated that at least

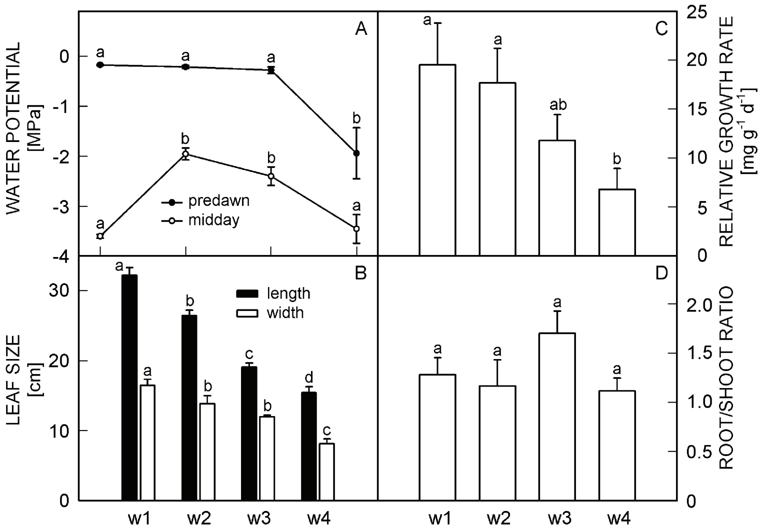

Fig. 1. Leaf water potential (*A*), leaf size (*B*), relative growth rate (*C*) and root/shoot ratio (*D*) of *Incarvillea delavayi* in four water regimes. Means ± SE ($n = 6$). Different letters above the columns indicate statistically different means at $P < 0.05$ as determined by LSD test.

Table 1. Photosynthetic parameters and leaf traits of *Incarvillea delavayi* in four water regimes. Means ± SE, $n = 3$. Different letters within the same row indicate mean values statistically different at $P < 0.05$ as determined by LSD test.

Parameters		w1	w2	w3	w4
P_{max} [μmol(CO_2) m^{-2} s^{-1}]	July	19.61 ± 1.60a	16.71 ± 0.66a	8.53 ± 0.50b	4.92 ± 0.12c
	August	15.81 ± 1.14ab	19.71 ± 1.90a	14.11 ± 1.02b	8.60 ± 0.56c
g_s [mol(CO_2) m^{-2} s^{-1}]	July	0.225± 0.041a	0.173± 0.042ab	0.106± 0.010bc	0.033± 0.002c
	August	0.127± 0.004ab	0.148± 0.013a	0.104± 0.009b	0.088± 0.019b
g_m [mol(CO_2) m^{-2} s^{-1}]	July	0.145± 0.015b	0.192± 0.018a	0.103± 0.004c	0.034± 0.009d
	August	0.207± 0.051a	0.222± 0.087a	0.187± 0.036a	0.121± 0.023a
V_{cmax} [μmol m^{-2} s^{-1}]	July	56.00 ± 3.21a	55.33 ± 2.73a	41.33± 1.33b	28.00 ± 1.00c
	August	54.00 ± 6.66ab	62.19 ± 2.45a	53.00 ± 6.56ab	43.67 ± 5.55b
J_{max} [μmol m^{-2} s^{-1}]	July	252.7 ±24.3a	230.0 ± 3.8a	162.3 ± 2.3b	94.30 ± 5.0c
	August	153.7 ±17.8b	189.0 ± 4.0a	178.7 ± 6.1ab	150.3 ± 6.8b
c_i [μmol mol^{-1}]	July	287.0 ±13.0a	260.0 ± 2.4ab	236.0 ± 8.0ab	212.0 ±19.0b
	August	233.0 ±14.0a	250.0 ±22.0a	223.0 ±21.0a	229.0 ±30.0a
LMA [g m^{-2}]	July	89.08 ± 5.16a	82.50 ± 5.18a	106.03 ±14.90a	110.00 ± 9.25a
	August	88.09 ± 8.79a	81.15 ±10.43a	92.57 ± 2.94a	123.37 ± 3.31b
Chl *a* [μg cm^{-2}]	July	61.09 ± 1.12a	65.70 ± 3.79ab	71.09 ± 0.79b	70.45 ± 0.28b
	August	57.81 ± 1.25a	62.45 ± 2.71ab	64.69 ± 0.01b	63.16 ± 1.60ab
Chl *b* [μg cm^{-2}]	July	18.86 ± 1.15a	24.10 ± 4.05ab	30.87 ± 1.99bc	35.06 ± 2.07c
	August	18.01 ± 0.65a	24.52 ± 2.96ab	28.96 ± 4.37b	27.79 ± 2.92ab
Chl *a/b*	July	3.26 ± 0.13a	2.83 ± 0.31ab	2.32 ± 0.12bc	2.02 ± 0.12c
	August	3.21 ± 0.06a	2.60 ± 0.23ab	2.33 ± 0.31b	2.32 ± 0.20b
LNC [g(N) m^{-2}]	July	2.656± 0.144a	2.752± 0.343a	3.459± 0.057b	4.293± 0.172c
	August	2.464± 0.250a	2.664± 0.060a	2.445± 0.120a	3.721± 0.171b
P_R [g(N) in RuBPCO g^{-1}(N)]	July	0.269± 0.014a	0.258± 0.006a	0.152± 0.021b	0.084± 0.009c
	August	0.277± 0.026a	0.297± 0.042a	0.274± 0.028a	0.150± 0.020b
P_B [g(N) in bioenergetics g^{-1}(N)]	July	0.090± 0.009a	0.080± 0.003a	0.044± 0.005b	0.021± 0.003c
	August	0.058± 0.006a	0.067± 0.009a	0.069± 0.002a	0.038± 0.003b
NUE [μmol(CO_2) g^{-1}(N) s^{-1}]	July	7.45 ± 0.82a	6.32 ± 1.01a	2.47 ± 0.20b	1.15 ± 0.07b
	August	6.62 ± 1.05a	7.38 ± 0.57a	5.76 ± 0.18b	2.33 ± 0.24c

Table 2. Relative limitations [%] to photosynthesis in *Incarvillea delavayi* in four water regimes.

		Total	S_L	NS_L	S_M	S_B
July	w1	0	0	0	0	0
	w2	7.43	7.43	0	0	0
	w3	34.77	19.12	15.65	10.78	4.87
	w4	74.63	36.65	37.98	31.91	6.07
August	w1	7.57	5.81	1.76	1.70	0.06
	w2	0	0	0	0	0
	w3	19.05	13.07	5.98	3.85	2.13
	w4	32.99	18.01	14.98	14.70	0.28

half of the decline in P_N could be attributable to stomatal limitation (S_L) from mild (w2) to moderate water stress (w3) (Table 2). The decreasing c_i with increasing water stress also supported this assumption. However, with the increasing water stress (w4), NS_L ($S_M + S_B$) increased and had nearly similar magnitude as S_L. It has been suggested that the plants under severe water stress had higher value of NS_L than S_L (Lawlor and Cornic 2002, Lawlor 2002). Our result showed that S_L was higher than NS_L throughout the experiment (Table 2). Within NS_L, S_M represented the main component. So diffusional limitation ($S_L + S_M$) was the predominant factor limiting photosynthesis of *I. delavayi* under water stress, which is in agreement with the result of Grassi and Magnani (2005). In our study, we also found that the value of S_B was low. It could be attributable to the complete functional development of the leaf, as biochemical limitations were quantitatively important only during leaf senescence or under severe drought (Grassi and Magnani 2005, Niinemets *et al.* 2005).

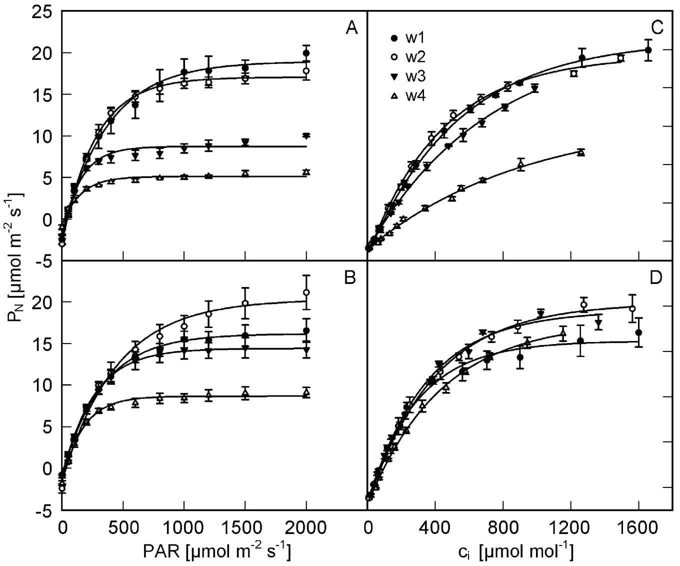

Fig. 2. P_N-PAR, P_N-c_i response curves of *Incarvillea delavayi* in four water regimes in July (*A*,*C*) and August (*B*,*D*). Means ± SE ($n = 3$).

The strong positive correlation between P_N and leaf nitrogen content per unit area (LNC) has been widely accepted because of the large proportion of leaf N presented in the photosynthetic apparatus (Evans 1989) but mostly when the plants are limited by nitrogen (Wang *et al.* 1998). However, in the present study, P_N and LNC showed an opposite trend along the water stress. Well watered plants had lower LNC and higher P_N than water-stressed plants. Furthermore, the well watered plants partitioned more nitrogen to photosynthetic apparatus (higher P_R and P_B) and thus had higher NUE (Table 1).

The changes in the Chl *a/b* ratio are related to the balance of the irradiance absorption capacity of the two photosystems. Increasing Chl *a/b* ratio associates with the decrease in the size of PS 2 light-harvesting antenna, ensure that the supply of electrons from PS 2 is sufficient to keep pace with the rate of excitation of PS 1 (Kitajima and Hogan 2003). So the rapid decrease in Chl *a/b* ratio with the increasing water stress (Table 1) might excite unbalance between PS 2 and PS 1, and could be considered as a component of overexcitation that might damage photosynthetic apparatus.

The photosynthetic acclimation to water stress: After irrigation was stopped, progressively increasing water stress declined P_N in parallel with the reduction of g_s and transpiration rate (E) while leaf-air vapour pressure difference (VPD) significantly increased (Fig. 3). However, those values were maintained approximately constant for 16 d without irrigation. When the plants are subjected to water stress, the limitations to CO_2 assimilation imposed by stomatal closure may promote an imbalance between photo- chemical activity of PS 2 and the electron requirement for photosynthesis, leading to an overexcitation and subsequent photoinhibitory damage of PS 2 reaction centers (Barber and Andersson 1992). Chlorophyll fluorescence analysis can give insights into the ability of plant to tolerate environmental stress and the

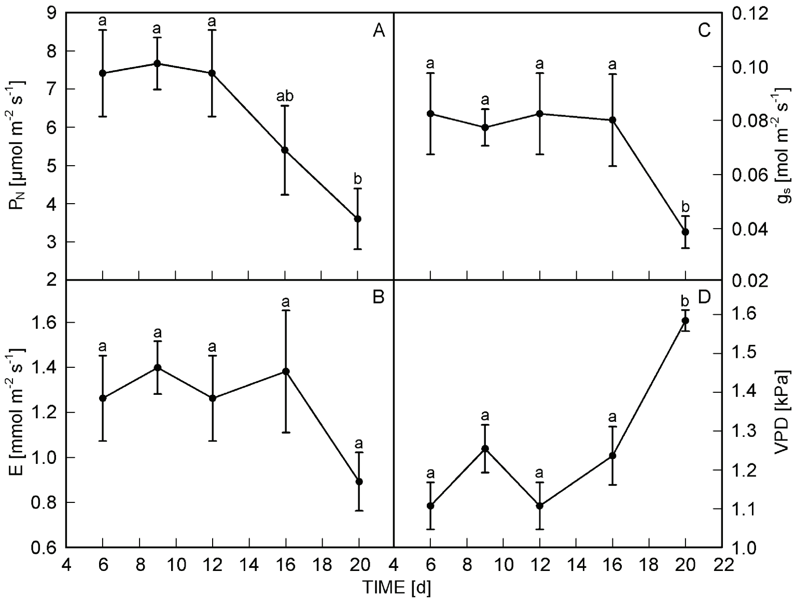

Fig. 3. The changes in net photosynthetic rate (*A*), transpiration rate (*B*), stomatal conductance (*C*) and leaf-air vapour pressure difference (*D*) of *Incarvillea delavayi* after irrigation was stopped. Means ± SE (n = 10). Different letters above the columns mean values statistically different at $P < 0.05$ as determined by LSD test.

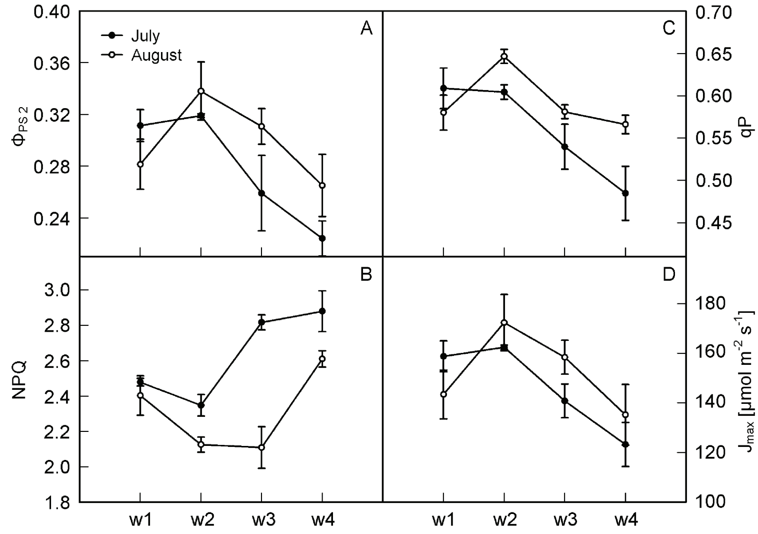

Fig. 4. PS 2 photochemical efficiency (*A*), non-photochemical quenching (*B*), photochemical quenching (*C*) and electron transport rate (*D*) in four water regimes in July and August. Means ± SE (n = 3).

extent to which those stresses damaged the photosynthetic apparatus (Maxwell and Johnson 2000). The observed decreases in Φ_2, qP and J_{max} with the increasing water stress (Fig. 4*A*,*C*,*D*) indicated an overexcitation of the photochemical system. Higher values of NPQ occurred in w4 treatment (Fig. 4*C*) as a result of energy dissipation that protected the leaf from high irradiance-induced damage (Maxwell and Jonhson 2000). The maximum quantum yield of PS 2, as revealed by F_v/F_m ratio, was maintained between 0.82 and 0.85 throughout the experiment (data not shown), indicating that PS 2 was quite resistant to water deficit. Nevertheless, water stress inevitably influenced the photosynthesis and following development of *I. delavay* but the occurrence of damages did not seem to be very serious. The plants of w3 and w4 treatments had higher P_{max}, V_{cmax}, J_{max}, P_B, P_R and NUE in August than in July (Table 1), which indicated the partial recovery of photosynthetic capacity. It could be attributed to significantly increased g_m and the optimal balance of g_s (Table 1). This increased the CO_2 transport from atmosphere to photosynthetic apparatus. The S_L, S_M of w3 seems to be beneficial during the early growth of fast growing species, as the fast growth allows the plant to cover the soil surface rapidly, thus substantially diminishes direct water evaporation from the soil and improves water use efficiency. Late leaf growth and development is likely to make better progress under mild water deficits, which benefit for higher water use efficiency (Fischer 1980).

In conclusion, progressively increasing water stress decreased the photosynthetic capacity *via* the decreases in g_s and g_m and the changes of leaf traits such as LMA, LNC, Chl *a/b*, P_R and P_B. S_L was the major limitation but NS_L increased quickly with the increasing water stress, and the S_M was the main component of NS_L. Although some impairments of photochemical activity were presented in water stressed plants, the plants had a substantial physiological plasticity and could tolerance long period of drought. However, well water condition (60 - 80 % of field soil capacity) is needed for the successful domestication and cultivation.

References

Barber, J., Andersson, B.: Too much of a good thing: light can be bad for photosynthesis. - Trends Biochem. Sci. **17**: 61-66, 1992.

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. **130**: 1992-1998, 2002.

Bowman, W.D., Theodose, T.A., Fisk, M.C.: Physiological and production response of plant growth forms to increases in limiting resources in alpine tundra: implications for differential community response to environmental change. - Oecologia. **101**: 217-227, 1995.

Boyer, J.S.: Plant production and environment. - Science **218**: 443-448, 1982.

Chandra, S.: Effects of leaf age on transpiration and energy exchange of *Ficus glomerata*, a multipurpose tree species of central Himalayas. - Physiol. mol. Biol. Plants **9**: 255-260, 2003.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osorio, M.L., Carvalho, L., Faria, T., Pinheiro, C.: How plants cope with water stress in the field: photosynthesis and growth. - Ann. Bot. **89**: 907-916, 2002.

Chen, S.T., Zhou, Z.K., Guan, K.Y., Nakata, M.: Karyomorphology of *Incarvillea* (Bignoniaceae) and its implications in distribution and taxonomy. - Bot. J. Linn. Soc. **144**: 113-121, 2004.

DaMatta, F.M., Loos, R.A., Silva, E.A., Loureiro, M.E.: Limitations to photosynthesis in *Coffea canephora* as a result of nitrogen and water availability. - J. Plant Physiol. **159**: 975-981, 2002.

Enquist, B.J., Ebersole, J.J.: Effects of added water on photosynthesis of *Bistorta vivipara*: the importance of water relations and leaf nitrogen in two alpine communities, Pikes Peak, Colorado, USA - Arct. Alpine Res. **26**: 29-34, 1994.

Evans, J.R.: Photosynthesis and nitrogen relationships in C_3 plants. - Oecologia **78**: 9-19, 1989.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Fischer, R.A.: Influence of water stress on crop yield in semiarid regions. - In: Turner, N.C., Kramer, P.J. (ed.): Adaptation of Plants to Water and High Temperature Stress. Pp. 323-339. Wiley, New York 1980.

Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T.D.: Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. - Plant Biol. **6**: 269-279, 2004.

Galmés, J., Medrano, H., Flexas, J.: Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. - New. Phytol. **175**: 81-93, 2007.

García, A.L., Marcelis, L., García - Sánchez, F., Nicolas, N., Martinez, V.: Moderate water stress affects tomato leaf water relations in dependence on the nitrogen supply. - Biol. Plant. **51**: 707-712, 2007.

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - Biochim. biophys. Acta **990**: 87-92, 1989.

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. **28**: 834-849, 2005.

Harley, P.C., Loreto, F., Marco, G. D., Sharkey, T.D.: Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. - Plant Physiol. **98**: 1429-1436, 1992.

Inskeep, W.P., Bloom, P.R.: Extinction coefficients of chlorophyll *a* and *b* in N, N-dimethylformamide and 80 % acetone. - Plant Physiol. **77**: 483-485, 1985.

Jones, H.G.: Partitioning stomatal and non-stomatal limitations to photosynthesis. - Plant Cell Environ. **8**: 95-104, 1985.

Kao, W.Y., Tsai, T.T.: Tropic leaf movements, photosynthetic gas exchange, leaf $\delta^{13}C$ and chlorophyll *a* fluorescence of three soybean species in response to water availability. - Plant Cell Environ. **21**: 1055-1062, 1998.

Kitajima, K., Hogan, K.P.: Increases of chlorophyll *a*/*b* ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. - Plant Cell Environ. **26**: 857-865, 2003.

Körner, C. (ed.): Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. - Springer-Verlag, Berlin - Heidelberg 1999.

Krall, K.E., Edwards, G.E.: Relationship between photosystem Ⅱ activity and CO_2 fixation in leaves. - Plant Physiol. **86**: 180-187, 1992.

Lawlor, D.W.: Limitation to photosynthesis in water stressed leaves: stomata *vs*. metabolism and the role of ATP. - Ann. Bot. **89**: 871-885, 2002.

Lawlor, D.W., Cornic, G.: Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. - Plant Cell Environ. **25**: 275-294, 2002.

Lin, M.J., Hsu, B.D.: Photosynthetic plasticity of *Phalaenopsis* in response to different light environments. - J. Plant Physiol. **161**: 1259-1268, 2004.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. - J. exp. Bot. **51**: 659-668, 2000.

Melgar, J.C., Syvertsen, J.P., Martínez, V., García-Sánchez, F.: Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. - Biol. Plant. **52**: 385-390, 2008.

Nakamura, M., Kido, K., Kinjo, J., Nohara, T.: Antinociceptive substances from *Incarvillea delavayi*. - Phytochemistry **53**: 253-256, 2000.

Niinemets, Ü., Cescatti, A., Rodeghiero, M., Tosens, T.: Leaf internal diffusion conductance limits photosynthesis strongly in older leaves of Mediterranean evergreen broad-leaved species. - Plant Cell Environ. **28**: 1552-1566, 2005.

Niinemets, Ü., Tenhunen, J.D.: A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. - Plant Cell Environ. **20**: 845-866, 1997.

Pearcy, R.W.: Acclimation of photosynthetic and respiratory carbon dioxide exchange to growth temperature in *Atriplex lentiformis* (Torr.) Wats. - Plant. Physiol. **59**: 795-799, 1977.

Pollock, K.M.: Aspects of the Water Relations of Some Alpine Species of *Chionochloa*. - Ph.D. Thesis, University of Otago, Otago 1979.

Sobrado, A., Turner, N.C.: Photosynthesis, dry matter accumulation and distribution in the wild sunflower *Helianthus petiolaris* and the cultivated sunflower *Helianthus annuus* as influenced by water deficits. - Oecologia **69**: 181-187, 1986.

Terashima, I., Araya, T., Miyazawa, S.I., Sone, K., Yano, S.: Construction and maintenance of the optimal photosynthetic systems of the leaf, herbaceous plant and tree: an eco-developmental treatise. - Ann. Bot. **95**: 507-519, 2005.

Von Caemmerer, S., Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange rates of leaves. - Planta **153**: 376-387, 1981.

Wang, J.R., Hawkins, C.D.B., Letchford, T.: Photosynthesis, water and nitrogen use efficiencies of four paper birch (*Betula papyrifera*) populations grown under different soil moisture and nutrient regimes. - Forest. Ecol. Manage. **112**: 233-244, 1998.

Watling, J.R., Press, M.C., Quick, W.P.: Elevated CO_2 induces biochemical and ultrastructural changes in leaves of C_4 cereal sorghum. - Plant Physiol. **123**: 1143-1152, 2000.

Yeo, A.: Molecular biology of salt tolerance in the context of whole plant physiology. - J. exp. Bot. **49**: 915-929, 1998.