Effects of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings

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Abstract

Two-month-old seedlings of *Sophora davidii* were subjected to a randomized complete block design with three water (80, 40, and 20 % of water field capacity, *i.e.* FC_{80}, FC_{40}, and FC_{20}) and three N supply [N0: 0, Nl: 92 and Nh: 184 mg(N) kg^{-1}(soil)] regimes. Water stress produced decreased leaf area (LA) and photosynthetic pigment contents, inhibited photosynthetic efficiency, and induced photodamage in photosystem 2 (PS2), but increased specific leaf area (SLA). The decreased net photosynthetic rate (P_N) under medium water stress (FC_{40}) compared to control (FC_{80}) might result from stomatal limitations, but the decreased P_N under severe water deficit (FC_{20}) might be attributed to non-stomatal limitations. On the other hand, N supply could improve photosynthetic capacity by increasing LA and photosynthetic pigment contents, and enhancing photosynthetic efficiency under water deficit. Moreover, N supply did a little in alleviating photodamages to PS2 caused by water stress. Hence water stress was the primary limitation in photosynthetic processes of *S. davidii* seedlings, while the photosynthetic characters of seedlings exhibited positive responses to N supply. Appropriate N supply is recommended to improve photosynthetic efficiency and alleviate photodamage under water stress.

Additional key words: adaptation; chlorophyll fluorescence; intercellular CO_2 concentration; leaf area; net photosynthetic rate; respiration rate; specific leaf area; stomatal conductance; water use efficiency.

Introduction

Regional and significant aggravation in water deficit and increase in nitrogen (N) deposition are predicted for the near future in many ecosystems due to global climate changes and human activities (Vitousek *et al.* 1997, Hura *et al.* 2007). To gain an early picture of how plant total C balance can be affected by foreseen environmental changes, it is necessary to investigate the photosynthetic responses of vegetation to such environment change variables.

Water deficit is one of the most important limitations to photosynthesis and then plant productivity (Boyer 1982, Tezara *et al.* 1999). There is now substantial consensus that reduced CO_2 diffusion from the atmosphere to the site of carboxylation—as a result of both stomatal closure and reduced mesophyll conductance—is the main cause of decreased photosynthesis under most water stress conditions (Chaves and Oliveira 2004, Flexas *et al.* 2004, 2006, Ennahli and Earl 2005, Grassi and Magnani 2005). On the other hand, many studies have well documented photosynthetic responses of plants to N availability, which included significant and positive correlation between photosynthetic capacity and leaf N content, suggesting that most of the N is used for the synthesis of components in the photosynthetic apparatus (Sugiharto *et al.* 1990, Egli and Schmid 1999, Shangguan *et al.* 2000, DaMatta *et al.* 2002, Makoto and Koike 2007). Furthermore, N application often brought about an increase in cell wall rigidity and osmotic adjustment (Fife and Nambiar 1997, Saneoka *et al.* 2004). The increase of N availability might improve photosynthetic capacity or stomatal control in water and N deficit conditions. But out of the expectation, N limitations *per se* affect stomatal behaviour in different ways: both increase (Livingston *et al.* 1999) and decrease (Lima *et al.* 1999) in stomatal conductance (g_s) have been noted. Radin and Ackerson (1981) showed that low-N cottons had the greatest

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stomatal sensitivity to water stress. They suggested that N deficient plants accumulated more abscisic acid and were therefore predisposed to respond more rapidly to water stress. Conversely, Morgan (1984, 1986) found that N-fertilized wheat responded more rapidly to increasing water stress by closing stomata and reducing net photosynthesis. Chen *et al.* (2005) mentioned that in two grasses, varying N addition did not alter the gas exchange characteristics including net photosynthetic rate (P_N), transpiration rate (g_s), and intercellular CO_2 concentration (C_i) under water stress. The inconsistent results might at least partly be induced by species characteristics, native environment, N supply, and water stress levels.

However, gas exchange measurement cannot completely represent the effects of abiotic stress on different processes such as the photochemistry and biochemistry of C fixation. In this respect, the modulation chlorophyll (Chl) fluorescence technique, which has been widely used to detect the impact of abiotic stresses on the light reactions of photosynthesis, is satisfactory (Wang and Kellomäki 1997, Bigras 2005). Under drought stress, disturbances of photosynthesis at the molecular level are connected with the low electron transport through photosystem 2 (PS2) and/or with structural injuries of PS2 and light-harvesting complexes (van Rensburg and Krüger 1993, Hura *et al.* 2007). Restricted CO_2 may lead to increased susceptibility to photodamage due to stomatal closure, and then to photoinhibition (Powles 1984). It is characterized by parallel decreases in P_N and quantum yield of PS2 (Φ_{PS2}), and is accompanied by a decline in maximum quantum yield of photosynthesis (F_v/F_m) associated with loss of PS2 activity (Powles 1984, Tezara *et al.* 2005) and an increase in minimal Chl fluorescence (F_0) (Osmond and Grace 1995). Chl fluorescence is a useful tool for quantification of the effect of abiotic stress on photosynthesis (Krause and Weis 1991, Tezara *et al.* 2005). N supply could increase F_v/F_m and the effective quantum yield of photochemical energy conservation in PS2 ($\Delta F/F_m$') (Wang and Kellomäki 1997). Chl content and photosynthetic rates might also be enhanced in plants by supplying N (Shaw *et al.* 2002). These authors suggested that N supply might alleviate photoinhibition and photodamage caused by water stress.

Sophora davidii is a native perennial shrub widely distributed in the dry valleys of the Hengduan Mountain Systems and other places of China (Wei 1994, Li *et al.* 2006). This species is often predominant on eroded slopes where it plays a vital role in retaining ecological stability in semi-arid and arid regions (Bu *et al.* 2004, Xue *et al.* 2004). In the Loess Region of China, it is better adapted to dry environment than other shrubs (Xue *et al.* 2004, Kang *et al.* 2005, Wang *et al.* 2005a,b), prompting its use for reclamation of arid degraded lands. However, the distribution of this shrub is rapidly reduced due to habitat destruction in recent decades (Kang *et al.* 2005, Wang *et al.* 2005b). Protection and restoration of this ecologically important shrub in arid and semi-arid regions is one of our goals in China. Because of serious drought stress and low availability of soil nutrients (N and P) (Wang *et al.* 2003), *S. davidii* has weak regeneration with poor seedling emergence and early growth. Fertilization could increase soil fertility, alleviate photodamage, facilitate photosynthetic efficiency and consequently plant growth, and thus improve the ability of plant adaptation to dry and infertile condition. But little information has been aimed on the effects of N supply and drought stress in this ecologically important shrub.

Our study was conducted in the dry valley of Minjiang River, which is one of the main branches of Yangtze River and is located in the north of the Hengduan Mountain Systems, China. It was focused on (*1*) the photosynthetic adaptation of *S. davidii* seedlings to various water and N supply conditions, and (*2*) whether or not N supply could improve photosynthetic capacity of seedlings under dry condition in the first year of growth.

Materials and methods

Plants and their growth: Seeds of *S. davidii* were collected in September 2005 in Maoxian County, Sichuan, China. After drying for one week in open sunlight, apparently healthy seeds were air-dried and then stored at ambient laboratory temperature until the experimental pre-treatment in March 2006. Surface soil from several collection sites was mixed thoroughly and used as growth substrate. Characteristics of this cinnamon soil are as follows: pH 8.06, bulk density 1 360 g m^{-3}, graval content 52 %, water FC 24.68 %, organic C 55.29 g kg^{-1}, Kjeldahl N 2.01 g kg^{-1}, total P 0.81 g kg^{-1}, NH_4-N 9.55 mg kg^{-1}, NO_3-N 17.63 mg kg^{-1}, available P 9.25 mg kg^{-1}. After 10 d, 7.5 kg soil was placed in each of forty-five 7 500 cm^3 plastic pots.

Before sowing, the seeds were pre-treated with 2.5 % sodium hypochlorite (NaOCl) for 1 h. Four seeds of similar size were sown in each pot on March 24, 2006. All pots were moved into a greenhouse at the Maoxian Mountain Ecosystem Research Station, Chinese Academy of Sciences (experimental site, 103°53′58″E, 31°41′07″N, 1 816 m a.s.l.) where temperature was 30/11 °C day/night and the relative humidity (RH) was kept between 45 and 85 %. Midday photosynthetic photon flux density was about 1 600 μmol^{-1}(photon) s^{-1} m^{-2} (Fig. 1). Pots were initially well-watered in order to ensure sprout establishment. Shortly after emergence, seedlings were thinned to one plant per pot.

Experimental design: The experiment was arranged in a Randomized Complete Block Design with five replicates for three water supply regimes [80, 40, and 20 % field water capacity (FC), *i.e.* FC_{80}, FC_{40}, and FC_{20}] and three

N supply (control N0, low N supply Nl, and high N supply, Nh) treatments. The watering and N treatments were initiated on June 28, 2006 after seedling establishment. 10 cm^3 solutions containing 0, 1.5, and 3.0 g urea (46 % N) [corresponding to 0, 92, and 184 mg(N) kg^{-1}(soil), respectively] were applied for the three different N treatments, respectively. To avoid rapid N loss, the solutions were supplied 5 cm under soil surfaces. Evaporation from the soil surface was minimized by covering with a 3-cm layer of quartz gravel. Transpiration water loss was measured gravimetrically by weighing all pots and re-watering by distilled water every other day at 18:00. The watering amount for each pot was determined according to the difference between the mass of a re-watered pot and mass of the same pot 48 h later. During the experiment, the average soil volumetric water contents were 19.7±0.3, 9.8±0.7, and 4.9±1.1 % under FC_{80}, FC_{40}, and FC_{20}, respectively. The experimental layout was surrounded with a single row of border plants to protect the experimental seedlings from external influences. All pots were randomized weekly. Following the period of rapid growth, increases in seedling mass were estimated from the regression of the relationship between seedling fresh matter (Y) [g], plant height (X_1) [cm], and seedling basal diameter (X_2) [mm]: $Y = -1.496 + 0.117\, X_1 + 0.998\, X_2$, $r^2 = 0.848$, $p<0.001$ (unpublished data). Mass of the pot plus seedling at field capacity was adjusted accordingly.

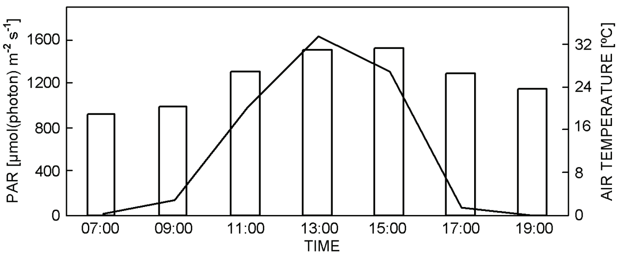

Fig. 1. Diurnal fluctuations of photosynthetically active radiation (PAR) (*line*) and air temperature (*columns*).

Chl fluorescence was determined on fully expanded exposed leaves (one leaf each plant) using a modulated fluorometer (*PAM 2100*, *Walz*, Effeltrich, Germany) on September 2, 2006 according to Roháček and Barták (1999) and Yin *et al.* (2006). Initial fluorescence (F_0) and maximal fluorescence (F_m) were measured after a 30-min dark adaptation. The intensity of saturation pulses to determine the maximal fluorescence emission in the presence (F_m’) and absence (F_m) of quenching was 4 000 μmol(photon) m^{-2} s^{-1}, 0.8 s, whereas the “actinic light” was 600 μmol(photon) m^{-2} s^{-1}. Steady-state fluorescence (F_s), basic fluorescence after light induction (F_0’), maximal PS2 photochemical efficiency (F_v/F_m), effective quantum yield of PS2 (Φ_{PS2}), and photochemical (q_P) and non-photochemical (q_N) fluorescence quenching coefficients were also recorded. The effective quantum yield of photochemical energy conservation in PS2 ($\Delta F/F_m$’) was calculated as (F_m’ – F_s)/F_m’ according to Roháček and Barták (1999).

Gas exchange was measured on the same leaves as Chl fluorescence using a portable computerized open gas system IRGA (*LI-6400*, *LiCor*, Lincoln, NE, USA). P_N, g_s, C_i, ambient CO_2 concentration (C_a), and PAR were measured at 07:00, 09:00, 11:00, 13:00, 15:00, 17:00, and 19:00 on a fully sunny day (September 3, 2006) under natural conditions to determine diurnal variation of gas exchange. Intrinsic water use efficiency (WUEi) was calculated by dividing P_N by g_s, and stomatal limitation value (L_s) was calculated using the following formula: $L_s = 1 - C_i/C_a$ according to Yin *et al.* (2006). P_N-PAR response curves were measured at 1 800, 1 500, 1 200, 1000, 800, 500, 200, 100, 50, 20, and 0 μmol(photon) m^{-2} s^{-1} of PAR under uniform conditions (25 °C, 330–350 μmol(CO_2) mol^{-1}, and 71–75 % RH) at 10:00-11:30 on two sunny days (September 5 and 7, 2006). Linear regressions of irradiance and P_N over the range of 0–200 μmol(photon) m^{-2} s^{-1} of PAR were applied to determine dark respiration rate (R_D), compensation irradiance (CI), and apparent quantum yield (AQY) (Yin *et al.* 2006). Maximum net photosynthetic rate (P_{max}) and saturation irradiance (SI) were estimated according to Walker (1989). All the measurements were recorded 5 times.

Leaf area (LA) and photosynthetic pigments: After the determination of photosynthetic activity, all leaves were harvested. 0.1 g fresh leaves were taken for the determination of Chl content. Leaves were ground in 80 % acetone for the extraction of Chl and carotenoids (Car). Pigment amounts were determined according to Li *et al.* (2000). The images of remaining leaves were recorded with a scanner (model *F6580*, *Founder Electronics Co.*, Beijing, China) and then digitized by the *Arcview 3.2a* software (*Environmental Systems Research Institute*, New York, USA) in order to determine LA. Finally, leaves were oven dried for 48 h at 70 °C for biomass determination. Specific leaf area (SLA) was calculated as leaf area/leaf mass.

Statistical analysis: All the variables measured were analyzed using *Univariate* process of General Linear Model (GLM) with water and N supply regimes and their interaction as factors ($n = 5$). When significant differences were noted, LSD multiple range test was used to determine where differences existed. All of the statistical analyses were performed using *SPSS* software package (standard released version *11.5 for Windows*, *SPSS*, IL, USA).

Results

LA and contents of photosynthetic pigments exhibited significant ($p<0.05$) responses to water stress and the interaction of water and N, although N supply only statistically ($p<0.05$) affected total LA (Table 1). Water stress decreased LA, contents of Chl *a* and *b* and Car, the ratios Chl *a/b* and Car/Chl, but increased SLA. On the other hand, all these measurements showed higher values in Nl compared to the other two water stresses (FC_{40} and FC_{20}), while Nl did not enhance Chl *a/b* and Car/Chl.

Table 1. Total leaf area (LA), specific leaf area (SLA), chlorophyll (Chl) *a* and *b* contents, carotenoid (Car) content, and the ratios of Chl *a/b* and Car/Chl of *Sophora davidii* seedlings at different water (FC) and N supply regimes. Dry mass (DM), fresh mass (FM). Means±SD, *n* = 5, $^{*}p<0.05$, $^{**}p<0.01$.

	LA [cm^2]	SLA [$m^2\ kg^{-1}$(DM)]	Chl *a* [$g\ kg^{-1}$(FM)]	Chl *b* [$g\ kg^{-1}$(FM)]	Car [$g\ kg^{-1}$(FM)]	Chl *a/b*	Car/Chl
FC_{80}N0	8.89±0.33	75.16±4.19	2.82±0.27	0.63±0.05	0.71±0.07	4.55±0.13	0.20±0.01
FC_{80}Nl	10.41±0.94	65.11±3.98	2.66±0.46	0.68±0.12	0.69±0.09	4.64±0.20	0.21±0.01
FC_{80}Nh	9.89±0.92	61.12±6.78	2.66±0.31	0.59±0.08	0.69±0.07	4.51±0.09	0.21±0.00
FC_{40}N0	5.07±0.27	62.31±1.21	2.29±0.19	0.55±0.04	0.56±0.05	4.16±0.04	0.20±0.00
FC_{40}Nl	5.35±0.40	74.33±4.89	2.63±0.21	0.59±0.05	0.63±0.05	4.45±0.04	0.20±0.00
FC_{40}Nh	4.40±0.28	68.32±2.52	2.57±0.24	0.57±0.07	0.62±0.06	4.50±0.21	0.20±0.00
FC_{20}N0	3.38±0.33	83.46±2.19	2.39±0.02	0.52±0.01	0.54±0.01	4.38±0.08	0.19±0.00
FC_{20}Nl	3.79±0.94	90.25±0.96	2.67±0.08	0.62±0.01	0.59±0.01	4.33±0.21	0.18±0.00
FC_{20}Nh	3.66±0.92	89.54±1.05	2.66±0.10	0.54±0.01	0.49±0.01	4.03±0.25	0.18±0.00
F_{water}	390.65^{**}	43.44^{**}	6.30^{*}	3.25^{*}	17.79^{**}	6.22^{**}	58.36^{**}
F_N	3.18*	0.57	0.05	0.04	0.01	0.77	0.15
$F_{water×N}$	3.87^{*}	5.31^{*}	3.10^{*}	3.05^{*}	3.20^{*}	5.78^{**}	2.86^{*}

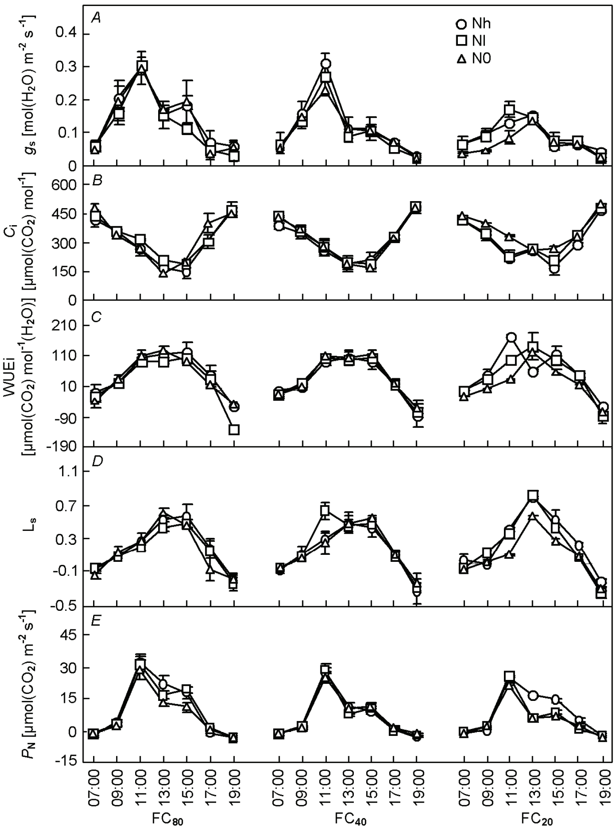

Fig. 2. Diurnal changes of stomatal conductance, g_s (*A*), intercellular CO_2 concentration, C_i (*B*), intrinsic water use efficiency, WUEi (*C*), stomatal limitation value, L_s (*D*), and net photosynthetic rate, P_N (*E*) in *Sophora davidii* seedlings at different water (FC) and N supply regimes. *Bars* indicate SD (*n* = 5).

Diurnal changes of leaf gas exchange: Gas exchange parameters changed with the daytime (Fig. 2). Each parameter shared respective similar dynamics pattern during daytime under different water and N supply regimes. Water stress significantly decreased P_N; maximum values were observed in Nh at corresponding water condition. All the nine treatments showed P_N peak at about 11:00 and the other apparent peak was observed at about 13:00 except for Nh regardless of water conditions (Fig. 2*E*).

g_s paralleled with P_N under FC_{80} and FC_{40}. Under severe water stress (FC_{20}), N supply enhanced g_s in the morning, and $g_s>0.1$ mol(H_2O) $m^{-2}\ s^{-1}$ was only observed at 11:00 in Nh and Nl and at 13:00 in all three N treatments (Fig. 2*A*).

C_i decreased with the increase of P_N and g_s during daytime. Compared to well-watered condition, medium water stress decreased C_i, but C_i kept higher between 11:00 and 15:00 under severe water stress (Fig. 2*B*). N supply had no significant effects on C_i.

Diurnal changes of WUEi and L_s displayed a similar pattern. All nine treatments showed their peaks at 13:00 (Fig. 2*C*,*D*). Although no significant differences were observed, N supply enhanced WUEi under all three water conditions, and WUEi showed higher values under severe water stress than that under the other two. L_s increased with the increase of water stress, and N supply also increased L_s under water stress whereas decreased it under well-watered conditions.

***P_N*-PAR response curves**: Water stress and N supply also affected the P_N-PAR response curves (Fig. 3): P_N showed pronounced decreasing tendency with the increase of water stress while an increasing tendency with the increase in N supply above 500 μmol(photon) m^{-2} s^{-1} of PAR. Both water stress and N supply significantly ($p<0.01$) influenced P_{max}, but their interaction had no statistically ($p>0.05$) significant effects. P_{max} decreased with the increase of water stress, but increased with the increase of N supply. AQY, R_D, SI, and CI were significantly ($p<0.05$) affected by water stress, N supply, and their interaction (Table 2). Under severe water stress, AQY was lower than that under the other two water conditions, and the lowest values were observed in Nh at corresponding water condition. Both water stress and N supply enhanced R_D. Water stress increased CI, but N supply decreased it under water stress conditions (FC_{40} and FC_{20}). However, water stress decreased SI whereas N supply increased it at corresponding water condition.

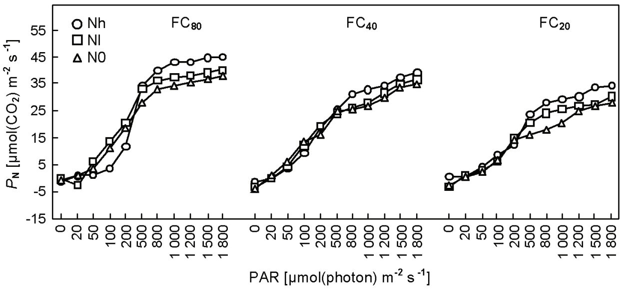

Fig. 3. Photosynthesis-PAR response curves in *Sophora davidii* seedlings at different water (FC) and N supply regimes.

Table 2. The maximum net photosynthetic rate (P_{max}) [μmol(CO_2) m^{-2} s^{-1}], apparent quantum yield (AQY) [μmol(CO_2) $μmol^{-1}$], dark respiration rate (R_D) [μmol(CO_2) m^{-2} s^{-1}], saturation irradiance (SI), and compensation irradiance (CI) [μmol(photon) m^{-2} s^{-1}] of *Sophora davidii* seedlings at different water (FC) and N supply regimes. Means±SD, $n = 5$, $^*p<0.05$, $^{**}p<0.01$.

	P_{max}	AQY	R_D	SI	CI
FC_{80}N0	43.32±1.32	0.0990±0.0110	–1.55±0.10	258.60±7.99	9.61±0.44
FC_{80}Nl	45.24±1.60	0.1135±0.0040	–1.85±0.06	278.00±6.71	13.76±0.50
FC_{80}Nh	48.52±1.93	0.0972±0.0068	–2.03±0.16	300.00±9.49	15.02±0.30
FC_{40}N0	38.04±1.83	0.1152±0.0038	–1.97±0.05	239.60±6.80	16.46±0.57
FC_{40}Nl	39.26±2.01	0.1146±0.0107	–2.00±0.08	234.20±6.83	14.70±0.60
FC_{40}Nh	41.33±1.06	0.0992±0.0090	–2.23±0.09	269.60±15.37	14.23±0.58
FC_{20}N0	33.75±1.54	0.0993±0.0033	–2.03±0.14	206.40±7.63	18.66±0.83
FC_{20}Nl	36.87±1.20	0.0996±0.0046	–2.17±0.15	221.60±15.04	17.98±1.29
FC_{20}Nh	38.10±1.17	0.0922±0.0062	–2.24±0.05	230.40±10.59	18.58±0.78
F_{water}	142.36^{**}	30.25^{**}	20.93^{**}	128.83^{**}	237.04^{**}
F_N	28.34^{**}	53.56^{**}	6.79^{*}	38.75^{*}	8.05^{**}
$F_{water×N}$	1.01	19.92^{**}	55.88^{**}	3.50^{*}	43.41^{**}

Table 3. The maximum quantum yield of photosystem 2 (PS2) photochemistry (F_v/F_m), effective quantum yield of PS2 (Φ_{PS2}), photochemical quenching (q_P), non-photochemical quenching (q_N), and effective quantum yield of photochemical energy conservation in PS2 ($\Delta F/F_m$’) of *Sophora davidii* seedlings at different water (FC) and N supply regimes. Means±SD, $n = 5$, $^*p<0.05$, $^{**}p<0.01$.

	F_v/F_m	Φ_{PS2}	q_P	q_N	$\Delta F/F_m$’
FC_{80}N0	0.85±0.01	0.69±0.01	0.90±0.02	0.35±0.04	0.69±0.00
FC_{80}Nl	0.86±0.00	0.73±0.01	0.96±0.01	0.41±0.02	0.73±0.01
FC_{80}Nh	0.82±0.06	0.72±0.00	0.94±0.01	0.41±0.03	0.72±0.00
FC_{40}N0	0.85±0.01	0.69±0.02	0.91±0.01	0.44±0.05	0.65±0.01
FC_{40}Nl	0.83±0.05	0.71±0.03	0.92±0.02	0.42±0.06	0.70±0.02
FC_{40}Nh	0.81±0.05	0.71±0.05	0.87±0.05	0.36±0.12	0.69±0.03
FC_{20}N0	0.80±0.06	0.64±0.07	0.89±0.05	0.52±0.10	0.62±0.03
FC_{20}Nl	0.83±0.03	0.62±0.04	0.81±0.05	0.52±0.12	0.63±0.07
FC_{20}Nh	0.72±0.12	0.46±0.15	0.93±0.02	0.63±0.01	0.63±0.08
F_{water}	2.88^{*}	12.48^{**}	6.73^{**}	8.61^{**}	2.41^{*}
F_N	2.59^{*}	4.26^{*}	0.93	0.92	1.88
$F_{water×N}$	1.19	2.41	8.19^{**}	3.20^{*}	0.72

Chl fluorescence: Water stress had significant ($p<0.05$) effects on Chl fluorescence parameters (Table 3). However, N supply only had significant ($p<0.05$) effects on F_v/F_m and Φ_{PS2}, although q_P and q_N showed significant ($p<0.05$) responses to the interaction of water and N. Water stress decreased F_v/F_m and Φ_{PS2}, while Nl increased them under FC_{40} and FC_{80}. Both Nl and Nh decreased Φ_{PS2} under severe water stress, and Nh also decreased F_v/F_m regardless of water conditions. q_P was slightly decreased with the increase of water stress while q_N was increased. $\Delta F/F_m'$ was decreased with the increase of water stress, while it slightly positively responded to Nl.

Discussion

As expected, the photosynthetic characters showed strong responses to water stress and N supply, which agreed with many previous studies (DaMatta *et al.* 2002, Flexas *et al.* 2006, Makoto and Koike 2007). Nevertheless, we found that water stress seemed to play the primary limitation roles in photosynthetic capacity and N supply could improve it but could not alter the declining tendency.

Lower photosynthetic performance of seedlings may be associated with the decreasing LA and Chl and Car contents under water stress. Decreased Chl *a/b* and Car/Chl ratios were also observed under water stress, decline of the former showing that the damage of light-harvesting complex (LHC) was serious because of relatively high amount of Chl *b* in the LHC (Jeon *et al.* 2006); decline of the latter suggested that the development of LHC and the dissipation of thermal energy might be strongly affected by water stress since Cars of chloroplast are major components of antenna systems (Jeon *et al.* 2006). Our study also showed that SLA increased with increasing water stress. This could be another adaptation mechanism for *S. davidii* seedlings. A greater SLA implies thinner leaf (Carter *et al.* 1997, Cordell *et al.* 1998), which could partly reduce CO_2 diffusion pathway from stomata to carboxylation site (Vitousek *et al.* 1992), thus counteracting stomatal and mesophyll resistances (Castro-Diez *et al.* 2000). Similar to the above analysis, the observed highest LA and photosynthetic pigment contents in Nl treatment under water stress implied that low N supply could alleviate the damages caused by water stress and improve photosynthetic performance under water deficit.

Both water stress and N supply slightly affected diurnal fluctuation patterns of *S. davidii* seedlings, which were greatly related to the biological rhythm of plant. An apparent P_N “midday depression” phenomenon was observed at 13:00 when PAR and temperature reached the diurnal maximum (Figs. 1 and 2). High PAR and temperature might inhibit P_N by controlling stomata closure because similar pattern happened to g_s and inversely to C_i. Bilger *et al.* (1989) observed a similar phenomenon in cotton. Diurnal changes of Ls and WUEi were very similar, because of their dependent linkages to C_i/C_a (Ripullone *et al.* 2004). Even then, water stress pronounced decreased P_N but N supply had a little effect.

Reductions in P_N and g_s were accompanied by reductions in C_i at medium water stress. However, reductions in P_N and g_s, but relatively higher C_i values were observed under severe water stress. The former would indicate that under medium water stress g_s was the dominating factor which limits assimilation, irrespective of any metabolic impairment (Chaves and Oliveira 2004, Flexas *et al.* 2006, Lopes and Araus 2006). The latter suggested that decreased CO_2 availability at mesophyll level caused by stomatal closure was not the principal cause of decreased assimilation at severe water stress. According to Rouhi *et al.* (2007), the increase in C_i indicates decreased carboxylation efficiency. This would mean that non-stomatal limitations prevailed for *S. davidii* seedlings at severe drought stress. Additionally, both water stress and N supply increased WUEi and Ls, which was in agreement with the results of Yin *et al.* (2006) and the general theory, that is, supply one limited resource could enhance the use efficiency of other resources.

Theoretically, P_{max}, which determines the plant potential photosynthetic capacity, is proportional to the number of active catalytic sites in chloroplasts that are involved in the reductive assimilation of CO_2 (Jia and Gray 2003). In our study, decreased P_{max} under water stress and increased P_{max} at N supply were respectively associated with stomatal closure and improvement of carboxylation efficiency as discussed above, suggesting that N supply could enhance potential photosynthetic capacity under water deficit. In agreement with the study of Yin *et al.* (2006), water stress decreased AQY in our study. Increasing CI and decreasing SI would reduce the time of effective P_N, and the increase of R_D would result in more consummation at night. Therefore, water stress caused assimilation decrease at daytime and dissimilation increase at night might be the main reason of reducing plant growth and productivity. Accordingly, N supply might improve photosynthetic efficiency under water stress by increasing P_{max} and SI and decreasing CI in our study.

The efficiency and stability of PS2 have been widely monitored through the measurement of F_v/F_m in dark-adapted leaves (Wang and Kellomäki 1997). In our study, water stress increased F_v/F_m, which implies that water stress affected more the energy cycling between the reaction centre (RC) and the Chl pool, F_m, than the energy absorption rate of leaves, F_0 (Havaux *et al.* 1991). Low N supply led to apparent modifications of F_0, F_m, and F_v/F_m, which might alleviate the photoinhibition or other kind of injury to PS2 caused by water stress. This is consistent with the findings in Scots pine (Wang and Kellomäki 1997).

For monitoring the efficiency of photochemical processes in PS2 in light-adapted state, Φ_{PS2} and $\Delta F/F_m$' are usually used (Roháček and Barták 1999). The former is called the efficiency of excitation energy capture by open PS2 RCs, and the latter is considered to be closely related to the quantum yield of linear electron flow *in vivo* (Wang and Kellomäki 1997, Roháček and Barták 1999). In our measurements, both parameters exhibited negative responses to water stress but positive responses to Nl under relatively well-watered condition, suggesting that water stress decreased the efficiency of excitation energy capture of open PS2 RCs and N supply might improve it under medium water stress. Similar results were also observed in *Pisum sativum* responding to water stress (Havaux *et al.* 1991) and sugar beet affected by N supply (Shaw *et al.* 2002). However, N supply (especially excess N supply) might aggravate the hurt caused by water stress. On the other hand, the coupled changes of Φ_{PS2} and $\Delta F/F_m$' imply that F_m' is much sensitive to water and N disturbances since both Φ_{PS2} and $\Delta F/F_m$' would be determined by F_m'. This is also consistent with the findings of Wang and Kellomäki (1997).

Two basic parameters describe the quenching of maximum variable Chl fluorescence yield during the inducing irradiation period: q_P and q_N. q_P quantifies the photochemical capacity of PS2 in light-adapted leaves and corresponds to the steady-state fraction of oxidized PS2 RCs, and $1/(1 - q_P)$ is called the degree of PS2 RC closure. q_N reflects the influences of non-photochemical processes on Chl fluorescence emission during the transition of sample from dark-adapted to light-adapted state (Krause and Weis 1991, Roháček and Barták 1999). Decreased q_P under water stress in our study suggested that water stress might hurt PS2 RCs and lead to higher closure of PS2 RCs. Higher q_N under water stress indicated that plants efficiently dissipated energy trapped at PS2 in the form of heat. It is the photoprotective mechanism under stress (Bigras 2005).

In conclusion, water stress not only results in the decreases of LA, contents of photosynthetic pigments, gas exchange, and P_N-PAR curves, but also affects the efficiency of PS2. However, LA, photosynthetic pigments, and gas exchange show positive responses to N supply and N supply also alleviates the degree of photoinhibition and the injury under water stress by improving F_v/F_m and slightly increasing $\Delta F/F_m$'. Thus, appropriate N supply is recommended for *S. davidii* seedlings to improve photosynthesis processes inhibited by water stress and contributes to seedling establishment under water deficit. Measurements of combined fluorescence and gas exchange provide valuable complementary information on photosynthetic performance, but the results of these measurements reflect only relative states of photosynthetic processes because they depend strongly on measurement conditions.

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ERRATUM

J.T. Tsialtas, N. Maslaris: Leaf shape and its relationship with Leaf Area Index in a sugar beet (*Beta vulgaris* L.) cultivar. − Photosynthetica 45: 527-532, 2007.

Please correct:

On p. 528, right column, replace the last sentence as follows:

Analogous to LAI trends were found for LA, L, W, and AR. For all these parameters, the highest values were found in 2006, the lowest ones in 2003, and moderate ones in 2005.

Fig. 2 – replace the legend as follows:

Seasonal changes of leaf shape parameters. For abbreviations see Table 2.

The publisher and authors apologize for these errors and for inconveniences they may have caused.