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BRIEF COMMUNICATION

Leaf morphological and physiological responses to drought and shade in two *Populus cathayana* populations

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Abstract

Cuttings from two contrasting *Populus cathayana* Rehder populations originating from Hanyuan (wet climate) and Ledu (dry climate) in western China were grown in a greenhouse to determine the effects of drought, shade and their interaction on the morphological and physiological traits of leaves. The dry climate population was more drought-tolerant than the wet climate population, as indicated by smaller decreases in the leaf relative water content (RWC) and net photosynthetic rate (P_N), as well as by greater increases in antioxidative enzyme activities and free proline content under drought. On the other hand, the negative effects of shade on leaf traits were more pronounced in the dry climate population, which suggested that the dry climate population was more light-demanding. In addition, moderate shade alleviated the drought stress of *P. cathayana* not only by improving the leaf RWC but also by maintaining the positive carbon balance. In contrast, severe shade aggravated drought stress as indicated by a pronounced decrease in leaf size, carbon and nitrogen contents, maximum P_N, free proline content and antioxidative enzyme activities.

Additional key words: antioxidative enzymes, leaf area and thickness, net photosynthetic rate, nitrogen-use efficiency, proline.

In a natural environment, the availability of water and light are the two most important resources for plant survival and growth. The plasticity of seedlings to adjust leaf morphological and physiological performance in response to water deficit and light limitation plays an increasingly important role in the forest ecosystem (Aranda *et al.* 2005, Čaňová *et al.* 2008), as drought is becoming more severe and common worldwide. According to the trade-off mechanism (Smith and Huston 1989), a shaded plant has a higher leaf area than sun plants and invests more biomass in leaves but less in roots for efficient light capture (Niinemets and Valladares 2006), which may decrease its drought tolerance. On the other hand, there is increasing evidence that shade lessens the impact of drought by indirect effects, such as reduced leaf and air temperature, vapour pressure deficit and oxidative stress (Holmgren 2000). However, Sack and Grubb (2002) have proposed that the impacts of shade and drought on seedlings are independent. At the whole-plant level, drought stress usually leads to a decrease in photosynthesis and growth, which is associated with alterations in carbon and nitrogen metabolism (Raven *et al.* 2004). Moreover, to prevent the accumulation of reactive oxygen species under environmental stress, antioxidative enzymes, such as SOD, CAT, APX and GPX, are activated (Sivritepe *et al.* 2008). However, plants possess lower amounts of antioxidants due to a lower photosynthetic capacity under low irradiance as compared with high irradiance (Lermontova and Grimm 2006). Thus the antioxidant enzymes activities are important indexes for drought or shade tolerance of a plant. Additionally, free proline accumulates under stress,

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; C_{mass} - leaf carbon content per mass; GPX - guaiacol peroxidase; LCP - light compensation point; LS - leaf size; N_{mass} - leaf nitrogen content per mass; P_N - net photosynthetic rate; P_{Nmax} - maximum photosynthetic rate; PNUE - photosynthetic nitrogen-use efficiency; R_d - dark respiration rate; RWC - relative water content; SLA - specific leaf area (leaf mass/area ratio); SOD - superoxide dismutase; TLA - total leaf area.

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lowers osmotic potential and so contributes to osmotic adjustment (Misra and Gupta 2005, Su and Bai 2008). However, relatively little is known about how free proline responds to combined drought and shade effects.

In this study, pot-grown cuttings from two contrasting populations of *Populus cathayana* Rehder were employed to compare leaf traits in response to combinations of two stresses. The objectives of the study were *1*) to compare the shade and drought tolerance between the two populations, and *2*) to analyze, whether the effects of drought and shade are independent or not.

The mean annual rainfall in Hanyuan and Ledu are 750 and 335 mm, respectively. Therefore, the populations from Hanyuan and Ledu represent wet and dry climate populations, respectively. The experimental layout was completely randomized with three factors: two populaions, two water regimes (well-watered and drought) and three levels of irradiance (full sunlight, moderate shade and severe shade). Shade was provided by neutral shade clothes, which have no effect on spectral composition (Yates 1989). During the experiment, the photosynthetic photon flux density (PPFD) was measured every 2 h from 10:00 to 16:00 on non-cloudy days throughout the growing season with a *Li-Cor 190SA* sensor (*Li-Cor*, Lincoln, NE, USA). PPFD of the moderate and severe shade were approximately 33 and 12 % of full sunlight, respectively. In the well-watered treatment, the pots were re-watered to the 100 % field capacity by replacing the amount of water transpired every second day. In this case, the volumetric soil water content was kept at 24 %, as measured by a moisture probe meter (*MPM-160*, *ICT International*, Australia). In the drought treatment, the pots were watered to 50 % field capacity every second day. In this case, the volumetric soil water content was kept at 13 %. The cuttings were grown at the Maoxian Field Ecological Station in a naturally lit greenhouse in a semi-controlled environment with a day temperature range of 22 - 31 °C, a night temperature range of 9 - 15 °C and the relative humidity range of 55 - 85 %. The plants were subjected to stress for 4 months, from 1 June to 30 September. The measurements of various physiological and biochemical traits were performed within a 2-week period in September.

The relative water content (RWC) of leaves was calculated as: RWC [%] = [(fresh mass - dry mass)/water saturated mass - dry mass)] × 100. Water saturation of leaves was reached after incubation of them in water for 24 h at room temperature. Dry mass was measured following oven-drying at 80 °C to constant mass. The total leaf area (TLA) was measured with a portable laser area meter (*CI-203*, *CID*, Camas, USA), and the average leaf size (LS) was calculated. Leaf samples used for specific leaf area (SLA) measurements were oven-dried at 80 °C for 36 h after measuring the leaf area. SLA was calculated as the ratio of leaf area to dry mass. The samples of leaves were ground and passed through a mesh screen after being dried at 80 ºC for 36 h. The total contents of nitrogen (N) and carbon (C) were determined by the semi-micro Kjeldahl method and the rapid dichromate oxidation technique (Nelson and Sommers 1982), respectively.

Gas exchange parameters were determined using an open system (*LI-6400*, *Li-Cor*). The net photosynthetic rate was measured at 10 different PPFD (from 0 to 2 000 μmol m^{-2} s^{-1}) using a *LI-6400* artificial light source unit coupled to a leaf chamber. The temperature inside the leaf chamber was maintained at 25 °C and air humidity of 55 - 75 %. The dark respiration rate (R_d) and the light compensation point (LCP) were determined by fitting the nonrectangular hyperbola to the data (Prioul and Chartier 1977). We recalculated P_{max} assuming a PPFD of 1 400 μmol m^{-2} s^{-1} (Zhang *et al.* 2005). The photosynthetic N use efficiency (PNUE) was estimated as P_{max}/N content (Field and Mooney 1986).

The total superoxide dismutase (SOD, EC 1.15.1.1) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) (Beuchamp and Fridovich 1971). The ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured using a modification of the procedure of Nakano and Asada (1981). The catalase (CAT, EC 1.11.1.6) activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm, as described by Aebi (1984). The guaiacol peroxidase (GPX, EC 1.11.1.7) activity of leaves was measured as described by Chance and Maehly (1955). Free proline was measured as described by Bates *et al.* (1973).

Statistical analyses were performed with the statistical software package for social science (*SPSS version 11.0*) Among all treatments, the means were compared by Duncan's multiple range tests at $P < 0.05$.

For both populations, drought-stressed plants exhibited reduced leaf RWC, TLA, LS, SLA, P_{max}, R_d, PNUE, and increased SOD, CAT, APX and GPX activities and free proline content than well-watered plants (Table 1). The decrease in SLA of a plant under drought can reduce water loss and improve water use efficiency (Nobel 1991). Additionally, the higher activities of antioxidant enzymes could effectively scavenge the reactive oxygen species resulting from drought stress (Sivritepe *et al.* 2008). In this study, the decreases in the leaf RWC, P_{max}, LCP and N_{mass} were smaller, while the activities of GPX, CAT, APX and the content of free proline were higher in the dry climate population than in the wet climate population under drought (Table 1). This suggested that the dry climate population was more drought-tolerant. On the other hand, the negative effect of shade was more pronounced in the dry climate population as indicated by the smaller increases in SLA and PNUE as well as the higher decreases in TLA, N_{mass} and proline content in the dry climate population than in the wet climate population (Table 1).

When plants are exposed to environmental stress, there is a large set of parallel changes in morphological and physiological responses, which could enhance their capability to survive and grow (Li *et al.* 2003a, 2003b). These responses can be different in populations and

Table 1. The leaf relative water content (RWC), total leaf area (TLA), leaf size (LS), specific leaf area (SLA), leaf carbon content per mass (C_{mass}), leaf nitrogen content per mass (N_{mass}), maximal photosynthetic rate (P_{Nmax}), dark respiration rate (R_d), light compensation point (LCP), photosynthetic nitrogen-use efficiency (PNUE), superoxide dismutase activity (SOD), catalase activity (CAT), ascorbate peroxidase activity (APX), guaiacol peroxidase activity (GPX), and free proline content of two *P. cathayana* populations (WC - wet climate population, DC - dry climate population) when exposed to full sunlight, moderate shade and severe shade and were well-watered or drought-stressed. Means ± SE, *n* = 5.

Parameters	Popul.	Full sunlight well-watered	drought	Moderate shade well-watered	drought	Severe shade well-watered	drought
RWC	WC	91.27±1.24a	70.94±1.27d	93.79±1.55a	80.00±2.72c	92.40±1.55a	77.93±2.34c
[%]	DC	83.25±0.99b	70.27±0.81d	84.90±1.63b	78.34±2.90c	85.30±2.52b	73.98±1.81d
TLA	WC	20.55±5.41b	7.58±1.81de	24.99±3.01a	6.59±0.90de	10.19±1.16d	3.64±0.91ef
[dm^2]	DC	22.52±3.74ab	9.59±1.33d	15.02±1.87c	5.24±0.53ef	6.10±1.13de	1.87±0.41f
LS	WC	63.73±12.82b	48.07±7.63c	90.12±6.02a	48.17±5.16c	65.15±2.66b	32.79±5.29d
[cm^2]	DC	59.02±6.77b	28.15±3.08de	48.89±1.41c	33.40±2.60d	21.71±3.34ef	17.37±2.10f
SLA	WC	180.01±16.56cde	166.14±5.75de	242.48±18.64b	196.14±4.98c	265.24±12.29a	187.15±25.56cd
[cm^2 g^{-1}(d.m.)]	DC	158.96±7.59ef	118.56±6.57g	162.58±4.68ef	141.85±6.41f	160.94±6.85ef	101.76±14.55g
C_{mass}	WC	418.93±9.67a	395.78±8.71cd	403.28±7.06bc	413.89±5.91ab	396.04±3.36cd	383.21±4.80e
[mg g^{-1}(d.m.)]	DC	414.11±7.21ab	398.20±4.32cd	405.37±11.09bc	396.00±16.45cd	389.51±4.46de	378.40±2.20e
N_{mass}	WC	35.65±2.72a	32.42±0.91b	33.58±2.80ab	31.17±1.08b	30.86±0.72b	27.84±1.37c
[mg g^{-1}(d.m.)]	DC	32.13±3.46b	31.35±1.56b	28.28±1.20c	27.91±1.74c	26.07±0.94cd	24.53±0.79d
P_{Nmax}	WC	19.54±0.39a	12.91±0.30e	17.06±1.14bc	14.90±0.69cde	13.94±0.61de	13.04±0.71e
[μmol(CO_2) m^{-2} s^{-1}]	DC	17.53±1.03b	15.65±1.97bcd	16.02±0.41bcd	15.34±1.55cd	15.03±1.00cde	14.48±1.14de
R_d	WC	1.28±0.10a	1.07±0.13cd	1.09±0.26cd	0.93±0.03ef	1.05±0.10cde	0.97±0.11de
[μmol(CO_2) m^{-2} s^{-1}]	DC	1.32±0.10a	1.06±0.02cd	1.23±0.05ab	1.14±0.02bc	0.82±0.09f	0.57±0.01g
LCP	WC	21.90±0.29bc	22.96±1.61ab	18.81±0.47e	15.91±0.47f	16.22±0.66ef	15.98±0.27f
[μmol(phot.) m^{-2} s^{-1}]	DC	23.63±1.32a	20.52±0.46cd	22.46±1.75ab	19.95±0.63de	16.52±0.88f	15.30±0.47f
PNUE	WC	9.92±0.82b	6.61±0.90e	12.42±1.63a	9.38±0.40bc	11.98±0.42a	8.77±0.25cd
[μmol(CO_2) g^{-1}(N) s^{-1}]	DC	8.72±0.67cd	5.94±0.84e	9.22±0.40bc	7.83±0.97d	9.29±0.77bc	6.00±0.25e
SOD	WC	10.00±1.02bc	13.12±1.15a	9.65±0.70bcd	10.88±0.87b	8.75±1.03cd	7.73±1.15de
[U mg^{-1}(prot.)]	DC	7.94±0.82de	12.92±1.57a	7.75±1.25de	10.20±1.10bc	6.76±0.82ef	5.67±0.73f
CAT [μmol(H_2O_2)	WC	14.41±0.23bc	15.85±0.72ab	14.45±1.54bc	14.54±0.86bc	11.70±1.17de	8.97±1.28f
mg^{-1}(prot.) min^{-1}]	DC	10.65±0.92ef	16.88±1.88a	10.48±1.08ef	12.69±1.12cd	9.44±0.65f	8.75±0.74f
APX [μmol(H_2O_2)	WC	54.03±5.69bc	69.21±3.85a	53.01±4.42bc	59.43±7.14b	47.39±7.40c	47.32±7.89c
mg^{-1}(prot.) min^{-1}]	DC	51.56±2.86bc	74.28±3.39a	53.63±7.61bc	58.05±3.87b	51.19±3.66bc	44.94±5.00c
GPX [μmol(gua.)	WC	22.45±5.00def	34.00±4.38b	23.13±2.94def	27.43±3.48cd	19.83±2.82ef	17.30±4.26f
mg^{-1}(prot.) min^{-1}]	DC	25.93±1.38cde	41.67±3.33a	24.86±3.98cde	30.79±1.94bc	23.71±3.73def	21.03±2.41def
Proline	WC	16.42±1.56de	25.06±1.34b	15.34±2.08def	22.25±1.98bc	16.20±2.34de	18.36±1.63cd
[μg g^{-1}(f.m.)]	DC	13.83±1.63efg	29.17±3.39a	11.67±2.97fg	22.04±4.54bc	7.35±0.38h	9.72±1.12gh

genotypes originating from different climate regions (Li *et al.* 2003c, Lu *et al.* 2007, Toumi *et al.* 2008). In this study, the dry climate population was more drought-tolerant and shade-sensitive than the wet climate population, and such different responsive patterns in the two contrasting populations are strongly related to their habitats.

In addition, our results showed that SLA decreased and RWC increased in the droughted plants, but the P_{Nmax} and C_{mass} did not decrease under moderate shade compared with full sunlight conditions (Table 1), which suggested moderate shade did not significantly limit the C acquisition and assimilation of drought-treated cuttings. Such effects may enable plants growing in shade to continue gaining C longer during the drought period (Prider and Facelli 2004). Furthermore, the PNUE value of drought-treated cuttings was higher under moderate shade than under full sunlight. According to the facilitation mechanism, shade can reduce leaf and air temperatures, vapour pressure deficit and oxidative stress that would alleviate the drought impact on seedlings (Holmgren 2000). The lower activities of antioxidant enzymes and free proline content in shaded plants compared with sun plants during drought, which may be related with the less need for reactive oxygen species removal and osmotic adjustment, also suggested that moderate shade mitigates the potential deleterious effects of drought (Duan *et al.* 2005, Kloeppel *et al.* 1993). The trade-off hypothesis suggests that deep shade will aggravate the impact imposed by drought and that both drought and severe shade negatively impact the carbon assimilation of plants. This was also observed in our study. The results obtained from *P. cathayana* for the TLA, LS, and C and N contents showed that the decreases induced by drought were more pronounced under severe shade than under full sun or moderate shade (Table 1). The photosynthetic parameters, including P_{Nmax}, R_d and LCP, were lower under drought plus severe shade than in any other treatment (Table 1). Thus, extreme limitation in radiation could severely lower C assimilation. Besides these effects, *e.g.*, ability for osmotic adjustment may be decreased in shade environments (Mendes *et al.* 2001). The proline content and activities of SOD, GPX, APX and CAT under severe shade plus drought stress, were even lower than in the well-watered plants grown under full sun (Table 1). Therefore, the drought effects on cuttings were exacerbated under severe shade, as was also documented in previous studies (Valladares and Pearcy 2002, Climent *et al.* 2006).

Therefore, the irradiance modified the leaf responses of *P. cathayana* to drought in different ways. There was an apparent alleviation of drought effects under moderate shade but an aggravation under severe shade. Thus, the trade-off and facilitation effects of drought and shade are complementary rather than exclusive. More research in field will be needed to verify the present results and to estimate their implications to ecosystems and practical forestry under current climate change.

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