Effects of water stress on photosynthetic activity, dry mass partitioning and some associated metabolic changes in four provenances of neem (*Azadirachta indica* A. Juss)

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Abstract

Chlorophyll (Chl) content, dry mass, relative water content (RWC), leaf mass per area (LMA), proline (Pro) content, malondialdehyde (MDA) content, superoxide dismutase (SOD) and peroxidase (POD) activity, P_N-PAR response curves and gas exchange were studied to determine the effects of water stress on photosynthetic activity, dry mass partitioning and metabolic changes in four provenances of neem (*Azadirachta indica* A. Juss). The results indicated that provenance differences existed in the adaptation response to water stress that included changes to growth strategies coupled with ecophysiological and metabolic adjustments. As water stress increased, stomatal conductance (g_s), net photosynthetic rate (P_N), transpiration rate (E), and leaf RWC decreased while LMA increased in all provenances. Dry mass was reduced in droughted plants and the percentage increased in dry mass allocated to roots, and enzyme activities of SOD and POD were highest in neem originating from Kalyani (KA) provenance and lowest in neem originating from New Dehli (ND) provenance. In contrast, water stress increased MDA content least in KA and most in ND. Furthermore, neem originating from ND also had the greatest decrease in Chl *a/b* ratio while the ratio was least affected in neem originating from KA. These findings suggest neem originating from KA may have more drought resistance than neem originating from ND. The data from P_N-PAR response curves are less clear. While these curves showed that drought stress increased compensation irradiance (I_c) and dark respiration (R_D) and decreased saturation irradiance (I_s) and maximum net photosynthetic rate (P_{max}), the extent of decline in P_{max} was provenance dependent. P_{max} under non-water-limiting conditions was higher in neem originating from Jodhpur (MA) (about 14 μmol m^{-2} s^{-1}) than in the other three provenances (all about 10 μmol m^{-2} s^{-1}), but mild water stress had minimal effect on P_{max} of these three provenances whereas P_{max} of MA provenance declined to 10 μmol m^{-2} s^{-1}, *i.e.* a similar value. However, under severe water stress P_{max} of MA and KA provenances had declined to 40% of non-stressed values (about 6 and 4 μmol m^{-2} s^{-1}, respectively) whereas the decline in P_{max} of neem originating from Kulapachta (KU) and ND provenances was about 50% of nonstressed values (about 5 μmol m^{-2} s^{-1}). These data suggest the P_N responses of KU and ND provenances are most tolerant, and KA and MA least tolerant to increasing water stress, but also suggest MA provenance could be the most desired under both non-water-limiting and water-limiting conditions due to highest P_{max} in all conditions.

Additional key words: chlorophyll contents; dry mass partitioning; maximum net photosynthetic rate; P_N-PAR response curves; provenance differences.

Introduction

Water deficit is probably the most important stress factor affecting plant growth and productivity worldwide (Boyer 1982). Plant response to water stress involves changes in carbon assimilation and metabolism

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Abbreviations: Chl – chlorophyll; F_P – ANOVA of provenance; F_W – ANOVA of water treatment; $F_{W\times P}$ – ANOVA together with interactions between water and provenance treatments; FC – field water capacity; g_s – stomatal conductance; I_c – compensation irradiance; I_s – saturation irradiance; KA – Kalyani; KU – Kulapachta; LMA – leaf mass per area; MA – Jodhpur; MDA – malondialdehyde; ND – New Dehli; PAR – photosynthetically active radiation; P_{max} – maximum net photosynthetic rate; P_N – net photosynthetic rate; POD – peroxidase; Pro – proline; R_D – dark respiration rate; RWC – relative water content; SOD – superoxide dismutase; E – transpiration rate; WUE – water-use efficiency; Φ – apparent quantum yield.
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(Miyashita *et al*. 2005). There is a substantial consensus now 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). On the other hand, one of the major mechanisms causing plant damage during arid environmental conditions is the excess production of active oxygen species, such as the superoxide radical, hydrogen peroxide, and the hydroxyl radical, a general phenomenon that creates oxidative stress (Bowler and Van 1992). To defend against oxidants, plants increase contents of their antioxidant defensive enzymes such as superoxide dismutase (SOD), peroxidase (POD), and other compounds such as malondialdehyde (MDA). Generally, plants in arid areas have developed physiological responses, in addition to ecological strategies, to cope with water shortages by either stress avoidance or stress tolerance. These responses allow plants to survive and even to continue growth under adverse conditions.

Neem (*Azadirachta indica* A. Juss) (Meliaceae), an important species of the subcontinent of southern Asia, is the source of unique natural products including those for integrated pest management, medicine, and industrial purposes. It is wellknown not only for its medical and bioactive properties, but also as a versatile agroforestry species of the semiarid and arid tropics (Koul *et al*. 1990, Ketkar and Ketkar 1995). The report by the US National Academy of Sciences (1992) stated neem trees show desirable properties for assisting with global environmental concerns. Neem was introduced in the dry-hot valley areas of southwest China in 1995 and large-scale plantations were established there for its multipurpose utilization (Zhang 2008). These dry-hot valleys characterized by drought, high temperature, high irradiance, and dry air are among the most serious desertification areas of China. A better understanding of both the ecological strategies and the mechanisms of physiological and biochemical responses to water stress which enable plants to adapt and maintain growth, development, and productivity during stress periods, would help in breeding and selecting for drought resistance in the dry-hot valley areas.

There is only limited understanding of the effect of provenance on growth, morphological characteristics, or azadirachtin content of neem trees growth in southern China (Zhang 2008). Additionally, the impact of water stress on growth strategies, physiological and biochemical properties is scarce. The specific objective of the present study was to investigate the ecophysiological strategies and biochemical adaptations of neem provenances during water stress to attempt to find the possible differences in adaptation to varying water stress in order to establish a drought tolerance index system of neem provenances.

Materials and methods

Plants and experimental design: Our study was conducted in the dry-hot valley of Yuanjiang River, Yunnan province, China. The site was located at Yuanjiang experimental station of Research Institute of Resource Insects (101°59´E, 23°36´N, 490 m a.s.l.), Chinese Academy of Forestry. The climate is characterized by a mean annual air temperature of 23.9°C and mean annual rainfall of 764.6 mm, which occurs from May to October, with the dry season occurring from November to April.

Four neem provenances introduced from India [Kalyani (KA), Kulapachta (KU), Jodhpur (MA), and New Dehli (ND)] in 1995 were studied. One-year-old half-lignified cuttings which were individually collected from four provenances at Yuanjiang experimental station were pricked in July 2006. After sprouting and growing for about seven months, forty-eight healthy cuttings (12 cuttings per provenance) of uniform height (about 50 cm) were chosen and transferred to plastic pots of 50-cm diameter and 80-cm depth filled with homogenized soil [a total of 1,000 g of a cattle manure and 50 g of a slow-release fertilizer (10% N, 10% P, and 10% K) was added to the soil in each pot and fully mixed] (1 cutting per pot), and grown in a naturally lit greenhouse under the semicontrolled environment (only shelter from rainfall). The bottoms of the plastic pots were mulched with plastic to avoid the roots spreading into ground and thereby absorb moisture from it. The treatments were started on March 12 and plants were harvested on June 4, 2007.

The experiment was arranged in a Randomized Complete Block Design with three replicates for four water-supply regimes [95–100, 80–85, 50–55, and 35–40% field water capacity (FC)], designated as L1, L2, L3, and L4 respectively. During the experiment, the average soil volumetric water contents were 28.9 ± 0.8, 24.5 ± 0.7, 15.5 ± 0.7, and 11.7 ± 0.7% under L1, L2, L3, and L4, respectively. The pots were weighed once every second day and rewatered after 17:30 by replacing the amount of water transpired.

P_N-PAR response curves and gas-exchange measurements: The fully expanded leaves from the middle canopy of the cuttings were sampled, using a portable photosynthesis system (*LI-6400p, LiCor*, Lincoln, NE, USA). P_N-PAR response curves were measured at 1,600; 1,400; 1,300; 1,200; 1,000; 900, 800, 600, 400, 200, 100, 50, and 0 μmol(photon) m^{-2} s^{-1} of PAR under uniform conditions [ambient CO_2 concentration of 330–360 μmol(CO_2) mol^{-1}, leaf temperature of 30°C, and 50–55% relative humidity inside the leaf chamber)] at 09:30–11:30 (local time) on two sunny days (May 30 and 31,

2007). Both leaf g_s measurements and photosynthetic gas exchange were measured at 1,000 μmol(photon) m^{-2} s^{-1} of PAR under uniform conditions as P_N-PAR response curves measurements. 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 R_D, I_c, and apparent quantum yield (Φ) (Yin *et al.* 2006). P_{max} and I_s were estimated according to Walker (1989). Water use efficiency (WUE) was calculated as P_N/E. The measurements were made of 2 cuttings per water treatment and 5 replicate leaves per cutting and 5 replicate readings per leaf at each PAR value.

Leaf samples and plant dry mass: After leaf samples for chlorophylls content, leaf relative water content and biochemical analysis were collected, then plant dry mass was assessed during which all remaining leaves were collected and measured for leaf area. The leaves similar to those used for photosynthetic measurements from the middle portion of the plant with similar age between treatments were sampled for leaf relative water content and biochemical analysis.

Leaf relative water content was determined in leaf discs taken at 08:00 [(June 4, 2007) as RWC = (FM − DM)/(TM − DM)], where FM is the leaf fresh mass, TM is the turgid mass after 6 h of rehydration in distilled water, and DM is the dry mass of the leaf segment after being oven-dried at 70ºC for 48 h (Li *et al.*2000).

Biochemical analysis: POD activity was measured by following the change in absorption at 470 nm due to guaiacol oxidation (Li *et al.* 2000). The reaction solution (3 cm^3) was composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 20 mm^3 H_2O_2, and 100 mm^3 of enzyme extract. SOD activity was based on the method described by Li *et al*. (2000). One unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction of nitroblue tetrazolium (NBT) by 50%. The reaction mixture (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.8) with 0.1 mM ethylene diaminotetraacetic acid (EDTA), 2.25 μM NBT, 39 mM methionine, 2 μM riboflavin, and 25 mm^3 of enzyme extract. MDA content was measured according to Li *et al*. (2000). Samples (0.1 g) were ground in liquid nitrogen and dissolved in 2 cm^3 of 20% trichloroacetic acid (TCA) and 0.5% thiobarbituric acid. The solution was heated for 30 min at 90°C and then cooled on ice. The homogenate was centrifuged at 10,000 × *g* for 10 min at 4°C and the supernatant was measured at 532 and 600 nm using MDA as a standard. Pro was extracted from leaves in 3% aqueous sulphosalicylic acid and its content estimated using ninhydrin reagent (Li *et al.*2000).

Plant DM: Plants were harvested at the end of the water-deficit treatment (June 4, 2007). The above-ground parts were separated into leaves and shoots. Images of remaining leaves were recorded with a scanner (model *Li3000*, *LiCor*, Lincoln, NE, USA) and then digitized by the *Arcview 3.2a* software (*Environmental Systems Research Institute*, New York, USA) in order to determine leaf area. LMA was calculated as leaf DM/leaf area. Roots were excavated from pool and subsequently washed by water. Then DM of all the parts was determined after 48 h in an oven at 85°C.

Chlorophylls content: After determining photosynthetic activity, the measurement leaves were harvested and 0.1 g of the fresh ones sampled for determining chlorophyll (Chl) composition and content according to Li *et al*. (2000). The cleaned leaf disks (6 mm in diameter) were extracted with 80% acetone. Absorption was measured at 663 and 645 nm using an ultraviolet (UV)-visible spectrophotometer (*Unico*, *UV-3802*, China).

Statistical analyses were performed using the *SPSS* statistical package (version *13*, *SPSS*, Chicago, IL, USA). Differences between means of treatments were performed by the *Duncan*'s test with means considered significantly different at $p<0.05$.

Results

***P_N*-PAR response curves and gas exchange**: Water stress affected the P_N-PAR response curve with P_{max} and P_N above 500 μmol(photon) m^{-2} s^{-1} of PAR decreasing as water stress increased (Fig. 1). Although both level of water stress and provenance affected P_{max}, their interaction was not statistically significant ($p<0.05$) (Table 1). P_{max} under no water stress (L1) was highest for MA and similar for the remaining provenances, but a mild water stress (from L1 to L2) had no effect on KA, KU, and to a lesser extent on ND provenances, while this mild water stress decreased P_{max} in MA. At the highest water stress, (L4), P_{max} was highest in KU and least in ND. Differences between provenances can be seen as (be explained) the reason for the percentage decline in P_{max}. P_{max} of MA and KA at L4 was about 40% of P_{max} at L1, whereas P_{max} of KU and ND was about 50% of P_{max} value at L1 (Table 1). This suggests that KU and ND are most tolerant, and KA and MA least tolerant to increasing water stress. However it is noted that P_{max} was highest in MA in all conditions. Additionally, water stress increased I_c and R_D but decreased I_s in all provenances (Table 1). The interaction between water stress and provenance was significant for these traits. Water deficit also reduced Φ in all provenances, but MA was most affected by water stress. Further more, Water deficit significantly ($p<0.01$) influenced g_s, P_N, E, and WUE (Table 2). With the increase of water stress from L1 to L4 treatment, P_N, g_s and E decreased dramatically while WUE showed

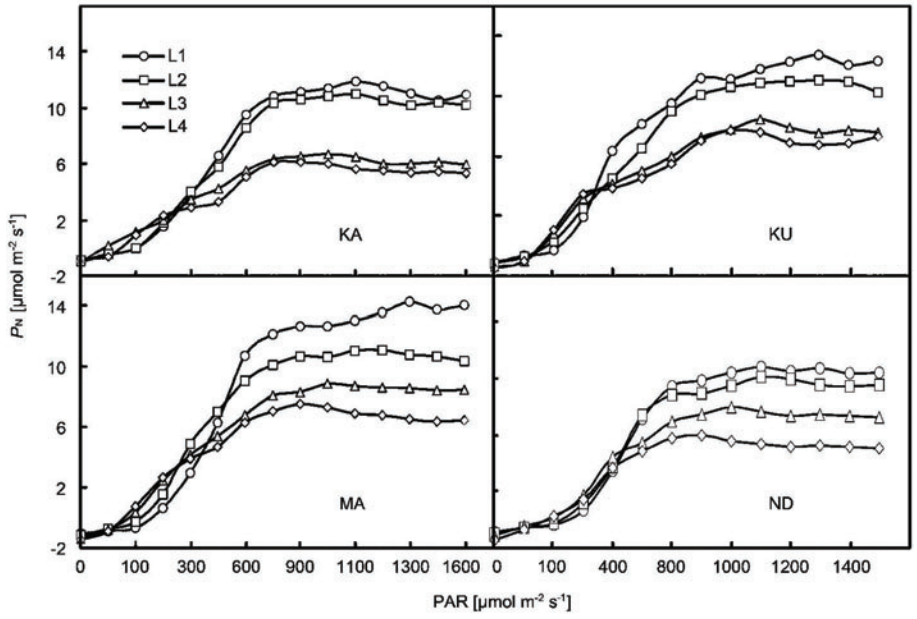

Fig. 1. Photosyntheis-PAR response curves for four *Azadirachta indica* provenances (KA, KU, MA, and ND) at different water supply regimes (L1–L4). All the values are means of ten replications.

Table 1. Saturation irradiance (I_s), compensation irradiance (I_c), the maximum net photosynthetic rate (P_{max}), apparent quantum yield (Φ), and dark respiration (R_D) of four neem provenances (KA, KU, MA, and ND) at different water supply regimes (L1–L4). Water treatment (W), provenance (P). Means ± SE, n = 5, $^{*}p<0.05$, $^{**}p<0.01$. F_W – ANOVA of water treatment; F_P – ANOVA of provenance treatment; $F_{W\times P}$ – ANOVA together with interactions between water and provenance treatments.

Treatment		I_s [μmol m^{-2} s^{-1}]	I_c [μmol m^{-2} s^{-1}]	P_{max} [μmol m^{-2} s^{-1}]	Φ [μmol μmol^{-1}]	R_D [μmol m^{-2} s^{-1}]
L1	KA	1076.44 ± 23.43	42.53 ± 2.93	11.39 ± 0.94	0.0746 ± 0.0061	–1.01 ± 0.13
	KU	1162.12 ± 22.93	41.81 ± 1.67	13.45 ± 0.89	0.0708 ± 0.0087	–1.19 ± 0.07
	MA	1239.51 ± 27.75	38.80 ± 1.49	14.15 ± 1.03	0.0735 ± 0.0072	–1.17 ± 0.03
	ND	1085.75 ± 22.50	37.83 ± 1.39	11.04 ± 0.72	0.0732 ± 0.0044	–0.98 ± 0.04
L2	KA	1074.27 ± 26.48	48.14 ± 1.87	10.89 ± 0.87	0.0693 ± 0.0029	–1.34 ± 0.09
	KU	1149.88 ± 21.39	46.77 ± 2.23	11.06 ± 0.71	0.0744 ± 0.0065	–1.24 ± 0.05
	MA	1205.47 ± 24.87	41.16 ± 2.17	11.49 ± 0.81	0.0729 ± 0.0046	–1.35 ± 0.14
	ND	1021.82 ± 14.46	37.95 ± 2.24	9.57 ± 0.49	0.0753 ± 0.0018	–1.07 ± 0.13
L3	KA	904.17 ± 19.62	61.18 ± 2.45	7.31 ± 0.55	0.0595 ± 0.0017	–1.49 ± 0.21
	KU	941.85 ± 17.42	57.67 ± 1.74	8.57 ± 0.26	0.0563 ± 0.0051	–1.56 ± 0.15
	MA	981.80 ± 17.79	53.65 ± 2.01	8.85 ± 0.53	0.0647 ± 0.0008	–1.57 ± 0.16
	ND	915.62 ± 9.54	48.55 ± 1.23	7.24 ± 0.34	0.0598 ± 0.0021	–1.25 ± 0.09
L4	KA	846.82 ± 18.76	69.32 ± 2.05	6.42 ± 0.61	0.0573 ± 0.0023	–1.67 ± 0.15
	KU	872.97 ± 18.61	58.42 ± 1.93	7.86 ± 0.44	0.0529 ± 0.0014	–1.72 ± 0.08
	MA	900.69 ± 11.27	59.90 ± 1.60	7.50 ± 0.66	0.0643 ± 0.0008	–1.66 ± 0.19
	ND	823.33 ± 17.17	51.74 ± 1.85	5.59 ± 0.48	0.0531 ± 0.0009	–1.41 ± 0.05
F_W		1130.88**	289.95**	22.10**	32.69*	217.33**
F_P		186.59**	74.76**	3.41*	4.10*	58.83**
$F_{W\times P}$		12.62**	5.56**	0.19	1.90	3.80**

a similar trend of initially decreasing, then increasing, and decreasing again. From L1 to L4 treatment, P_N and g_s decreased by 35.8 and 62.1%, respectively, in KU, 43.3 and 61.7% in ND, while they decreased by 47.0 and 69.1%, respectively, in KA, 44.8 and 59.9% in MA.

Leaf RWC: Soil water availability greatly affected leaf RWC. Both water and provenance treatments, and their interaction significantly ($p<0.01$) affected RWC (Table 3). RWC dramatically decreased as water stress increased with the highest RWC (73.7–77.9%) in L1 and the lowest RWC (56.2–61.9%) in L4.

Metabolic changes: Reduced irrigation and the interaction of water deficit and provenance significantly ($p<0.05$) affected Pro and MDA content, and SOD and POD activity (Table 3). However, no significant ($p>0.05$) differences of POD activity were observed between

Table 2. Stomatal conductance (g_s), net photosynthetic rate (P_N), transpiration rate (E), and water use efficiency (WUE) of four neem provenances (KA, KU, MA, and ND) at different water supply regimes (L1–L4). Water treatment (W), provenance treatment (P). Means ± SE, $n = 5$, $^{*}p<0.05$, $^{**}p<0.01$. F_W – ANOVA of water treatment; F_P – ANOVA of provenance; $F_{W\times P}$ – ANOVA together with interactions between water and provenance treatments.

Treatment		g_s [mol m^{-2} s^{-1}]	P_N [μmol m^{-2} s^{-1}]	E [μmol m^{-2} s^{-1}]	WUE [μmol μmol^{-1}]
L1	KA	0.615 ± 0.045	11.154 ± 1.026	4.517 ± 0.368	2.469 ± 0.246
	KU	0.508 ± 0.050	11.713 ± 1.178	4.104 ± 0.497	2.854 ± 0.196
	MA	0.518 ± 0.039	12.497 ± 1.110	4.459 ± 0.351	2.803 ± 0.246
	ND	0.576 ± 0.027	10.402 ± 1.211	4.962 ± 0.293	2.096 ± 0.183
L2	KA	0.537 ± 0.042	10.780 ± 0.996	4.420 ± 0.501	2.439 ± 0.296
	KU	0.432 ± 0.019	10.617 ± 1.114	3.876 ± 0.305	2.739 ± 0.302
	MA	0.452 ± 0.026	10.504 ± 0.998	4.027 ± 0.410	2.609 ± 0.314
	ND	0.506 ± 0.038	9.463 ± 0.968	4.533 ± 0.229	2.087 ± 0.211
L3	KA	0.355 ± 0.031	6.656 ± 1.014	2.655 ± 0.331	2.507 ± 0.187
	KU	0.272 ± 0.034	8.072 ± 0.965	2.358 ± 0.410	3.423 ± 0.367
	MA	0.329 ± 0.022	8.143 ± 0.896	2.258 ± 0.291	3.606 ± 0.412
	ND	0.406 ± 0.016	7.014 ± 0.913	2.601 ± 0.197	2.697 ± 0.264
L4	KA	0.190 ± 0.015	6.015 ± 0.889	2.347 ± 0.202	2.563 ± 0.315
	KU	0.193 ± 0.011	7.517 ± 0.953	2.931 ± 0.109	2.565 ± 0.267
	MA	0.207 ± 0.019	6.901 ± 0.836	2.534 ± 0.112	2.723 ± 0.202
	ND	0.221 ± 0.020	5.897 ± 0.872	2.292 ± 0.147	2.573 ± 0.183
F_W		189.975**	34.273**	498.034**	55.861**
F_P		10.690**	4.692*	61.734**	95.258**
$F_{W\times P}$		1.763	0.264	1.593	2.879*

Table 3. Changes of leaf relative water content (RWC), free proline (Pro) content, superoxide dismutase (SOD) activity, peroxidase (POD) activity, and malondialdehyde (MDA) content of four *Azadirachta indica* provenances (KA, KU, MA, and ND) at different water-supply regimes (L1–L4). Fresh mass (FM), active unit (U), optical density (OD), water treatment (W), provenance treatment (P). Means ± SE, $n = 5$, $^{*}p<0.05$, $^{**}p<0.01$. F_W – ANOVA of water treatment; F_P – ANOVA of provenance treatment; $F_{W\times P}$ – ANOVA together with interactions between water and provenance treatments.

Treatment		RWC [%]	Pro [mg kg^{-1} (FM)]	SOD [(U g^{-1}(FM)]	POD [OD g^{-1}(FM) min^{-1}]	MDA [nmol g^{-1}(FM)]
L1	KA	77.1 ± 2.3	193.38 ± 9.88	30.39 ± 1.08	218.62 ± 1.89	17.50 ± 1.19
	KU	73.7 ± 3.2	197.57 ± 7.67	28.72 ± 3.12	216.43 ± 4.32	19.87 ± 0.86
	MA	77.9 ± 4.1	209.26 ± 12.01	29.67 ± 1.94	223.61 ± 2.96	18.25 ± 0.99
	ND	76.2 ± 2.9	189.24 ± 7.68	27.33 ± 1.99	209.34 ± 7.14	19.63 ± 0.89
L2	KA	76.5 ± 3.1	225.52 ± 10.22	31.29 ± 1.65	254.38 ± 2.03	17.41 ± 1.02
	KU	72.8 ± 2.7	209.73 ± 8.21	27.35 ± 1.26	231.91 ± 1.87	18.54 ± 1.02
	MA	74.9 ± 3.4	213.73 ± 8.96	28.48 ± 0.98	229.42 ± 3.22	17.61 ± 1.16
	ND	71.4 ± 3.6	200.95 ± 10.04	27.85 ± 1.62	214.54 ± 9.26	19.54 ± 1.16
L3	KA	63.8 ± 5.4	347.41 ± 14.21	55.31 ± 4.07	345.06 ± 3.87	31.28 ± 1.92
	KU	66.5 ± 3.3	362.96 ± 13.27	56.52 ± 3.94	369.59 ± 3.57	32.09 ± 0.98
	MA	67.3 ± 2.8	350.70 ± 15.21	57.63 ± 3.16	373.22 ± 4.65	31.24 ± 1.08
	ND	63.6 ± 4.2	334.14 ± 16.37	52.63 ± 5.23	316.20 ± 11.03	35.26 ± 2.01
L4	KA	58.3 ± 4.4	311.69 ± 9.92	40.52 ± 3.46	293.17 ± 2.41	34.86 ± 2.91
	KU	61.9 ± 3.7	257.32 ± 11.16	34.50 ± 2.15	287.30 ± 2.95	36.41 ± 2.17
	MA	57.8 ± 2.1	322.58 ± 13.34	39.17 ± 2.05	279.83 ± 2.04	35.44 ± 1.64
	ND	56.2 ± 3.9	247.19 ± 14.21	36.40 ± 2.68	277.38 ± 6.76	39.43 ± 2.26
F_W		766.67**	305.37**	959.97**	277.29**	392.09**
F_P		16.67**	10.86**	17.56**	0.88	66.07**
$F_{W\times P}$		15.56**	5.09**	3.47**	2.57*	10.88**

provenance treatments. With the increase of water stress from L1 to L4 treatment, Pro content, and SOD and POD activity showed a similar trend of initially increasing, then decreasing, while MDA content displayed a gradually increasing content. The relative rankings of provenances differed with the measured trait as water

Table 4. Variance analysis of dry mass partitioning of shoot, root, and leaf under different provenances (KA, KU, MA, and ND) and water supply regimes (L1–L4). W – water treatment, P – provenance treatment, P×W – interactions between water and provenance treatments. $^{*}p<0.05$, $^{**}p<0.01$.

Source	Shoot		Root		Leaf	
	Mean square	F	Mean square	F	Mean square	F
P	25.62	160.10**	19.24	120.24**	123.72	63.12**
W	371.73	2323.31**	188.22	1176.40**	978.57	499.27**
W×P	28.79	179.94**	35.32	220.73**	65.55	33.44**

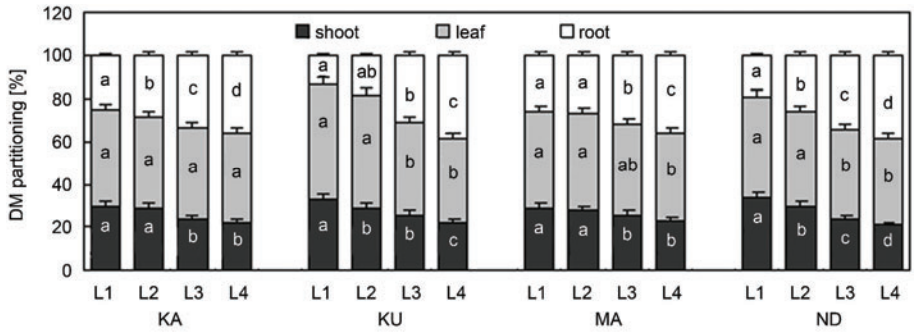

Fig. 2. Dry mass (DM) partitioning for four *Azadirachta indica* provenances (KA, KU, MA, and ND) at different water supply regimes (L1–L4). *Different letters* indicate significant differences between water treatments within a provenance ($p<0.05$). *Error bars* are ± SE, $n = 5$.

Table 5. Changes of leaf mass per area (LMA), chlorophyll (Chl) *a, b* and Chl (*a*+*b*) contents and Chl *a/b* ratio of four *Azadirachta indica* provenances (KA, KU, MA, and ND) at different water supply regimes (L1–L4). Dry mass (DM), fresh mass (FM), water treatment (W), provenance treatment (P). Means ± SE, $n = 5$, $^{*}p<0.05$, $^{**}p<0.01$. F_W – ANOVA of water treatment; F_P – ANOVA of provenance treatment; $F_{W\times P}$ – ANOVA together with interactions between water and provenance treatments.

Treatment		LMA [mg cm^{-2}(DM)]	Chl *a* [g kg^{-1}(FM)]	Chl *b* [g kg^{-1}(FM)]	Chl (*a*+*b*) [g kg^{-1}(FM)]	Chl *a/b*
L1	KA	0.69 ± 0.12	1.43 ± 0.12	0.94 ± 0.01	2.37 ± 0.11	1.61 ± 0.16
	KU	0.84 ± 0.14	1.39 ± 0.17	0.91 ± 0.04	2.30 ± 0.14	1.51 ± 0.12
	MA	0.69 ± 0.16	1.64 ± 0.14	1.04 ± 0.05	2.68 ± 0.21	1.63 ± 0.07
	ND	0.82 ± 0.13	1.49 ± 0.16	1.03 ± 0.11	2.52 ± 0.18	1.51 ± 0.14
L2	KA	0.93 ± 0.17	1.44 ± 0.10	0.86 ± 0.03	2.30 ± 0.09	1.68 ± 0.09
	KU	0.97 ± 0.19	1.32 ± 0.09	0.90 ± 0.02	2.22 ± 0.08	1.48 ± 0.03
	MA	0.84 ± 0.09	1.41 ± 0.11	0.96 ± 0.07	2.37 ± 0.14	1.52 ± 0.13
	ND	0.98 ± 0.08	1.44 ± 0.12	1.01 ± 0.08	2.45 ± 0.13	1.46 ± 0.09
L3	KA	1.10 ± 0.13	1.19 ± 0.06	0.76 ± 0.02	1.95 ± 0.07	1.57 ± 0.03
	KU	1.43 ± 0.21	1.09 ± 0.18	0.81 ± 0.06	1.90 ± 0.07	1.39 ± 0.08
	MA	1.20 ± 0.12	1.21 ± 0.09	0.84 ± 0.03	2.05 ± 0.15	1.47 ± 0.04
	ND	1.22 ± 0.11	1.15 ± 0.08	0.88 ± 0.04	2.03 ± 0.06	1.33 ± 0.03
L4	KA	1.58 ± 0.09	1.07 ± 0.14	0.69 ± 0.07	1.76 ± 0.16	1.52 ± 0.10
	KU	1.76 ± 0.15	0.94 ± 0.07	0.74 ± 0.04	1.68 ± 0.12	1.29 ± 0.02
	MA	1.39 ± 0.16	1.01 ± 0.05	0.72 ± 0.03	1.73 ± 0.06	1.40 ± 0.02
	ND	1.49 ± 0.16	0.97 ± 0.10	0.80 ± 0.01	1.77 ± 0.09	1.22 ± 0.05
F_W		77.87**	443.07**	89.32**	2005.00**	2.22*
F_P		23.56**	27.14**	20.38**	132.00**	9.25**
$F_{W\times P}$		0.91	11.39**	1.48	25.00**	14.96**

stress increased (from L2 to L4). Rankings of SOD and POD were similar, that is KA>MA>KU>ND for SOD and KA>KU>MA>ND for POD, while the rankings of MDA were the reverse of the ranking for SOD, that is ND>KU>MA>KA.

DM partitioning: Both water and provenance treatments, and their interaction significantly ($p<0.01$) affected DM partitioning of shoot, root, and leaf of neem (Table 4). Total plant DM declined significantly as water supply decreased. Additionally, irrigation affected percentage DM allocation in all provenances (Fig. 2). DM allocation to leaf and shoots decreased, whereas the allocation in roots increased along with increasing of water stress. As such, the ratio of root/aboveground part dry matter partitioning increased from 15 to 61% for KA, from 33 to

56% for KU, from 23 to 61% for MA, and from 35 to 56% for ND from L1 to L4 treatment. Thus, with increasing of the drought stress, the increase of the ratio of root/aboveground part DM is the highest in KA and the lowest in ND.

LMA and Chl content exhibited significant ($p<0.05$) responses to different water- and provenance treatments, although the interaction of water and provenance only statistically ($p<0.01$) affected Chl *a*, Chl (*a*+*b*) and the ratio of Chl *a/b* (Table 5). Water stress deceased contents of Chl *a*, *b*, Chl (*a*+*b*), and the ratios of Chl *a/b*, but increased LMA for all provenances although the provenance rankings differed depending on the measured trait. For example as water stress increased, that is, L1 to L4, provenance rankings for the increase in LMA were KU, KA, and MA being higher than ND while the provenance rankings for the decrease in Chl (*a*+*b*) and Chl *a/b* were MA>ND>KU>KA and ND>MA>KU>KA, respectively.

Discussion

Water stress is one of the most important environmental factors that regulate plant growth and development, and limit plant production (Mokhtar *et al*. 2009). Water deficit greatly affected P_N and g_s in neem plants. Our results showed that both P_N and g_s decreased dramatically with the increase of water stress. The close association between them revealed that the decline in net photosynthesis was largely a consequence of stomatal limitation. Flexas *et al.* (1999) and Cornic (2000) showed that stomatal closure was one of the earliest responses of plants to water stress, and it was the main cause for drought-induced suppression in photosynthesis, because stomatal closure in plants decreased CO_2 diffusion into leaf thereby perturbing photosynthesis. Additionally, in an investigation of grapevine under water stress, Flexas *et al.* (2002) reported that stomatal regulation is the major factor limiting photosynthesis. Similarly, a positive relationship between g_s and P_N was earlier observed in fig and peach trees (Clifford *et al*. 1997).

Water stress also decreased Chl content in neem plants. Such water-deficit-induced reduction in Chl content has been ascribed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation and the appearance of lipid droplets (Kaiser *et al*. 1981) and restrained Chl *a/b*-protein synthesis (Alberte *et al*. 1977). In addition, because of the relatively high amount of Chl *b* in the light-harvesting complex (LHC) (Jeon *et al*. 2006), the maintenance of high Chl *a/b* ratios has been associated with stronger drought resistance (Wu *et al*. 1990). This experiment showed Chl *a/b* ratio declined with increasing water stress, but provenances differed in the extent of decline (Table 5). Neem originating from ND had greater decrease in Chl *a/b* ratio than did neem originating from KA with increasing water stress. Accordingly using these criteria of pigment content and composition it is suggested drought resistance of KA provenance may be greater than ND one.

The drought stress also brought about other biochemical responses in neem plants in order to minimize its deleterious effects. MDA is a decomposition product of polyunsaturated fatty acid hydroperoxides and is widely used as an indicator of lipid oxidative damage (Halliwell and Chirico 1993). Our results showed that there were specific MDAs in four provenances under water stress, indicating the occurrence of different degrees of oxidative stress and membrane damage in arid conditions. Water stress increased MDA content least in KA and most in ND provenance, suggesting membrane damage in ND was more severe than in KA. Additionally, plants use their available machinery to combat oxidative stress by scavenging excess reactive oxygen species through enhancing activity of various antioxidant enzymes (Seel *et al*. 1992). SOD activity is positively correlated with plant antioxidative ability (Bor *et al.* 2003). SOD activity was significantly higher in KA than in ND under severe water stress (L4) (Table 3). Thus, neem originating from KA had a greater ability to regulate oxidative stress than neem originating from ND and might be influenced much less by drought than ND. There are disagreements over the defensive functions of POD to peroxidation. In an investigation of wheat species under water stress, Zhang and Kirkham (1994) showed that hexaploid wheats had higher POD activities than tetraploid and diploid ones with increasing water stress. Del-Longo *et al.* (1993) showed that drought-resistant plants exhibited higher POD activity than did drought-sensitive plants and Guo *et al.* (1997) also demonstrated similar results. However, Gong *et al.* (2002) showed that POD activity in water-tolerant species (*e.g. Artemisia ordosica*) decreased with increasing water stress. Our study showed that POD activity in four provenances significantly increased under water-limiting condition with the highest value in KA and the lowest value in ND under severe water stress (L4) (Table 3), similar to the results of Zhang and Kirkham (1994). These findings also suggested neem originating from KA provenance might have more drought resistance than neem originating from ND.

RWC reflects the deficit in maximum tissue water content. It is a measure of the mass of water in the sample divided by the maximum possible water in the sample. RWC is often used as a parameter to assess the severity of drought owing to the typically high correlations between declining RWC and declining photosynthetic capacity (Tardieu and Simmonneau 1998, Lawlor and Cornic 2002). In our study, leaf RWC was significantly reduced by the decreasing water supplies from L1 to L4,

which indicated the sensitive response of neem to soil water and their higher regulation capability for photosynthesis, and comparative effectiveness of their root systems to acquire and transport water.

As water supply declines, live cells accumulate osmotically active compounds that reduce the osmotic potential and, therefore, help maintain turgor and enable the plants to continue to acquire water from the soil at low water potentials. We found Pro content to increase in neem as water stress increased. The accumulation of Pro might contribute to maintaining proper balance between extracellular and intracellular osmolarity under water stress. However, the significance of Pro accumulation is controversial. Our results were different to the findings of Clifford *et al.* (1998) and Bajji *et al*. (2001), suggesting that the magnitude of accumulation of Pro was dependent on the degree of drought stress and plant species.

In agreement with the findings of Colom and Vazzana (2003), P_{max}, I_s, and Φ of four neem provenances all pronounced decreased, while R_D and I_c gradually increased with increasing water stress (Table 1). However, there was marked difference among parameters of plants from different provenances. For example, under severe water stress (*i.e.* L4), both P_{max} and R_D were the highest in KU and the lowest in ND. In theory, P_{max} determines the plant potential photosynthetic capacity, and increasing I_c and decreasing I_s will reduce the time of effective P_N, while increasing R_D will mean plants consume more carbohydrate at night thereby reducing plant growth and productivity. According to these analyses, although KU had greater photosynthetic potential capacity than the other three provenances, they might consume more photosynthate at night.

As discussed above, inhibition of photosynthesis associated with drought stress often affects plant growth and yield. Our study indicated that a larger proportion of photosynthates were allocated to the belowground plant parts under water deficit, which meant the percent increase in root allocation was at the expense of both leaf and shoot DM. The changed ratio of root/aboveground part DM reflected the adaptation strategies in DM distribution pattern under different water supply (Rodrigues *et al*. 1995). Under lower soil water availability, the seedlings invested more DM in root growth in order to absorb more water thereby enhancing higher survival competitiveness. Contrariwise, the seedlings grown under well water supply invested more DM in leaf and shoot in order to capture more light and enhance photosynthesis. We found the ratio of root/aboveground part DM showed significant variation with soil moisture and with the four provenances had different sensitivities to water deficit. The percent increase in root allocation was the highest in KA and the lowest in ND with increasing of water stress, which meant KA might have a well developed root system with the stronger drought resistance while ND might be contrary. This was consistent with experimental results.

In conclusion, the photosynthetic characters, DM allocation, and associated metabolism of four neem provenances showed strong responses to water stress. However, there were obvious differences in adaptation to varying water stress through changed growth strategies coupled with ecophysiological and metabolic adjustments among four provenances. The differences of adaptation mechanisms among provenances would contribute to the better selection of neem provenances and cultivars in the dry-hot valley areas of southwest China. Thus, future work should establish an index system on comprehensive judgement of drought resistance ability to estimate the magnitude of variability of neem provenances and obtain better provenances to meet the demand of neem industrialization in China.

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