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# Anatomical and morphological characteristics of *Populus euphratica* in the lower reaches of Tarim River under extreme drought environment

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**Abstract:** *Populus euphratica* Oliv. is an old desert tree species that has been naturalized and invades zones along the watercourses in many arid and semiarid regions. The plant species developed some plasticity to adapt to the gradual environmental gradients. The aim of this study was to test the hypothesis that the changes in leaf morphology of *P. euphratica* reflect the adaptability of the plant to the unique environment of the lower reaches of Tarim River in China. The foliar architecture, blade epidermal and internal anatomies of *P. euphratica* were analyzed at different sites along the Tarim River. Compared with the abaxial surface of the leaves, their adaxial surface has more hairs, a greater stomatal density and opening, higher mesophyll proportion, and increased blade thickness, palisade width, and epidermal thickness. The long trichome of the roots found at site 6 in the Yinsu section may be an adapted structure of the plants in arid areas. The mature leaves of *P. euphratica* have comparatively more epidermis and cuticles, well developed palisades and more chloroplasts at different sites compared to the young leaves. Foliar morphological and anatomical variability in *P. euphratica* may be considered an adaptive advantage that enables leaves to develop and function in different habitats, marked by strong variations in solar radiation, air temperature, humidity and water table.

**Keywords:** *P. euphratica*; ecological adaptation; leaf morphology; water stress; Tarim River

In order to survive in different environments, the morphological traits of widely distributed plant species often vary considerably. Leaves are exposed to aerial conditions more than any other plant organs, and the changes in their characters have been interpreted as adaptations to specific environments (Leymarie *et al.*, 1999; Charles *et al.*, 2003). The variations in the morphological and anatomical features of leaves and with the relationships of the amount of sun exposure, the degree of salinity, or water availability have been reported for many species (Gu *et al.*, 1999; Maria, 2000; Shin, 2000).

*Populus euphratica* Oliv. is widely distributed in the desert regions of Central Asia, and developed along the watercourse in the Tarim River valleys of China. The species is known for its ability to grow in a wide range of environmental conditions, such as arid, high

salinity, or windy and dusty abominably environments. The area of *P. euphratica* woodlands in the Tarim River basin accounts for 54% of the species in the world (Zhang *et al.*, 1996; Wang *et al.*, 1999; Li *et al.*, 2006; Wang *et al.*, 2009; Zhuang *et al.*, 2010). The researches of the capacity for *P. euphratica* to establish and grow in extreme environments might favor its recovery and spread.

The size and shape of *P. euphratica* leaves varies at different times in the growing season, which is beneficial for the evaluation of the environmental heterogeneity in the lower reaches of Tarim River. The anatomical and morphological characters of *P. euphratica* leaves were studied in order to analyze the relationship between the external environmental factors and the observed

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characteristic differences. Therefore, the hypothesis that the anatomical and morphological features of *P. euphratica* leaves are developed in response to the environmental spatial heterogeneity, especially to the changes of water table in extremely arid regions.

## 2 Materials and methods

### 2.1 Plant material and growth conditions

In 2008, *P. euphratica* leaf samples were collected from the following sections: Yahepu (836 m a.s.l., 40°29.516'N, 87°50.172'E), Yingsu (832 m a.s.l., 40°29.208'N, 87°56.337'E), Abudali (828 m a.s.l., 40°24.879'N, 88°03.003'E), Kardayi (829 m a.s.l., 40°22.339'N, 88°10.243'E), Alagan (817 m a.s.l., 40°08.769'N, 88°21.575'E), Yiganbjima (811 m a.s.l., 39°47.288'N, 88°22.671'E), and Kaogan (926 m a.s.l., 41°19.722'N, 88°25.241'E), which are all located along the Tarim River (Fig. 1). This species has been important to the conservation of the Tarim River for the last 30 years. Five or six plots were established in 50 m, 100 m, 200 m, 300 m, 400 m and 500 m distances from the river in accordance to the position of the groundwater monitoring wells in each section. The climate in the lower reaches of the Tarim River belongs to the continental warm temperature zone, with dry weather. The average temperature during the coldest month (January) is  $-15^{\circ}\text{C}$ , and the hottest month (July) is  $25^{\circ}\text{C}$ . The annual pan evaporation is 2,671.4–2,902.2 mm, with a negative water balance throughout the year. The annual precipitation is 17.4–42.0 mm, most occurring from April to September. The average annual solar radiation is 1,740

$\text{kW}\cdot\text{h}/(\text{m}^2\cdot\text{a})$ , with about 3,000 h of sunlight. The landform is a composite mode with barchan chains, and longitudinal and shrub dunes.

### 2.2 Methods

The air temperature, humidity and soil moisture were recorded using SAP FLOW RELATIVE RATE SENSORS (PHYECH, plant physiology and ecology monitoring system, Israel). The soil water content was measured by the gravimetric method. The groundwater data was monitored monthly using universal meter. The salinity was measured monthly by *in-situ* soil salinity. Leaf water potential was measured by HR-33T (Dew-point water potential meter, WESCOR Company, Logan, UT, USA).

In each plot, fully expanded and undamaged leaves of *P. euphratica* from 2.5–3 m height were collected in all directions.

Leaf water content (LWC) during the growing period was measured by collecting 100 g of leaf material in each direction of three trees. The fresh weight was determined, and then the plant tissues were dried for 48 h at  $60^{\circ}\text{C}$  to determine the dry weight.

The leaf material for the scanning electron microscopy (SEM) was fixed by formal-acetic acid-alcohol (FAA). Samples were dehydrated using increasing strengths of 75% alcohol and cleared using an ultrasonic wave for 10 min. The samples were then frozen in liquid nitrogen and coated with a film of gold and a sputter coater SCT620. Both the epidermis and the cross-section of each sample were examined under a scanning electron microscope (LEO1430VP SEM), respectively. Foliar architecture was observed using

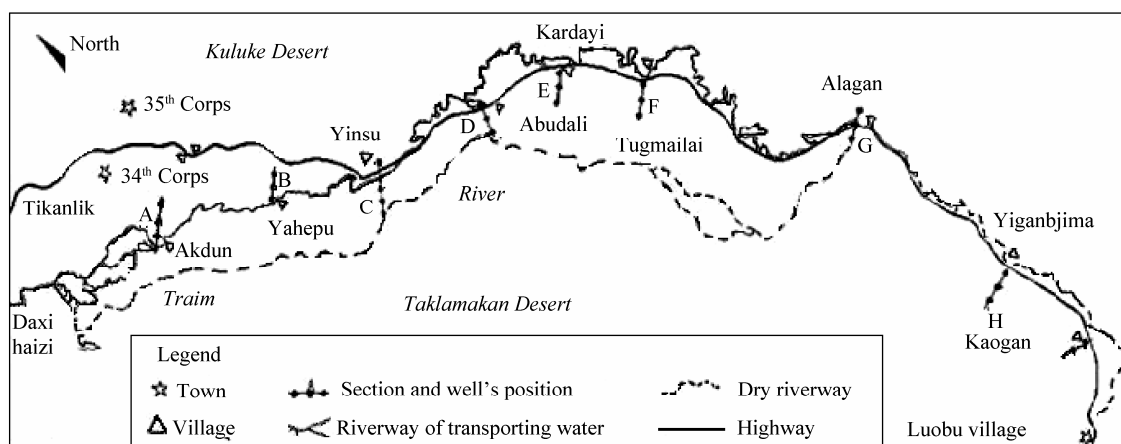


Fig. 1 The sketch of investigation sections in the lower reaches of Tarim River

transmission electron microscopy (TEM). Cleared leaves were fixed in FAA, rinsed thoroughly in a phosphate buffer, then fixed with osmic acid and rinsed with a phosphate buffer again. The samples were dehydrated in increasing strengths of alcohol from 30% (15 min), 50% (15 min), 70% (12 h), 85% (15 min), 95% (15 min), to 100% (15 min, twice). After a series of alcohol-acetone to pure acetone wash and acetone-epoxy resin to pure epoxy resin, the samples were embedded with an epoxy resin and baked in oven from 35–60°C (in 5°C intervals), after which the samples were cut using a microtome. The microtome sections were stained by uranium acetate and citric acid lead. The cellular structure of each sample was examined using Hitachi H-600X TEM (Tokyo, Japan).

### 2.3 Data analysis

The statistical analysis was performed with SAS software (version 6.12, SAS Institute Inc., Cary, USA). All data were subject to a one-way analysis of variance (ANOVA) and the means were separated by least significant difference (LSD) test at 0.05 level.

## 3 Results

The results of monitoring environmental factors during the period of 2006–2008 in the study area are listed in Table 1. Soil salinity values reached the highest at 5 cm depth, up to a maximum of 8.73 g/mol at the site 4 of the Yinsu section (Y4) in August 2006. The air temperature in Yinsu section of the lower reaches of Tarim River reached a maximum of 42°C in July 2007. The air humidity at the Y1 sampling site reached a minimum of 10% and, at the Y3 site attained a maximum of 100%. The measured soil moisture values were from zero to the maximum of 69.2%.

The average annual water table in the five sampling

sites of the Yinsu section is shown in Fig. 2. During spring and summer, the water table decreased corresponding to the increase in the distance from the river course in 2006, 2007 and 2008, except in the Y2 site in April 2006 and April 2008. Water tables of the Y1–Y5 sites ranged from 2.55 m to 6.49 m, and 50% of them were over 5 m.

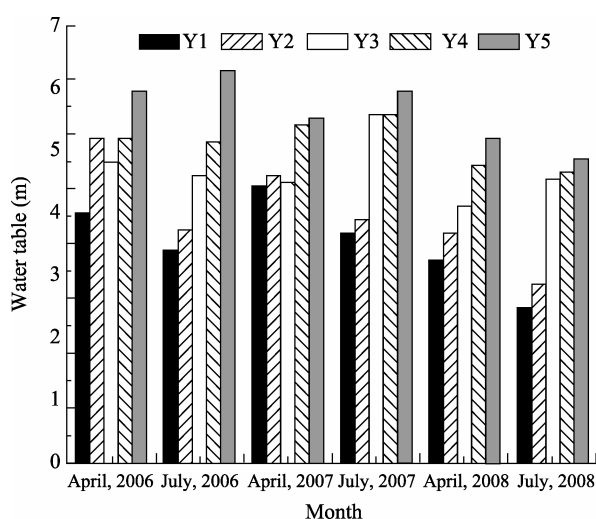
The average dry weight of *P. euphratica* leaves is shown in Table 2. A total of 50 leaves of three plants in each direction were collected and measured during August of 2006. The average dry weights of *P. euphratica* leaves at the sites 1 of every section were higher than other sites except Yinsu section where the highest and the lowest values were 1.024 g and 0.1367 g in the site 2 and site 4, respectively.

Daily water potentials of the *P. euphratica* leaves were found to be higher in the morning and evening while the values were lower at noon. The highest value of the water potential was measured in the leaves of the species that occurred 300 m away from the river (Table 3). The average relative water capacity measured in the *P. euphratica* trees in the Yinsu section are shown in Fig. 3. The Y6 site had the highest average relative water capacity, with an increase of 61.62%.

The leaves of *P. euphratica* are deciduous and become golden yellow color in autumn before senescing. New leaves grow during early spring. Most of the leaves of a healthy *P. euphratica* tree may wither under extremely environments, such as water stress. When the environment is too harsh, only a small cluster of leaves can survive, along with fewer flowers and fruits. Fully expanded leaves are simple and symmetrical, with an entire margin; however, *P. euphratica* leaves can differ based on their location in the tree, or based on the different growing periods of the plant species. Most of the upper leaves of a mature tree are

**Table 1** Environmental factors of Yinsu section during the growing period of *P. euphratica*

Site	Soil depth (cm)				Air temperature (°C)			Air humidity (%)			Soil moisture (%)		
	5	10	20	40	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
	Soil salt (g/mol)												
Y1	6.40	6.09	3.57	4.97	42.0	20.0	31.43	62	12	26.80	11.1	4.6	9.69
Y2	7.41	3.50	3.55	4.09	40.5	22.5	31.76	54	10	20.95	15.6	6.1	12.68
Y3	3.62	2.34	4.89	1.23	34.0	13.5	22.82	100	26	71.93	69.2	0	49.39
Y4	8.73	7.49	5.31	2.11	41.5	18.0	31.48	54	10	21.93	15.2	4.6	8.70
Y5	7.11	2.49	3.15	1.84	38.5	20.5	30.74	56	16	30.09	16.3	4.6	15.63



**Fig. 2** Mean water tables (m) in Yinsu section during the period of April, 2006 to August, 2008

**Table 2** The average dry weights of *P. euphratica* leaves at sites 1–6 of different sections

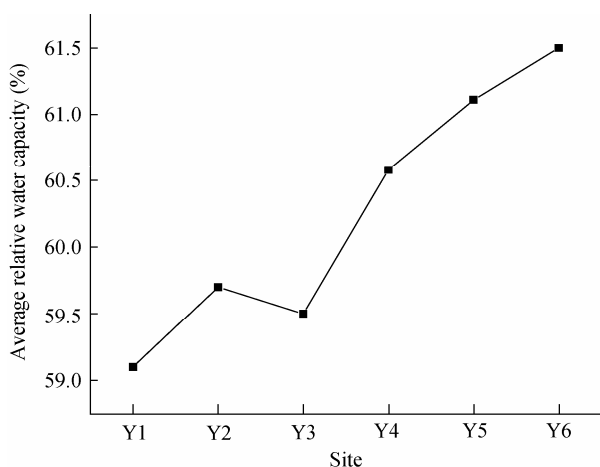
Section	Average dry weight (g)					
	1	2	3	4	5	6
Yahepu	0.7201 <sup>a</sup>	0.4697 <sup>b</sup>	0.4859 <sup>c</sup>	0.4762 <sup>d</sup>	—	—
Yinsu	0.6225 <sup>a</sup>	1.0247 <sup>b</sup>	0.7995 <sup>c</sup>	0.1367 <sup>d</sup>	0.5123 <sup>c</sup>	0.6351 <sup>f</sup>
Abudali	0.7588 <sup>a</sup>	0.5524 <sup>b</sup>	0.6204 <sup>c</sup>	0.4817 <sup>d</sup>	0.5505 <sup>c</sup>	0.6532 <sup>f</sup>
Kardayi	0.7082 <sup>a</sup>	0.6332 <sup>b</sup>	0.5889 <sup>c</sup>	0.6454 <sup>d</sup>	0.6019 <sup>c</sup>	0.3258 <sup>f</sup>

Note: For each row, the values with the same letter are not significantly different at 0.05 level.

**Table 3** Water potential (WP) of *P. euphratica* leaves at different distances from Tarim River course in Yinsu section

Distance (m)	Water potential (bar)			Range	Standard deviation	Coefficient of variation
	Max.	Min.	Mean			
100	−15.00	−23.67	−20.96a	8.67	4.94	16.69
200	−15.13	−25.20	−22.13ab	10.07	4.31	19.47
300	−18.00	−30.00	−22.76b	12.00	4.94	21.69

Note: The values are the mean for 5 samples. The values with different letters are significantly different at 0.05 level.

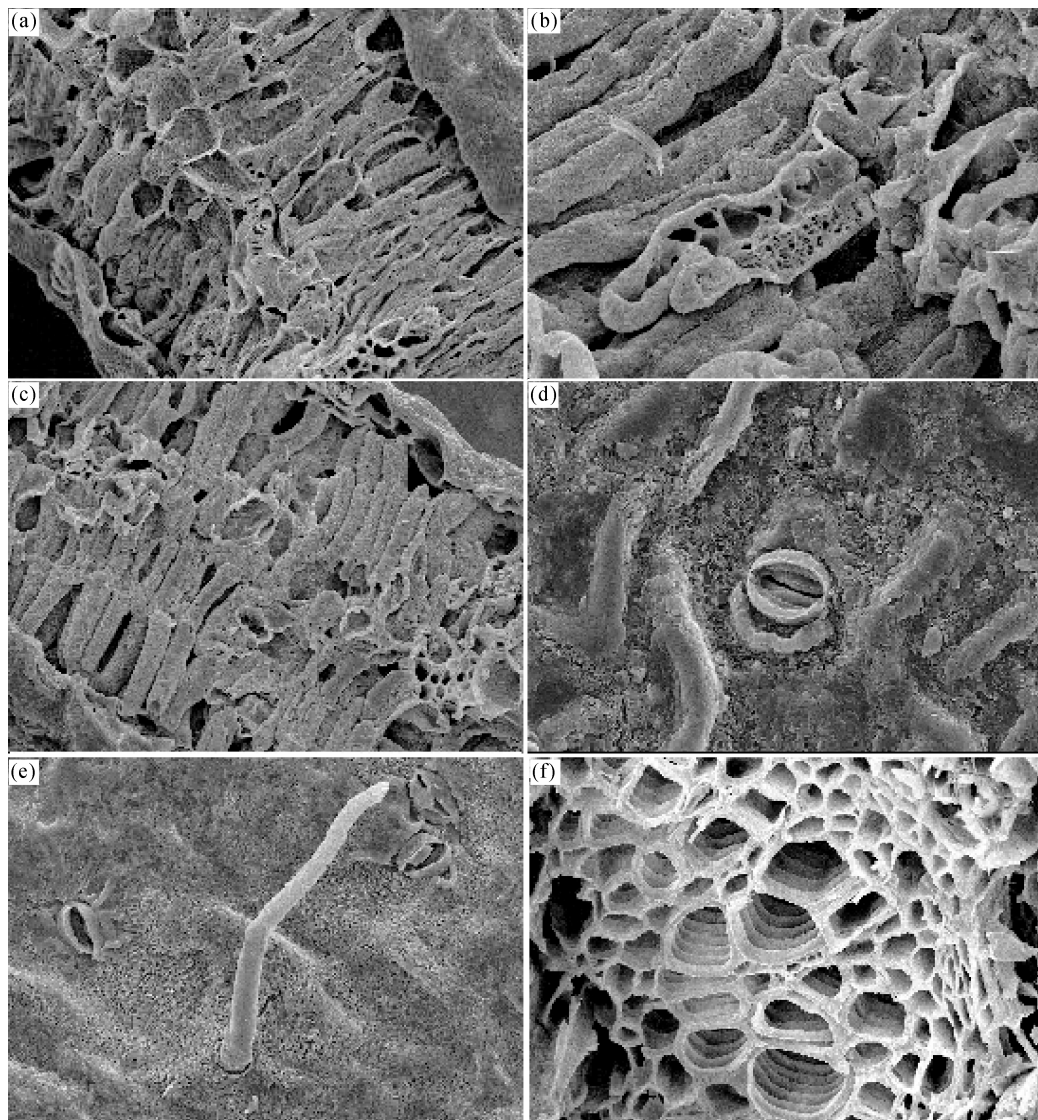


**Fig. 3** Average relative water capacity of *P. euphratica* during the period of August, 2006–2008 in Yinsu section

ovate with an entire margin or vandyke, and the sizes and shapes are very different, ranging from oblong to ellipse. Lanceolate leaves occur on the base of the stem. Therefore, the tree was again named as “*Populus* with heteromorphous leaves”. The texture of the lamina is leathery, but the color varies, with dark green adaxial surfaces and the abaxial surfaces having a woolly appearance and being lighter color, a feature that is especially evident in mature leaves.

The leaves are bifacial, with a biseriate palisade in most sites, but a third, poorly organized stratum was observed in the Y4 site (Fig. 4a). Palisade cells have cylindrical and spongy tissue, arranged regularly and inseparably (Fig. 4b). Spongy parenchyma is randomly oriented (Fig. 4c). The proportion of mesophyll tissue in the highest (81.4%) was Y6 site and lowest (62.5%) in Y5 site. Stomata are presented below the

level of neighboring ordinary epidermal cells, and there are three layers of shield-like structures outside, sinking the stomata (Fig. 4d). The pentagonal or quadrilateral epidermal cells are smaller in the lower epidermis than that in the upper epidermis. The volume of the epidermal cells had decreased and the shape of the epidermal cells became irregular as the distance to the river increased. Stomata density was higher in the abaxial surface than that in the adaxial surface. The abaxial surface was always younger than the adaxial, which can be cleared by an ultrasonic wave. The hollow, hairy root structure cavity found in the leaf epidermis of Y6 site was connected to the epidermal cells (Fig. 4e). When the leaf catheter structures from different sampling sites were compared, all internal



**Fig. 4** Ultra-structure of *P. euphratica* leaves in Yinsu section

(a), (b) mesophyll tissue; (c) spongy parenchyma; (d) stomata; (e) long epidermal hair; and (f) spiral catheter

layers of the cylindrical catheter exhibited a spiral twist (Fig. 4f).

#### 4 Discussion and conclusion

The results of the study confirmed that the leaves of *P. euphratica* in the different sections of Tarim River Basin show great variation. The lower reaches of Tarim River, located between the Taklamakan Desert and Kulu Desert, lies in the lowest region of Tarim Basin, therefore, it becomes an ideal location for salt accumulation, due to the soluble salt taken by the surface and groundwater from the upper and middle reaches of Tarim River. Because the area is located in an arid environment, the air temperature is much higher in the summer. The differences in humidity between

day and night were significant, with a lower value during day and sometimes reaching saturation at night.

Since there is an exponential relationship between light absorption and the cumulative foliage area, the changes in the sizes of *P. euphratica* leaves can be considered functionally significant, related to the natural conditions where they developed. The upper wider ovate leaves, with thick cuticles, are useful for light absorption and reducing evaporation. The lower, young leaves are exposed to lower light, higher air humidity and are protected from wind. Thus, the leaf shape of *P. euphratica* changes gradually from a narrow lanceolate to a wider ovate upon maturation. The expansion of leaf diameter increases photosynthesis,

which contributes to the production of some defensive metabolites, when subjected to stress. The phenomenon of survival of a small cluster of leaves, along with the stems, branches, and having enough flower and fruits, could be related to programming cell death under stress.

In the lower reaches of Tarim River, the scarcity of the soil water resources and the high evaporative demand of the atmosphere during the warmer seasons induce stress conditions. The survival of the plants in these adverse conditions requires long- and short-term plasticity responses and plants could develop stress avoidance mechanisms by changing its morphology and architecture (Xing, 2000), root development (Niinemets, 1996) or leaf anatomy (Long *et al.*, 1994). When soil surface water becomes the limiting factor, trees have the potential to reach groundwater, and the adaptability of the root architecture may be an important determining factor of the competitive strength compared to foliar plasticity (Niinemets, 1996). However, the species was distributed at different sites, which was exposed to varying water tables. The various environments led to the leaf morpho-anatomical plasticity with respect to the combined conditions of soil salt, air temperature and humidity. One or all of these features could confirm the survival of the species, and enable them to compete with other species.

Bicolor is a common phenomenon among species that occupy shaded habitats; the leaf side facing away from the sun is lighter in color than the leaf surface facing the sun. The lighter surface may act as a reflective surface and enhance the participation of the spongy mesophyll in leaf photosynthesis (Maria, 2000). The abaxial surface of *P. euphratica* mature leaves is lighter than the adaxial surface because it is densely covered with hairs. Pubescence is another characteristic that confirms stress resistance (Ehleringer, 1980), because the presence of hairs on the aerial parts of the plants is regarded as an adaptation to arid conditions (Fahn, 1986) because the substantial increase in leaf reflectance and reduced radiation absorption which results in the reduction of leaf temperature. The long hair found in the site 6 of Yinsu section could be a special structure of epidermis that may be related to increased aridity. Water stored in hollow hairs could help to reduce the degree of injury with increasing stress due to drought.

The changes in the sizes, especially the shapes of the *P. euphratica* leaves, can be considered to have a

functional significance related to the different natural conditions. The narrow, lanceolate leaves in the new branches help to reduce evaporation, while the wider, ovate leaves on the mature branches are capable of capturing as much light as possible, protecting them from the wind by thick cuticle and the presence of wax on them. In addition, self-orientation of the *P. euphratica* can decline leaf temperature and minimize water consumption. These characteristics may be related to the high solar irradiation of the region as increased light has a detrimental impact on the photosynthetic performance (Smith *et al.*, 1997).

Smith *et al.* (1997) suggested that bicolor laminar leaves containing stomata only in the abaxial epidermis may have evolved as a result of selective pressure to enhance light capture, while avoiding the detrimental effect of exposure to sunlight and minimizing water-loss due to transpiration. In terms of *P. euphratica*, the difference in color between the mature leaf surfaces is marked and the stomata density of the abaxial surface is higher than that of the adaxial surface. The higher stomata density in the adaxial surface of *P. euphratica* may be a response to the more arid habitat. The proportionally large leaf size of mature leaves could mean an additional mechanical advantage under stressed conditions.

However, the plasticity of the leaves' shape and morphology is insufficient, and the anatomical features, such as the volume and organization of the mesophyll tissue, should be taken into consideration (Fahn *et al.*, 1992). The abundance of plastids in the cytoplasm and amyloid in the plastids was observed, and the osmophilic particles were obviously displayed in the cytoplasm and in the posterior margin of the vacuole. The chloroplast is very important and unique plant organ which has the ability to convert light energy into chemical energy, and then store the energy as carbohydrates. The carbohydrates are mainly stored as starch granules in the chloroplasts. It can release enormous energy after hydrolysis and also provide carbon skeletons for protein synthesis at the same time (Hostak *et al.*, 1986). These characteristics are defined as the structural mechanisms that increase photosynthesis per unit leaf area and enable greater water-use efficiency. The palisade tissue in the *P. euphratica* leaves is well developed and the palisade tissue cells are arranged perpendicular to the direction of the leaf epidermis cells and parallel to the direction of light. These characteristics can guarantee normal

photosynthesis by leaves and also protect them from intense sunlight.

There are two epidermal cells in the mature leaves of the *P. euphratica* with a proportionally greater cuticle, which helps in reducing the diffusion of the water vapor from the leaf interior to the atmosphere. In addition, there is an obvious gap between the cuticle and the epidermal. This gap can play a role in storing water. On the other hand, it could lead to adaptation of leaves to the relatively large temperature ranges by isolating cell interior and exterior air. There are no significant relationships among the anatomical characteristics, such as stomata density and stomata opening, blade thickness, catheter lumen diameter and lumen wall thickness, epidermal thickness and palisade width, and the water table. However, the opening and density of the stomata of the abaxial surface was larger than those of the adaxial surface except blade thickness, palisade width and epidermal outer wall thickness. These characteristics indicate that water table is not the only inhibiting factor in the lower reaches of Tarim River and, therefore, the differences in the morphological and anatomical structures of *P.*

*euphratica* could be related to other environmental factors.

Leaf variability in *P. euphratica* can be considered an adaptive advantage of the leaves in different habitats, marked by strong variations in solar radiation, air humidity, salinity, water table, temperature, and wind exposure. The leaves show many xeromorphic characters that enable the trees to maintain their normal development even under unfavorable conditions. These results confirmed the hypothesis that variations in the developmental responses of the *P. euphratica* leaves to spatial heterogeneity are related to its ecological strategies. This species relies, at least partially, on its foliar plasticity to overcome the environmental changes in different places, and even compete against other species in places where spatial environment heterogeneity can easily manifest, such as in a river valley of the lower reaches of Tarim River.

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