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Variation in *Populus euphratica* foliar carbon isotope composition and osmotic solute for different groundwater depths in an arid region of China

Jianhua Si · Qi Feng · Tengfei Yu · Chunyan Zhao · Wei Li

Abstract Water use efficiency (WUE) is an important trait associated with plant acclimation caused by water deficits, and $\delta^{13}C$ is a good surrogate of WUE under conditions of water deficits. Water deficiency also enhances the accumulation of compatible solutes in the leaves. In this study, variations in foliar $\delta^{13}C$ values and main osmotic solutes were investigated. Those included total soluble sugar (TSS), sucrose, free proline, glycine betaine (GB), and inorganic ionic (K^+, Ca^{2+}, and Cl^-) content of *Populus euphratica* for different groundwater depths in a Ejina desert riparian forest, China. Results indicated that foliar $\delta^{13}C$ values in the *P. euphratica* for different groundwater depths ranged from -29.14 ± 0.06 to -25.84 ± 0.04‰. Foliar $\delta^{13}C$ signatures became richer as groundwater levels declined. TSS, sucrose, free proline, GB, and K^+ were accumulated in *P. euphratica* foliage with developing plant growth and increasing groundwater depth. Ca^{2+} and Cl^- content increased under stronger *P. euphratica* transpiration rates for shallower groundwater depths (1–2.5 m) and decreased for deeper groundwater depths (greater than 3.0 m). Moreover, correlations between $\delta^{13}C$, osmotic solutes, and groundwater depths showed that the primary osmotic solutes were TSS, sucrose, proline, GB, and K^+. Correlations also showed that $\delta^{13}C$ was not only a useful measure for *P. euphratica*-integrated WUE but also could be used as an indicator reflecting some physiological osmotic indexes.

Introduction

Drought stress is widely considered to be the primary environmental factor that limits plant growth and yield in arid regions, and it is predicted to become increasingly more severe in certain regions due to developing changes in global climate conditions (Chaves et al. 2003). Plant species native to arid environments are typically subject to salt and drought stress conditions caused by high evaporative demand, low water availability, and high mineral content in groundwater. These plant species have evolved various physiological acclimation processes in response to such environmental factors. Osmotic adjustment (OA) is one such drought avoidance mechanism to counteract turgor loss by increasing and maintaining higher levels of intracellular compatible solutes and has proven to be the most effective among all drought acclimation mechanisms (Cushman 2001). It involves the active accumulation of organic and inorganic solutes in cells in response to deceases in cell environment water potential. Consequently, cell osmotic potential is lowered, which, in turn, attracts water to enter cells, thereby maintaining turgor pressure. Soluble sugar (Rekika et al. 1998) and proline (Mattioni et al. 1997) levels have been demonstrated to increase under water stress and are therefore potentially important contributors to osmotic adjustment.

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Water use efficiency (WUE) is another important trait associated with the adaptive capacity of plant species to water deficits (Condon et al. 2002). WUE is traditionally defined either as the quantity of carbon (C) biomass produced per unit water transpired by plants or the ratio of net photosynthesis to stomatal conductance over a period of second or minute (Wright et al. 1988). Recently, stable carbon isotope composition ($\delta^{13}C$) has been developed as a tool to measure WUE because there is a strong positive correlation between the two (Farquhar et al. 1989). Foliar $\delta^{13}C$ is partly determined by Ci/Ca, the ratio of CO_2 concentration in intercellular spaces of foliage to that in the atmosphere (Farquhar et al. 1982, 1989). This ratio differs between plant species due to variations in stomatal opening (affecting the CO_2 supply rate) and chloroplast demand for CO_2. Therefore, $\delta^{13}C$ values do not only integrate physiological and environmental properties that influence the interplay between all aspects of plant carbon and water relationships throughout the growth period but also have been instrumental in revealing how plant species adjust their gas exchange metabolism and resource acquisition strategies in a given habitat (Dawson et al. 2002). In general, species native to arid or semi-arid environments show either no change or an increase in WUE with decreasing water supplies; that is, their water use patterns could become more conservative during times of drought (Toft et al. 1989).

Water stress could limit plant growth more significantly than any other factor. Subjected to stress caused by water deficits, plant species can not only enhance WUE (Farquhar et al. 1989), but they can accumulate osmoticum that is widely considered to be the key mechanism that maintains turgor through osmotic adjustment by plants forced to confront increasing water stress (Rekika et al. 1998; Cushman 2001). Hence, it is surmised that certain correlations must exist between water stress and WUE. Gao et al. (2006) reported on the existence of multiple variable relationships between content of different osmotic solutes and $\delta^{13}C$ values for *Ammopiptanthus mongolicus* foliage under two distinct water regimes. Furthermore, numerous studies have focused on the relationship between plant species and environments using $\delta^{13}C$ (Merah et al. 2001; Su et al. 2003), and osmotic adjustments induced under artificial conditions (treated with NaCl, PEG, etc.) have also traced the course of osmotic adjustments in field plants (Evans et al. 1992; Martínez-Ballesta et al. 2004). However, relationships between WUE and other physiological properties in plant species remain largely

unknown. What's important, however, is that even though many researches have taken interest in OA mechanisms and WUE, few studies have been conducted on physiological responses to water stress, which occurs in native riparian plant species inhabiting hyper-arid environments. For example, Chen et al. (2003) only investigated free proline accumulation in *Populus euphratica* in response to variable groundwater tables in the lower reaches of the Tarim River. Francisco et al. (2001)) reported on the effects of pulse floods on native tree species regeneration and vegetation tolerance in the delta region of the Colorado River. To date, however, there remains an ever greater deficient in research related to WUE, osmotic solute, and their relationship as it pertains to desert riparian plant species.

P. euphratica is a tree species that grows at the edge of barren deserts. Its high survival rate and biomass production in arid regions of Mongolia, China, Pakistan, Iraq, and Iran are widely acclaimed (Sharma et al. 1999). On account of its tolerance to severe drought conditions and high salinity and alkaline soils, *P. euphratica* is important in maintaining ecosystem function in arid regions. However, worldwide changes in climate and irrational utilization of water resources in the upper and middle reaches of inland rivers in China (such as the Tarim and Heihe rivers) over the past several decades have resulted in a decline of forested areas and degradation of ecological environments along both riverbanks. *P. euphratica*, as the primary constructive species in desert riparian ecosystems for such regions, has subsequently decreased and at present is primarily comprised of over-matured forests. Its degradation has seriously threatened the safety of ecological sensitive desert oases in the lower reaches of inland rivers in China. To prevent *P. euphratica* from continued degeneration and to aid in its recovery, a number of researchers have studied *P. euphratica* response to salt stress (Watanabe et al. 2000; Ma et al. 2002) and ecophysiological response to water release (Chen et al. 2003; Wang et al. 2007; Ruan et al. 2008). Moreover, according to results reported by Zhu (2007) and Zhao et al. (2008) on water use sources, suggesting that *P. euphratica* growing in an Ejina desert riparian forest in the lower reaches of the Heihe River primarily used Heihe River groundwater recharge for its development. Hence, to further explore mechanisms of *P. euphratica* adaptation to arid environments and to determine a rational groundwater depth for its continued survival (given that moderate water deficits benefit plant WUE), it is vital to focus research on variations in foliar

P. euphratica osmotic solutes and $\delta^{13}C$ values for different groundwater depths throughout the growing season.

The objectives of this study were (1) to determine seasonal changes in foliar $\delta^{13}C$ and various osmotic solutes for different groundwater depths, and (2) to determine the primary osmotic solutes and a rational range of groundwater depths for which *P. euphratica* will experience moderate water stress through correlations between $\delta^{13}C$, osmotic solute content, and groundwater depth. Findings could have important implications for a better understanding of desert riparian *P. euphratica* forest protection and restoration.

Materials and methods

Experimental sites

Experiments were conducted within the Ejina Oasis in the lower reaches of the Heihe River, Northwestern China. The experimental area was located within the *P. euphratica* Reserve (41° 50′ 28.7″ N~42° 1′ 40.86″ N, 100° 59′ 24.5″ E~101° 05′ 00.9′ E) at an elevation of 980 m (Fig. 1). The study site is considered one of the most extreme arid regions in China. Mean annual precipitation is 37.4 mm, 75 % of which occurs between the July and September growing season. Monthly average precipitation is less than 10 mm. Potential evaporation ranges between 36.01 and 555.75 mm. Annual average temperature ranges from −11.3 to 26.8 °C with the minimum and maximum values of −7.0 and 29.8 °C, respectively. Relative humidity ranges between 22.36 and 49.38 % (Table 1). The direction of prevailing winds is northwest in winter and spring and southwest to south in summer and autumn. The dominant plant species is *P. euphratica*, which is found in high densities (148 stems ha^{-1}) and contributes approximately 75 % of the total basal area in the study site.

Sampling process

P. euphratica foliage for purposes of sampling was collected on May 18, June 21, July 23, August 20, and September 20, 2008, within the *P. euphratica* Reserve. To objectively assess WUE and each physiological index of *P. euphratica*, five typical sampling plots were selected in April with the area of 50×50 m, which represent the widespread habitats that *P. euphratica* presently grows in, such as the Gobi Desert, dunes, riparian lowlands, typical *P. euphratica* flatlands, and riparian dry sandpiles. In addition, to conduct a comparison between the different natural plots stated above, another riparian flatland plot irrigated during the winter of 2007 was selected in order to obtain the shallowest groundwater depth required for the experiment (Table 2). Distances between plots were less than 20 km, and airborne C isotopes near the selected sites were assumed to not be significantly different due to the absence of roads (vehicles) or any other fossil fuel burning processes or practices near the selected sites. Five individuals of each of the two species were selected and tagged in each plot, all exhibiting similar diameter at breast height (DBH) and coverage. Mature *P. euphratica* foliage was collected for all sampling times. Although each stand exhibited wide differences in height and density, the following procedure was applied to avoid dissimilarity in effects of leaf maturity and light exposure: Mature foliage from a sunlight-facing aspect, from similar heights (6–7 m), and from similar branch positions were collected between 8 and 10 a.m., and approximately 20 leaves from each tree were collected as samples. Samples were first ultrasonically washed in distilled water and then air-dried. At this point, they were oven-dried at 70 °C for at least 48 h to a constant mass and then ground with a mill (1093 Sample Mill, Sweden) into uniformly fine powder. Finally, samples were sieved using a 0.25 mm mesh screen.

At the same time, groundwater depth was measured twice weekly using the electronic conductance method in a groundwater well imbedded into each plot.

Determination of foliar $\delta^{13}C$

From the same sample stock, the dried powder was weighed in small tin cups for stable C isotope analysis (0.06–0.09 mg C). C analysis samples were combusted using an elemental analyzer (Finnigan MAT DELTAplus, IRMS, Germany). The isotopic signature was obtained via a continuous-flow mass spectrometer (Finnigan MAT DELTAplus, IRMS, Germany). The resultant isotopic signature was expressed in δ notation as follows:

$$\delta^{13}C(‰) = \left[\frac{R_{sample}}{R_{stardand}} - 1\right] \times 1000$$

where $\delta^{13}C$ is the C isotope ratio in parts per mile (‰); $R = {}^{13}C/{}^{12}C$ for samples (R_{sample}) and for the
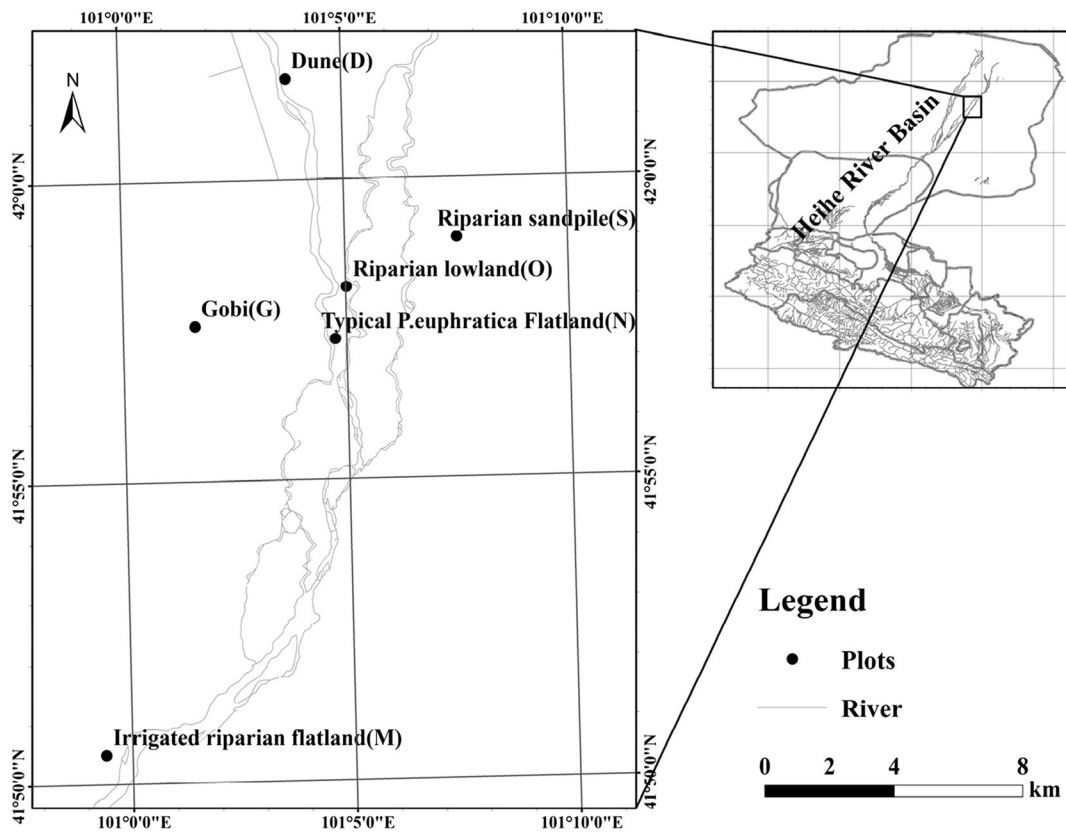
Fig. 1 Map of the Heihe River and the location of the study site

international standard ($R_{standard}$: PDB (Pee Dee Belemnite) for C. Overall long-term sample preparation and analysis error between repeated analyses of the same ground tissue was less than ±0.1‰.

Table 1 Monthly average of main climate variables for Ejina (1961–2005)

Month	Air temperature (°C)[a]	Maximum air temperature (°C)	Precipitation (mm)	Relative humanity (%)	Evaporation (mm)[b]	Wind speed (m/s)
Jan.	−11.3	−7.0	0.2	48.22	36.01	2.58
Feb.	−6.3	−1.4	0.2	36.38	69.17	2.83
Mar.	2.1	6	1.1	28.33	180.34	3.38
Apr.	11.3	15.7	1.2	23.33	342.22	4.14
May	19.2	21.4	3.1	22.36	495.03	4.13
Jun.	24.8	26.7	5.2	26.22	553.08	3.92
Jul.	26.8	29.8	9.4	32.69	555.75	3.64
Aug.	24.6	27.3	8.4	34.91	482.82	3.36
Sept.	17.7	20.3	3.6	33.11	339.14	2.98
Oct.	8.2	10.5	2.3	35.09	200.53	2.87
Nov.	−1.8	2.8	0.5	40.67	92.58	3.12
Dec.	−9.9	−4.6	0.2	49.38	39.99	2.74

[a] Measured at 1 m above the surface

[b] Water evaporation measured at the station

Table 2 The condition of six typical plots

Plots	Latitude and longitude	Altitude (m)	Soil type	Org. matter (g/kg)	Org. carbon (g/kg)	Total nitrogen (g/kg)	C/N	Available potassium (mg/kg)
Gobi (G)	41° 57′ 35.3″ N, 101° 01′ 36.5″ E	970	Coarse sand	0.63	0.37	0.07	5.29	87.61
Riparian sandpile (S)	41° 59′ 00.0″ N, 101° 07′ 30.7″ E	975	Fine sand	1.09	0.63	0.11	5.73	99.94
Riparian lowland (O)	41° 58′ 11.8″ N, 101° 05′ 00.9″ E	986	Fine sand	1.18	0.68	0.13	5.23	153.26
Typical *P. euphratica* flatland (N)	41° 57′ 20.5″ N, 101° 04′ 44.6″ E	995	Clay loam	4.99	2.89	0.41	7.05	170.61
Dune (D)	42° 1′ 40.86″ N, 101° 3′ 45.12″ E	990	Fine sand	0.89	0.52	0.08	6.5	80.16
Irrigated riparian flatland (M)	41° 50′ 28.7″ N, 100° 59′ 24.5″ E	982	Fine sand	1.29	0.68	0.19	3.58	115.56

Estimation of proline and glycine betaine content

Proline content was estimated according to Bates (Bates et al. [1973]). Of the leaf tissue, 5.0 g was homogenized in 30 % sulfosalicylic acid and then filtered. Added to the solution were 2 ml of filtrate, 2 ml of acid ninhydrin, and 2 ml of glacial acetic acid, and the solution was incubated for 1 h in a boiling water bath followed by an ice bath. At this point, 4 ml of toluene was added, and the solution was mixed vigorously. The chromophore containing toluene was aspirated during the aqueous phase, and absorbance was measured at 575 nm.

Glycine betaine (GB) content in leaf extracts was determined according to Storey and Jones ([1977])). Of the leaf tissue, 5.0 g was homogenized in 25 ml of isopropyl alcohol and centrifuged at $3000 \times g$ for 10 min. The supernatant was dried in a vacuum at 40 °C. The resultant residue was washed twice consecutively with chloroform (20 ml) and distilled water (5 ml). The two phases of six bulked washings were partitioned by centrifugation, and the upper aqueous layer was removed. The remaining lipid layer was washed twice in 20 ml of MeOH/H_2O (1:1). Combined aqueous layers were evaporated to dryness in a hot water bath and then re-dissolved in distilled water (5 ml). A potassium triiodide solution (0.2 ml) was added to 1 ml of the above extract, and the mixture was incubated for 90 min on an ice bath with intermittent shaking. Two milliliter of ice-cold distilled water was added to the mixture followed by 2 ml of 1,2-dichloroethane. The two layers were then mixed by a constant stream of air bubbles for 5 min, while temperature was maintained at 4 °C. Absorbance of the lower organic layer was measured at 365 nm.

Analysis of soluble sugars

Dried leaves (50 mg) were grounded in 2 ml of 80 % (*v/v*) ethanol and incubated at 75 °C for 10 min followed by centrifugation at $5000 \times g$ for 10 min. The supernatant was collected, and the residue was extracted twice as stated by the method above. Three extracts were assembled and diluted into volumes of 10 ml each. After extracts were laid on a worktable for 30 min and filtered, the filtrate was analyzed for total soluble sugars (TSS) and sucrose.

The content of TSS and sucrose was estimated according to Dubois et al. ([1956])) with minor changes. Specifically, 0.1 ml ethanol extract was drawn out, and 3 ml anthracene ketone reagent was added, which was confected by 150 μg anthracene ketone resolving in 100 ml diluted vitriol (76 ml vitriol in pouring into 30 ml of distilled water). The mixture was incubated at 90 °C for 15 min, cooled, and then, absorbance was measured at 620 nm in a 752-C spectrophotometer. Sucrose content was estimated by degrading reactive sugars present in a mixture of 0.1 ml extraction and 2 M NaOH boiled at 100 °C for 5 min. Three milliliters of freshly prepared anthrone reagent were then added to the cooled reaction product, and the mixture was boiled for 10 min, cooled, and read at 480 nm.

Determination of K^+, Ca^{2+}, and Cl^- concentrations

Foliar K concentrations were measured by means of flame ionization using the Model 6400-A analyzer (Shanghai, China), and concentrations were uniformly expressed as per 100 g of dry weight sample for ease of study. Ca^{2+} measurements were conducted by means of

an atomic absorption spectrophotometer according to the method by Issac (1980). Cl⁻ was colorimetrically determined with ferric ammonium sulfate and mercuric thiocyanate following the procedure by Guerrier and Patolia (1989)). Every measurement was replicated three times, and the mean was used for analysis.

Statistical analysis

Because there are at least three factors, season, habitat, water depth, involved in the experimental design for this study, in reality, the factors are not independent. However, water depth belongs to an implicit factor affected by season and habitat in the experiment. So, we firstly did a two-factor (season × habitat) variance analysis. Results showed that seasonal factor had a significant ($p<0.05$) impact on all physiological indexes, including proline, glycine betaine, total soluble sugars, and so on. For habitat factor, every indexes except for Ca^{2+} were also significantly affected ($p<0.05$). However, interaction of season and habitat only significantly affected Ca^{2+} and sucrose ($p<0.05$). Therefore, an analysis of variance (ANOVA) for variables obtained from measurements was used to test month differences in the same habitat. Pearson correlation coefficients were calculated to determine relationships between variables using individual species data. Statistical analysis was conducted using the SPSS 13.0 for Windows statistical software package (SPSS Inc., Chicago, USA).

Results

Changes in groundwater depth

Groundwater depth was shallowest in May for all plots, and it deepened during the advancement of the growth period (Fig. 2). Apparent differences existed between plots. For example, groundwater depth for the dune plot was deepest with a mean depth of −5.32 m followed in descending order by the Gobi Desert (−3.86 m mean), the riparian sandpile (−3.00 m mean), typical *P. euphratica* flatland (−2.33 m mean), riparian lowland (−2.15 m mean), and the irrigated riparian flatland plot (−1.88 m mean).

Changes in foliar $\delta^{13}C$ signature

Figure 3 showed that levels of $\delta^{13}C$ values in the *P. euphratica* for different plots throughout the whole

growing season ranged from −29.14±0.06 to −25.84±0.04‰ with a mean of −27.80±0.04‰. Moreover, $\delta^{13}C$ signatures from the deepest groundwater depth were significantly richer than those from other groundwater depths in plots during the same period. Changes in mean $\delta^{13}C$ signatures for each plot during the growing season indicated that $\delta^{13}C$ signatures became richer with a decline in groundwater depth. Although an overall decreasing trend was found for different plots, there was also a slight trend in increasing richness between June and September. Trends were apparently the highest in May and the lowest in July for all plots during the growing season with the exception of the Gobi Desert plot where the lowest $\delta^{13}C$ value appeared in August. Significant site differences were determined each month ($p<0.05$).

Variation in sugar levels

TSS and sucrose exhibited different trends for different plots during the growing season (Fig. 4a, b). The content of TSS and sucrose during the whole growing season ranged between 2.69±0.25 mg/g dry weight (DW) and 8.35±0.11 mg/g DW (5.59±0.13 mg/g DW mean) and between 2.46±0.05 and 6.24±0.19 mg/g DW (4.79±0.11 mg/g DW mean), respectively. For the irrigated riparian flatland, riparian lowland, and typical *P. euphratica* flatland plots, a decreasing trend was found between May and September. However, in the riparian sandpile, Gobi Desert, and dune plots, TSS, and sucrose content increased between May and July or August and then declined thereafter until the season ended. For the dune plot, TSS content was the highest in June. Significant monthly differences were found for TSS and sucrose between the six plots ($p<0.05$) (Fig. 4a, b). The highest mean TSS and sucrose content was found in the dune plot (7.09±0.15 and 5.62±0.16 mg/g DW, respectively) followed in descending order by the Gobi Desert, riparian sandpile, and riparian flatland plots, but the lowest TSS content was found in the riparian lowland plot with a mean value of 4.36±0.16 mg/g DW. The lowest sucrose content was found in the irrigated riparian flatland plot with a mean value of 3.70±0.05 mg/g DW. Overall, the mean content of TSS and sucrose for the different plots investigated during the growing season increased with deepening groundwater depth. In other words, they were accumulated in foliage when *P. euphratica* experienced water deficient;

Fig. 2 Change in groundwater depth for different plots throughout the 2008 growing season. *M*, *O*, *N*, *S*, *G*, and *D* represent the irrigated riparian flatland, riparian lowland, typical *P. euphratica* flatland, riparian sandpile, Gobi Desert, and dune plots, respectively
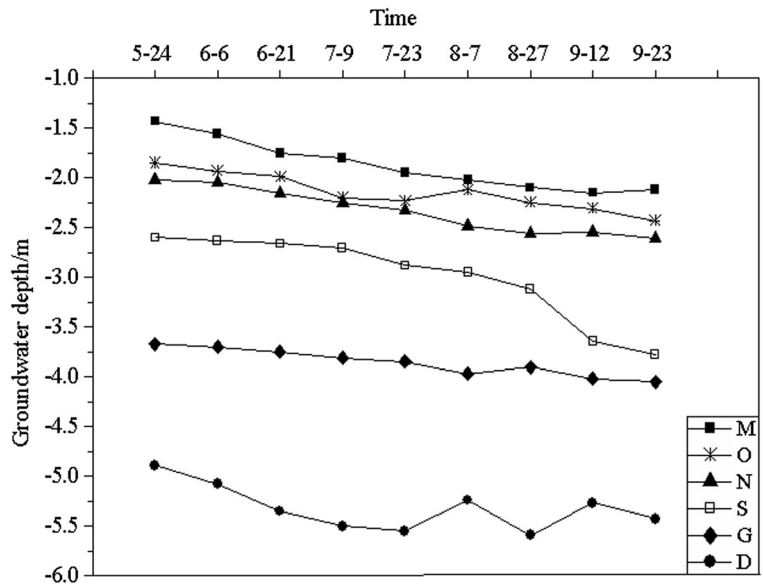
otherwise, TSS and sucrose decomposed and their content decreased.

Changes in proline and glycine betaine

Proline and GB content during the whole growing season ranged between 0.03 ± 0.00 and 0.77 ± 0.01 mg/g DW (0.24 ± 0.00 mg/g DW mean) and between 1.12 ± 0.05 and 3.76 ± 0.07 mg/g DW (1.80 ± 0.05 mg/g DW mean), respectively (Fig. 5a, b). Foliar proline and GB content in the *P. euphratica* during each stage were definitively lower in shallower groundwater depth plots and continually increased between May and September

in the different plots investigated, especially in the riparian sandpile, Gobi Desert, and dune plots ($p<0.05$). Furthermore, proline and GB gradually accumulated with increasing groundwater depth. Proline and GB content in the dune plot was respectively higher by a factor of 4.23 and 1.82 compared to the irrigated riparian flatland plot.

Variations in inorganic ions

Foliar K^+ content throughout the whole growing season ranged between 0.32 ± 0.001 and 1.78 ± 0.11 % (1.09 ± 0.05 % mean) (Fig. 6a). Concentrations of foliar K^+ in

Fig. 3 *P. euphratica* foliar $\delta^{13}C$ value trends for different groundwater depths. Means of data were taken from three replicates in 2008. *Vertical bars* denote SE of means. *Different lowercase letters* denote significant differences ($p<0.05$). *M*, *O*, *N*, *S*, *G*, and *D* represent irrigated riparian flatland, riparian lowland, typical *P. euphratica* flatland, riparian sandpile, Gobi Desert, and dune plots, respectively
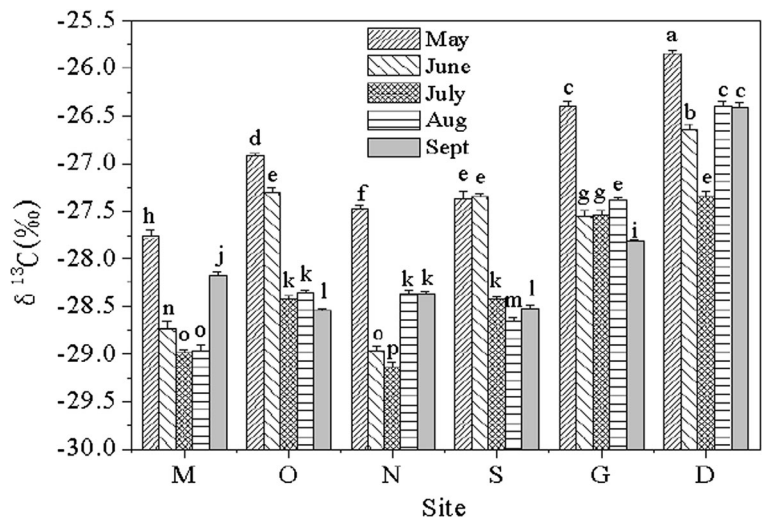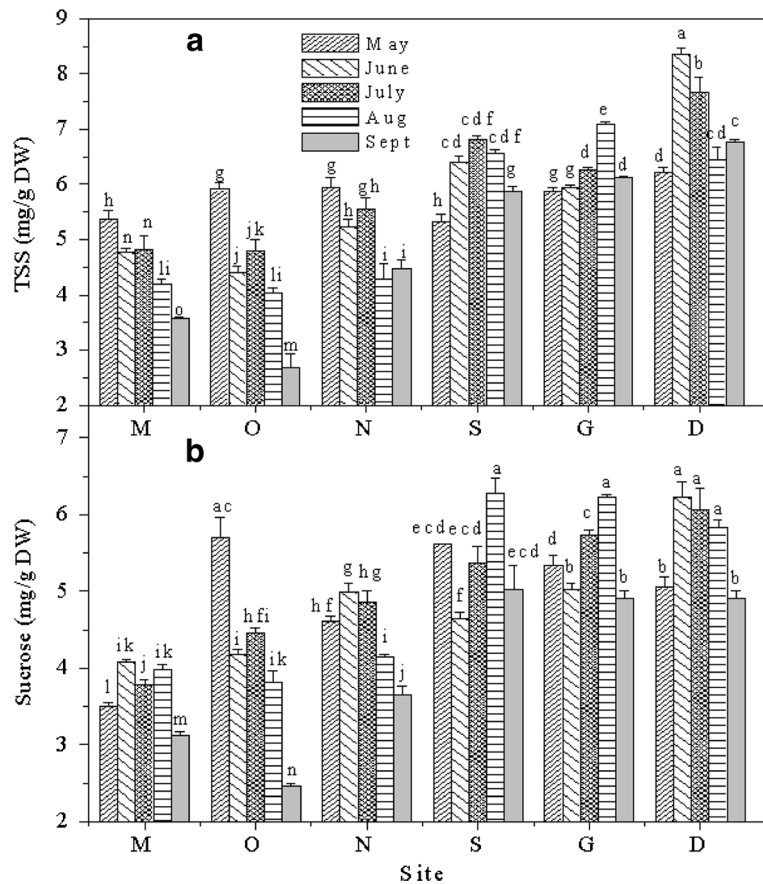
Fig. 4 Changes in content of TSS (**a**) and sucrose (**b**) in *P. euphratica* foliage for different groundwater depths. Symbols and abbreviations the same as per Fig. 3


the *P. euphratica* for each month was definitively lower for shallower groundwater depth plots and continually decreased between May and September in the different plots ($p < 0.05$). Furthermore, K^+ concentrations significantly increased during declining processes of groundwater depth. Mean K^+ content for the dune plot was higher by a factor of 2.24 compared to the irrigated riparian flatland plot.

As Fig. 6b showed, foliar Ca^{2+} content in the *P. euphratica* was higher in the shallowest groundwater depth plot, ranging between 1.36 ± 0.04 and 5.73 ± 0.02 mg/g DW with a mean of 3.74 ± 0.03 mg/g DW. Seasonal variation in Ca^{2+} content for each plot was similar. An increase was found between May and September before accumulating at the end of the growing season. The lowest Ca^{2+} content was found in the dune plot with a mean value of 2.50 ± 0.03 mg/g DW, while the highest was found in the irrigated riparian flatland plot in September with a value of 5.73 ± 0.02 mg/g DW. Mean Ca^{2+} content for other plots closely matched the plots above. At the same time,

Ca^{2+} content for each month in shallower groundwater depth plots increased more pronouncedly than for the other deeper groundwater depth plots.

Cl^- content values for the six plots during the whole growing season ranged between 15.99 ± 0.07 and 32.27 ± 0.11 mg/g DW with a mean value of 23.04 ± 0.09 g/g DW (Fig. 6c). Seasonal content of *P. euphratica* foliar Cl^- for different plots indicated that the Cl^- level obtained from the water-deprived dune plot was significantly lower than those from plots with sufficient water resources, such as the irrigated riparian flatland, riparian lowland, and typical *P. euphratica* flatland plots. The mean Cl^- content for the irrigated riparian flatland plot was higher by a factor of 1.56 compared to the dune plot. However, mean Cl^- content for the Gobi Desert plot, having a deeper groundwater depth than the abovementioned three sites, was clearly higher than those of the riparian lowland and riparian flatland plots. Seasonal trends between May and September were similar between the six plots. Cl^- was generally accumulated in foliage by the end of the growing season.

Fig. 5 Changes in content of proline (**a**) and glycine betaine (**b**) in *P. euphratica* foliage for different groundwater depths. Symbols and abbreviations the same as per Fig. 3
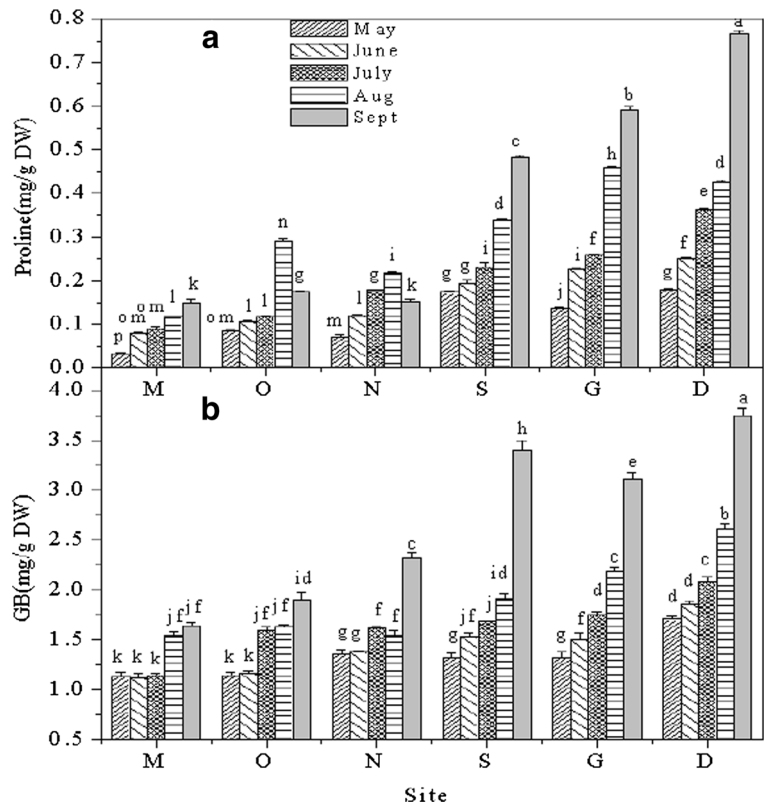
Concentrations were therefore obviously higher during September with the exception of the irrigated riparian flatland plot where the highest Cl^- content was found in August.

Correlations between $\delta^{13}C$, physiological properties, and groundwater depth

Table 3 showed correlations between $\delta^{13}C$, each osmotic solute, and groundwater depth during the growing season. Correlation analyses showed that foliar $\delta^{13}C$ values in *P. euphratica* were significantly and positively correlated to TSS and sucrose ($p<0.05$) and had an extreme significant positive relationship with K^+ content ($p<0.0001$). No significant correlations were found between both proline and GB to $\delta^{13}C$, but significant negative correlations were found between $\delta^{13}C$ to Ca^{2+} and Cl^- ($p<0.01$). Foliar TSS content was extremely and significantly positively correlated to sucrose and K^+ ($p<0.0001$). Furthermore, a significant positive correlation was found between TSS and proline ($p<0.05$), and a negative correlation was found between Ca^{2+} ($p<0.05$) and Cl^- ($p<0.01$). Extreme and significant

positive correlations ($p<0.0001$) were also found between sucrose and K^+, proline and GB, and Ca^{2+} and Cl^-, respectively. GB was positively correlated to Ca^{2+} ($p<0.05$). The relationship between Ca^{2+} and K^+ was significantly negative ($p<0.05$) (Table 3). Furthermore, extreme significant negative correlations ($p<0.0001$) were found between $\delta^{13}C$, TSS, sucrose, proline, GB, and K^+ to groundwater depth, respectively. That is to say, these values increased with a decline in groundwater depth. However, Ca^{2+} and Cl^- contents were weakly and positively correlated to groundwater depth.

Discussion

Foliar $\delta^{13}C$ values in the *P. euphratica* for the six study plots were investigated, and all exhibited a declining seasonal trend with the highest values to be found in spring, which was probably due to the more positive $\delta^{13}C$ signature connected with storage-derived C as observed in previous studies (Damesin and Lelarge 2003; Helle and Schleser 2004) and the degree of leaf maturity (Garten and Taylor 1992; Su et al. 2003).

Fig. 6 Changes in content of K^+ (**a**), Ca^{2+} (**b**), and Cl^- (**c**) in *P. euphratica* foliage for different groundwater depths. Symbols and abbreviations the same as per Fig. 3
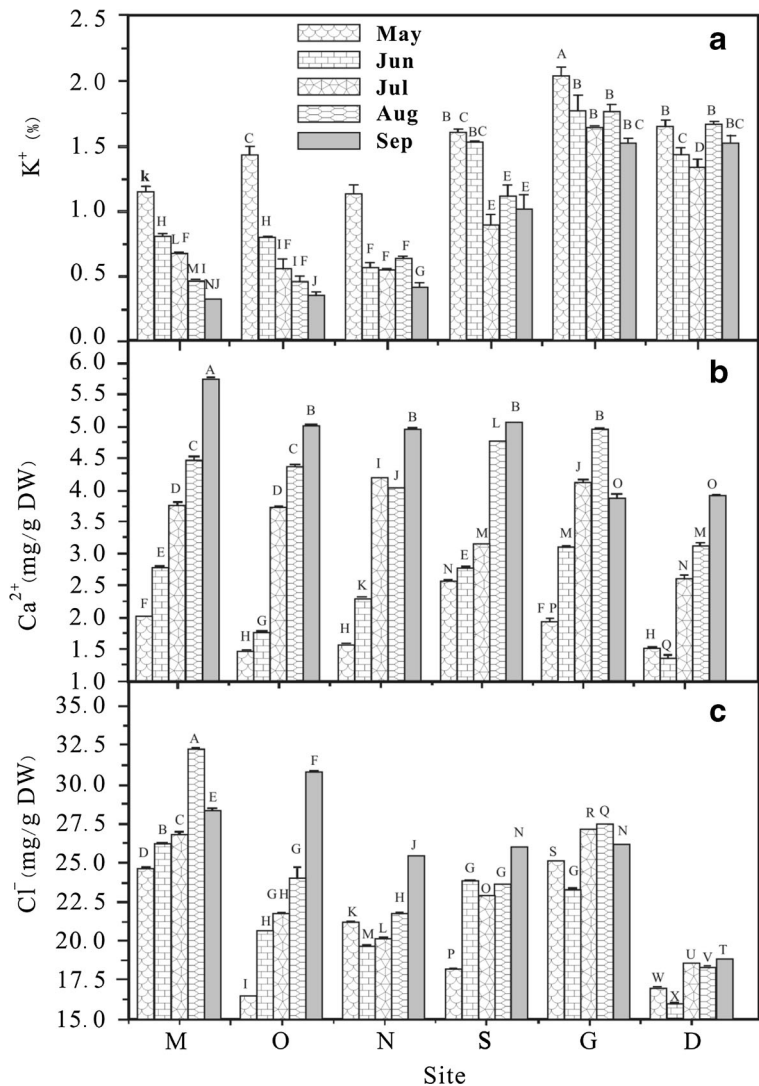
Furthermore, the present study found significant variations in foliar $\delta^{13}C$ values among the six plots (Fig. 3). The highest $\delta^{13}C$ value was found in the plot with the deepest groundwater depth, and the lowest was found in the plot with the shallowest groundwater depth. Foliar $\delta^{13}C$ values gradually increased with a decline in groundwater depth, indicating that *P. euphratica* WUE increased as water availability decreased and its water use strategy abated and became more conservative. This observation is consistent with the predicted response of $\delta^{13}C$ to water deficits as it is in agreement with previous studies (Sandquist and Ehleringer 2003; Seghatoleslami et al. 2008). The cause for this can be attributed to stomatal regulation of gas exchange under drought conditions. First, increases in $\delta^{13}C$ values along with

deepening groundwater tables are likely caused by simple stomatal control of transpiration loss. Second, any additional stomatal effect (rubisco activity and photosynthetic capacity) on photosynthesis could be invoked to concurrently influence $\delta^{13}C$ in *P. euphratica* foliage under conditions of prolonged drought conditions. Such effects would result in greater negative $\delta^{13}C$ values, partially counterbalancing increases in intrinsic WUE due to stomatal control of transpiration. During photosynthesis, plant species discriminate against $^{13}CO_2$ over the lighter and more abundant $^{12}CO_2$, and discrimination is greatest when stomatal conductance (g_s) is high (Farquhar et al. 1989). In contrast, when the stomata is partially or completely closed, nearly all CO_2 inside foliage reacts by activating ribulose-bisphosphate

Table 3 Correlations among $\delta^{13}C$, osmotic solutes, and groundwater depths

Index	$\delta^{13}C$	TSS	Sucrose	Proline	GB	K^+	Ca^{2+}	Cl^-	GWD
$\delta^{13}C$	1.000								
TSS	0.453*	1.000							
Sucrose	0.530**	0.869***	1.0000						
Proline	0.273	0.453*	0.387*	1.000					
GB	0.183	0.309	0.196	0.912***	1.000				
K^+	0.791***	0.727***	0.71***	0.373*	0.193	1.000			
Ca^{2+}	−0.551**	−0.391*	−0.318	0.350	0.430*	−0.447*	1.000		
Cl^-	−0.539**	−0.519**	−0.533**	−0.08	0.007	−0.355	0.662***	1.000	
Groundwater depth (GWD)	−0.640***	−0.700***	−0.608***	−0.717***	−0.649***	−0.625***	0.075	0.372*	1.000

*$p<0.05$, **$p<0.01$, ***$p<0.0001$

(RuBP) carboxylase, and there is little fractionation of C isotopes. It is for this reason that plant tissue $\delta^{13}C$ is directly related to average g_s during growth, providing an integrated measure of WUE. In addition, differences in the air vapor pressure deficit (VDP) between plots would also cause considerable differences in g_s and therefore WUE regardless of differences in groundwater depth. Although this requires further study, the present study concluded that in the absence of other physiological measurements, results obtained by C isotope analysis could lead to underestimations related to effective stress levels experienced by the *P. euphratica*.

Water stress is considered the primary environmental factor limiting plant growth and yields worldwide, especially in arid and semi-arid regions. Exposed plant species have to minimize water losses and thereby maintain a favorable water status for development. Cells must then develop a sufficiently low osmotic potential to reverse the flow of water, either through the uptake of ions from the medium or by synthesis and transport of organic compounds. Thus, osmotic adjustment helps plant cells to withstand water deficits by maintaining sufficient turgor for growth (Carvajal et al. 1999). Results from this study indicated that one such mechanism of *P. euphratica* response to drought conditions, especially to water stress, was to accumulate osmotic solutes to resist water deficiency for an environment under sustained drought conditions. Water conditions can affect accumulation levels of soluble sugars. Figure 4a, b showed that the content of TSS and sucrose in the foliage was higher for plots with deeper groundwater depths compared to those with shallower groundwater depths, which may contribute to the fact that water deficiency often inhibits starch synthesis and accelerates decomposition (Chen and Burris 1990). According

to a previous report, it has been proposed that soluble sugars in higher plant species have two primary functions: (1) as a transportable and storable form of sugar derived through photosynthesis and (2) as a compatible solute that can maintain cell turgor during osmotic adjustment in plant tissues in response to water deficiency (Chen and Burris 1990). In this study, both functions may in fact be relevant for foliar soluble sugars as it pertains to *P. euphratica*. These functions may have initially reacted to the shallower groundwater depth plots, such as the irrigated riparian flatland, riparian lowland, and typical *P. euphratica* flatland plots, given that some TSS (primarily sucrose) was found to have accumulated and lower concentrations at later stages, which were lower than that found in the dune, Gobi Desert, and riparian sandpile plots. However, the response of the latter three plots (the dune, Gobi Desert, and riparian sandpile plots) was the reverse. Therefore, TSS and sucrose perhaps played a more important role in osmotic adjustment (Fig. 4a, b).

Proline is an important osmoticum in higher plant species. Many plant species accumulate proline under a broad range of stress conditions, such as water shortages, salinity, extreme temperatures, and high sunlight intensity (Maggio et al. 2000; Bano et al. 2009). The potential benefit of proline accumulation has been proposed in several ways: as a compatible osmotic solute (Handa et al. 1986), as a protein-stabilizing or solubilizing factor under limited cell water conditions (Blackman 1992), and as a source of reduced nitrogen (N) and C (Xie et al. 1997). The beneficial role of proline as it relates to plant stress tolerance as suggested by previous correlative studies has been recently confirmed by genetic as well as transgenic studies, which demonstrated that proline could increase plant tolerance to abiotic

stresses (Xiong and Zhu 2002). In this study, foliar proline concentration in *P. euphratica* during each stage was definitively lower for shallower groundwater depth plots. This was especially true in riparian sandpile, Gobi Desert, and dune plots for which proline content increased continually between May and September (Fig. 5a). Proline content in the dune plot was the highest. Furthermore, proline gradually accumulated as groundwater depth increased during the growing season. This indicated that water availability could result in plant water deficits and further lead to differing proline levels. Barker et al. (1993) reported that proline accumulated exponentially in foliage of five forage grasses as soil water content decreased during each dry-down period. That study and results from the present study both agreed that plant proline content could clearly reflect water availability in soil. Water deficit is the primary factor that controls proline accumulation. However, in this study, proline accumulation was lower. In the driest plot investigated (the dune plot), mean proline content was only 0.40 mg/g DW, taking into account that its value was 0.09 mg/g DW for the wettest plot. Ruan et al. (2008) reported a similar trend. This also indicated that proline must act as an osmotic solute, but the role of proline as it relates to *P. euphratica* response to water stress may be less significant due to its lower content. Further quantitative research must be conducted to verify such findings.

Apart from proline, GB has also been shown to protect enzymes and membranes and is known to stabilize photosystem II protein pigment complexes under stressful conditions (Papageorgiou and Morata 1995). In the present study, GB content throughout the whole growing season ranged between 1.12 ± 0.05 and 3.76 ± 0.07 mg/g DW (1.80 ± 0.05 mg/g DW mean) (Fig. 5b). *P. euphratica* foliar GB content during each stage was definitively lower for the shallower groundwater depth plots. Especially for the riparian sandpile, Gobi Desert, and dune plots, GB content continually increased between May and September. At the same time, GB was significantly accumulated with an increase in groundwater depth throughout the growing season. This indicated that GB accumulation resulted in the enhanced tolerance of *P. euphratica* to water deficits through the adjustment and protection of the thylakoid membrane, thereby maintaining photosynthetic efficiency.

A sharp seasonal increase in K^+ concentration was detected under drier conditions (Fig. 6a), which perhaps facilitated osmotic adjustment, and at the latter stages of the growing season, especially for extensions and

various movements of individual cells in leaf tissues through the regulation of g_s, which stimulated photosynthesis and reduced transpiration to maintain turgor pressure at lower foliar water potentials while improving the ability of plants to tolerate drought stress (Andersen et al. 1992; Khan et al. 2009). Foliar K^+ concentration for *P. euphratica* during each month was definitively higher for deeper groundwater depth plots, and *P. euphratica* K^+ concentration was significantly accumulated with a decline in groundwater depth (Fig. 6a). These findings indicated that sufficient potassium (K) nutrition uptake enhanced drought resistance and WUE capacity of *P. euphratica* to maintain its minimum growth rates under drought conditions.

However, larger increases in calcium (Ca) and chloride (Cl) ion content were found in wetter plots with seasonal changes, but the reverse was true for drier plots (Fig. 6b, c). This showed that the two chemical elements accumulated while coupling with stronger transpiration during the growth period and did not readily leach from living and senesce foliage owing to their relative immobilization in pectate and on membranes (Adamec 2002). Furthermore, Cl content was significantly higher than Ca^{2+} content, suggesting that Cl^- may exist in forms of other chloride, such as NaCl and $MgCl_2$. On the other hand, a reduction in Ca^{2+} and Cl^- content was associated with a decline in groundwater level. This showed that higher salinity in groundwater can induce an increase in Ca^{2+} and Cl^- concentration in the foliage. According to a study by Liu et al. (2008), the soil-water chemical type was high Ca^{2+}-Mg^{2+}-Cl^--SO_4^{2-}. For shallower groundwater depth plots, where stronger soil evaporation and plant transpiration occurred, leading to salinity, Ca^{2+} and Cl^- were accumulated in plant foliage. Accumulation of high concentration of ions from external mediums can produce a toxic effect on normal biochemical activities of cells. For example, Cl^- in expanded foliage is associated with chlorosis and mortality (Greenway and Munns 1980). Thus, although inorganic ions are sequestered in vacuoles or cytoplasms (Voetberg and Sharp 1991), their higher concentrations, especially related to Ca^{2+} and Cl^-, may have a toxic effect on the healthy growth of *P. euphratica*. Moreover, correlations between groundwater depth to K^+, Ca^{2+}, and Cl^- also showed that K^+ was a key inorganic osmotic ion but Ca^{2+} and Cl^- were not.

The extreme and significant negative correlations were found between groundwater depths, and each physiological index also demonstrated the validity of the preceding discussion (Table 3). TSS, sucrose, proline, GB, and K^+

were the primary osmotic solutes activated when *P. euphratica* encountered water stress. As a consequence of such net accumulation, cell osmotic potential decreased while turgor pressure tended to be maintained. This allowed *P. euphratica* to uptake more groundwater while maintaining turgor and cell function for a longer period of time under drought conditions. At the same time, the significantly negative relationship between $\delta^{13}C$ values and groundwater depths and the positive TSS, sucrose, and K^+ found not only indicated that WUE increased with decreasing water supplies (that is, *P. euphratica* water use patterns became more conservative when faced with water deficits), it also verified that $\delta^{13}C$ itself is a comprehensive physiological osmotic index. The weakly positive relationship between proline, GB, and $\delta^{13}C$ showed that proline levels had no obvious effect on WUE. This result was in accordance with reports by McCree and Richardson (1987) and Ludlow et al. (1990), indicating that proline and GB did not directly improve the water use capacity of plant species. The fact of the matter is that it may have indirectly positive effects on WUE by controlling stomatal conductance, maintaining leaf area, and facilitating water absorption (Xiong and Zhu 2002). These findings are in opposition to other studies that state that proline accumulation could enhance plant WUE under water stress conditions (Naidu et al. 2000). Thus, correlations between proline, GB, and $\delta^{13}C$ as well as their effects must be further demonstrated.

In summary, WUE enhancement and the accumulation of osmotic solutes in the *P. euphratica* foliage are its adaptive means to hyper-arid environments. The primary osmotic solutes found were TSS, sucrose, proline, GB, and K^+. Ca^{2+} and Cl^- content only showed that the *P. euphratica* was a high salinity-tolerant species, and their function related to osmotic adjustments may be less than first thought. However, their quantitative contribution to cell osmotic potential reduction must be further studied. Moreover, recommending that groundwater depths should be maintained at a range between 2.5 and 3.5 m to attain the healthy growth of *P. euphratica*. However, results from this study must be further investigated since observed differences may also have a genetic basis rather than simply a representation of plant adjustment.

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