Seasonal and annual variation of osmotic solute and stable carbon isotope composition in leaves of endangered desert evergreen shrub *Ammopiptanthus mongolicus*

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Abstract

In order to elucidate the seasonal and annual variation, leaves of *Ammopiptanthus mongolicus* were collected in 3 consecutive years from natural (drought stressed) and irrigated (water supplemented) fields at Shapotou in Tengger Desert, in which stable carbon isotope composition ($\delta^{13}C$) and major osmotic solute such as proline, total soluble sugar (TSS), especially sucrose and glucose, as well as inorganic cations ($K^+$, $Ca^{2+}$, $Mg^{2+}$, $Na^+$) were measured. Contents of osmotic solutes involved in osmotic adjustment varied with growth periods. At the early stage, they were mainly soluble sugars and inorganic cations, especially $Ca^{2+}$ and $Mg^{2+}$; among them, glucose was the principal osmotic agent. At the late stage, besides soluble sugar, inorganic cations, free amino acids, especially proline, also play an important role in osmoregulation due to contents of proline and potassium increased continually under both natural and water supplemented conditions. In all growth stage, relatively higher $\delta^{13}C$ can be observed in natural site of *A. mongolicus* rather than in irrigated ones. Variations between contents of osmotic solute and $\delta^{13}C$ value under two water conditions have been revealed by multiple variable regression analysis. Variation trends of osmotic solute and $\delta^{13}C$ value coincided with precipitations in 3 years. The results obtained by the present study would help understand the seasonal and annual change of osmotic solutes and adaptive mechanism to severe environment of the plant as well as strengthen the protection of this endangered evergreen species in this area.

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Keywords: *Ammopiptanthus mongolicus*; Endangered species; Evergreen; Osmotic solute; $\delta^{13}C$; Seasonal and annual variation

1. Introduction

*Ammopiptanthus mongolicus* (Maxim. ex Kom) Cheng f., an evergreen xerophyte of Mesquite in Mid-Asian desert, an ancient relic from the Tertiary period, has been listed as a primary protected plant by Chinese government. The species shows strong tolerance to high and low temperature, extreme salinity and drought, prevents sand-drift triggered by strong north–west and north–east winds, and provides fuel, fodder and timber for local people and livestock. In addition, the species has many ecological and economic values and act as an ornamental and medicinal desert plant (Li et al., 1999). Due to increasing population and consequent demand for fuel, it has been over-exploited, resulting in the decrease of its population, desertification and destruction of its surrounding arable land (Wu, 1983).

Many studies have focused on the relationship between plant and environment using $\delta^{13}C$, which reflects not only a photosynthetic pathway, but is also a good indicator of environmental factors, especially the long-term water status of plant in physiology and growth (Feng et al., 2003; Merah et al., 2001; Su et al., 2003), and osmotic adjustment induced under artificial conditions (treated with NaCl, PEG, etc.) has also traced the course of osmotic adjustment in field plants (Evans et al., 1992). As for *A. mongolicus*, although some work has been conducted on its morphology (Yang and Wang, 1991), photosynthesis, respiration (Li et al., 1999), water use and nitrogen metabolism
(Huang, 1979; Xu et al., 2002), little is known about variations in osmotic solutes and the adaptive mechanism to extreme environment especially under water stress.

Only two stable carbon isotopes, $^{12}\text{C}$ and $^{13}\text{C}$, exist in nature. During the process of photosynthesis, the heavier $^{13}\text{C}$ isotope of atmospheric CO$_2$ is discriminated against the lighter $^{12}\text{C}$, leading to the poorer ratios of $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$ values) in plant tissues, organs or chemical compositions. Therefore, it is essential to study these variations of osmotic solute and $\delta^{13}\text{C}$ value in leaf tissue systematically, which would indicate water status and adaptive mechanisms of this species to harsh environment and for the protection of the endangered plant.

In order to investigate the adaptive mechanisms of this evergreen desert shrub around the Yellow River that supported the ancient Silk Road in the Tenger Desert. In particular, we sought to quantify the seasonal and annual changes in leaf osmotic solute and $\delta^{13}\text{C}$ value. Leaves of $A.\ mongolicus$ living in natural and water supplemented environments at Shapotou in western China (one of the world’s most harsh environments owing to its location) were used at the end of every month during the vegetation period in 2002, 2003 and 2004 to assess seasonal and annual variations in different field conditions through the analysis of free amino acids (especially proline), total soluble sugars (TSS) (especially sucrose and glucose), inorganic cations (especially K$^+$, Ca$^{2+}$, Mg$^{2+}$, Na$^+$) and $\delta^{13}\text{C}$ value ($\delta^{13}\text{C} / \delta^{12}\text{C}$ ratio). Three major objectives were included in the present investigation: (a) to study the seasonal and annual changes in different kinds of osmotic solutes under the deficient (natural) and sufficient (irrigated) water conditions; (b) to attempt to quantify variation between the $\delta^{13}\text{C}$ and the contents of osmotic solute; and (c) to make clear the adaptive mechanisms of the species to extremely droughty environment and search for the protective strategy.

2. Materials and methods

2.1. Site description and sampling

The study site is located at the southeast edge of the Tenger Desert in Zhongwei County, Ningxia Autonomous Region (104°57'6"E, 37°47'40"N, 1140 m. alt.) in western China. Due to the Mongolian high pressure system, the climate of Shapotou tends to be of temperate arid desert with strong winds. It receives a very small amount of precipitation each year and is subject to large annual temperature ranges and frequent sandstorms, with a mean annual temperature of 9.7 °C (35 °C in summer, −20 °C to −30 °C in winter), an annual average rainfall of 186.2 mm, mostly in June to August, and an annual evaporation of 3000 mm. Water from rainfall accounts for 3.1% of the available soil moisture and the low level of groundwater, the highly permeable calcareous sandy soil with scarce clay particles and the high evaporation determines the area to be of arid desert. This is clearly a hostile environment in which only a few perennial species can survive.

$A.\ mongolicus$ (Maxim. ex Kom.) Cheng f. is a relic dominant xerophyte shrub in Tenger Desert and accompanied by $Caragana korshinskii$, $Artemisia ordosica$, $Calligonum spp.$, $Zygophyllum xanthozylon$, $Haloxylon ammodrendron$ and $Agriphyllum squarrosum$. $A.\ mongolicus$ flowers from April to May and the seed matures in June (Wu, 1983).

Table 1

<table>
<thead>
<tr>
<th>Osmotic solute (%)</th>
<th>Year</th>
<th>Seasons</th>
<th>2004</th>
<th>2003</th>
<th>2002</th>
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<tbody>
<tr>
<td>Glucose$^1$</td>
<td>May</td>
<td>Natural</td>
<td>40.20±0.0938$^a$</td>
<td>33.03±0.8841$^b$</td>
<td>40.30±0.4918$^a$</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>Water supplemented</td>
<td>40.30±0.4918$^a$</td>
<td>33.05±0.4719$^b$</td>
<td>40.27±0.7775$^b$</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>Water supplemented</td>
<td>40.27±0.7775$^b$</td>
<td>33.02±0.7909$^d$</td>
<td>40.28±0.9938$^b$</td>
</tr>
<tr>
<td>Sucrose$^2$</td>
<td>May</td>
<td>Natural</td>
<td>7.56±0.2176$^a$</td>
<td>12.47±0.6148$^a$</td>
<td>7.52±0.2734$^a$</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>Water supplemented</td>
<td>7.52±0.2734$^a$</td>
<td>12.41±0.6240$^a$</td>
<td>7.54±0.2154$^a$</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>Water supplemented</td>
<td>7.54±0.2154$^a$</td>
<td>12.44±0.3385$^a$</td>
<td>7.56±0.2176$^a$</td>
</tr>
<tr>
<td>Proline$^3$</td>
<td>May</td>
<td>Natural</td>
<td>19.68±0.6789$^b$</td>
<td>24.45±0.9083$^b$</td>
<td>19.40±0.4969$^c$</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>Water supplemented</td>
<td>19.40±0.4969$^c$</td>
<td>24.10±0.5902$^b$</td>
<td>19.54±0.2738$^c$</td>
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<td>Water supplemented</td>
<td>19.54±0.2738$^c$</td>
<td>24.28±0.6097$^b$</td>
<td>19.68±0.6789$^b$</td>
</tr>
<tr>
<td>Potassium$^4$</td>
<td>May</td>
<td>Natural</td>
<td>30.70±0.6304$^a$</td>
<td>49.65±1.5254$^a$</td>
<td>30.38±0.7722$^a$</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>Water supplemented</td>
<td>30.38±0.7722$^a$</td>
<td>49.63±1.3786$^a$</td>
<td>30.34±0.6469$^a$</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>Water supplemented</td>
<td>30.34±0.6469$^a$</td>
<td>49.60±1.0446$^a$</td>
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</tr>
<tr>
<td>Magnesium$^5$</td>
<td>May</td>
<td>Natural</td>
<td>37.19±0.8638$^a$</td>
<td>57.66±1.2349$^a$</td>
<td>37.14±0.9923$^a$</td>
</tr>
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<td></td>
<td>July</td>
<td>Water supplemented</td>
<td>37.14±0.9923$^a$</td>
<td>57.58±1.2259$^a$</td>
<td>37.16±1.1173$^a$</td>
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<tr>
<td></td>
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<td>Water supplemented</td>
<td>37.16±1.1173$^a$</td>
<td>57.62±1.4447$^a$</td>
<td>37.19±0.8638$^a$</td>
</tr>
<tr>
<td>Sodium$^7$</td>
<td>May</td>
<td>Natural</td>
<td>3.34±0.1159$^a$</td>
<td>3.33±0.1237$^a$</td>
<td>3.33±0.0706$^a$</td>
</tr>
<tr>
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<td>July</td>
<td>Water supplemented</td>
<td>3.33±0.0706$^a$</td>
<td>3.32±0.1386$^a$</td>
<td>3.32±0.0798$^a$</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>Water supplemented</td>
<td>3.32±0.0798$^a$</td>
<td>3.32±0.0713$^a$</td>
<td>3.34±0.1159$^a$</td>
</tr>
</tbody>
</table>

1: Glucose (%)=(glucose): (total soluble sugar)×100%; 2: sucrose (%)=(sucrose): (total soluble sugar)×100%; 3: proline (%)=(proline): (free amino acid)×100%; 4: potassium (%)=(potassium): (inorganic cation)×100%; 5: calcium (%)=(calcium): (inorganic cation)×100%; 6: magnesium (%)=(magnesium): (inorganic cation)×100%; 7: sodium (%)=(sodium): (inorganic cation)×100%. The same letter at superscript of each numerical value in each osmotic solute represents no significant difference between them but different letter of those in each osmotic solute means significant difference between them; $p<0.05$.
Two sampling sites were selected: (a) at the north side of the Bao-Lan railway, where all individuals with 1.0 m average height are local species in natural (drought stressed) conditions. The precipitation was only 226.2 mm (2002), 294.4 mm (2003) and 121.0 mm (2004) during the growth period, which determines the plants to be drought stressed in this area; and (b) in the nursery garden of Shapotou Desert Experimental Research Station of the Chinese Academy of Sciences close to the Yellow River, to where 10 plants with their soil had been transplanted from their natural site (described above) in spring 2001 and been irrigated twice a month on the 1st and 16th day (water supplemented) to ensure enough water supply.

Sixty leaves or so were cleaned and collected from each of the six similar plants growing in fully expanded canopy on sunny days (4 to 6 days after irrigation) in May, July and September of 2002, 2003 and 2004, frozen in liquid nitrogen immediately after being separated into portions of 0.5 g fresh weight (FW) and then taken to lab.

2.2. Determination of $\delta^{13}C$

The samples were dried at 80 °C in oven to a constant weight. After being ground into powder with 20–40 mesh sieves, 7 mg samples were combusted to CO$_2$ in the presence of pre-cleaned cupric oxide in evacuated and sealed tubes at 600 °C for 3 h. The CO$_2$ was dried cryogenically (cold ethanol, −80 °C) and collected with liquid nitrogen. Following mass-spectrometric analysis of the CO$_2$, the $^{13}C/^{12}C$ ratios were expressed as $\delta^{13}C$ (per mil unit) with respect to the VPDB (Vienna Pee-Dee Belemnite) calcite standard (Craig, 1957):

$$\delta^{13}C (\%e) = \left( \frac{R_{sa}}{R_{st}} - 1 \right) \times 1000$$

where $R_{sa}$ and $R_{st}$ are the ratios $^{13}C/^{12}C$ of the sample and the standard, respectively. The overall precision is less than ±0.1%.

2.3. Contents of proline, sugar and inorganic cations

Samples frozen in liquid nitrogen were lyophilized and weighed. Proline was determined according to Branett and Naylor (1966) with some modification. 1 g samples were extracted in ethanol (v/v=80%) for 30 min and centrifuged at 1500×g for 15 min at 4 °C four times. After the compounded supernatant was reduced to 5 ml in water at 80 °C, 5% (v/v) aqueous sulphosalicylic acid was added in the proportion of 1:1 (v/v). The supernatant centrifuged (18,000×g, 4 °C, 15 min) was used to determine the proline content by using an amino acid analyzer (Amino Sys A200, Germany).
Sugars were extracted by grinding 0.1 g lyophilized leaves into powder in 7 ml diethyl ether in mortar with pestle, adding 70 °C deionized water to 7 ml, and it was centrifuged at 1500×g for 5 min. TSS were analyzed through chemical reaction between 0.5 ml of the diethyl ether extract and 5.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 76% (v/v) H₂SO₄) bathed in boiling water for 3 min, and the cooled samples were read at 625 nm in a 752-C spectrophotometer. Glucose and sucrose estimations were performed according to the procedure of Riaze et al. (1985).

Contents of selected inorganic cations (K⁺, Na⁺, Ca²⁺, Mg²⁺) were analyzed with a Perkin-Elmer-5000 Programmable Atomic Absorption Spectrophotometer according to the method of Issac (1980).

2.4. Statistical analysis

Multivariate ANOVA, Duncan’s multiple comparisons and correlation analysis were used to test the significant difference at \( p<0.05 \) level (SPSS Ver.12.0).

3. Results

Table 1 showed the average percentage contribution of each osmotic solute measured, and there was no significant difference among these values in 2004, 2003 and 2002, but a significant difference was observed in May, July and September, respectively, under two water conditions (\( p<0.05 \)).

3.1. Changes of sugar levels

In this study, soluble sugar (especially TSS, sucrose and glucose) showed the same trends in May, July and September during 3 years under natural and water supplemented conditions and there were significant annual differences for TSS, sucrose and glucose in May, July and September in 2003 and 2004, for TSS and sucrose in May and July, and for glucose in July and September in 2002, 2003 and 2004 (Fig. 1). Glucose was the principal osmotic solute (Table 1) to the detected total soluble sugar in natural environment in May, July and September, respectively (\( p<0.05 \)).
3.2. Variations of proline and inorganic cations

Concentrations of proline and K⁺ (Fig. 2a,b) in each stage were absolutely lower in the water supplemented plants than in natural ones, increased continually from May to September under both water conditions. Significant annual differences for proline (Fig. 2a) and Ca²⁺ (Fig. 3b) in May, July and September in 2002, 2003 and 2004 under both water conditions, for K⁺ (Fig. 2b) in May, July and September in 2003 and 2004, and for Na⁺ (Fig. 3a) in 2002, 2003 and 2004 in natural environmental condition (p<0.05).

Na⁺ and Ca²⁺ were highest in May, especially in 2004, while lower in July and lowest in September, especially in 2003. Under water supplemented conditions, there were no significant differences in 2002, 2003 and 2004 (Fig. 3). There were significant differences for Ca²⁺ in May, July and September between 2003 and 2004, and for Mg²⁺ in May, July and September in 2002, 2003 and 2004 under natural and water supplemented conditions. There was a sharp decrease in September in 2002, 2003 and 2004 for Mg²⁺ under both natural and water supplemented conditions, especially in September 2004 (Fig. 3) (p<0.05).

3.3. Changes of δ¹³C

Fig. 2c showed seasonal and annual changes in δ¹³C value of *A. mongolicus* leaves in different water conditions, which indicates that, in all growth season, the levels of δ¹³C values from water supplemented condition were lower than those from natural condition during the same periods and showed a decreasing trend from May to September under the two water conditions, whereas they were obviously higher in May and lower in September between the seasons and also higher in 2004 lower in 2003 between the years. Significant annual difference occurred in May, July and September between 2002 and 2003, 2003 and 2004 under two water conditions, and significant difference between treatments under water conditions occurred in September in 2002 and 2003 (p<0.05).

Table 2 indicated the equations of the optimum curve estimation and regression analysis between osmotic solute measured and carbon isotope composition (δ¹³C). They were all significant (p<0.05) except that between proline and δ¹³C in both natural and water supplemented condition, potassium and...
In present study, relatively increased contents of free amino acid, especially proline, and relatively decreased contents of soluble sugar especially glucose have been shown in natural conditions. From Fig. 2a, the concentration of proline was highest in September at the later stage of the growth season in every experimental year, especially in 2004, with the same trends in May, July and September during 3 years, while lower in July, lowest in May in 2002, 2003 and 2004, especially in 2004, which indicated that proline is implicated with osmotic regulation of plants in response to drought stress (Hare and Cress, 1997). The proline accumulation within its leaves further indicated that it might be an active response to tardy growth of the species’ foliar tissue for its evergreen with the winter coming and the sharp decrease of water supply (Yoshida et al., 1997). This might be regulated by both the activation of its biosynthesis and the inactivation of its degradation when rainfalls decreased until autumn ended as reported in other stress species (Hare et al., 1999). Probably, our data showed that the variation of seasonal and water condition is one of the reasons for the accumulation of proline in A. mongolicus leaves. Consequently, perhaps proline is a primary osmotic solute when the soluble sugars continuously decreased from May to September under both natural and water supplemented conditions.

A sharp seasonal increase of K⁺ concentration could be detected in natural and water supplemented conditions (Fig. 2b), which perhaps facilitates osmotic adjustment when the soluble sugars continuously decrease from May to September and, at the late stage of growing season in every experimental year, especially the extensions and various movements of individual cells in leaf tissues by regulating stomatal conductance, stimulating photosynthesis (Marschner, 1995) and reducing transpiration (Andersen et al., 1992a,b) to maintain turgor pressure at lower foliar water potentials and improve the ability of plants to tolerate drought stress (Lindhauer, 1985; Munns et al., 2000).

In September, less than 20 mm monthly rainfall put the species in an extreme dry condition, hence K⁺ concentration in leaves highest, especially in 2004, showing the same increasing trends in September during 3 years, while lower in July and

### Table 2

<table>
<thead>
<tr>
<th>Osmotic solute</th>
<th>Water conditions</th>
<th>Model</th>
<th>Regression equation</th>
<th>F value</th>
<th>P value</th>
<th>R²</th>
<th>S.E.</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>Natural</td>
<td>Inverse</td>
<td>( Y = -18.259 - 86.625X )</td>
<td>29.837</td>
<td>0.000</td>
<td>0.365</td>
<td>1.987</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Water supplemented</td>
<td>Linear</td>
<td>( Y = -50.475 + 2.667X )</td>
<td>37.136</td>
<td>0.000</td>
<td>0.417</td>
<td>2.614</td>
<td>52</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Natural</td>
<td>Linear</td>
<td>( Y = -38.198 + 6.655X )</td>
<td>35.600</td>
<td>0.000</td>
<td>0.406</td>
<td>1.921</td>
<td>52</td>
</tr>
<tr>
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<td>Water supplemented</td>
<td>Inverse</td>
<td>( Y = -8.187 - 32.891X )</td>
<td>63.421</td>
<td>0.000</td>
<td>0.549</td>
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<td>52</td>
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<tr>
<td>Glucose</td>
<td>Natural</td>
<td>Linear</td>
<td>( Y = -41.401 + 2.624X )</td>
<td>42.898</td>
<td>0.000</td>
<td>0.452</td>
<td>1.845</td>
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<td>Logarithmic</td>
<td>( Y = -49.102 + 13.287lnX )</td>
<td>61.724</td>
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<td>0.543</td>
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<td>Proline</td>
<td>Natural</td>
<td>Logarithmic</td>
<td>( Y = -28.210 - 1.048lnX )</td>
<td>0.796</td>
<td>0.377</td>
<td>0.015</td>
<td>2.474</td>
<td>52</td>
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<td>Logarithmic</td>
<td>( Y = -29.980 - 2.478lnX )</td>
<td>1.777</td>
<td>0.188</td>
<td>0.033</td>
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<td>52</td>
</tr>
<tr>
<td>Potassium</td>
<td>Natural</td>
<td>Cubic</td>
<td>( Y = -22.444 - 3.507X + 0.137X^3 )</td>
<td>1.557</td>
<td>0.221</td>
<td>0.058</td>
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<td>51</td>
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<tr>
<td></td>
<td>Water supplemented</td>
<td>Cubic</td>
<td>( Y = -34.358 - 15.951 - 11.064X^2 + 1.960X^3 )</td>
<td>10.175</td>
<td>0.000</td>
<td>0.379</td>
<td>2.750</td>
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<td>Sodium</td>
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<td>Linear</td>
<td>( Y = -31.375 - 35.865X )</td>
<td>20.581</td>
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<td>0.284</td>
<td>2.110</td>
<td>52</td>
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<tr>
<td></td>
<td>Water supplemented</td>
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<td>( Y = -29.823 + 11.059X )</td>
<td>0.058</td>
<td>0.810</td>
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<td>21.554</td>
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<td>52</td>
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<td>0.543</td>
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<td>( Y = -29.719 + 7.276X )</td>
<td>9.722</td>
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<td>( Y = -26.969 - 0.357X )</td>
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<td>0.000</td>
<td>0.292</td>
<td>2.880</td>
<td>52</td>
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</tbody>
</table>

\( Y = \delta^{13}C \) (‰), \( X = \) osmotic solute measured (g/100 g, DW), \( n = 54 \).
lowest in May. Thus, sufficient potassium nutrition enhanced the species' drought resistance and water use efficiency for maintaining its minimum growth under drought conditions with the winter approaching (Eakes et al., 1991a).

Na⁺ content under water supplemented condition showed no significant variation but it decreased in natural condition from May to September (Fig. 3a). To our knowledge, desert plants can compartmentalize a major portion of Na⁺ of the cytoplasm into vacuoles; thus, cytosolic Na⁺ concentration is maintained at non-toxic levels (Binzel et al., 1988), which allows the species to use suitable Na⁺ as an osmotic solute and maintains the osmotic potential that increases the water content within the cells (Blumwald et al., 2000). From May to September, due to antagonistic effect, sharply increased K⁺ (Fig. 2b) inhibited excessive accumulation of Na⁺ in that excessive Na⁺ disturbs intracellular ion homeostasis and this can lead to membrane dysfunction, attenuation of metabolic activity and secondary effects that cause growth inhibition and ultimately cell death (Hasegawa et al., 2000). That is, the decline in Na⁺ content from May to September is an adaptive mechanism of the species to drought stress in Tengger Desert.

Fig. 3c indicated that, under water supplemented condition, Mg²⁺ content increased continually and maximized in July but it decreased sharply in September. Probably, some reasons should be noted, one of which is related to weather factors and the biomass of leaves. Nearly 80% rainfall is in July in study site and the biomass increases at the end of May to its maximum value in July, especially greater under water supplemented condition than in natural environment. In September, the intensity and photosynthetic rate of light, temperature and rainfall become gradually weaker, shorter, lower and less, as compared to those in May and July, which inhibit stomata opening to some extent. The decline of Mg²⁺ (an essential component of chlorophyll molecule, ribosome and many enzymes) affects ion homeostasis and the stomatal conductance, retards metabolism, which probably caused the damage of chlorophyll molecules and, as a result, photosynthesis decreased, the growth of cell and tissue within leaves slowed down. It was reported that the growth of the plants under Mg²⁺ deficiency reduced photochemical yield of needles by 8% as compared with the control Mg²⁺-grown needles (Laing et al., 2000). In addition, inorganic cations within leave tissues are redistributed and reallocated to other parts of the plant as a reliable source of nutrients before it starts to senesce and defoliate with winter approaching. The retranslocation of Mg²⁺ from leaves to other organs maintains intracellular osmotic adjustment as autumn ends (Popp and Smirnoff, 1995). Finally, the active absorption of inorganic cations within leave tissues relatively decreased because of low biosynthesis and basal metabolism after autumn. Thus, the decrease in Mg²⁺ content maybe is an adaptation to slow growth and a preparation for the species to live through winter.

Fig. 3b showed that leaf Ca²⁺ underwent a more significant increase in natural environment than under water supplemented condition every month in each experimental year, suggesting that, under natural conditions, possibly, a little higher content of Ca²⁺ maintains a higher tissue water status by condensing the lipid bilayer. Thus, making it impervious to water and electrolysis, which ensures the stability of cell wall-plasma-lemma interface, enhances the thickness of the membranes (Legge et al., 1982), and inhibits the influx and accumulation of ion in guard cells during water stress (Berkowitz et al., 2000), particularly regulates the ion transporters that control Ca²⁺ homeostasis and maintain the ion compartment (localization) as well as membrane integrity and stability (Volotovski et al., 1998). This can be done by inducing, to some extent, biosynthesis and regulating metabolism when the species is under stress (Christiansen and Foy, 1979).

There is a little decrease in calcium content in natural conditions with seasonal changes, because Ca²⁺ might serve as a secondary message and a signal molecule; therefore, it also plays an essential role in stress signaling and stress protection (Knight and Knight, 2001). In addition to the reasons stated above related to magnesium, other possible reasons might be due to the fact that calcium is not leached readily from living and senescing foliage because of its relative immobilization in pectates and on membrane (Adamec, 2002). According to our data, the decrease in Ca²⁺ content was less dramatic than that of magnesium from May to September; rather, it was of a very small degree. More noticeable is on Fig. 3b that showed little variation in the content of Ca²⁺ in every month of each year under water supplemented conditions, which means that Ca²⁺ is relatively immobile in leaf tissues under favorable conditions.

Fig. 2c reflected the increased tendency for δ¹³C in natural environment in contrast to water supplemented condition. This observation is consistent with the response of δ¹³C to water stress. It could be attributed to the stomatal regulation of reducing transpiration to maintain lower photosynthesis and growth rate during dry periods with its high δ¹³C, and sharply increase its water use efficiency in the face of severe conditions and show a conservative strategy (Brooks et al., 1997; Livingston et al., 1999).

δ¹³C values, an indicator of plant water status, demonstrated significant annual variations, the highest δ¹³C in 2004 and lowest δ¹³C in 2003 corresponded to the rainfalls lowest in 2004 and highest in 2003, Fig. 2c reflected a declining trend of δ¹³C values from May to September. Although no significant difference was observed in two water conditions at p<0.05 level but it was significant at p<0.10 level. In fact, a little difference of 1% in δ¹³C and indeed lighter higher δ¹³C value in leaves of A. mongolicus is considered sufficient to unambiguously differentiate the species under water supplemented and natural environment conditions as reported in another desert species (Ehleringer, 1993b). Notwithstanding some difference in δ¹³C values of A. mongolicus leaves under two water conditions, we speculated that variation in δ¹³C observed probably could not entirely explain the genetically predetermined variation because the water supplemented species was introduced from its surrounding native desert population which is naturally drought stressed. These seasonal and annual variations in δ¹³C may largely be related to the species' different patterns of physiological response to two water requirements and more conservative water use strategies (Brooks et al., 1997; Ponton et al., 2006; Smedley et al., 1991).

Our results, along with those of others, showed that the species living in drought environment exhibits lower biomass
(unpublished data) than in irrigated environment in order to continue its life span through slow growth that is required for survival in a more severe environment. Thus, *A. mongolicus* has evolved into a unique long-living evergreen shrub with ascleroophyllous and conservative water use strategies in arid desert of northwest China since the receding of ancient Mediterranean (Li et al., 1999; Xu et al., 2002).

It is noteworthy that in our study the contents of main osmotic solute showed different seasonal trends: from May to September, soluble sugar, magnesium, sodium and calcium decreased, whereas proline and potassium increased. Annual variations in the contents of main osmotic solute were related to the changes in yearly precipitation. Other measurements (total proteins, cation, anion, organic acid, carbohydrate, glycinebetaine, starches, chlorophyll a/b ratios, membrane permeability, etc.) related to the senescence will be published separately.

### 5. Conclusion

The accumulation of osmotic solutes in the leaves of *A. mongolicus* is an adaptation to its severe environment. Variation trends in osmotic solute content and δ13C value coincided with the rainfall in 3 consecutive years and with the shifts of seasons. Variations of osmotic solute contents and δ13C value are closely related to the drought-adaptive mechanism. This species has the capacity to increase cytochyloma concentration by accumulating osmotic solutes, inducing the closure of stoma and decreasing the evaporation for maintaining minimum foliar growth under rigorous, arid and cold conditions. The adaptive mechanisms indicate the species has very conservative water use strategies to adapt to an extremely severe environment, especially the drought stress. Artificial irrigation further promotes the growth, alleviates drought stress and is propitious to the protection of the endangered species. Consequently, watering is one of the most important measures for the protection of the evergreen shrub.

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