

## MORPHO-PHYSIOLOGICAL RESPONSES OF *ALHAGI SPARSIFOLIA* SHAP. (LEGUMINOSAE) SEEDLINGS TO PROGRESSIVE DROUGHT STRESS

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### Abstract

Water is a key limiting factor influencing plant growth and development in arid ecosystem. To explore the mechanisms of the desert plant *Alhagi sparsifolia* seedlings to tolerate drought stress in extreme desert, an experiment was conducted from July to September in 2010 with four water treatments: 100% (W<sub>100</sub>), 80% (W<sub>80</sub>), 60% (W<sub>60</sub>) and 45% (W<sub>45</sub>) of water holding capacity (WHC). Plant growth, photosynthesis, nutrient content and water use efficiency (WUE) were measured. The results showed that plant growth, branch number, biomass allocation, number of leaves and area per leaf as well as leaf area ratio with drought stress treatments (W<sub>80</sub>, W<sub>60</sub> and W<sub>45</sub>) decreased than W<sub>100</sub> treatment, while root/shoot ratio and specific leaf area increased gradually throughout the experimental duration. Furthermore, photosynthetic pigment content, light-saturated photosynthetic rate, and concentration of carbon and nitrogen in plant significantly decreased with increasing drought stress. The WUE at W<sub>100</sub> and W<sub>80</sub> treatments increased significantly at the beginning of drought stress treatment and then reduced with stress prolonged. In conclusion, the desert plant *A. sparsifolia* can tolerate the progressive drought stress due to the strong plasticity of morphological and physiological traits. The critical level of soil WHC to limit the growth and dry mass production of *A. sparsifolia* seedlings in the southern fringe of the Taklimakan Desert was approximately at 45%.

**Key words:** *Alhagi sparsifolia*, Plant growth, Photosynthesis, Nutrient content, Water holding capacity.

### Introduction

All plant life in extreme deserts with annual precipitation less than 70 mm is suffering several drought stress (Walter & Box, 1983; Shmida, 1985; Le Houérou, 1986; Li *et al.*, 2015; Liu *et al.*, 2015). The Taklamakan Desert, located in the Central-Asian Tarim Basin (Xinjiang province, north-west China), is a hyper-arid desert having a mean annual precipitation to mean annual evapotranspiration ratio less than 0.03. In the central part of its southern fringe, mean annual precipitation is approximately 33 mm (Thomas *et al.*, 2000; Liu *et al.*, 2016), and potential annual evaporation is approximately 2600 mm (Xia *et al.*, 1993; Liu *et al.*, 2013). Water supply in the oases of this region is mainly from the melting of snow and glaciers in the adjacent Kunlun mountain range. In the transition zone between the oasis and the open desert, perennial plant species form stands of varying density and composition (Bruelheide *et al.*, 2003). These plant species bear ecological and economical importance for the human population because they constitute a shelter to prevent the sand drift (Xia *et al.*, 1993; Li *et al.*, 2015) and are used for grazing, forage, construction and fuel (Thomas *et al.*, 2000). Among the perennial plants, *Alhagi sparsifolia* Shap. (Leguminosae) is a dominant perennial shrub, which are widely distributed in the desert-oases transition zone, and plays an important role in sand stabilization and animal husbandry. *A. sparsifolia* originated and largely distributed in northwestern China and Central Asia (Zeng *et al.*, 2012; Liu *et al.*, 2013).

Previous studies showed that perennial plants in extreme deserts must tolerate the drought stresses due to low soil water availability. The plant's adaptations to drought stress are frequently linked to phenotypic plasticity (Alpert and Simms, 2002), which is widely considered as a major evolutionary response to temporal environmental stress (Alpert & Simms, 2002; Picotte *et al.*, 2007). Plant phenotypic plasticity involves changes in physiology, morphology or development of the same genotype growing in different environments. Moreover, plant success in a given environment is often dependent on water availability (Volaire *et al.*, 1998; Galmes *et al.*, 2004). Many plants have characteristics to tolerate drought (Schmidt & Levin 1985). For example, plants under drought stress increase biomass allocation to roots in order to increase their capacity to take up soil water, and decrease leaf area ratio to prevent excessive water loss and allow them to survive in arid environments (Capon *et al.*, 2009; Li *et al.*, 2011). It has been found that the water-use efficiency (WUE) is lower in water-stressed plants than in well-watered plants (Rodyyati *et al.*, 2005; Bacelar *et al.*, 2007). If plants do not have adequate access to water, then rates of photosynthesis are reduced (Boyer, 1976). Plants may also be able to alter characteristics of above-ground (shoots and leaves) and/or belowground (roots) organs throughout the growing season (plasticity) as an adaptation in response to water stresses (Koike *et al.*, 2003; Chidumayo, 2006). Although plastic responses of phenotypic traits may increase plants' ability to survive drought stress, there may be a fitness cost. Since morphological modifications incur the use of

both nutrients (nitrogen, phosphorous, etc.) and energy (carbohydrates) (van Kleunen & Fischer, 2005), it is important to understand the adaptive mechanisms of such trait changes in response to drought stress.

Hence, we used an experimental approach to investigate the growth and development of *A. sparsifolia* seedlings under drought stress over a period of 3 months. The *A. sparsifolia* was selected due to its broad distribution, ecological significance and the paucity of knowledge regarding its recruitment. We explored ontogenetic patterns in physiological and morphological traits in response to drought stress. Finally, we assessed the ability of the *A. sparsifolia* seedlings to tolerate drought stress and identified the critical value of soil water content to limit or stop growth. Besides, we also tested whether *A. sparsifolia* seedlings exhibit plastic responses to environmental stress, in terms of showing a fixed pattern in biomass allocation or selected physiological and morphological traits.

## Materials and Methods

**Seed collection and field site description:** The mature seeds of *A. sparsifolia* were collected in October 2009, from native plants in the desert-oasis transition zone. The transition zone lies in Qira on the southern fringe of the Taklimakan Desert, Xinjiang, China. The seeds were taken from a large sample with more than 20 randomly selected plants. Plump seeds of similar size were picked out, dried in a ventilated room, and stored in a closed cotton bag in a refrigerator at 4°C. The mean mass of six groups of 1000 seeds each was  $5.06 \pm 0.02$  g (mean  $\pm$  SD).

The experiment was carried out in Cele National Field Research Station for Desert Steppe Ecosystems, Chinese Academy of Sciences (80°03'24" ~ 82°10'34" E; 35°17'55" ~ 39°30'00" N). This area is characterized by a typical continental arid climate, with an average annual precipitation of only 35.1 mm and annual evaporation as high as 2595.3 mm. Rainfall is highly erratic and occurs mainly between May and July. The extreme maximum and minimum air temperatures are 41.9°C and -23.9°C, respectively. The difference between diurnal and nocturnal air temperature is high, with an annual average temperature range of more than 15°C. The mean wind speed is 1.9 m/s. The altitude is 1340~1380 m. The Qira oasis is surrounded by natural vegetation clockwise from its western part to eastern part, while border on mobile dunes and gravel desert in its southern part. Sand drift and sand storm are common ecological threats. The oasis and the surrounding vegetation act as a shelter to prevent the sand-drift and also provide fuel, fodder and timber for local people and livestock.

**Plant material and experimental design:** The experimental soils were collected from the natural habitat where *A. sparsifolia* population grew (sampling depth of 0~20 cm). They were homogeneous loose aeolian sediments with the water holding content (WHC) of  $17.78 \pm 0.49\%$ . The soils were then air dried to the WHC of  $0.24 \pm 0.03\%$ , and mixed evenly before being put into the plastic pots (height 28 cm; top diameter 28 cm and bottom diameter 23 cm). The predominant fraction of

particle size was silt (> 88%), with sand (particle size 63 ~ 2000  $\mu\text{m}$ ) and clay (particle size < 2  $\mu\text{m}$ ) contributed less than 5% (Thomas *et al.*, 2006). The soil had pH 8.52, bulk density  $1.49 \text{ g/cm}^3$ , total salinity 1.75 g/kg, organic carbon 0.85 g/kg, organic matter 1.46 g/kg, total N 0.10 g/kg, total P 0.48 g/kg, total K 15.93 g/kg, available N 10.51 mg/kg, available P 2.29 mg/kg, available K 148 mg/kg. Each pot was filled with 16 kg soil.

The seeds of *A. sparsifolia* were pre-treated with 2.5% sodium hypochlorite for 20 min. Five seeds with similar size were sowed in each pot on April 10, 2010. Soil in the pots were well-watered to keep the WHC at 80% to stimulate the seed germination. Drought stress treatments were initiated after plants were grown for 60 days. During the experimental period, the average day and night temperatures were 32°C and 19°C, respectively. The *A. sparsifolia* seedlings were sampled and dried at 70°C for 48 h for measuring aboveground dry mass determination ( $n = 120$ ). Plants were harvested at the beginning of July, August and September, respectively to take further measurements of growth, photosynthesis and nutrient contents.

The experiment was originally designed as a random complete block design with four drought stress treatments: 100% ( $W_{100}$ ), 70% ( $W_{70}$ ), 50% ( $W_{50}$ ) and 30% ( $W_{30}$ ) of WHC. However, the two-month-old seedlings all died in the  $W_{30}$  treatment (with the soil volumetric water content of  $6.67 \pm 0.35\%$ ) and withering phenomenon was observed at  $W_{40}$  treatment. Therefore we modified the design with the soil WHC of 100% ( $W_{100}$ ), 80% ( $W_{80}$ ), 60% ( $W_{60}$ ) and 45% ( $W_{45}$ ). Soil volumetric water content was maintained at  $16.00 \pm 1.78\%$ ,  $12.45 \pm 1.77\%$ ,  $9.34 \pm 1.33\%$  and  $7.63 \pm 0.46\%$  for the  $W_{100}$ ,  $W_{80}$ ,  $W_{60}$ ,  $W_{45}$  treatments, respectively. To maintain the soil water content, the pots were weighted per day at 18:00 ~ 20:00, and were watered to the designed level. There were thirty replications per treatment. A shelter was used to prevent the influence of rainfall.

**Measurements of growth:** Plant total biomass, height, number of stems and leaves, relative weight of roots, stems and leaves, average area per leaf (APL), leaf area ratio (LAR) and specific leaf area (SLA) were recorded monthly from July to September, 2010 (Table 1).

**Table 1. Measured or calculated plant traits and allocation variables.**

Variable and units	Formula
Root biomass (g)	$W_R$
Stem biomass (g)	$W_S$
Leaf biomass (g)	$W_L$
Plant height (mm)	$H$
Number of stems	SNo.
Number of leaves	LNo.
Total leaf area ( $\text{cm}^2$ )	LA
Total biomass (g)	$W - W_R + W_S + W_L$
Relative root weight (%)	$\%R - (W_R/W) \times 100$
Relative stem weight (%)	$\%S - (W_S/W) \times 100$
Relative leaf weight (%)	$\%L - (W_L/W) \times 100$
Average area per leaf ( $\text{cm}^2$ )	$APL - LA/LNo.$
Specific leaf area ( $\text{cm}^2 \text{g}^{-1}$ )	$SLA - LA/W_L$
Leaf area ratio ( $\text{cm}^2 \text{g}^{-1}$ )	$LAR - LA/W$

**Measurements of WUE:** The WUE of each seedling was evaluated by determining the ratio of dry mass production to water loss throughout the experiment. Average dry matter of seedlings at the beginning was subtracted from dry matter of final seedlings for WUE calculation. For calculating the amount of water loss during the experiment, evaporative loss from the pots was taken into account by subtracting the average amount of water loss from the control pots without plants.

**Measurements of photosynthetic pigment contents and light-saturated photosynthetic rate:** Photosynthetic pigment contents and photosynthetic parameters were analyzed in mid-September. Photosynthetic pigment contents, chlorophyll a, b and carotenoids, were extracted with 80% acetone. The clear supernatant obtained after centrifugation at  $480 \times g$  for 3 min was used for estimation of Chl a, Chl b and Car in the same solution using the extinction coefficients and equations re-determined by Lichtenthaler (1987).

Light-saturated photosynthetic rate was determined with a portable steady-state photosynthetic system (Li-6400; Li-Cor, Lincoln, NE). The responses of leaf photosynthesis to photosynthetic photon flux density ( $A/PPFD$ ) were measured. The  $A/PPFD$  curves were produced under ambient  $CO_2$  concentration (38 Pa), leaf temperature of around  $25 \pm 2^\circ C$  and a relative humidity of around 30%. The light intensity was raised from 0, 50, 100, 200, 400, 600, 800, 1000, 1200, 1500, 1800, 2100 to  $2400 \mu mol photons m^{-2} s^{-1}$ . All measurements were conducted in the morning (8:00–10:00) throughout the experiment to avoid afternoon stomatal closure. Because the size of measured leaves were too small to match the settled size of  $6 cm^2$  in the leaf chamber, a scanner was used to scan the measured leaves into pictures, and then we used the software of Delta-T Scan (CB50EJ, Cambridge, UK) to analyze the pictures and calculate the actual photosynthetic rate.

The response of leaf photosynthesis to photosynthetic photon flux density ( $A/PPFD$ ) can be described by the following function (Harley *et al.*, 1986):

$$A = \Phi PPFD / \sqrt{(1 + \Phi^2 PPFD^2 / A_{max}^2 - Rd)}$$

where  $A$  is the net photosynthetic rate,  $PPFD$  is the incident photon flux density,  $A_{max}$  is the light-saturated photosynthetic rate,  $Rd$  is the rate of respiration in the light, exclusive of photorespiration, and  $\Phi$  is the initial slope (quantum yield).

**Measurements of nutrient in plants:** Carbon (C) and nitrogen (N) concentrations were analyzed monthly from July to September. Tissues from leaves, stems and roots were dried at  $70^\circ C$  for 48 h. Concentrations of C and N in the plant were determined using an elemental analyzer (CNS-2000, LECO Corporation, St Joseph, MI, USA). Results were expressed as dry matter based.

**Statistical analysis:** Analysis of variance was performed on all experimental data. Differences between means were declared to be statistical significant ( $p < 0.05$ ) judged by

the Duncan's New Multiple Range Test using SPSS software 17.0 (Windows XP). All data were expressed as means  $\pm$  S.D.

## Results

**Responses of growth parameters to progressive drought stress:** Drought stress significant decreased plant dry biomass, height and number of stems (Fig. 1a-c). Plant height and number of stems for all treatments increased with stress duration ( $p < 0.05$ ). However, there was no significant differences on plant dry biomass between  $W_{100}$  and  $W_{80}$  treatments in September ( $p > 0.05$ ). All these three parameters at  $W_{100}$ ,  $W_{80}$  and  $W_{60}$  treatments increased from July to September ( $p < 0.05$ ), but there was no significant change at  $W_{45}$  treatment after three months of drought treatment ( $p > 0.05$ ). Besides, seedling height and stems number increased significantly at  $W_{100}$ ,  $W_{80}$  and  $W_{60}$  treatments ( $p < 0.05$ ), but there was no significant increase at  $W_{80}$  and  $W_{60}$  treatments in July and August ( $p > 0.05$ ). Seedling height and number of stems rarely changed at  $W_{45}$  treatment and no seedling withering or death were observed.

In addition, the biomass allocation of leaves and stems decreased with the progressive drought stress ( $W_{100}$ ,  $W_{80}$ ,  $W_{60}$  and  $W_{45}$ ) in July and August, while the biomass allocation of root and R/S ratio increased throughout the experimental duration (Fig. 2a-d). However, there was no significant difference in these parameters was observed between treatments in September. Besides, the biomass allocation of plant and R/S ratio with the stress duration increased from July to August, and then decreased from August to September. The biomass of leaves and stems with drought stress duration significantly reduced from July to September ( $p < 0.05$ ), while there was significant increase of root biomass and the R/S ratio ( $p < 0.05$ ).

**Response of leaves to progressive drought stress:** The leaf numbers increased by 154%, 111%, 223% and 5% at  $W_{100}$ ,  $W_{80}$ ,  $W_{60}$ ,  $W_{45}$  treatments, respectively (Fig. 3a). The SLA parameter with  $W_{40}$  treatment was much higher than that with  $W_{100}$  treatment in August and September ( $p < 0.05$ ; Fig. 3b). Significant differences of APL and LAR parameter were found across the progressive drought stress ( $p < 0.05$ ; Fig. 3c and 3d). The APL parameter with all the treatments reduced from July to August and then increased significantly from August to September ( $p < 0.01$ ). Moreover, the LAR parameter was similar from July to August ( $p > 0.05$ ) and then decreased from August to September ( $p < 0.05$ ).

**Responses of photosynthetic pigment contents and light-saturated photosynthetic rate to progressive drought stress:** The photosynthetic pigment content and light-saturated photosynthetic rate significantly decreased with the increasing drought stress ( $p < 0.01$ ). However, there was no difference in pigment contents and light-saturated photosynthetic rate between the  $W_{80}$  and  $W_{60}$  treatments (Table 2). The Chl a:b value only showed a significant decrease at  $W_{45}$  treatment ( $p < 0.01$ ). However, there was no significant differences in the Chl:Car value among four treatments ( $p > 0.01$ ).

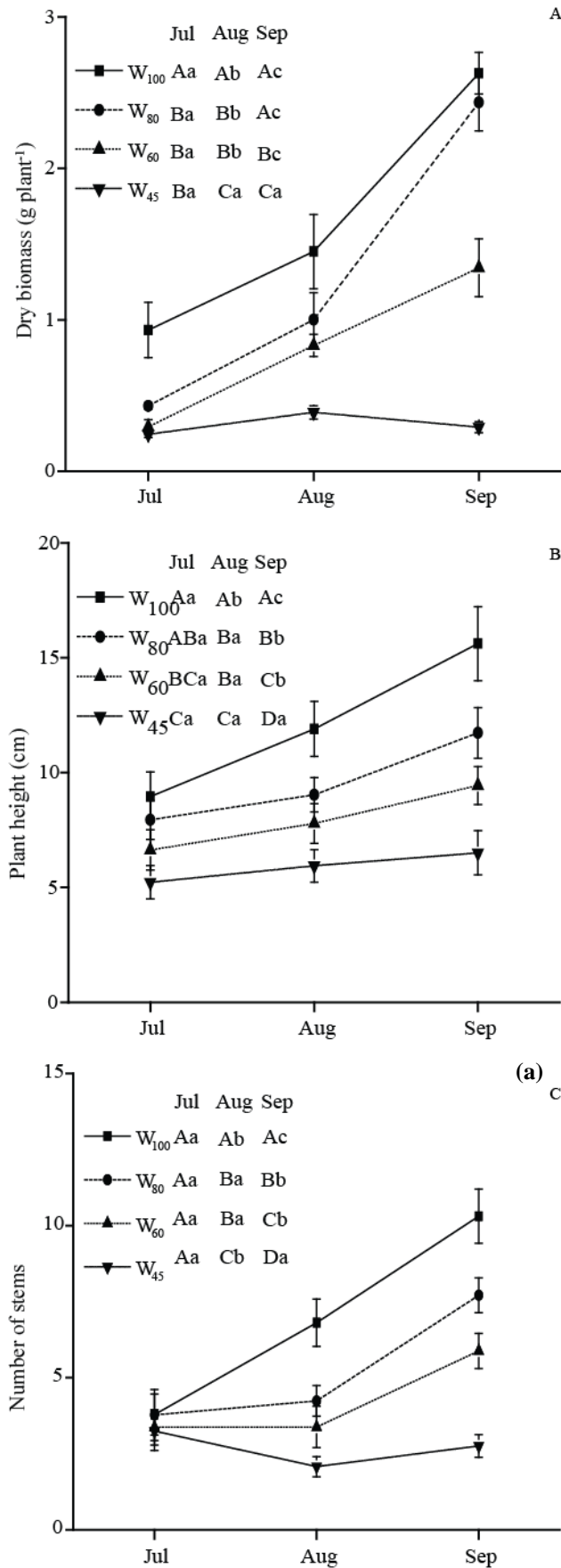


Fig. 1. Dynamics on total biomass, plant height and number of stems of *A. sparsifolia* seedlings to progressive drought stress (W<sub>100</sub>, W<sub>80</sub>, W<sub>60</sub> and W<sub>45</sub>). Each point represents the mean value  $\pm$  SD of five replicate plants.

**Responses of C and N concentrations to progressive drought stress:** The C content in seedling leaves, stems and roots generally decreased with the drought stress treatments from W<sub>100</sub> to W<sub>45</sub> except there was one high root carbon content for the W<sub>45</sub> treatment in July (Fig. 4a-c). With the increasing duration of drought stress, there was no significant changes of leaf C content and stem C content among W<sub>100</sub>, W<sub>80</sub>, W<sub>60</sub> treatments from July to September ( $p > 0.05$ ). The C content of leaves, stems and roots at W<sub>45</sub> treatment decreased from July to August and increased from August to September.

The N content of leaves and roots reduced with the progressive drought stress, while the N content of stems significantly increased ( $p < 0.01$ ; Fig. 4d-f). With the increasing duration of drought stress, the N content in leaves and stems decreased significantly ( $p < 0.01$ ). The decreasing slope from August to September was smaller compared to that from July to August. The N content in roots at W<sub>100</sub>, W<sub>80</sub> and W<sub>60</sub> treatments significantly increased from July to August ( $p < 0.01$ ) and then decreased. For W<sub>45</sub> treatment, however, N content in roots significantly increased from July to September ( $p < 0.01$ ).

**Responses of WUE to progressive drought stress:** The WUE of the W<sub>80</sub> treatment remained the highest in August and September (Fig. 5). The WUE with W<sub>100</sub> and W<sub>80</sub> treatments was significantly higher than that with W<sub>60</sub> and W<sub>45</sub> treatments ( $p < 0.01$ ). With the increasing drought stress duration, the WUE value of the W<sub>100</sub> and W<sub>80</sub> treatments significantly increased from August to September, while that of the W<sub>60</sub> and W<sub>45</sub> treatments kept similar.

## Discussion

**Growth limit and tolerance of seedling to drought stress:** Plant seedlings showed sensitive response to drought stress, which was the main limiting factor for plant to survive and reproduce in desert areas (Taiz & Zeiger, 2002; Aziz & Khan, 2003; Liu *et al.*, 2015). In the present study, *A. sparsifolia* seedlings died at W<sub>30</sub> treatment and could hardly grow at W<sub>45</sub> treatment. These results implied the lowest critical level of soil WHC for survival and growth of *A. sparsifolia* seedlings was approximately 45%. Moreover, there was significant growth decline in seedling dry biomass, height, stem/leaf number, area per leaf and leaf area ratio starting at W<sub>80</sub> treatment (Fig. 1a-c and Fig. 3c-d), suggesting that the soil WHC in this case might be the initial critical level for seedling growth. Besides, the *A. sparsifolia* seedlings with W<sub>80</sub> and W<sub>60</sub> treatments could grow well as the experiment continued, so that W<sub>80</sub> and W<sub>60</sub> treatments were moderate drought stress for *A. sparsifolia* seedlings. Numerous studies have shown that the initial critical level of soil WHC for *A. sparsifolia* seedling is much lower than other drought-enduring species, such as *C. polyantha* (Li *et al.*, 2012), *Bauhinia faberi* var. *microphylla* (Li *et al.*, 2008) and *Sophora davidii* (Wu *et al.*, 2008) with the initial critical level of 25.6%, suggesting that *A. sparsifolia* seedlings are more tolerant to severe drought stress.

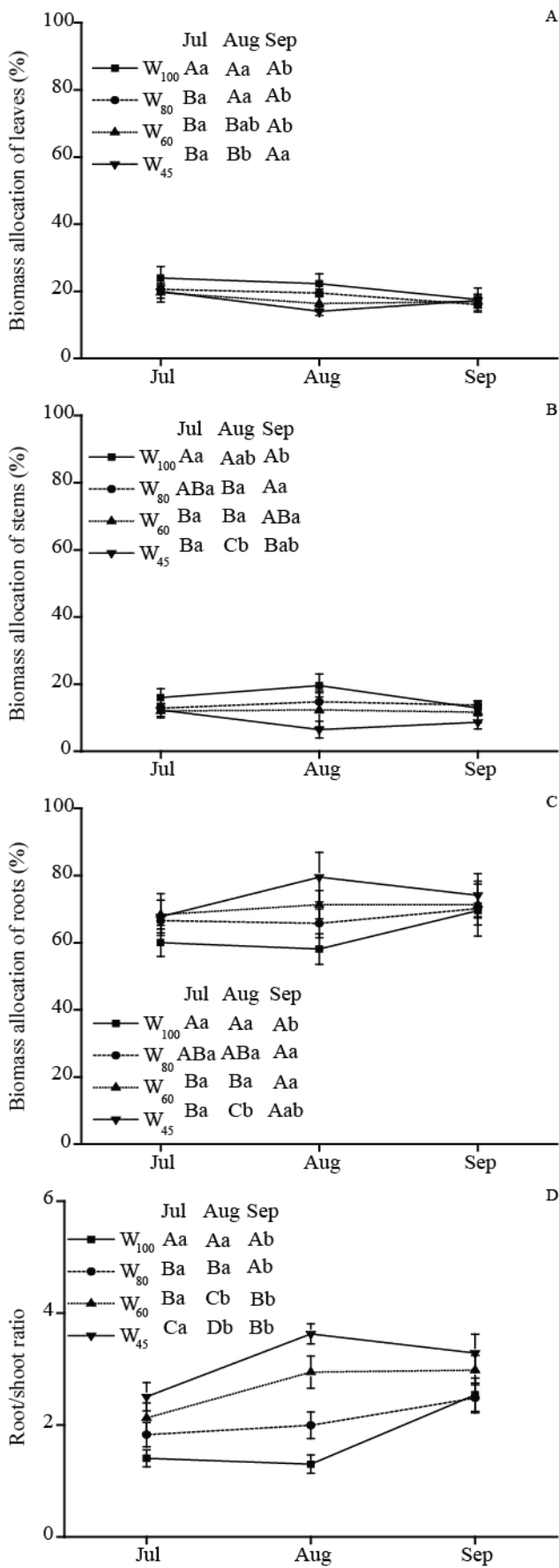


Fig 2. Dynamics on relative weights of leaves, stems, roots and root/shoot ratio in *A. sparsifolia* seedlings to progressive drought stress (W<sub>100</sub>, W<sub>80</sub>, W<sub>60</sub> and W<sub>45</sub>). Each point represents the mean value ± SD of five replicate plants.

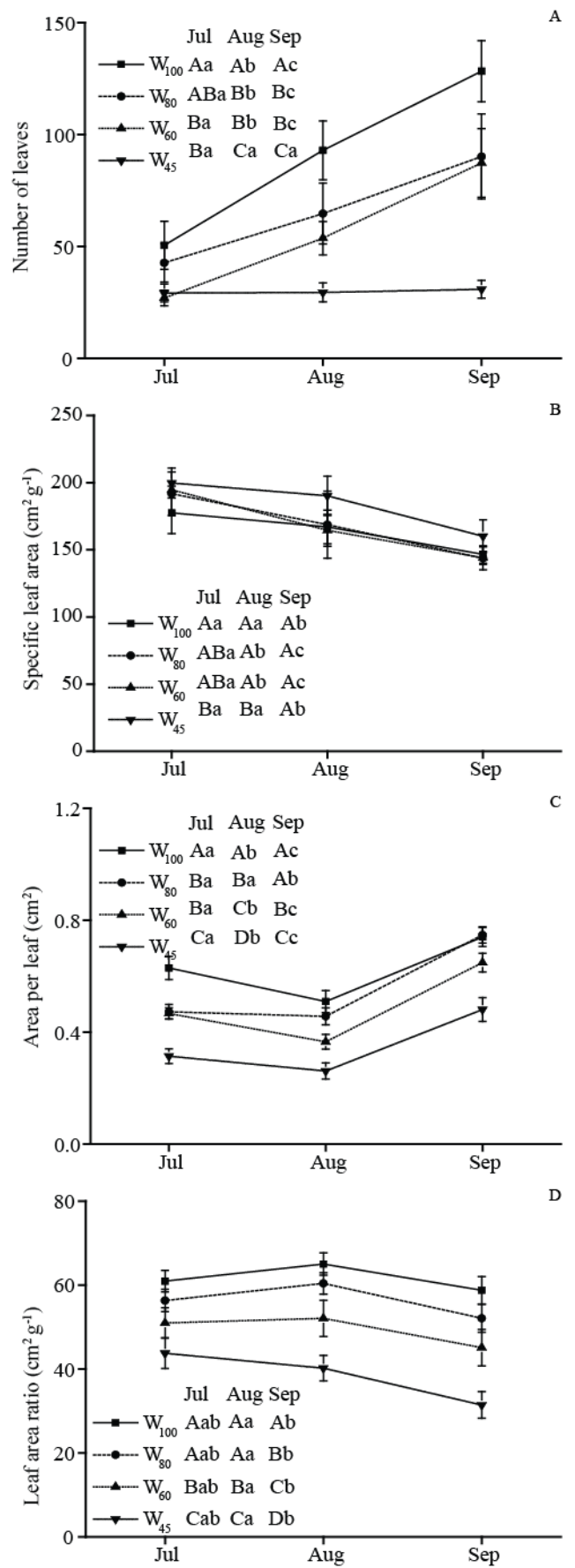
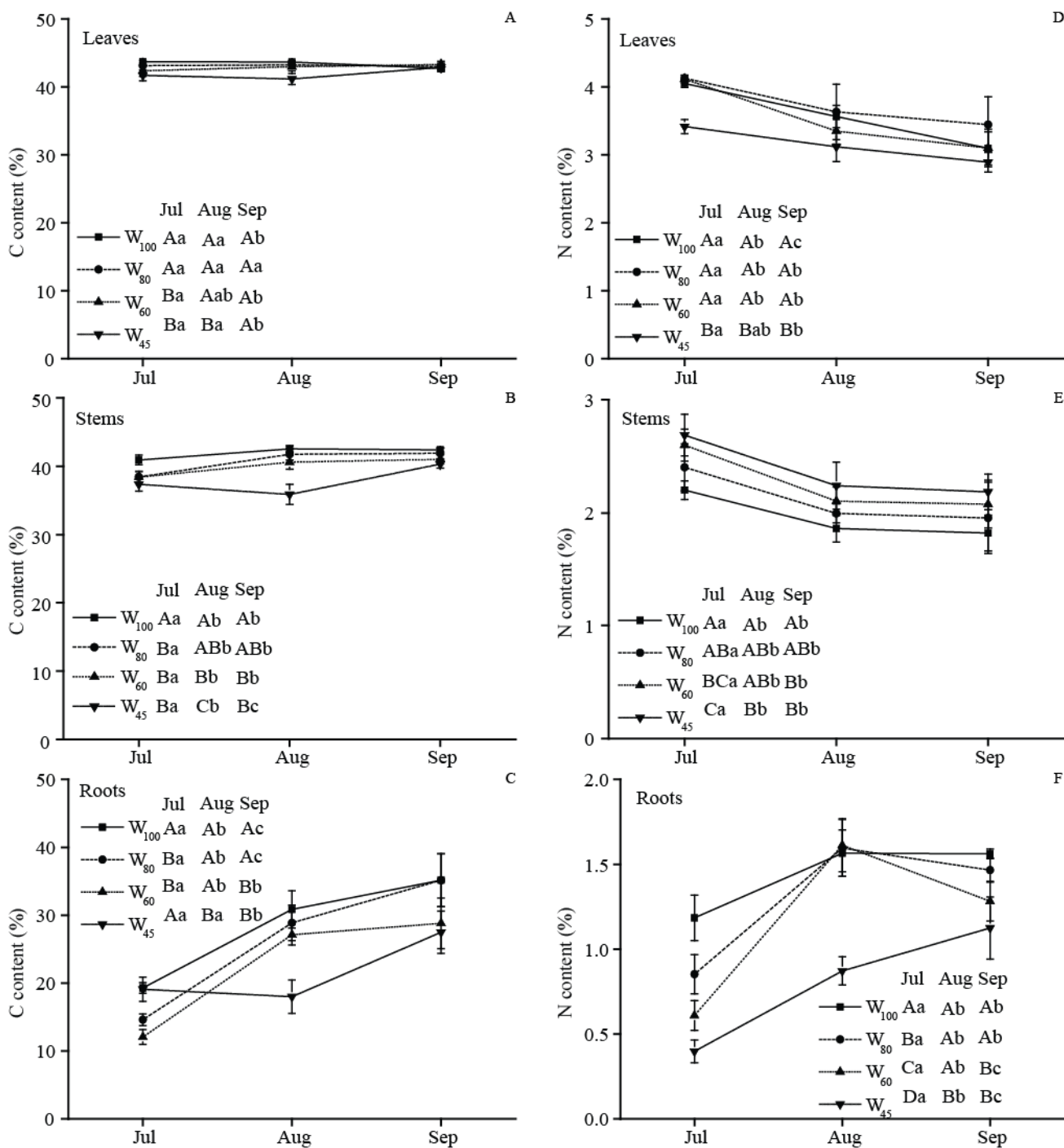


Fig 3. Dynamics on LNo., APL, SLA and LAR in *A. sparsifolia* seedlings to progressive drought stress (W<sub>100</sub>, W<sub>80</sub>, W<sub>60</sub> and W<sub>45</sub>). Each point represents the mean value ± SD of five replicate plants.

**Table 2. Responses of photosynthetic pigment contents and light-saturated photosynthetic rate in leaves of *A. sparsifolia* seedlings to progressive drought stress ( $W_{100}$ ,  $W_{80}$ ,  $W_{60}$  and  $W_{45}$ ). Each point represents the value  $\pm$  SD of five replicate plants. Different letters within a column indicate significant differences across four water conditions according to LSD test,  $p < 0.05$ .**

Water conditions	Chl a ( $\text{mg g}^{-1}$ FW)	Chl b ( $\text{mg g}^{-1}$ FW)	Chl a + b ( $\text{mg g}^{-1}$ FW)	Chl a:b	Car ( $\text{mg g}^{-1}$ FW)	Chl: Car	$A_{max}$
$W_{100}$	$1.42 \pm 0.06$ a	$0.48 \pm 0.02$ a	$1.90 \pm 0.08$ a	$2.96 \pm 0.04$ a	$0.31 \pm 0.01$ a	$6.06 \pm 0.06$ a	$17.81 \pm 3.19$ a
$W_{80}$	$1.20 \pm 0.05$ b	$0.41 \pm 0.02$ b	$1.61 \pm 0.07$ b	$2.95 \pm 0.09$ a	$0.26 \pm 0.02$ b	$6.12 \pm 0.11$ a	$12.76 \pm 2.17$ b
$W_{60}$	$1.17 \pm 0.07$ b	$0.40 \pm 0.02$ b	$1.58 \pm 0.09$ b	$2.94 \pm 0.06$ a	$0.26 \pm 0.02$ b	$6.07 \pm 0.09$ a	$10.56 \pm 2.02$ b
$W_{45}$	$0.90 \pm 0.08$ c	$0.33 \pm 0.02$ c	$1.22 \pm 0.10$ c	$2.74 \pm 0.06$ b	$0.20 \pm 0.01$ c	$6.10 \pm 0.20$ a	$6.29 \pm 0.89$ c



**Fig. 4. Dynamics on C and N concentrations in leaves, stems and roots in *A. sparsifolia* seedlings to progressive drought stress ( $W_{100}$ ,  $W_{80}$ ,  $W_{60}$  and  $W_{45}$ ). Each point represents the mean value  $\pm$  SD of five replicate plants.**

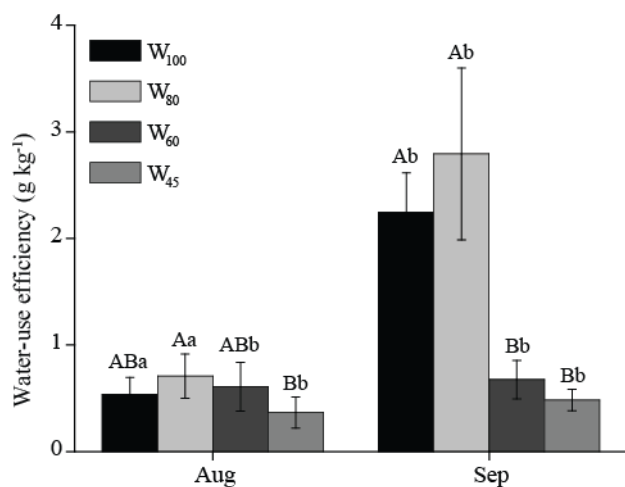


Fig. 5. Dynamics on WUE in *A. sparsifolia* seedlings to progressive drought stress (W<sub>100</sub>, W<sub>80</sub>, W<sub>60</sub> and W<sub>45</sub>). Each point represents the mean value  $\pm$  SD of five replicate plants.

#### Morphological and physiological modifications:

Drought stress caused morphological and physiological modifications on *A. sparsifolia* seedlings, such as biomass allocation variation on shoot and root, physiological and photosynthetic changes in leaves, carbon and nitrogen concentration difference in organs, which were considered as major means of plants to cope with environmental changes (Chaves *et al.*, 2002; Chaves *et al.*, 2003; Valladares *et al.*, 2007). Drought stress significantly decreased dry biomass, plant height, stems number and leaves number at W<sub>80</sub>, W<sub>60</sub>, W<sub>40</sub> treatments, and the reducing slope became greater with the increasing drought stress duration (Fig. 1a-c). The biomass allocation of leaves and stems reduced in severe drought stress treatment (W<sub>40</sub>), while that of root and R/S ratio increased (Fig. 2a-b and d). The R/S ratio increased with the increasing drought stress, and the variation became significantly greater from July to August (Fig. 2d). All these results implied that more metabolism products were distributed to root to gain water, and the diminishment of shoot would decrease water losses by transpiration. Previous studies suggested that reduced LAR under drought situations is mainly due to shedding of lower leaves and reduced formation of new leaves (Ogbonnaya *et al.*, 1998; Bacelar *et al.*, 2007). In this study, no shedding leaves were found. Therefore, the significantly lower LAR were possibly caused by reduced formation of new leaves. Significant decrease of LAR from August to September was likely due to the increasing root biomass and the slower production of new leaves.

Drought stress leads to decrease of leaf photosynthesis, but it varies in different plant species and with stress levels in different habitats (Lawlor, 2009; Cornic and Massacci, 2004). In most cases, drought stress decreased leaf chlorophyll content, carotenoid content, LNo, APL and LAR would decrease and increased SLA (Flexas *et al.*, 2004; Yin *et al.*, 2004; Lobato, 2008; Pandey *et al.*, 2012; ). We confirmed such observation in this study that photosynthesis-related parameters LNo., APL, Chl a, Chl b, Car,  $A_{max}$  significantly decreased with the deficit degree of water (Fig. 3c-d and Table 2), suggesting that the photosynthesis declined with the

severe drought stress. Such negative effect of drought stress on photosynthesis was similar to the previous findings in *Campylotropis polyantha* (Franch.) Schindl. Seedlings (Li *et al.*, 2011) and other species (Chaves *et al.*, 2009). In addition, significant decrease of Chl a:b was found only at W<sub>40</sub> treatment, suggesting that Chl a is more sensitive to severe drought than Chl b. Some previous works mentioned that carotenoids were less sensitive to drought stress than chlorophyll (Sestak & Pospisilova, 1986; Chernyad'ev, 2005). However, our results showed the parameters of Chl a, Chl b and Car diminished by 36.62%, 31.25% and 35.48% respectively (Table 2) and there was no significant difference of Chl:Car, which were in agreement with the results in *Campylotropis polyantha* (Franch.) Schindl. Seedlings in response to drought stress (Li *et al.*, 2011).

Recent work has shown that drought stress leads to C starvation resort to stoma closure, declined photosynthesis and blocked CO<sub>2</sub> assimilation affected by water deficit (Cornic, 2000; Chaves *et al.*, 2009). However, according to Muller (2011), most C compounds often accumulate in organs resulting in increased C concentrations. Such accumulation under water deficit has been reported in several species, various plant parts, and for different (i.e. soluble or structural) C forms. In our research, the C content of leaves, stems and roots with W<sub>45</sub> treatment was significantly lower than that in other three treatments, but C content in root was significantly increased with all the treatments (Fig. 4a-c).

Drought stress showed significant impact on N content in plant. Drought stress always leads to nutrient stress. Because the function of nutrient acquirement and transfer may be easily blocked by drought stress (Karaki *et al.*, 1998; Nahar & Gretzmacher, 2002). Moreover, root nodules numbers and activities may be largely reduced with the decreased soil water content (Spent, 1972; Eugene & Spent, 2006; Onuh & Donald, 2009). In the present study, the N content of leaves and roots with severe drought stress treatment (e.g. W<sub>45</sub>) was much lower than other three treatments (Fig. 4d and f), which is in agreement with drought stress in tomato (Nahar & Gretzmacher, 2002). However, the N content in stems increased with the progressive drought stress, which might be a sign of nutrient transfer strengthening in stems.

Although many cases showed that the WUE of seedlings increased with the increasing drought stress (Kramer *et al.*, 1995; Li *et al.*, 2000; Akhter *et al.*, 2005; Li *et al.*, 2011), it's still not clear how it would be in severe water deficit conditions and low water-holding sandy soils. Our results showed an increase of WUE with the progressive drought stress (from treatment W<sub>100</sub> to W<sub>80</sub>). However, the WUE was not significant different between August and September (Fig. 5). The WUE significantly decreased with the increasing levels of drought stress (e.g. W<sub>80</sub>, W<sub>60</sub> to W<sub>45</sub> treatments). With the further growth and development of seedling in September, the WUE with W<sub>100</sub> and W<sub>80</sub> treatments were much higher than with W<sub>60</sub> and W<sub>45</sub> treatments, implying that the low metabolic productivity, instead of evaporation and transpiration, was the main factor of low WUE under the long-term water deficit situation. Moreover, the WUE with W<sub>60</sub> and W<sub>45</sub> treatments remained low from August to September. Overall, the severe drought stress would lead to lower WUE.

**Adaptive strategies in response to drought stress duration:** Plants had their own strategies to adjust water usage for survival and growth in the desert environment. Specific changes on R/S ratio, SLA, C concentration and N concentration would be of great importance for seedlings to cope with the water deficit situation and to identify the tendentious strategies (Wilson *et al.*, 1999; Chaves *et al.*, 2003; Bartels *et al.*, 2005).

Increasing R/S ratio was an efficient way for plants to exploit the limiting water resource (Li *et al.*, 1999, Li *et al.*, 2000, Zhang *et al.*, 2004, Duan *et al.*, 2005). In our experiments, the R/S ratio was significantly higher with severe drought stress (e.g. W<sub>45</sub> treatment) and the R/S ratio increased dramatically with W<sub>45</sub> treatment than the moderate and abundant water treatments (Fig. 2d). Moreover, the increase of C and N content in roots from July to September provided a hint that cells metabolism such as respiration and enzymatic activities in roots might be more active as the drought prolonged (Fig. 4a-f). The increase of N content in root from July to August alleviated the drought stress, which is in agreement with previous findings that higher rate of nitrogen effectively alleviated the adverse effect of drought stress (Wu *et al.*, 2008; Brueck *et al.*, 2010)

In an arid environment, higher SLA related to CO<sub>2</sub> assimilation can result in less dry matter accumulation and lower growth rate (Grime *et al.*, 1997; Wilson *et al.*, 1999), suggesting the trade-off between survival and biomass obtain under drought stress. The SLA exhibited a significantly higher value with W<sub>45</sub> treatment (Fig. 3b), therefore *A. sparsifolia* seedlings chose the surviving strategy to save water and slow down the CO<sub>2</sub> assimilation. The SLA with abundant water treatment (e.g. W<sub>100</sub> treatment) was significantly lower than moderate water treatments (e.g. W<sub>80</sub> and W<sub>60</sub> treatments) in July, but there was no significant differences from August to September. The graduate change might be caused by seedling acclimation in response to drought stress as reported by Yordnov *et al.* (2000), Kozłowski (2002), Chaves *et al.* (2003) and Page *et al.* (2016).

## Conclusions

The desert plant *A. sparsifolia* can tolerate the progressive drought stress in an arid-desert area. The *A. sparsifolia* seedlings showed the changes in morphological and physiological traits in responses to progressive drought stress, suggesting a strong plasticity adaption to drought stress. It coped with drought stress by avoidance mechanisms such as significant decrease in plant dry biomass and height, leaf development, branch number and also by tolerance mechanisms such as significant increase in R:S ratio, water use efficiency and decrease in photosynthetic parameters along with C and N contents in plant. The soil volumetric water content around 7.63% (45% of water holding capacity) was the critical soil WHC level restricting the optimal growth and dry mass production of the *A. sparsifolia* seedlings.

## Acknowledgments

This work was supported by the Key Program of Joint Funds of the National Natural Science Foundation of China and the Government of Xinjiang Uygur Autonomous Region of China (U1203201) and National Natural Science Foundation of China (No. 41371516, 31070477, 31500367). We are also grateful to the anonymous referees for their reviewing this manuscript.

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(Received for publication 9 August 2015)