

# Comparison of photosynthesis and antioxidative protection in *Sophora moorcroftiana* and *Caragana maximovicziana* under water stress

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**Abstract:** This study aims to investigate the protective roles of photosynthetic characteristics and antioxidative systems in the desiccation tolerance of *Sophora moorcroftiana* and *Caragana maximovicziana* as they adapt to arid environments. A variety of physiological and biochemical parameters in the leaves of two Leguminosae species were monitored for 1, 7, 14, 21 and 28 d of drought stress. Soil water content decreased from 38.58% to 7.33% after exposure to 28 d of water stress. The photosynthetic carbon-assimilation rates of the two Leguminosae plants decreased for non-stomatal limitation with processing water stress. The malondialdehyde content and cell membrane relative conductivity of the two species increased significantly from 1 to 21 d and then decreased. *S. moorcroftiana* showed higher superoxide dismutase and peroxidase activities than *C. maximovicziana* during the 28 d treatment period. However, the catalase activities and proline content of *C. maximovicziana* were higher than those of *S. moorcroftiana* before the water stress treatment reached 21 d. Nine physiological and biochemical parameters were selected to comprehensively evaluate the two species' drought-resistance by the membership function values (MFV). The mean MFV indicated that *S. moorcroftiana* has a relatively stronger drought defense capability than *C. maximovicziana*. *S. moorcroftiana* mainly uses carbon-assimilation rate and osmotic adjustment to combat water deficiency.

**Keywords:** *Sophora moorcroftiana*; *Caragana maximovicziana*; photosynthesis characteristic; antioxidative protection; drought stress

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Water stress is an important environmental factor that inhibits plant survival and growth (Mao et al., 2009; Verlinden et al., 2013). The effects of changes in gas exchange parameters, especially the net photosynthetic rate (Pn), protective enzyme activity and osmoregulation substance, on plant growth has elicited much attention (Alfonso et al., 2012; Habibi et al., 2012; Hu et al., 2013). Understanding the mechanisms of plant tolerance under water stress is therefore a crucial research topic. Water limiting is also one of the main limiting factors of vegetation restoration and reconstruction in arid and semi-arid regions of the Tibet Plateau. The introduction of exotic species not only

made the maintenance of the afforesting survival rate difficult but also made the destruction of the original vegetation ecosystem easy (Mooney et al., 2001; Dreiss et al., 2013). Studying the drought resistance mechanism of native species is the fundamental principle of selecting adaptable afforestation tree species in the Tibet Plateau.

Plants can reduce the transpiration rate (Tr) by partly closing the stoma and allowing some leaves to wither under drought stress (Carmo-Silva et al., 2012). This scenario obviously limits carbon fixation (Tenhunen et al., 1990; Halsey et al., 2013). Water use efficiency (WUE) is determined by the ratio of Pn and

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Tr. Plants commonly increase their WUE to ease the damage of water limitation, which is related to the degree of water stress (Donovan et al., 2007).

Stress factors, such as drought, trigger common reactions in plants and lead to cellular damages mediated by the antioxidative protection system (Shaheen et al., 2013). Reactive oxygen species (ROS) have been the inevitable companions of aerobic life since the appearance of O<sub>2</sub>-evolving photosynthetic organisms. In contrast to O<sub>2</sub>, ROS are highly reactive and toxic and can lead to oxidative damage. Plants develop a complex antioxidant protection system to defend themselves against the injurious effects of ROS; this system consists of antioxidant enzymes (superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)), non-enzymatic solutes such as free proline (Pro), malondialdehyde (MDA) and cell membrane relative conductivity (Bailly et al., 1996; Shah et al., 2001; Del et al., 2005; He et al., 2007). Previous studies have shown that many plant species significantly accumulate free amino acids, such as Pro, in response to water stress. These compatible solutes of plants not only perform osmotic adjustment, but also stabilize and protect the structure of enzymes and proteins, maintain membrane integrity and scavenge ROS (Reddy et al., 2004; Huang et al., 2010). The value of cell membrane relative conductivity shows the degree of damage of cell membranes in water stress (Farooq et al., 2006). These solutes are also helpful in sustaining tissue metabolic activity under water stress (Chen et al., 2005; Anjum et al., 2011).

*Sophora moorcroftiana* and *Caragana maximovicziana* are Leguminosae plants; they are dominant shrub species distributed in the arid and semi-arid regions of the Tibet Plateau. *S. moorcroftiana* grows in the dry valley region in the middle and upper reaches of the Yalu Tsangpo River at high altitudes of 2,800 m to 4,400 m. It is a unique *Sophora* characterized by strong drought resistance. *C. maximovicziana* is mainly distributed in the Lhasa River basin at the altitudes of 3,900 to 4,600 m. They are the preferred drought-resistant afforestation tree species in the arid and semi-arid regions of the Tibet Plateau. However, the study on the physiological response mechanism of *S. moorcroftiana* and *C. maximovicziana* under water stress has not yet been published. Photosynthesis, antioxidative protection and osmoregulation substances

were analyzed in this study. The present work aims to (1) compare the photosynthesis characteristics (Pn, intercellular CO<sub>2</sub> concentration (Ci), stomatal conductance (Gs), Tr, stomatal limitation value (Ls) and WUE), antioxidant enzymes (SOD, POD and CAT) and accumulation of major solutes (such as Pro, MDA) in *S. moorcroftiana* and *C. maximovicziana* under water stress; (2) characterize and provide experimental evidence to confirm the physiological response mechanism and survival strategies of native shrub species in the arid and semi-arid regions of the Tibet Plateau; and (3) use the combined variables method to compare the drought resistance of the two Leguminosae plants.

## 1 Materials and methods

### 1.1 Study area

The study area is located in the Lhasa River valley (the central part of the Tibet Plateau in the Tibet autonomous region) at an altitude of 3,720 m (29°35'–29°36'N, 91°01'–91°03'E). The area is characterized by an arid and cold climate, with an average annual precipitation of 450 mm and evaporation of 2,184 mm. The mean annual sunshine hours and annual mean temperature is above 3,000 h and 7.5°C, respectively. The soil texture is sandy, which has a poor capacity for water and fertilizer conservation.

### 1.2 Plant materials and experiment design

Five-year-old individuals of *S. moorcroftiana* and *C. maximovicziana* were collected from the study area in May 2010. Eighteen healthy individuals of each species (approximately 20 cm in height; basal diameter of 1.0 cm) were selected and transferred to 5,000 cm<sup>3</sup> plastic pots filled with homogenized soil. These samples were grown in a naturally lit greenhouse at the nursery of the Lhasa Forestry Bureau for 2 month prior to the experiments. The greenhouse had a semi-controlled environment (sheltered from rainfall), with day temperatures of 15°C to 30°C, night temperatures of 8°C to 12°C and relative humidity (RH) of 45% to 85%. The plants were watered with distilled water twice a week. After the two-month acclimation period, the plants developed 8 to 10 pairs of leaves. Twenty four hours after watering the pots to field capacity, the experiments were conducted on 1 July 2010. Three control plants of each species were selected randomly

to be watered with distilled water to the field capacity twice a week. The remaining pots received no water for 28 d (Marshall et al., 2000).

Soil water content was measured with a soil moisture measurement system (TDR-200, England) from 9:00 to 10:00 each day. Pn, Tr, intercellular CO<sub>2</sub> concentration (Ci), Gs and air CO<sub>2</sub> concentration (Ca) were measured with a calibrated portable gas exchange system (Li-6400, Lincoln, NE, USA) from 9:30 to 11:00 each day. All measurements were conducted under relative humidity of 50% to 60%, leaf temperature of 23°C to 25°C, Ca of 380 to 400 mg/kg, and photosynthetic photon flux density of 1,500 μmol/(m<sup>2</sup>·s) (approaching the saturation light intensity; detailed data not shown) provided in the leaf chamber by red and blue LED light sources. A scanner was used to scan the measured leaves because the size of the measured leaves was too small to meet the settling size of 6 cm<sup>2</sup> in the leaf chamber. The software Delta-T Scan (CB50EJ, Cambridge, UK) was employed to read the surface area of the measured leaves and calculate the actual values of the gas exchange parameters. The stomatal limitation value (Ls) and WUE were calculated separately with the following formula:  $Ls = 1 - Ci/Ca$  and  $WUE = Pn/Tr$  (Berry et al., 1982; Ritchie, 1983).

The fresh leaves were sampled randomly, frozen in liquid N immediately after their fresh weight was measured, and then stored at -80°C until further use. SOD and POD activities were measured according to the methods of Shahbazi et al. (2009) and Cakmak et al. (1991), respectively. CAT activities and Pro content were determined according to the method of Habibi et al. (2010). Lipid peroxidation was assayed by determining the MDA content, which is a product of lipid peroxidation (Ohkawa et al., 1979). MDA content was measured with the method of Halliwell et al. (1993).

Relative conductivity was measured as described by Lutts et al. (1996) with minor modifications. The plant materials (0.3 g) were washed with distilled water, placed in tubes with 15 mL of deionized water, and incubated for 2 h at 25°C. The relative conductivity of the solution (L1) was determined. The samples were then autoclaved at 120°C for 20 min, and final conductivity (L2) was measured after equilibration at 25°C. Relative conductivity was defined as follows:  $\text{relative conductivity (\%)} = (L1/L2) \times 100\%$ .

### 1.3 Comparison of drought resistance of *S. moorcroftiana* and *C. maximovicziana*

Physiological indices, namely, Pn, Tr, WUE, SOD, POD, CAT, Pro, MDA and relative conductivity, are closely correlated to plant drought resistance (Karkanis et al., 2011) and are usually utilized to evaluate the drought resistance of species (Mittler, 2002; Reddy et al., 2004). Therefore, the membership function values of drought tolerance (MFVD) of the nine indices were used as a comprehensive index to evaluate the drought resistance of the species. MFVD was calculated by Eq. 1 because Tr and relative conductivity were negatively correlated with drought resistance (Belko et al., 2012; Karatas et al., 2012). The other seven indices were positively correlated with drought resistance; membership function value (MFV) was thus calculated by Eq. 2 (Chen et al., 2012).

$$MFVD = \frac{X - X_{min}}{X_{max} - X_{min}}, \quad (1)$$

$$MFV = 1 - \frac{X - X_{min}}{X_{max} - X_{min}}. \quad (2)$$

Where MFV is the membership function value of the species to drought tolerance,  $X$  is the mean value of each index, and  $X_{min}$  and  $X_{max}$  are the maximum and minimum values of the corresponding index of the two species, respectively.

### 1.4 Statistical analysis

The mean and standard error (SD) values of three replicates were calculated. All data were diagnosed for the normality of distribution and homogeneity of variance prior to the parametric statistical tests. All data passed the normality and equal variance tests. Measurements were subjected to the analysis of variance (ANOVA) to discriminate significant differences at  $P \leq 0.05$  or  $0.01$  using SPSS (version 17.0, SPSS Inc., Chicago, IL).

## 2 Results and discussion

### 2.1 Response of soil water content to water stress

The values in soil water content for *S. moorcroftiana* and *C. maximovicziana* species declined gradually during the process of water stress (Table 1). The difference between control and drought-stressed soil water content was significant at the same time intervals with the exception of 1 d. By contrast, the

drought-stressed soil water contents of the two species were not significantly different on the same stress days. The different stress stages and reduction rates of soil water contents varied. A similar characteristic was also observed in the experiment. Soil water content in pots decreased by approximately 21.8% from the first 14 d; however, the reduction percentage of the final 14 d was only 9.41% and 7.62% for *S. moorcroftiana* and *C. maximovicziana*, respectively. Water in sandy soil dissipates easily because this type of soil is loose (Wang et al., 2009; Oostindie et al., 2011). The soil water dissipation rate decreased rapidly during the early stages but gradually during the last stages of water stress (Zotarelli et al., 2009).

**Table 1** Soil water content (%) in pots of *S. moorcroftiana* and *C. maximovicziana* grown for 28 d under water stress

Stress day	<i>Sophora moorcroftiana</i>		<i>Caragana maximovicziana</i>	
	Control	Treatment	Control	Treatment
1	37.84±3.42 <sup>a</sup>	38.58±3.76 <sup>a</sup>	38.12±3.75 <sup>a</sup>	37.43±2.56 <sup>a</sup>
7	38.45±6.42 <sup>a</sup>	25.64±4.22 <sup>b</sup>	37.54±4.21 <sup>a</sup>	26.17±3.35 <sup>b</sup>
14	36.53±5.31 <sup>a</sup>	16.74±2.41 <sup>b</sup>	36.97±3.84 <sup>a</sup>	15.63±2.53 <sup>b</sup>
21	38.42±4.13 <sup>a</sup>	10.73±1.43 <sup>b</sup>	36.34±2.64 <sup>a</sup>	11.04±3.15 <sup>b</sup>
28	36.97±3.75 <sup>a</sup>	7.33±1.22 <sup>b</sup>	37.21±3.63 <sup>a</sup>	8.01±2.43 <sup>b</sup>

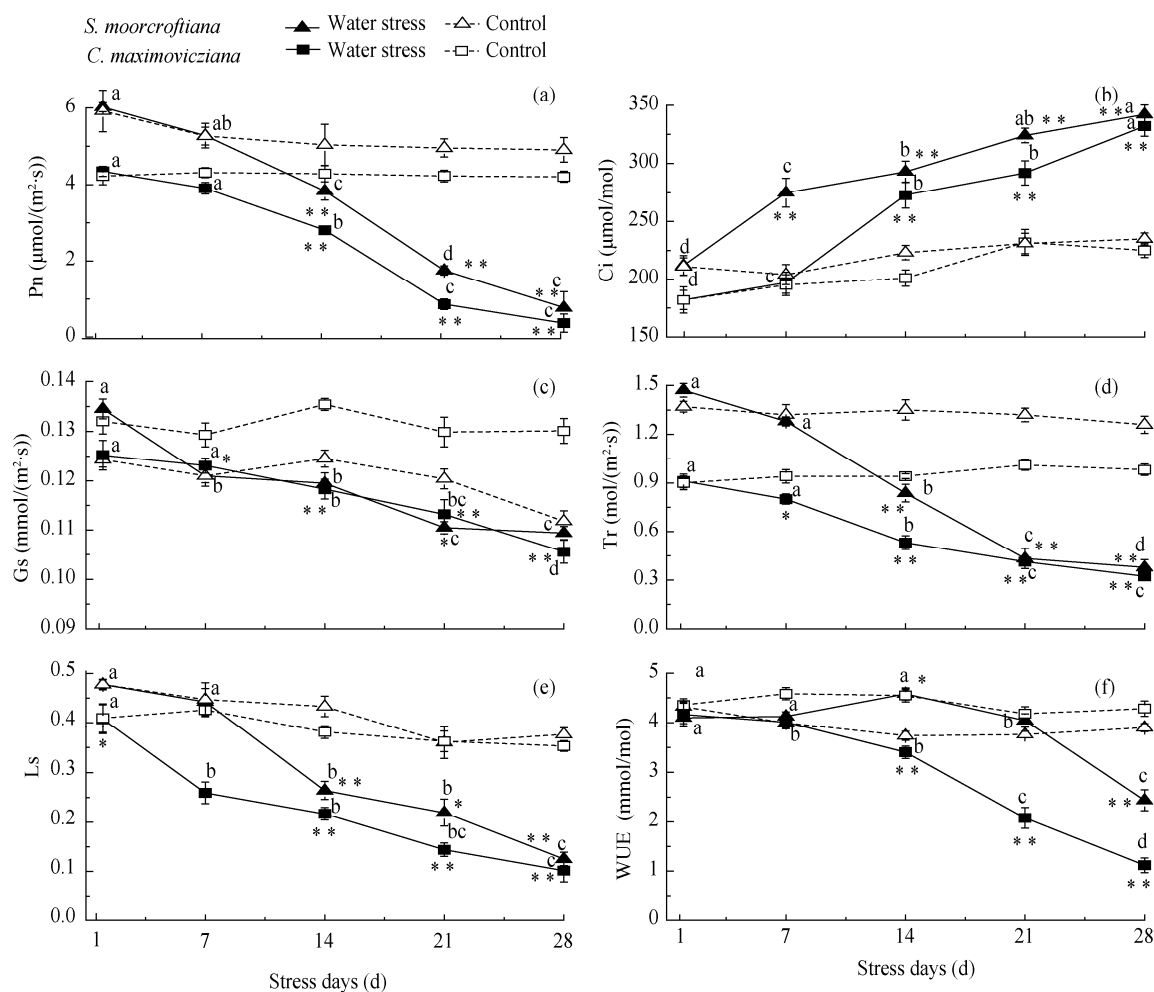
Note: Data for each parameter within each species followed by a different letter are significantly different ( $P < 0.05$ ) for the same number of days of water stress. Data are expressed as means±SD ( $n=9$ ).

## 2.2 Response of the gas-exchange parameters to water stress

Water stress caused the reduction in the gas-exchange parameters in both treatment species except for  $C_i$  (Fig. 1). The response of the gas-exchange parameters to water stress was exhibited as a significant reduction after 7 d in both species. The  $P_n$  values of *S. moorcroftiana* and *C. maximovicziana* declined significantly during water stress from 14 to 28 d (Fig. 1a); however, the  $P_n$  of *S. moorcroftiana* was always higher than that of *C. maximovicziana* during the same water stress period. By contrast, the concentrations of  $C_i$  for both species increased with prolonged water stress time (Fig. 1b). The  $C_i$  value of *S. moorcroftiana* was significantly higher than that of *C. maximovicziana* from 1 to 21 d, whereas the  $C_i$  values of both were insignificant at 28 d. Response of the plants to the soil water condition was regulated by slight opening or closing of the stomata. Thus, water stress

caused reduction in the values of  $G_s$  and  $T_r$  in both species (Figs. 1c and d). The  $G_s$  value of *S. moorcroftiana* was significantly higher than that of *C. maximovicziana* at 1 d. It then decreased gradually compared with the control and did not exhibit a significant difference from 7 to 28 d. The  $T_r$  value of *S. moorcroftiana* was significantly higher than that of *C. maximovicziana* from 1 to 14 d; however, both were not significantly different from 21 to 28 d.  $L_s$  value decreased significantly with water stress from 1 to 21 d. The  $L_s$  values of both species were significantly different on the same water stress days (Fig. 1e). The WUE value of *S. moorcroftiana* declined consistently during the entire water stress period; that of *C. maximovicziana* remained unchanged from 1 to 21 d and was significantly reduced at 28 d (Fig. 1f).

The changes in the gas-exchange parameters in both Leguminosae species were analyzed. The findings would improve our understanding of the photosynthetic characteristics of the two species under water stress, especially the adaptability to drought stress. The photosynthetic capacity of a plant is determined to a large extent by its natural habitat and by itself; some photosynthesis parameters, such as  $P_n$ ,  $G_s$ ,  $T_r$  and WUE, are important factors that help plants resist water stress (Karkanis et al., 2011; Huseynova, 2012). *S. moorcroftiana* and *C. maximovicziana* grow at the arid river valley region of the Yalu Tsangpo River; their long-term adaptability to their natural habitat kept them alive during the 28 d of water stress (Lytle et al., 2004). However, some of the gas-exchange parameters of these plants decreased significantly compared with the control, especially  $P_n$  and  $T_r$ . The  $P_n$  and WUE values of *S. moorcroftiana* were higher than that of *C. maximovicziana* in the 28 d of water stress, indicating that the former has a stronger capability to use light energy and limited soil water than the latter (Blum, 2009). We can conclude that the decline of  $P_n$  value was mainly caused by non-stomatal limitation because the reduction in the  $G_s$  and  $L_s$  values of both species led to the simultaneous increase in the value of  $C_i$  (Farquhar et al., 1982). The result that  $G_s$  values of the two species reduced by approximately 15% from 1 to 28 d implies that both species are characteristic xerophytes.

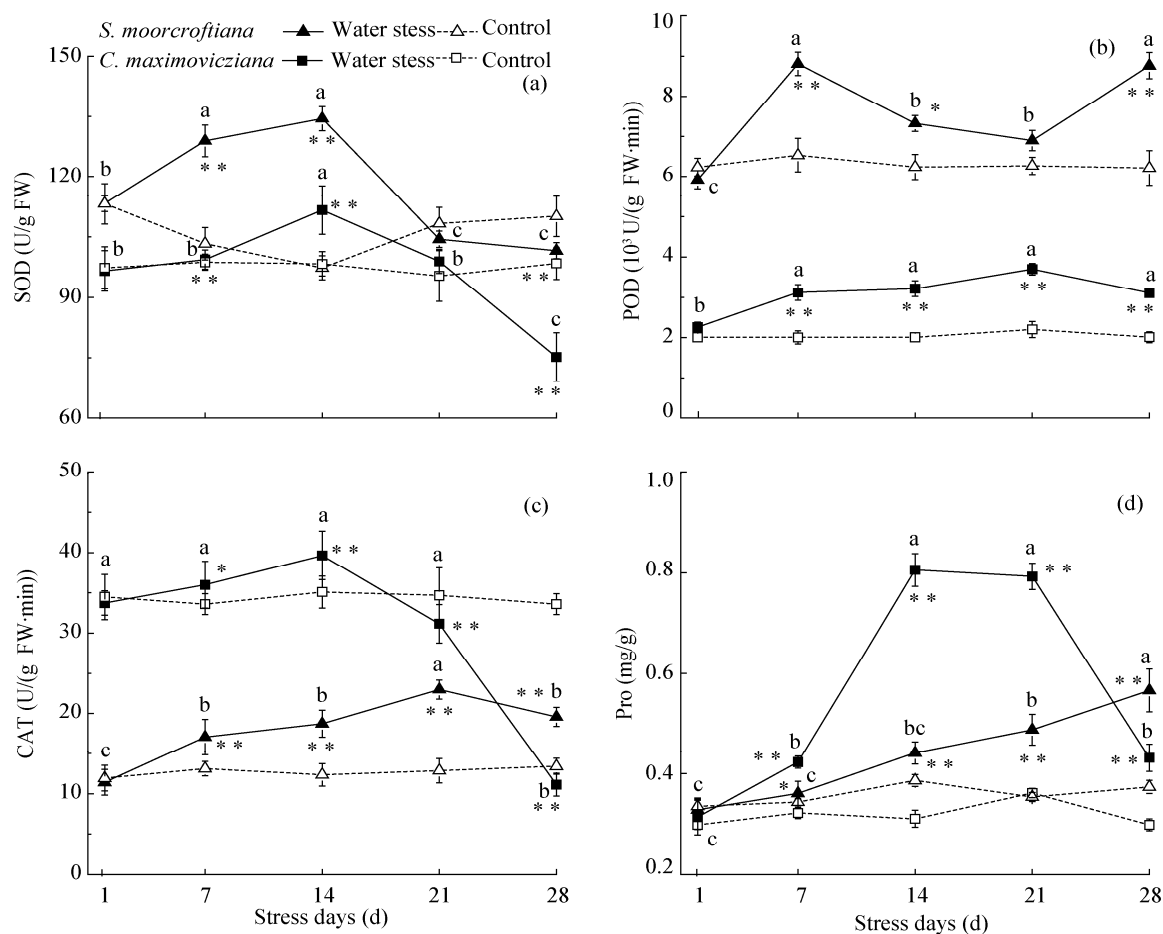


**Fig. 1** Net photosynthetic concentration (Pn), intercellular  $\text{CO}_2$  concentration (Ci), stomatal conductance to water vapor (Gs), transpiration rates (Tr), stomatal limitation value (Ls) and water use efficiency (WUE) of *S. moorcroftiana* and *C. maximovicziana* grown under drought treatment. Data for each parameter of the same species within different treatment days followed by a different letter are significantly different ( $P < 0.05$ ). Data for each parameter between treatment plants and the control in the same phase followed by \* or \*\* are significantly ( $P < 0.05$ ) or very significantly ( $P < 0.01$ ) different, respectively. Data are expressed as means  $\pm$  SD ( $n=9$ ).

### 2.3 Response of antioxidant enzyme activity and Pro content to water stress

The activities of several key antioxidant enzymes throughout the 28 d experiment were examined considering that oxidative stress can be derived from water limitation stress (Finkel et al., 2000; Mittler, 2002). The SOD, POD and CAT activities in the treatment seedlings increased sharply from 7 d onwards. The SOD activities of both species reached the maximum value at 14 d, increased by 18.75% and 15.60%, respectively, compared with 1 d, and then declined significantly after 14 d (Fig. 2a). The POD activities of both species also increased significantly from 1 to 7 d.

The POD activities in *S. moorcroftiana* decreased at 21 d and increased at 28 d, but those in *C. maximovicziana* remained relatively constant during the same period (Fig. 2b). The change in the CAT activities of *S. moorcroftiana* was not significantly different from 1 to 21 d. However, that in *C. maximovicziana* increased gradually from 1 to 21 d (Fig. 2c). The amount of accumulated Pro in the leaves revealed the resistance of the plants to water stress (Delauney et al., 1993; Matysik et al., 2002). With progressive water stress, the Pro content of *S. moorcroftiana* increased rapidly from 1 to 21 d (Fig. 2d) and that of *C. maximovicziana* accumulated at the same time. The increasing amount



**Fig. 2** Specific activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and free proline (Pro) at different water stress time in *S. moorcroftiana* and *C. maximovicziana* grown under drought treatment. Data for each index of the same species within different treatment days followed by a different letter are significantly different ( $P < 0.05$ ). Data for each parameter between treatment plants and the control in the same phase followed by \* or \*\* are significantly ( $P < 0.05$ ) or very significantly ( $P < 0.01$ ) different, respectively. Data are expressed as means  $\pm$  SD ( $n=9$ ).

of Pro in *S. moorcroftiana* was more than that in *C. maximovicziana*. In the entire water stress period, the antioxidant enzyme activity and Pro content of *S. moorcroftiana* were mostly significantly higher than that of *C. maximovicziana*, except for CAT activity and Pro content at 28 d.

Water stress is known to induce plants to produce ROS, both of which can damage tissues (Sharma et al., 2011). To ensure survival, plants generally enhance the production of ROS scavenging enzymes, such as SOD, CAT and osmoprotectants like Pro (Wahid et al., 2007). Increased Pro content, which has been reported in many water-stressed species, could contribute to the maintenance of turgor under conditions of limited water (Sofa et al., 2004; Yamada et al., 2005). The water-stressed plants had a higher Pro content than in the control (Fig. 2d). *C. maximovicziana* had greater cell

protoplasm stability to drought tolerance than *S. moorcroftiana* according to the Pro contents of both species from 1 to 21 d of treatment. However, the low Pro content from 21 to 28 d suggested that serious water shortage was beyond the plants' physiological self-regulation limit. This result is similar to the response of the antioxidative systems of six *Caragana* species to drought stress (Kang et al., 2012).

## 2.4 Response of MDA content and relative conductivity to water stress

The MDA content of leaf tissues indicates the degree of damage of adverse conditions to plants (Nikolaeva et al., 2010). In this study, the values of MDA content and relative conductivity of both species changed significantly because of water stress. The values of *S. moorcroftiana* and *C. maximovicziana* increased con-

tinuously from 1 to 21 d and then decreased (Figs. 3a and b). However, the results showed that the MDA content and relative conductivity of *C. maximovicziana* were significantly higher than those of *S. moorcroftiana*. This finding indicates that the former suffered from a higher degree of water stress than the latter.

MDA is the decomposition product of membrane lipid peroxidation under adversity stress, and its content reflects the damaged degree of plants (Yin et al., 2005). Meanwhile, relative conductivity represents the water content of leaf tissues. Therefore, MDA content and relative conductivity are the important physiological parameters of plants under water stress. During the treatment, MDA content increased gradually at the first 14 d but did not reach the maximum because the intense activities of SOD, POD, and CAT eliminated ROS from 1 to 14 d. However, the decrease in SOD and POD activities resulted in accumulation of ROS in plants; thus, the MDA content increased continuously and reached the maximum from 14 to 28 d.

## 2.5 Comparison of the drought resistance of *S. moorcroftiana* and *C. maximovicziana*

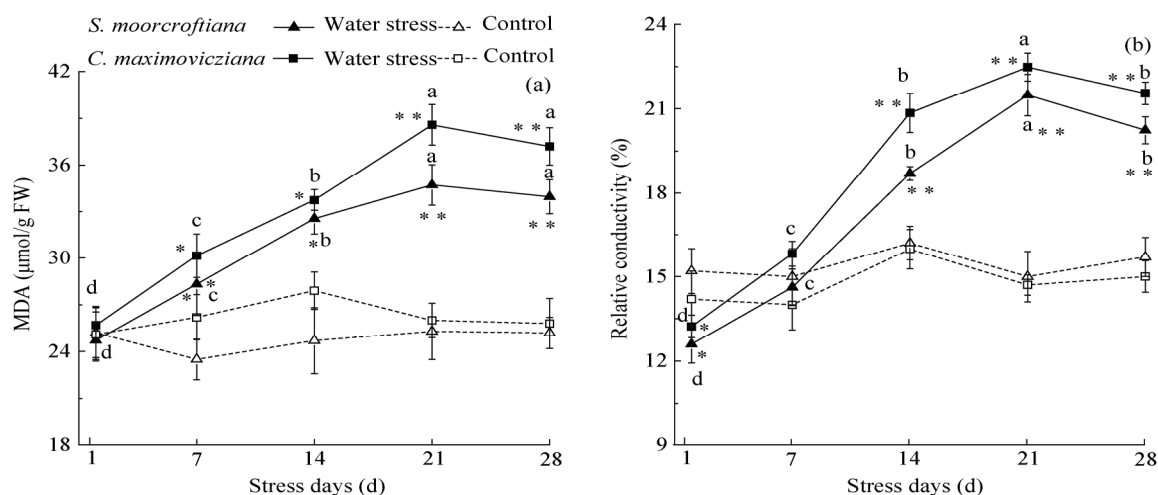
Numerous literatures reported the comparison of drought-resistance of different plant species. For instance, Chaitanya et al. (2009) and Kang et al. (2012) evaluated the physical response of different plant species to drought stress using the biochemical index. Galle et al. (2011) compared leaf gas exchange regu-

lation in evergreen *Quercus ilex* and the semi-deciduous *Cistus albidus* under drought stress. However, the treatment plants in the present study were assumed to adopt integrated physiological responses to resist stress conditions. The parameters include photosynthetic performance, antioxidant defense, and accumulation of compatible solutes.

According to the opinions mentioned above, nine indices were selected to compare the drought resistance of the two Leguminosae plants. The indices of *S. moorcroftiana* and *C. maximovicziana* exhibited different responses to 28 d of drought resistance. The Pn, WUE, SOD, POD, MDA and relative conductivity of *S. moorcroftiana* and the Tr, CAT and Pro of *C. maximovicziana* exhibited strong drought resistance at the same degree of water stress (Table 2). The mean MFV value suggests that the drought resistance of *S. moorcroftiana* is higher compared with that of *C. maximovicziana*.

## 3 Conclusion

Drought stress affects many of the physiological and biochemical processes, including the gas-exchange parameters, antioxidant enzyme activities, Pro content, MDA content and relative conductivity, of *S. moorcroftiana* and *C. maximovicziana*. The photosynthetic carbon-assimilation rates of the two species gradually decreased with drought-induced lipid peroxidation as the drought stress time increased. SOD-POD and Pro



**Fig. 3** Malondialdehyde (MDA) and relative conductivity (RC) at different water stress time in *S. moorcroftiana* and *C. maximovicziana* grown under drought treatment. Data for each index of the same species within different treatment days followed by a different letter are significantly different ( $P < 0.05$ ). Data for each parameter between treatment plants and the control in the same phase followed by \* or \*\* are significantly ( $P < 0.05$ ) or very significantly ( $P < 0.01$ ) different, respectively. Data are expressed as means  $\pm$  SD ( $n = 9$ ).

**Table 2** Comprehensive appraisal of drought resistance of *S. moorcroftiana* and *C. maximovicziana* (membership function)

Species	MFV									Mean MFV	Order
	Pn	Tr	WUE	SOD	POD	CAT	Pro	MDA	Relative conductivity		
<i>Sophora moorcroftiana</i>	1	0	1	1	1	0	0	1	1	0.67	1
<i>Caragana maximovicziana</i>	0	1	0	0	0	1	1	0	0	0.33	2

in the two Leguminosae species are effective protective mechanisms against oxidative damage caused by drought stress. According to the mean MFV value obtained after comprehensive appraisal, *S. moorcroftiana* has a relatively stronger drought defense capability than *C. maximovicziana*; the former uses carbon assimilation and osmotic adjustment to combat water deficiency.

This study provides useful information for the better use of native tree species in afforestation. In the past, *S. moorcroftiana* and *C. maximovicziana* were planted randomly for their similar appearance, and this led to high seedling mortality rates. According to our results, *S. moorcroftiana* other than *C. maximovicziana* should be selected for planting in arid land. Moreover, *S. moorcroftiana* and *C. maximovicziana* should be treated differently in suitable planting area for different soil water demand, which is very important to improve the survival rates of the planted trees.

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