



Size- and leaf age-dependent effects on the photosynthetic and physiological responses of *Artemisia ordosica* to drought stress

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Abstract: Drought is one of the most significant natural disasters in the arid and semi-arid areas of China. Populations or plant organs often differ in their responses to drought and other adversities at different growth stages. At present, little is known about the size- and leaf age-dependent differences in the mechanisms of shrub-related drought resistance in the deserts of China. Here, we evaluated the photosynthetic and physiological responses of *Artemisia ordosica* Krasch. to drought stress using a field experiment in Mu Us Sandy Land, Ningxia Hui Autonomous Region, China in 2018. Rainfall was manipulated by installing outdoor shelters, with four rainfall treatments applied to 12 plots (5 m×5 m). There were four rainfall levels, including a control and rainfall reductions of 30%, 50% and 70%, each with three replications. Taking individual crown size as the dividing basis, we measured the responses of *A. ordosica* photosynthetic and physiological responses to drought at different growth stages, i.e., large-sized (>0.5 m²) and small-sized (≤0.5 m²) plants. The leaves of *A. ordosica* were divided into old leaves and young leaves for separate measurement. Results showed that: (1) under drought stress, the transfer efficiency of light energy captured by antenna pigments to the photosystem II (PSII) reaction center decreased, and the heat dissipation capacity increased simultaneously. To resist the photosynthetic system damage caused by drought, *A. ordosica* enhanced its free radical scavenging capacity by activating its antioxidant enzyme system; and (2) growth stage and leaf age had effects on the reaction of the photosynthetic system to drought. Small *A. ordosica* plants could not withstand severe drought stress (70% rainfall reduction), whereas large *A. ordosica* individuals could absorb deep soil water to ensure their survival in severe drought stressed condition. Under 30% and 50% rainfall reduction conditions, young leaves had a greater ability to resist drought than old leaves, whereas the latter were more resistant to severe drought stress. The response of *A. ordosica* photosynthetic system reflected the trade-off at different growth stages and leaf ages of photosynthetic production under different degrees of drought. This study provides a more comprehensive and systematic perspective for understanding the drought resistance mechanisms of desert plants.

Keywords: drought stress; age difference; plant size; photosynthesis; physiological response; survival strategy

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1 Introduction

Due to the burning of fossil fuels and changes in land use, global surface temperatures have been rising in the past few decades and are expected to increase by 1.1°C–6.4°C by the end of this century (IPCC, 2013). Rising temperatures will change global air circulation patterns and hydrological cycles (Huntington, 2006), leading to more extreme drought events in the future (Easterling et al., 2000; Christensen et al., 2007; Yoon et al., 2015). Arid and semi-arid areas constitute about 50% of China's land territory, most of which are located in the northern and northwestern regions (Cheng et al., 2016). Regional characteristics, such as eco-fragility, sparse vegetation, dry land and low biological productivity make the arid and semi-arid areas of China extremely sensitive to climate change (Zhao et al., 2013). Therefore, investigating the response of typical vegetation in this region to rainfall changes is important for future regional vegetation management.

Photosynthesis allows plants to capture energy and synthesize organic matter, which directly affects plant survival and growth (Fermín et al., 1998). This process is particularly affected by drought stress, which can inhibit the activity of photosystem I (PSI) and PSII, and destroy the coordination between them (Kobayashi et al., 1995; Xiang et al., 2009; Wilhelm and Selmar, 2011). The destruction of PSI and PSII affects the electron transport chain and the original light energy conversion, resulting in a diminished photosynthetic rate, which hinders the synthesis of dark reactions, ultimately resulting in the declines in photosynthetic efficiency (Stefania et al., 2016; Li et al., 2018). Studies have shown that synergistic effect of reactive oxygen species (ROS) metabolism and PSII light energy conversion can stabilize the functioning of photosynthetic system (Fridovich, 1978; Bunce, 1982; Bavcon et al., 1996; Szabó et al., 2005; Alexandre et al., 2013).

Theoretically, the response of plants to environmental changes is developmentally different between individuals and organs (Chaves et al., 2002; del Valle et al., 2015). At the individual scale, the root structures and functions of small plants are not fully developed, which makes small plants more susceptible to water stress than large plants, thus affecting water uptake, transport and use (Gerhard et al., 2001). Pan et al. (2002) illustrates that the formation of individual size irregularity under competitive conditions is an evolutionary stabilization strategy for plant populations (Pan et al., 2002). However, the individual heterogeneity researchers for exploring the development and succession trends of species at the community scale are not enough now.

Studies have shown that there is heterogeneity of leaf age in plant resistance to stress. As the main functional organs of plant photosynthetic production, the structural and functional integrity of the leaves is particularly important for environmental response strategies of plants (Baghalian et al., 2011). For example, nitrogen (N) is transferred from old leaves to young leaves and the key chlorophyll element magnesium (Mg) is also transferred under stress conditions in order to maintain the photosynthetic activity of young leaves (Escudero and Mediavilla, 2003). In studies on the response mechanism of leaves to drought, it has been found that when plants are subjected to drought stress, the leaves slowly turn yellow, starting from old leaves until the whole plant dies (Wassmann et al., 2009). However, other studies have shown that environmental changes affect young leaves first and then the old ones (Huang et al., 2005). Therefore, it is necessary to fully consider the heterogeneity of individuals and organs when exploring the responses of plants to precipitation changes in sandy areas.

Artemisia ordosica Krasch is a sand-fixing semi-shrub that is widely distributed on fixed and semi-fixed sand dunes in temperate deserts and grasslands in northern and northwestern China (Qiu and Shi, 1991; Zhang et al., 2012). It can withstand being buried in the sand, wind erosion and barren soils, and is highly adapted to its arid and semi-arid sandy land habitat (Zhang et al., 2012). Accordingly, it plays an important role in the restoration of desert ecosystems and the succession of plant communities in sandy areas (Guo, 2000). And the ability of *A. ordosica* to tolerate rainfall changes has greatly affected the ecological security of sandy areas in northern China (Peters et al., 2012).

In this study, we explored two hypotheses for the response of *A. ordosica* populations to

rainfall changes. First, we hypothesized that decreased rainfall would accelerate the decline of *A. ordosica* populations, which will be reflected in the photosynthetic and physiological responses, chlorophyll fluorescence and antioxidant enzyme activity. Second, we assumed that growth stages and leaf maturity had different responses to drought stress. The aim of this study was to elucidate the adaptive mechanisms of *A. ordosica* to drought stress while considering the growth stages and leaf age differences. Our findings should help improve the cultivation and management of *A. ordosica* in arid and semi-arid areas and contribute to the understanding of the developmental trend of desert plants in response to environmental changes.

2 Materials and methods

2.1 Study area and plant species

The study was conducted at the Yanchi Research Station, located on the southwestern edge of the Mu Us Sandy Land, Ningxia Hui Autonomous Region, China in August 2018. The climate in this area is a mid-temperate and semi-arid continental monsoon climate with an annual mean temperature of 8.1°C. The average annual precipitation was 311.0 mm during 1956–2017, of which 83.3% occurred from May to September (She et al., 2016). The soil type is Quartisamment according to the US Soil Taxonomy (Gao et al., 2014). The dominant plant species in this region is *A. ordosica*, followed by semi-shrubs such as *Hedysarum mongolicum*, *Salix psammophila* and *Caragana korshinskii*. Grazing in this area has been prohibited since the late 1990s, and vegetation has been allowed to recover for over a decade.

A. ordosica is a xeric, deciduous, multi-stemmed and dwarf shrub with plumose, linearly lobate leaves and a height ranging from 50 to 100 cm. It has a sturdy main root that extends 1–3 m down and numerous lateral roots that are distributed mainly on the top 0–30 cm of the soil. Its main stem is not obvious and has many branches. The branch system configuration of *A. ordosica* is composed of stem, branch of previous year and branch of current year (She et al., 2016). The branch of current year sprouts from old branches (the branch of previous year) and stems and consists of vegetative and reproductive twigs. Most vegetative twigs survive the winter and bear new vegetative or reproductive twigs in the succeeding spring, while reproductive twigs die in winter (Li et al., 2011; She et al., 2017).

2.2 Experimental design

A site containing only *A. ordosica* growing on the flat terrain with abundant light, no occlusion and similar vegetation coverage was selected as the research site in April 2018. Twelve *A. ordosica* plots (5 m×5 m with a 1-m wide buffer zone) were laid out in a randomized block design. Given a maximum plant height of 1.4 m, we considered that the area of 5 m×5 m plot was reasonable. Polyvinyl chloride (PVC) plastic water-stop sheets were embedded 80 cm deep around each sample plot to prevent lateral infiltration from the soil seeping sideways and to ensure the independence of adjacent plots. At the same time, a rain shelter (a shed was constructed by the metal material with a transparent roof of PVC board) was erected in each sample plot, and rainfall in the sample plots was controlled by adjusting the cover area of the baffle on top of the shed. We designed four rainfall levels, i.e., natural rainfall (control treatment) and 30%, 50% and 70% reductions of natural rainfall. Each plot was tested in triplicate. According to the National Meteorological Center, China, we defined a general drought as a decrease of 25%–50% in rainfall over three consecutive months compared with the annual average, and a severe drought is defined as a decrease of 50%–80% in rainfall over a single station (Zhang and Feng, 2010).

2.3 Field sampling and measurements

In August 2018, the canopy width, height and vegetation coverage of *A. ordosica* in each sampled plot were measured. The size of the crown width is significantly correlated with the age of the shrubs (Ma et al., 2002; Wang et al., 2010). Based on our previous studies on *A. ordosica*, we divided *A. ordosica* individuals into two groups, including large-sized (>0.5 m²) and small-sized

($\leq 0.5 \text{ m}^2$) (Ma et al., 2002; Wang et al., 2010). Three representative plants in each group were selected from each sampling area for parameter measurements.

The leaves were divided into old leaves and young leaves for separate measurement. Small, light-colored leaves from the penultimate 4–6 upper leaves per plant were collected as young leaves (leaves with incomplete leaf function). Larger and dark-colored leaves from the lower leaves of the 4–6 pieces of the top of the whole plant were collected and considered as old leaves (Huang et al., 2018). The physiological parameters were measured daily from 26 July to 3 August, 2018.

2.3.1 Determination of net photosynthetic rate

Net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) of the plants were measured using a Li-COR 6400 photometer. Clear and cloudless weather was selected to measure these indicators from 08:30 to 12:00 (LST) (Maria et al., 2013). Illumination intensity was set to $2000 \mu\text{mol}/(\text{m}^2\cdot\text{s})$ in the transparent leaf chamber of the LI-6400 portable photosynthetic apparatus (Li-COR, Inc., Lincoln, NE, USA). Leaf area was measured by LI-3000C (Li-COR, Inc., Lincoln, NE, USA), and then the gas exchange parameters were corrected. Open air passage was adopted, and the flow rate was set at $500 \mu\text{mol}/\text{s}$.

Water use efficiency (WUE) was calculated from P_n and T_r . The formula is as follows:

$$\text{WUE} = P_n / T_r \quad (1)$$

2.3.2 Determination of chlorophyll fluorescence

The chlorophyll fluorescence kinetic parameters (maximum quantum yield of PSII photochemistry (F_v/F_m), non-photochemical quenching (NPQ), photochemical quenching (qP) and effective quantum yield of photochemical energy conversion in PSII (Φ_{PSII})) in the *A. ordosica* leaves were measured using a Fluor Cam portable chlorophyll fluorescence imager (PSI, Brno, Czech Republic) from 26 July 2018 to 3 Aug 2018. Before determination, the light source was blocked using an opaque black cloth. Dark adaptation was measured over 30 min to determine the chlorophyll fluorescence in the *A. ordosica* leaves under actinic light. Fluor Cam portable chlorophyll fluorescence imaging was used to measure fluorescence parameters and imaging *in vivo* using the following settings: shutter=1, sensitivity=40, light act1=60 and super=45 (Katarzyna et al., 2015).

$$F_v/F_m = (F_m - F_0)/F_m, \quad (2)$$

$$\text{NPQ} = (F_m - F'_m)/F'_m, \quad (3)$$

$$\text{qP} = (F'_m - F_s)/(F'_m - F_0), \quad (4)$$

$$\Phi_{\text{PSII}} = (F'_m - F_t)/F'_m, \quad (5)$$

where F_v is the variable fluorescence after dark adaptation; F_m is the maximum fluorescence after dark adaptation; F_0 is the initial fluorescence measured after dark adaptation; F'_m is the maximum fluorescence under light adaptation; F_0 is the minimum fluorescence measured after 5 s of far-red light; F_s is the actual fluorescence production; and F_t is the fluorescence at time t after onset of actinic illumination.

2.3.3 Determination of chlorophyll content

Fresh leaves (0.1 g) were cut into small pieces before being homogenously ground in a mortar with 10 mL ethanol solution. Another 5 mL ethanol was added before filtering the solution, and more ethanol was added to the filtrate to bring the total volume to 25 mL. The filtrate was placed in a 1-cm colorimetric dish, and pure ethanol was added to another dish of the same size as the control treatment. The optical density of the pigment solution was measured at wavelengths of 665 and 649 nm using a 721 spectrophotometer (Shanghai Spectrum Instrument Co. Ltd., China).

Chlorophyll content = (chlorophyll concentration \times extract volume \times dilution ratio of the extracted liquid) / sample fresh weight, (6)

$$\text{Chlorophyll } a/b = \text{chlorophyll } a \text{ content} / \text{chlorophyll } b \text{ content}. \quad (7)$$

2.3.4 Determination of leaf enzyme activity

Superoxide dismutase (SOD) activity was determined using the nitrogen blue tetrazolium method. Inhibition of 50% of nitroblue tetrazolium (NBT) photochemical reduction was considered as 1

enzyme activity unit (Wang et al., 1983). Peroxidase (POD) activity was determined by guaiacol colorimetry. A change in the absorbance at 470 nm of 0.10/min was considered as 1 enzyme activity unit (Amalo et al., 1994). Catalase (CAT) activity was measured by the ultraviolet absorption method, and a reduction in the absorbance at 240 nm of 0.1/min was considered as 1 enzyme activity unit (Li and Mei, 1989). Malondialdehyde (MDA) content was determined by two-component spectrophotometry (Lin et al., 1984).

2.3.5 Determination of soil moisture content

Real-time monitoring of soil moisture was conducted using the Watch Dog soil moisture meter (Spectrum Technolo, Plainfield, USA) in the sample plot at depths of 15 and 55 cm. A soil moisture meter was set in each sample plot. A soil moisture meter was selected at any point of the sample plot. Soil profiles with a depth of 55cm were dug, and probes were arranged vertically at 15 and 55 cm. The data were collected every 2 h.

2.3.6 Determination of rainfall, rainfall interception and environmental factor

To verify the effect of the rain shelter, we collected rainfall interception corresponding to the actual rainfall at each shelter. The air temperature (T_a), photosynthetic available radiation (PAR), net radiation (R_n), and relative humidity (RH) at the test time were obtained from a flux tower data near the sampling site.

2.4 Statistical analysis

The effects of different rainfall levels (four treatments) on the photosynthetic and physiological indices of *A. ordosica* were analyzed using general linear model. The response of the photosynthetic system to drought was analyzed at different growth stages and leaf age levels. Differences in the photosynthetic and physiological indices of *A. ordosica* at different plant growth stages and leaf ages were analyzed by 2-factor multivariate analysis of variance (MANOVA), including drought gradient (four levels) and plant size (two levels) or leaf age (two levels). For all analyses, statistical significance was determined at a level of $P < 0.05$. All analyses were done using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). All figures were plotted using Origin 2018 (Origina Lab., NY, USA).

3 Results

3.1 Photosynthesis, chlorophyll fluorescence and antioxidant responses of *A. ordosica* to drought stress

The increase in the degree of rain shelter led to significant decreases in soil water content (Fig. 1). The increase in the degree of rain shelter also led to significant decreases in WUE. WUE was significantly reduced by 1.26 $\mu\text{mol}/\text{mmol}$ in 70% rainfall reduction (Fig. 2).

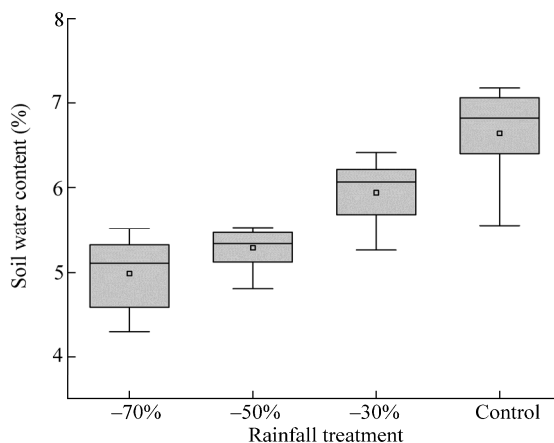


Fig. 1 Soil water content at a depth of 55 cm under different rainfall treatments. Rainfall treatments of -70%, -50% and -30% mean the rainfall reduction percentages of natural rainfall. Control, natural rainfall. Bars are standard errors.

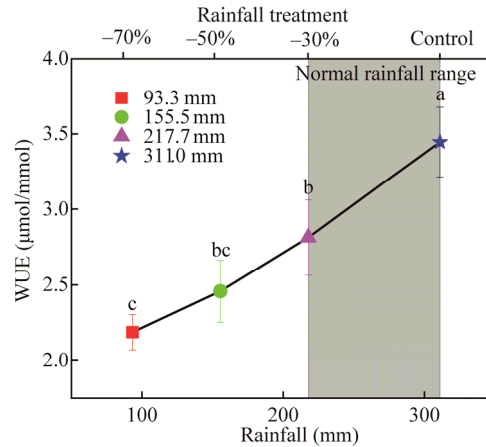


Fig. 2 Water use efficiency (WUE) in the leaves of *A. ordosica* under different rainfall treatments. Bars are standard errors. Different lowercase letters indicate significant differences among different rainfall treatments at $P < 0.05$ level.

Compared with control treatment, reducing rainfall decreased the chlorophyll content, P_n , T_r and G_s of *A. ordosica*. Chlorophyll content, P_n , T_r and G_s were the highest in control treatment and reached the lowest values in 70% rainfall reduction. Compared with control treatment, chlorophyll content, P_n , T_r and G_s in 70% rainfall reduction was significantly reduced by 2.37 mg/g, 1.95 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, 0.72 $\text{mmol}/(\text{m}^2\cdot\text{s})$ and 0.027 $\text{mmol}/(\text{m}^2\cdot\text{s})$, respectively. However, compared with control treatment, reducing rainfall increased C_i of *A. ordosica*. C_i was the highest in 50% rainfall reduction and reached the lowest value in control treatment. C_i was significantly reduced by 44.57 $\mu\text{mol}/\text{mmol}$ in 50% rainfall reduction (Fig. 3).

Drought stress had significant effects on the chlorophyll fluorescence index of the leaves of *A. ordosica*. Compared with control treatment, reducing rainfall decreased F_v/F_m , Φ_{PSII} and qP of *A.*

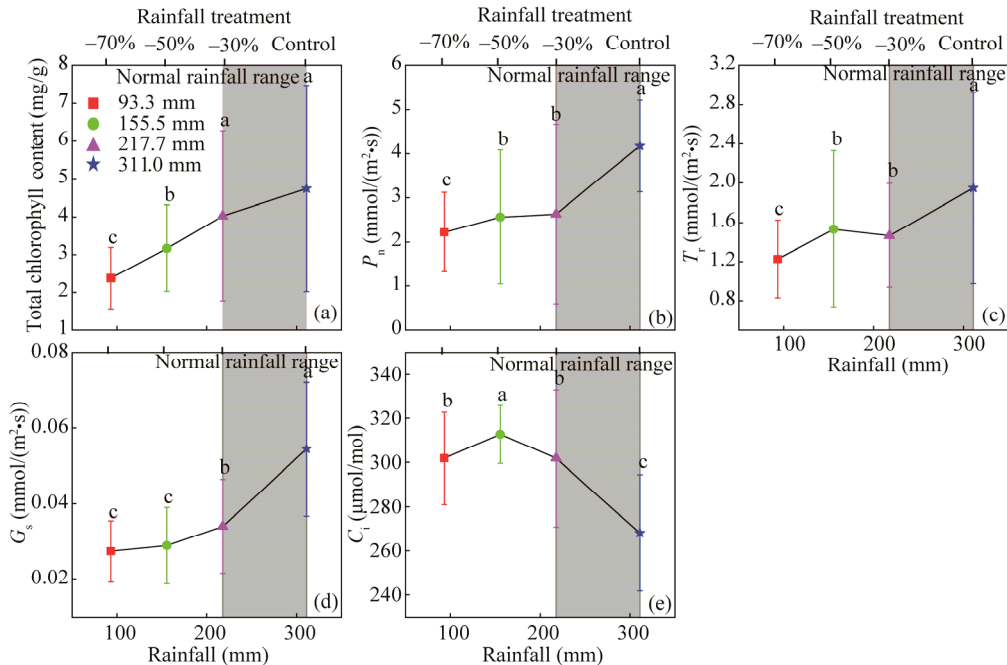


Fig. 3 Total chlorophyll content (a), net photosynthetic rate (P_n , b), transpiration rate (T_r , c), stomatal conductance (G_s , d) and intercellular CO_2 concentration (C_i , e) in the leaves of *A. ordosica* under different rainfall treatments. Bars are standard errors. Different lowercase letters indicate significant differences among different rainfall treatments at $P < 0.05$ level.

ordosica. F_v/F_m , ϕ_{PSII} and qP was significantly reduced by 0.007, 0.180 and 0.080, respectively in 70% rainfall reduction (Fig. 4). With the increase of drought stress, NPQ first increased and then decreased. And NPQ peaked in 50% rainfall reduction then it reduced by 0.670 in 70% rainfall reduction.

Drought stress had significant effects on the antioxidant enzyme activities in the *A. ordosica* leaves (Fig. 5). SOD, POD, CAT and MDA activities of the leaves peaked in 50% rainfall reduction. SOD, POD, CAT and MDA of the leaves were the lowest in control treatment. SOD, POD, CAT and MDA were significantly reduced by 793.51 U/L, 6.52 U/L, 1.16 U/L and 1.04 nmol/L, respectively in control treatment. Drought stress caused the greatest change in POD (gradient=0.494), followed by CAT (gradient=0.474) and SOD (gradient=0.346) activities, suggesting that POD is more sensitive to drought stress and is potentially the main antioxidant enzyme involved in the adaptive response of *A. ordosica* to drought stress (Fig. 5).

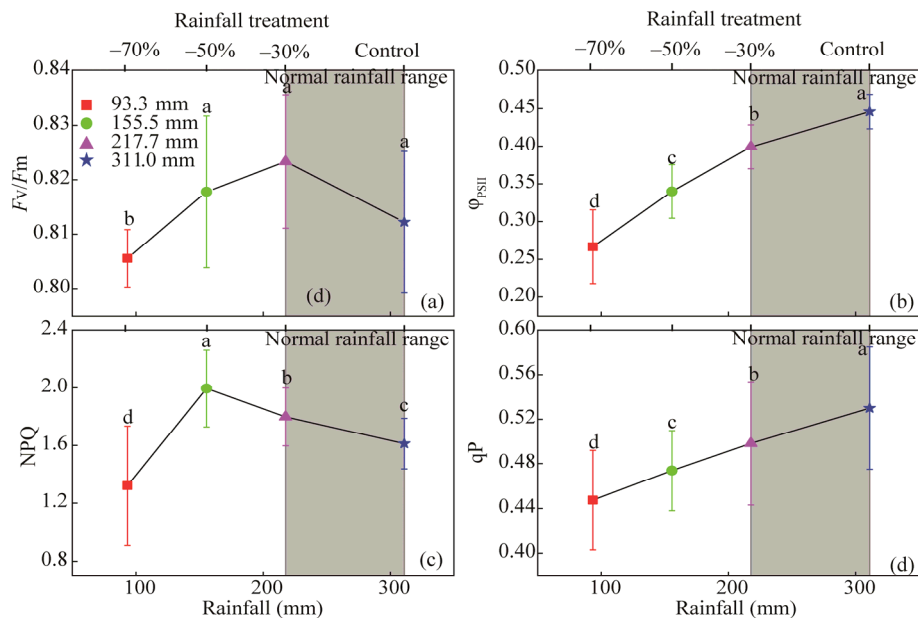


Fig. 4 Chlorophyll fluorescence parameters (F_v/F_m , ϕ_{PSII} , NPQ and qP ; a–d) in the leaves of *A. ordosica* under different rainfall treatments. F_v/F_m , maximum quantum yield of PSII photochemistry; ϕ_{PSII} , effective quantum yield of photochemical energy conversion in PSII; NPQ, non-photochemical quenching; qP , photochemical quenching. Bars are standard errors. Different lowercase letters indicate significant differences among different rainfall treatments at $P < 0.05$ level.

3.2 Responses of *A. ordosica* with different leaf age and growth stages to drought stress

Rainfall and leaf age had significant effects on chlorophyll content, chlorophyll fluorescence parameters (F_v/F_m , ϕ_{PSII} , NPQ and qP) and the antioxidant enzyme activities (SOD, POD, CAT and MDA). Growth stage had no significant effects on chlorophyll content, chlorophyll fluorescence parameters and antioxidant enzyme activities. However, there was significant effect between rainfall and leaf age on the above indices. Under drought stress, growth stage showed significant differences in the antioxidant enzyme activities and ϕ_{PSII} (Table 1).

For large plants, chlorophyll content of old leaves was significantly lower than those of young leaves in control treatment, 30% and 50% rainfall reductions. For small plants, chlorophyll content of old leaves was significantly higher than those of young leaves in 30% and 70% rainfall reductions (Fig. 6).

Different growth stages had no significant effect on chlorophyll content of old leaves. In 30% and 50% rainfall reductions, the chlorophyll content of young leaves was significantly different at the growth stage.

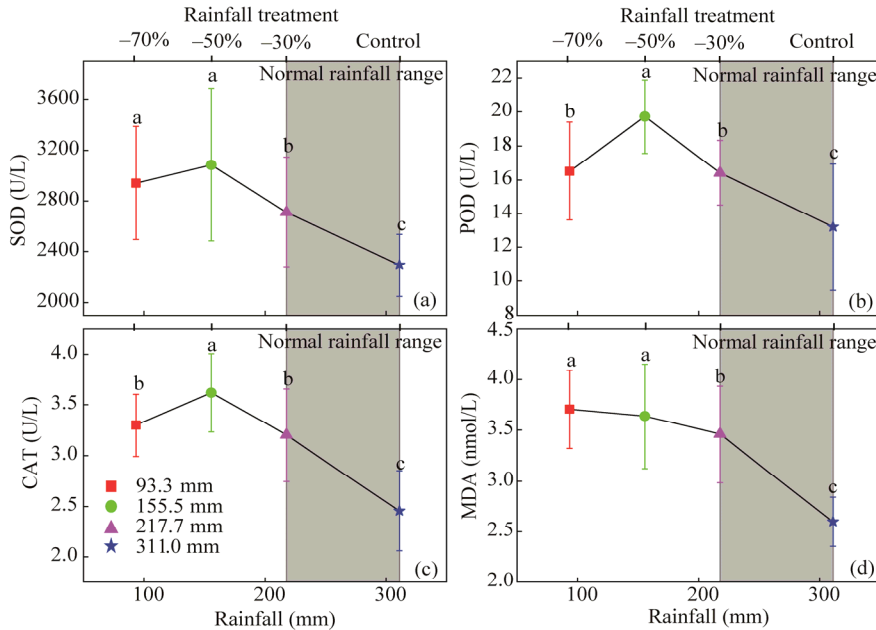


Fig. 5 Antioxidant enzyme activities in the leaves of *A. ordosica* under different rainfall treatments. (a), SOD (superoxide dismutase); (b), POD (peroxidase); (c), CAT (catalase); (d), MDA (malondialdehyde). Bars are standard errors. Different lowercase letters indicate significant differences among different rainfall treatments at $P < 0.05$ level.

Table 1 Effects of drought stress on WUE, chlorophyll content, P_n , T_r , G_s , C_i , F_v/F_m , Φ_{PSII} , NPQ, qP, SOD, POD, CAT and MDA of *A. ordosica*

	<i>F</i>	<i>P</i>		<i>F</i>	<i>P</i>
WUE	6.808	0.002	Φ_{PSII}	136.484	<0.001
Chlorophyll	10.192	<0.001	NPQ	28.819	<0.001
P_n	64.888	<0.001	qP	20.487	<0.001
T_r	32.230	<0.001	SOD	26.681	<0.001
G_s	26.619	<0.001	POD	41.528	<0.001
C_i	5.110	0.002	CAT	71.819	<0.001
F_v/F_m	25.437	<0.001	MDA	6.625	<0.001

Note: P_n , net photosynthetic rate; T_r , transpiration rate; G_s , stomatal conductance; C_i inter cellular CO_2 concentration; F_v/F_m , maximum quantum yield of PSII photochemistry; Φ_{PSII} , effective quantum yield of photochemical energy conversion in PSII; NPQ, non-photochemical quenching; qP, photochemical quenching; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; MDA, malondialdehyde.

In this study, the difference in chlorophyll *a/b* in old leaves and young leaves under each stress gradient was consistent with the differences in the chlorophyll fluorescence index and antioxidant enzyme activity (Fig. 7).

F_v/F_m , Φ_{PSII} and qP of old leaves were significantly lower than those in young leaves in control treatment, 30% and 50% rainfall reductions both in large and small plants (Fig. 8). However, in 70% rainfall reduction, F_v/F_m , Φ_{PSII} and qP were higher in old leaves than those in young leaves. Growth stages had significant differences in F_v/F_m , Φ_{PSII} and qP of old leaves. F_v/F_m , Φ_{PSII} and qP of large plants were significantly higher than those of small plants in old leaves in 70% rainfall reduction.

For large plants, NPQ of old leaves was significantly lower than those in young leaves in control treatment and 50% rainfall reduction, whereas it was significantly higher in old leaves than in young leaves in 70% rainfall reduction (Fig. 8). For small plants, NPQ of old leaves was significantly lower than those in young leaves in control treatment and 30% rainfall reduction.

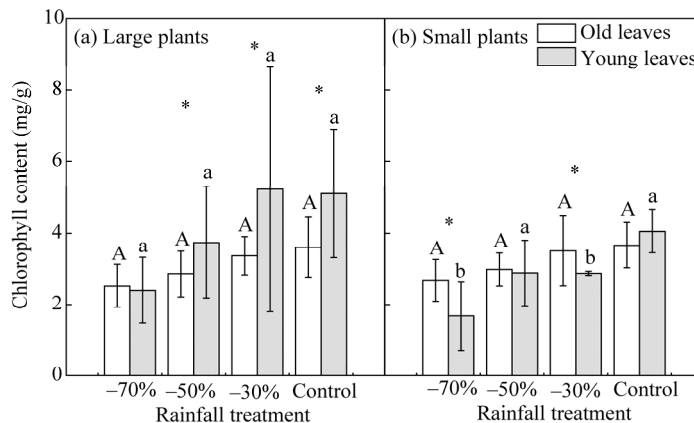


Fig. 6 Total chlorophyll contents of the leaves of large (a) and small (b) *A. ordosica* plants at different ages under different rainfall treatments. Bars are standard errors. Different uppercase letters indicate significant differences of old leaves between different growth stages within the same rainfall treatment at $P < 0.05$ level. Different lowercase letters indicate significant differences of young leaves between different growth stages within the same rainfall treatment at $P < 0.05$ level. * indicates significant difference between old and young leaves at $P < 0.05$ level.

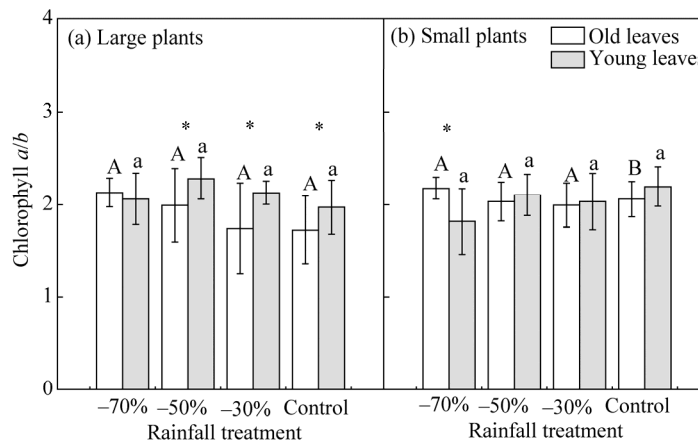


Fig. 7 Chlorophyll *a/b* of the leaves of large (a) and small (b) *A. ordosica* plants at different ages under different rainfall treatments. Different uppercase letters indicate significant differences of old leaves between different growth stages within the same rainfall treatment at $P < 0.05$ level. Different lowercase letters indicate significant differences of young leaves between different growth stages within the same rainfall treatment at $P < 0.05$ level. * indicates significant difference between old and young leaves at $P < 0.05$ level.

Leaves in different ages of large plants showed significant differences in response to drought stress. For large plants, SOD and POD of old leaves were significantly lower than those of young leaves in 30% and 50% rainfall reductions, whereas they were significantly higher in old leaves than in young leaves in 70% rainfall reduction (Fig. 9).

Leaves in different ages of small plants showed significant differences in response to drought stress. For small plants, SOD and POD of old leaves were significantly lower than those of young leaves in 50% rainfall reduction. For large plants, CAT of old leaves was significantly lower than those of young leaves in CK treatment and 30% rainfall reduction, whereas it was significantly higher in old leaves than in young leaves in 70% rainfall reduction (Fig. 9). For small plants, CAT of old leaves was significantly lower than those of young leaves in 30% and 50% rainfall reductions. For large plants, MDA of old leaves was significantly higher than those of young leaves in CK and 30% rainfall reduction, whereas it was significantly lower in old leaves than in young leaves in 70% rainfall reduction (Fig. 9). In 70% rainfall reduction, SOD, POD and CAT of old leaves had significant differences in each growth stage. SOD, POD and CAT of large plants were significantly higher than those of small plants.

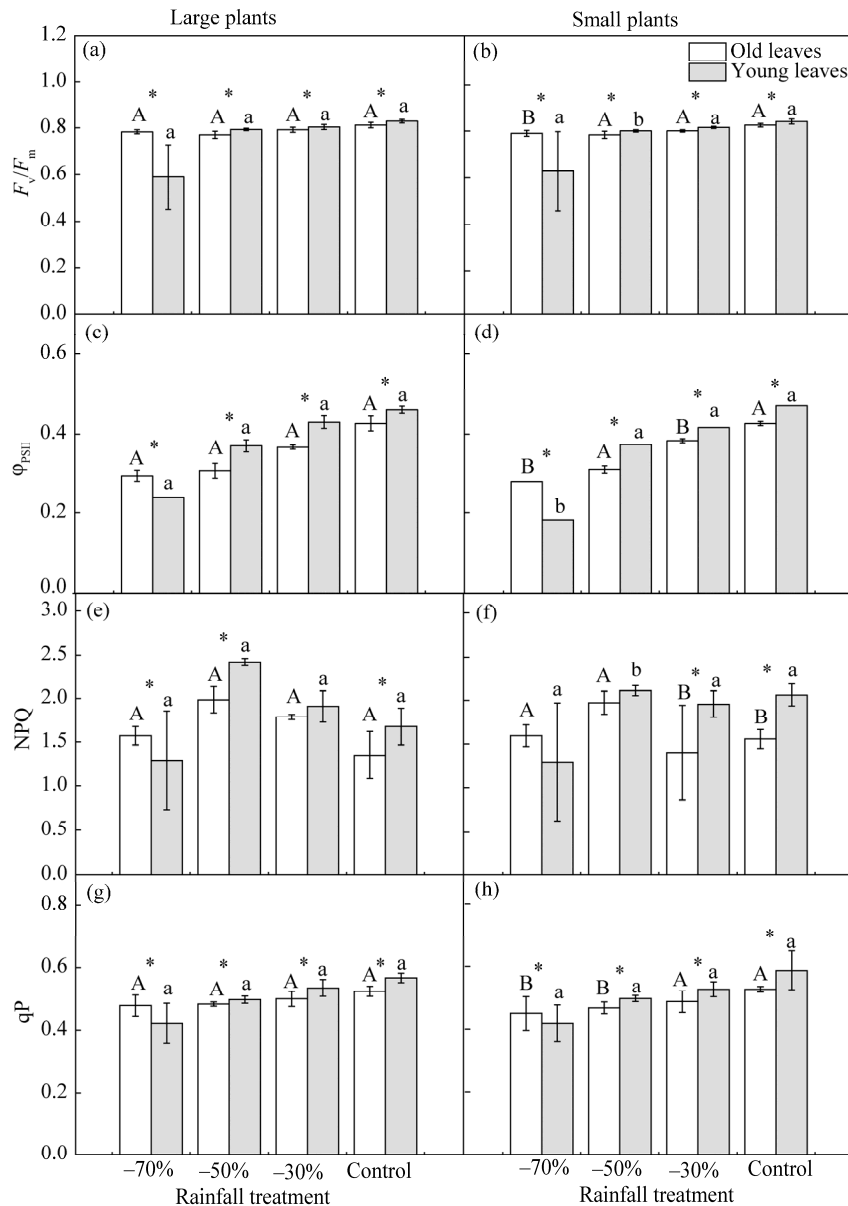


Fig. 8 Chlorophyll fluorescence parameters (F_v/F_m (a, b), Φ_{PSII} (c, d), NPQ (e, f) and qP (g, h)) of the leaves of large and small *A. ordosica* plants at different ages under different rainfall treatments. Bars are standard errors. Different uppercase letters indicate significant differences of old leaves between different growth stages within the same rainfall treatment at $P < 0.05$ level. Different lowercase letters indicate significant differences of young leaves between different growth stages within the same rainfall treatment at $P < 0.05$ level. * indicates significant difference between old and young leaves at $P < 0.05$ level.

4 Discussion

4.1 Photosynthetic response of *A. ordosica* leaves under drought stress

The decrease of rainfall significantly affected the photosynthetic physiological characteristics of *A. ordosica*. By regulating photosynthetic electron transport activity and heat dissipation capacity, the photoreaction center together with the antioxidant system resist drought (Sharkova, 2001; Tsonev and Kouki, 2003). In control treatment, 30% and 50% rainfall reductions, photosynthetic parameters and chlorophyll fluorescence parameters of *A. ordosica* decreased significantly with increasing drought stress. And, antioxidant enzyme activities of the leaves increased significantly

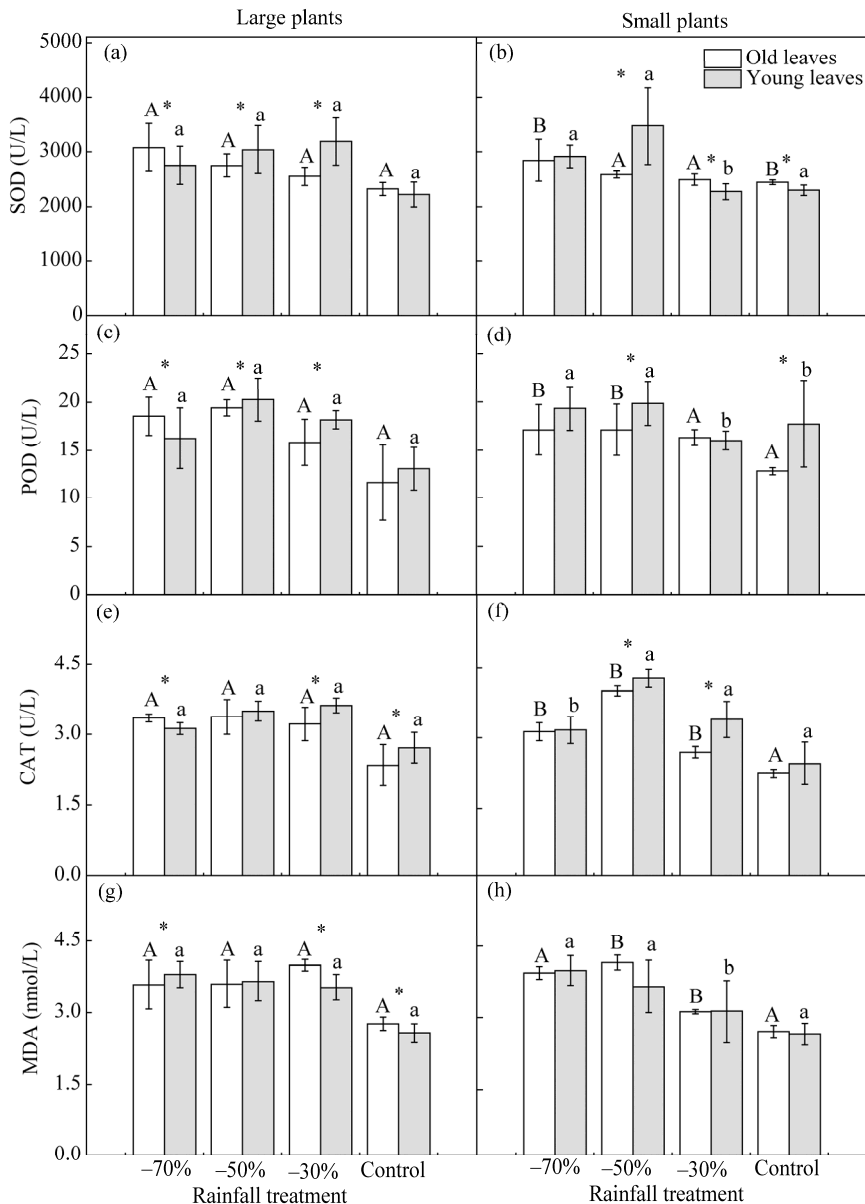


Fig. 9 Antioxidant enzyme activities of the leaves of large and small *A. ordosica* plants at different ages under different rainfall treatments. SOD, superoxide dismutase (a, b); POD, peroxidase (c, d); CAT, catalase (e, f); MDA, malondialdehyde (g, h). Bars are standard errors. Different uppercase letters indicate significance of old leaves between different growth stages within the same rainfall treatment at $P < 0.05$ level. Different lowercase letters indicate significance of young leaves between different growth stages within the same rainfall treatment at $P < 0.05$ level. * indicates significant difference between old and young leaves at $P < 0.05$ level.

with increasing drought stress, which corroborates the results of previous studies (Feng et al., 2004; Perez et al., 2014; Parra et al., 2015). The result indicated that under drought stress, the transfer efficiency of light energy in the leaves of *A. ordosica*, as captured by the antenna pigments to the PSII reaction center, decreased and the heat dissipation capability increased (Liu et al., 2019; Rudikovskii et al., 2019). In the meanwhile, the antioxidant enzyme system was activated, and the scavenging ability of free radicals was enhanced (Seel et al., 1992).

However, this mechanism of regulating the photosynthesis under drought stress was restricted under severe stress, i.e., 70% rainfall reduction in our study. Compared with mild drought stress, the photosynthetic electron transport activity of PSII reaction center under severe drought was

inhibited, the heat dissipation capacity was reduced and the antioxidant enzyme activity was significantly decreased, but MDA content remained at a high value. Eventually, the photosynthetic rate and chlorophyll content of the plants were reduced (almost 50% decline). That is, in 70% rainfall reduction, photosynthetic regulation system of *A. ordosica* lost its ability to resist drought. Studies have shown that under severe adverse conditions, the destruction of antioxidant system caused by excess energy of photochemical system leads to irreversible damage of photosynthetic system, which eventually leads to a decline in productivity (Huang et al., 2005; Dani and Dhawan, 2006; Verslues et al., 2006; Bhaduri and Fulekar, 2012). Thus, under future predicted patterns of decreased precipitation and increased frequency of extreme droughts, *A. ordosica* populations may experience an irreversible decline.

4.2 Responses of *A. ordosica* to drought stress at different growth stages

Studies have shown that the growth stages had a significant effect on the photosynthetic physiology of drought resistance (Norwood et al., 2000; Liu et al., 2016). Decreasing rainfall damaged the photosynthetic system of *A. ordosica*, and the damage showed heterogeneity among different growth stages of individuals. Plants regulate the photosynthetic membrane system via the PSII photosystem and antioxidant enzyme system, resulting in the stabilization of photosynthesis. And the photosynthetic system of large *A. ordosica* plant showed more stable under severe stress.

The difference in response to adversity at later growth stages has long been studied. For example, Gao et al. (2010) found that *Robinia pseudoacacia* had the best drought resistance in the early growth stages, followed by the vigorous growth stages and growth telophase. From the perspective of resource acquisition, mature plants generally have stronger resource competitiveness, and thus their ability to cope with adversity is advanced (Pan et al., 2015).

To figure out the resource acquisition ability of *A. ordosica* in different growth stages, we further studied the distribution of underground roots of *A. ordosica*. It was found that the large plants had a deeper root distribution and a larger biomass than the small ones. Surface moisture content (15 cm) showed more variable than the deep moisture content (55 cm) (data not shown), with the occurrence of extreme drought. Therefore, large plants with deep fine roots avoiding the adverse effects of water shortage caused by the moisture content variability of the surface. Therefore, it can be inferred that photosynthetic physiology of large plants is more stable under drought. In this study, it was observed that severe drought stress caused a significant decrease of surface water. Based on this, we speculated that under the trend of reduced precipitation, the small-sized plants of *A. ordosica* population would die first. As a result, population succession becomes biased towards old and larger plants, which is also known as a decline-type population pattern.

4.3 Responses of different aged *A. ordosica* leaves to drought stress

Studies have shown that the photosynthetic system damage caused by stress will depend on leaf age (Buxton et al., 2012). Our results showed that young leaves had a greater drought resistance capability than old leaves under mild drought stress. The internal causes for the differing responses of leaves of different ages to adversity have long been studied. Huang et al. (2005) found that young leaves of papaya are more sensitive to stress because the variation in membrane lipid composition is greater in young leaves than in old leaves (Huang et al., 2005). Wang et al. (2016) showed that the photosynthetic system of young leaves was less development and more vulnerability to stress (Wang et al., 2016).

However, in this study, when the drought intensified to severe stress, old leaves were better able to resist drought than young ones. Yue (2008) certificated that the value of chlorophyll *a/b* could reflect the stacking of the thylakoids in the chloroplasts. The higher the degree of thylakoid stacking, the less likely that photoinhibition would occur and the more drought resistant. The old leaves had stronger thylakoid stacking, so old leaves appear to be more resistant to drought than young ones. This can explain the strong resistance expression of old leaves under severe drought in this study.

The mutual conversion between old and young leaves in drought resistance mechanisms allows *A. ordosica* to cope with different gradients of drought stress conditions, and this is an important survival strategy for *A. ordosica* to adapt to the sandy habitats (Maseda and Fernandez, 2006).

5 Conclusions

When *A. ordosica* was subjected to drought stress, the transfer efficiency of light energy captured by the antenna pigments to the PSII reaction center decreased, whereas the heat dissipation capacity increased. To reduce the damage to the photosynthetic system caused by drought, *A. ordosica* enhanced the free radical scavenging capacity by activating its antioxidant enzyme system. However, when the rainfall was reduced by 70%, the photosynthetic regulation of *A. ordosica* failed. There were differences in the individual age and leaf age levels of plants in terms of the response of their photosynthetic system to drought. Severe drought damaged or even killed the small *A. ordosica* plants, whereas the larger *A. ordosica* plants resisted severe drought stress by absorbing water from deeper soil depths. Under mild drought stress, young leaves had a stronger ability to resist drought than old ones, whereas old leaves were more resistant to drought under severe stress. The response of the photosynthetic system of *A. ordosica* reflected the trade-off of photosynthetic production under different degrees of adversity. This study provides a more comprehensive and systematic perspective for understanding the drought resistance mechanisms of desert plants.

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