Physiological and Antioxidant Parameters in Two *Lycoris* Species as Influenced by Water Deficit Stress

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Abstract. From a field experiment, the changes in morphophysiological characters and antioxidant enzyme activities were studied in two *Lycoris* species (*Lycoris radiata* and *Lycoris aurea*) subjected to 16 days of water deficit stress. With the increase of water deficit stress processing time, leaf relative water content (RWC), membrane stability index (MSI), net photosynthesis (P_n), stomatal conductance (g_s), transpiration rate (E), and chlorophyll (Chl) content decreased in both studied species. The water use efficiency (WUE) showed an opposite tendency between the two species under water deficit stress, where WUE of *L. aurea* decreased moderately and WUE of *L. radiata* increased somehow. Inter-cellular CO_2 concentration (C_i) in *L. aurea* and *L. radiata* decreased in response to water deficit stress at early stages of stress treatment, then increased throughout the rest of the stress period, and reached levels higher than those in well-watered plants at the end of the treatment. In addition, there was a significant increment in soluble sugar content and proline accumulation under water deficit stress in both species, and *L. radiata* showed a much more accumulation. The activity of superoxide dismutase (SOD), guaiacol peroxidase (POD), and ascorbate peroxidase (APX) increased in both plants subjected to water deficit stress while declined as the stress time increased. In *L. aurea*, catalase (CAT) showed a sustained increment, but it responded later and after a transient increase declined again in *L. radiata* under water deficit stress. In conclusion, *L. radiata* was more tolerant to water deficit stress than *L. aurea* as evidenced by its relatively higher water status, higher levels of proline, soluble sugar and pigments, and stronger photo-protection. Moreover, relatively higher antioxidant enzyme activities and lower levels of thiobarbituric acid reactive substances (TBARS) in *L. radiata* were also associated with its better protection against water deficit stress-induced oxidative damage.

Water deficit is considered as a major environmental factor that influences various physiological and biochemical processes in plants (Tezara et al., 1999). Plants can exhibit either drought escape or drought resistance mechanisms, with resistance further classified

into drought avoidance and drought tolerance. Drought is manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell (Miller et al., 2010; Zhu, 2002), leading to increased accumulation of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot OH$) in plants thus causing an oxidative stress (Miller et al., 2010; Zhu, 2002). Plants possess well-developed antioxidant metabolites, enzymes, and nonenzymes to a variable extent, which have the ability to detoxify ROS (Miller et al., 2010). Antioxidant metabolites and nonenzymatic antioxidant systems include ascorbate acid (AsA), carotenoids (Cars), reduced glutathione, β -carotenes, and α -tocopherol. The antioxidant enzymes being the most efficient mechanisms against

oxidative stress, which mainly include SOD; CAT; various PODs; glutathione-ascorbate (GSH) cycle enzymes such as APX, dehydroascorbate reductase, monodehydroascorbate reductase, and glutathione reductase (Farooq et al., 2009).

Lycoris Herb. is a genus of Amaryllidaceae composed of ≈ 20 species and distributed in the moist warm temperate woodlands of eastern Asia such as China, Korea, and Japan (Hsu et al., 1994). *L. radiata* and *L. aurea* are two traditional groundcover plants and typical hysteroanthous geophyte appearing in autumn. Their floral stems and flowers start growing from August to September, and the leaves grow from September to October. In the wild, *L. radiata* and *L. aurea* usually distribute in sheltered moist slopes along streams in the mountains, edges of forests, paddy fields, and margins of plantations (Hsu et al., 1994). In addition, *L. radiata* seeds are often difficult to germinate and the seedlings take ≈ 10 years to reach flowering size. Therefore, these plants have been usually produced by clonal propagation (Zhou et al., 2007).

Many studies of *Lycoris* have been performed on karyotypes analysis (Hsu et al., 1994; Zhou et al., 2007), morphology (He et al., 2011), medicine (Toriizuka et al., 2008), photosynthetic characteristics (Liu et al., 2012; Meng et al., 2008), molecular aspect (He et al., 2010), and characterization of transcriptome (Chang et al., 2011; Wang et al., 2013). Although it has been considered that *Lycoris* bulbs are very durable, tolerating the extremes of drought and waterlogging, as well as poor soil conditions, little information is available in the literature on the underlying mechanisms of water deficit stress-induced oxidative stress in *Lycoris*. Meanwhile, the comparisons among different *Lycoris* species under water deficit stress were poorly investigated. Here, the effects of water deficit stress on various aspects of physiological responses in two *Lycoris* species *L. radiata* and *L. aurea* were examined, with the aim of identifying factors that influence tolerance to water deficit stress in *Lycoris* species.

Materials and Methods

Plant material and growth conditions. The bulbs of two species (*L. radiata* and *L. aurea*) with the same or similar sizes (1.8–2.2 cm) in diameter were planted and grown in a greenhouse at the research station of Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (118°83' E, 32°05' N), Nanjing, China. For the water deficit stress treatment, one seedling (with 2–3 leaves) was transferred into each plastic pot (15.0 cm top diameter and 14.5 cm deep), containing 1 kg of dry loamy soil of 25% sand, 25% organic matter, and 50% peat. Plants were placed in a chamber at a mean temperature 21.6 ± 1.4 °C, 53.7% $\pm$ 3.6% relative humidity, 14/10 h day/night rhythm with a photosynthetic photon flux (PPF) density of 150–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For water deficit stress, two watering regimes were set:

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75% field capacity (FC) as the control group and 35% FC as the stress group. FC was calculated according to: $FC = (SW - DW)/DW$, where SW was the weight of saturated soil and DW was the weight of dry soil. The pot masses were calculated for 75% and 35% FC according to the above equation. SW was determined after the pots were saturated for 24 h (the gravimetric water was allowed to drain). Then the soil was dried and DW was determined (Sun et al., 2013). During the experiment, the evapotranspiration loss was supplemented gravimetrically by weighing the pots every other day. Pots were randomly and periodically rotated to minimize the effect of environmental heterogeneity. At the indicated time of experiment (lasting for 16 d), three randomly selected seedlings from each treatment per species were harvested, and the leaves in the middle part of the plants were sampled to make physiological assays.

Measurement of leaf RWC. Leaf RWC was estimated gravimetrically according to the method of Shrestha et al. (2003). Briefly, three leaf discs (1 cm²) per plant were cut and weighed immediately to determine their fresh weight (FW). After being immersed in distilled water for 8 h under low irradiance, the leaves were reweighed as turgid weight (TW), and then dried at 70 °C for 48 h to obtain their dry weight (DW). RWC was calculated according to: $RWC (\%) = (FW - DW)/(TW - DW) \times 100$.

Photosynthetic traits measurements. P_n , g_s , C_i , and E were measured with a Li-6400 portable photosynthesis system analyzer (LI-COR, Lincoln, NE) under 25 ± 0.3 °C, 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ intensity of radiation, and 380 ± 2.83 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ concentration. Water use efficiency was calculated as the ratio of P_n/E . The photosynthetic light response curves were constructed after 16 d of water deficit stress treatment. Photosynthesis measurements were made between 9:00 AM and 11:00 AM on fully expanded leaves from each plant with a leaf temperature of 25 °C and a relative humidity of 70%. Leaves were exposed to a series of PPF density (PPFD) values: 2000, 1600, 1300, 1000, 800, 600, 400, 200, 100, 50, 20, and 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Leaf pigments content. Total photosynthetic pigments were extracted with 95% ethanol. Fresh leaf tissue (0.1 g) was placed in a test tube containing 10 mL 95% ethanol and left in the dark for 72 h. The absorbance of the extract was measured spectrophotometrically at 480, 665, 649 nm, and the turbidity of the extract was checked at 750 nm to be sure that it was always less than 0.01. The amounts of Chl and Car were calculated using the equations as described by Lichtenthaler (1987).

Determination of soluble sugar content. Leaves (0.5 g, FW) were ground in a mortar and pestle. Then 1 mL of 80% ethanol was added, and the mixture was filtered. The filtrates were recovered, and the residues were washed again with 80% ethanol and filtered. Both filtrates were mixed, and 3 mL of distilled water was added. The extract was centrifuged at 12,000 g_n for 15 min, and 1 mL

of supernatant was collected. Soluble sugar concentration was determined by the sulfuric acid anthrone method (Yemm and Willis, 1954).

Proline content. 0.5 g of FW leaves was extracted for free proline content measurements according to Bates et al. (1973). The reaction mixture consisted of 2 mL of leaf tissue extract, 2 mL of acid ninhydrin, and 2 mL of glacial acetic acid, and was boiled at 100 °C for 1 h. The reaction was then terminated in an ice bath, and the reaction mixture was extracted with 4 mL of toluene. The absorbance was read at 520 nm, and the free proline content was expressed on FW basis (micrograms per gram).

Measurements of TBARS content and MSI. Lipid peroxidation was estimated by measuring the concentrations of TBARS as described by Hodges et al. (1999). 0.5 g of leaves was homogenized in 5 mL of 80% (v/v) ethanol on ice. Following centrifugation at 10,000 g_n for 20 min at 4 °C, the supernatant (2 mL) was mixed with 2 mL of 20% (w/v) trichloroacetic acid containing 0.65% (w/v) thiobarbituric acid. The mixture was incubated at 95 °C for 30 min and then immediately cooled in an ice bath. After centrifugation at 10,000 g_n for 10 min, the absorbance of the supernatant was measured at 532 nm, subtracting the value for non-specific absorption at 600 nm. The TBARS concentration was calculated from the extinction coefficient 155 $\text{mm}^{-1}\cdot\text{cm}^{-1}$.

For membrane stability analysis, leaf samples (0.5 g) were cut into discs of uniform size and placed in test tube containing 20 mL of distilled water at 40 °C for 30 min. After the end of this period, their electrical conductivity (EC) was recorded by conductivity meter (C1). Subsequently, the same samples were placed on boiling water bath (100 °C) for 10 min and their EC was recorded (C2). The membrane stability index was calculated as $MSI (\%) = [1 - (C1/C2)] \times 100$ (Deshmukh et al., 1991).

Antioxidant enzymes assays. 0.5 g of leaves was homogenized in 10 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid and 1% polyvinylpyrrolidone for CAT (EC 1.11.1.6), POD (EC 1.11.1.7), and SOD (EC 1.15.1.1) assay, or combination with the addition of 1 mM ascorbic acid (ASC) in the case of APX (EC 1.11.1.11) assay. The homogenate was centrifuged at 12,000 g_n for 20 min at 4 °C, and the supernatant was used as the crude enzyme extract. Protein was determined by the method of Bradford (1976) with bovine serum albumin as the standard. CAT activity was spectrophotometrically measured by monitoring the consumption of H₂O₂ ($\epsilon = 39.4$ $\text{mm}^{-1}\cdot\text{cm}^{-1}$) at 240 nm for at least 3 min (Zhang and Kirkham, 1996). POD activity was performed by measuring the oxidation of guaiacol ($\epsilon = 26.6$ $\text{mm}^{-1}\cdot\text{cm}^{-1}$) at 470 nm within 2 min (linear phase) after the addition of H₂O₂ (Herzog and Fahimi, 1973). SOD activity was measured on the basis of its ability to reduce nitroblue tetrazolium (NBT) by O₂⁻ anion generated by the

riboflavin system under illumination. One unit of SOD (U) was defined as the amount of crude enzyme extract required to inhibit the reduction rate of NBT by 50% (Beauchamp and Fridovich, 1971). APX activity was measured by monitoring the decrease in absorbance at 290 nm as ASC was oxidized ($\epsilon = 2.8$ $\text{mm}^{-1}\cdot\text{cm}^{-1}$) for at least 1 min in 3 mL reaction mixture, as described by Nakano and Asada (1981). Each result was the mean of three replications.

Statistical analysis. Where indicated, results were expressed as the mean $\pm$ SE of three independent replicates. Analyses were performed with the software Statistical Package for the Social Science (SPSS) version 11.0 (SPSS Inc., Chicago, IL). For statistical analysis, the Duncan's multiple range test ($P < 0.05$) was chosen where appropriately. Significant intervariable correlations were determined with a Pearson's coefficient test at $P < 0.05$ or $P < 0.01$.

Results

Leaf RWC. Compared with the treatment of day 0, the leaf RWC reduced slightly but not significantly in both *L. radiata* and *L. aurea* under unstressed condition. There were also no significant changes in RWC between *L. radiata* and *L. aurea* (Fig. 1). With the water deficit stress, the obvious decreases of RWC were observed in both species when compared with their non-stressed controls at the different water deficit stress time. For example, after 4 d of water deficit stress, *L. radiata* leaves showed a slight decrease in the RWC from 82.75% to 77.75%, while a significant decrease from 85.53% to 75.09% in RWC of *L. aurea* was observed ($P < 0.05$). The longer the water deficit stress was imposed, the more RWC content declined. After 16 d of further water deficit stress, the RWC of *L. radiata* and of *L. aurea* declined with a 16.04% and 24.65% decrease of the initial values (day 0), respectively (Fig. 1).

Fig. 1. Changes of leaf relative water content (RWC) in *Lycoris radiata* and *Lycoris aurea* subjected to water deficit stress for the indicated time. Data are the means of three replicates with sds shown by vertical bars. Data of each parameter within each species followed by the same letter are not significantly different ($P < 0.05$).

Photosynthetic traits. Gas exchange measurements were conducted under defined conditions in the chamber for the experimental period. In the unstressed condition, P_n was higher in *L. radiata* than in *L. aurea* (Fig. 2A). Under water deficit stress, a gradual reduction in P_n was observed in the two species (Fig. 2A). The difference in the reduction in P_n between *L. aurea* and *L. radiata* was much more apparent under water deficit stress. For example, the extent of reduction in P_n after 4 d of water deficit stress was lower in *L. radiata* (7.6%) and more pronounced in *L. aurea* (26.2%). Further, 16 d withholding water caused 19.6% and 56.9% decrease in P_n , relatively to control value in *L. aurea* and *L. radiata*, respectively (Fig. 2A). Besides, water deficit stress strongly inhibited E (Fig. 2B). In both *L. radiata* and *L. aurea*, E was reduced to a similar extent by water deficit stress. For instance, after 4 d of treatment, it caused 16.60% and 15.43% reduction for *L. radiata* and *L. aurea*, respectively, compared with their unstressed controls (Fig. 2B). As water deficit stress treatment time increased, E continuously decreased up to day 16 (Fig. 2B).

Under unstressed condition, g_s was higher in *L. aurea* than in *L. radiata* at each time point, whereas the g_s of both *L. radiata* and *L. aurea* was reduced to a similar extent by water deficit stress (Fig. 2C). Similar to E , C_i

of *L. aurea* was slightly higher than *L. radiata* under the unstressed conditions at each time point (Fig. 2D). After water deficit stress, the tendency of C_i in the two species decreased first and then increased, but obvious differences existed. For example, the minimum C_i of *L. aurea* was observed at day 4 with water deficit stress (with a decrease of 13.3% compared with its unstressed sample), whereas the minimum of *L. radiata* appeared at day 8 (with a decrease of 20.6% compared with its unstressed samples). After 16 d of treatment, C_i of the two species increased greatly than their corresponding unstressed controls (with an increment of 11.8% in *L. aurea* and an increment of 15.9% in *L. radiata*). It is worthy of being noticed that the WUE in leaves of *L. aurea* showed an opposite tendency compared with WUE in *L. radiata* under water deficit stress (Fig. 2E). Under unstressed condition, WUE of *L. radiata* was higher than *L. aurea* at each time point, which is similar to the changes of P_n (Fig. 2E). During the treatment period, *L. aurea* showed a moderately decreased trend, while WUE of *L. radiata* increased somehow (Fig. 2E). Additionally, Changes in g_s were well correlated with P_n values ($r = 0.830$, $P < 0.01$ and $r = 0.947$, $P < 0.01$) only in water deficit-stressed *Lycoris* species (Fig. 3A). Similar results were also observed in the correlation between g_s and E (Fig. 3B).

Photosynthetic light response curves showed that P_n increased with the increment of PPFD and the patterns of the light response curves were similar between two species under both stressed and unstressed conditions (Fig. 4). Under water deficit stress, both the species recorded lower light saturation point (LSP) than those grown under unstressed condition (Table 1). The LSP for *L. radiata* under unstressed condition was found to be the highest. In contrast to LSP, it was observed that the light compensation point was higher under water deficit stress compared with the unstressed condition where there were no significant differences between the two species. In addition, the apparent

Fig. 2. (A) Net photosynthetic (P_n), (B) transpiration rate (E), (C) stomatal conductance (g_s), (D) intercellular CO_2 concentration (C_i), and (E) water use efficiency (WUE) changes in *Lycoris radiata* and *Lycoris aurea* as influenced by water deficit stress. Data are the means of three replicates with SDS shown by vertical bars. Data of each parameter within each species followed by the same letter are not significantly different ($P < 0.05$).

Fig. 3. Correlations between (A) stomatal conductance (g_s) and net photosynthesis (P_n) and between (B) g_s and transpiration rate (E) in *Lycoris radiata* and *Lycoris aurea* under water deficit stress. ** represents significance at the 1% levels of probability.

Fig. 4. Effect of water deficit stress on the light response curve of the two *Lycoris* species. Data are the means of three replicates with SDS shown by vertical bars.

Table 1. Effect of water deficit stress on light compensation point (LCP), light saturation point (LSP), and apparent quantum yield (AQY) in *Lycoris radiata* and *Lycoris aurea*.

Species	Treatment	LSP ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	LCP ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	AQY ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
<i>L. radiata</i>	Control	1,433.11 $\pm$ 113.14 a (100%) ^z	111.39 $\pm$ 11.31 ab (100%)	0.0249 $\pm$ 0.0021 a (100%)
	Stress	1,247.25 $\pm$ 84.85 ab (87.0%)	125.04 $\pm$ 7.78 ab (112.3%)	0.0217 $\pm$ 0.0017 ab (87.1%)
<i>L. aurea</i>	Control	1,402.82 $\pm$ 98.99 ab (100%)	106.86 $\pm$ 6.90 b (100%)	0.0222 $\pm$ 0.0018 ab (100%)
	Stress	1,142.14 $\pm$ 76.37 b (81.4%)	131.46 $\pm$ 7.07 a (123.0%)	0.0182 $\pm$ 0.0014 b (82.0%)

^zData in parentheses are relative values of the control.

The values represent the averages ($\pm$ SE) of three independent replicates followed by different letters within columns are significantly different at $P < 0.05$, according to Duncan's multiple range test.

Fig. 5. (A) Chlorophyll a, (B) chlorophyll b, and (C) carotenoid content changes in *Lycoris radiata* and *Lycoris aurea* as influenced by water deficit stress. Data are the means of three replicates with SDs shown by vertical bars. Data of each parameter within each species followed by the same letter are not significantly different ($P < 0.05$).

quantum yield (AQY) measured in the stress condition for the two species displayed lower values than those from the unstressed controls, and *L. aurea* under water deficit stress exhibits the lowest AQY (Table 1).

Pigments. The Chl contents were reduced in both species under water deficit stress (Fig. 5A). Under unstressed condition, the values of Chl were higher in *L. radiata* than in *L. aurea* (Fig. 5A). After 16 d of water deficit stress, the Chl contents decreased 33.95% and 39.95% in *L. radiata* and *L. aurea*, respectively, when compared with the unstressed sample. Meanwhile, water deficit stress also decreased the ratio of Chl a to Chl b in both *L. radiata* and *L. aurea* (Fig. 5B). However, there were no significant differences on the ratio of Chl a to Chl b between *L. radiata* and *L. aurea* under water deficit

Table 2. Soluble sugar and proline content in the two *Lycoris* species subjected to water deficit stress for the indicated time.

Species	Treatment	Soluble sugar content ($\text{mg}\cdot\text{g}^{-1}$ FW)	Proline content ($\mu\text{g}\cdot\text{g}^{-1}$ FW)
<i>L. radiata</i>	Day 0	10.89 $\pm$ 0.33 c (100%) ^z	19.73 $\pm$ 1.73 c (100%)
	Day 4 control	10.80 $\pm$ 0.44 c (99.2%)	19.04 $\pm$ 1.82 c (96.5%)
	Day 4 stress	12.84 $\pm$ 0.75 b (117.9%)	24.76 $\pm$ 2.04 b (125.5%)
	Day 16 control	11.93 $\pm$ 0.60 bc (109.6%)	21.01 $\pm$ 1.80 bc (106.5%)
	Day 16 stress	16.34 $\pm$ 0.6 a (150.0%)	49.25 $\pm$ 2.50 a (249.6%)
<i>L. aurea</i>	Day 0	11.31 $\pm$ 0.65 c (100%)	18.15 $\pm$ 1.64 c (100%)
	Day 4 control	11.36 $\pm$ 0.40 c (100.4%)	17.82 $\pm$ 1.50 c (98.2%)
	Day 4 stress	12.79 $\pm$ 0.65 b (113.1%)	21.08 $\pm$ 1.58 bc (116.1%)
	Day 16 control	12.05 $\pm$ 0.47 bc (106.5%)	19.85 $\pm$ 1.55 c (109.4%)
	Day 16 stress	13.15 $\pm$ 0.51 b (116.3%)	24.22 $\pm$ 0.48 b (133.4%)

^zData in parentheses are relative values of the control.

The values represent the averages ($\pm$ SE) of three independent replicates followed by different letters within columns are significantly different at $P < 0.05$, according to Duncan's multiple range test.

stress. In addition, under unstressed condition, *L. radiata* and *L. aurea* exhibited a slight but not significant increase on the ratio of Car to Chl (Fig. 5C). The peak of the ratio of Car to Chl in *L. radiata* was observed after 8 d treatment of water deficit stress, with a 16.9% increment when compared with the day 0. The maximum of *L. aurea* on the ratio of Car to Chl appeared at day 4.

Changes of soluble sugar and proline content under water deficit stress. The soluble sugar contents were increased in both species under water deficit stress (Table 2). Water deficit stress-induced soluble sugar increased by $\approx 17.9\%$ and 50.0% in *L. radiata* after 4 and 8 d of water deficit stress, respectively, when compared with day 0, whereas soluble sugar content in *L. aurea* only increased by $\approx 13.1\%$ and 16.3% ($P < 0.05$, Table 2). Similar to soluble sugar, the proline content in both species also showed increased tendency under water deficit stress (Table 2). The proline contents of *L. radiata* plants were higher than those of *L. aurea* at each time of water deficit stress treatment. In particular, after 16 d of water deficit stress, the proline content of *L. radiata* was ≈ 2 -fold that of *L. aurea*.

Lipid peroxidation and MSI. In Fig. 5A, TBARS contents of *L. radiata* and *L. aurea* seedlings subjected to water deficit stress for 16 d were shown. After 16 d treatment of water deficit stress, it was increased by $\approx 26.46\%$ in *L. radiata* and 63.86% in *L. aurea* when compared with their unstressed controls. In addition, MSI significantly decreased in both *L. radiata* and *L. aurea* under water deficit stress (Fig. 6B). There were no significant differences between *L. radiata* and *L. aurea* in MSI under unstressed condition. But there was a small but significant difference between the two at 12 d of water

deficit stress, with the *L. aurea* having slightly lower levels than *L. radiata*.

Antioxidant enzymes. Figure 7 showed the activity changes of SOD, POD, CAT, and APX in the two species. Activity of SOD in the two species showed a similar trend, but the SOD activity of *L. radiata* was greater than that of *L. aurea* under both unstressed and stressed conditions. SOD activity of *L. radiata* peaked after 12 d of water deficit stress treatment, up to $206.08 \text{ U}\cdot\text{g}^{-1}$ FW, and the maximum of *L. aurea* appeared at day 8, up to $139.25 \text{ U}\cdot\text{g}^{-1}$ FW, which were 167% and 143% as compared with their unstressed controls, respectively. Meanwhile, the highest rate of increase in SOD activity was still found in *L. radiata* on every indicated day of water deficit stress treatment (Fig. 7A).

POD activity of *L. aurea* was higher than *L. radiata* under unstressed conditions (Fig. 7B). Although at the early stage of water deficit stress, the POD activity of *L. aurea* was greater than *L. radiata*. POD activities were 130% and 129% at day 4, and 160% and 160% at day 8 as compared with their unstressed controls in *L. aurea* and *L. radiata*, respectively. Compared with *L. radiata*, the peak of POD activity in *L. aurea* displayed earlier, which appeared at day 8 with water deficit stress treatment. After 12 d of further water deficit stress, POD activity in *L. aurea* reached the maximum, while the POD activity in *L. aurea* began to decline. At the last stage of water deficit stress treatment, POD activities of *L. radiata* and *L. aurea* decreased, and there was even no difference detected between the unstressed control and the stressed sample in *L. aurea* (Fig. 7B).

When treated with water deficit stress, CAT activity of *L. aurea* obtained the maximum height at day 8, whereas the maximum CAT activity of *L. radiata* displayed at day

16, 24.47% and 34.82% greater than their unstressed controls, respectively. CAT activities in two species were still greater than their unstressed controls at day 12. However, *L. aurea* showed a moderate trend, and even after 16 d of water deficit stress, CAT activity of *L. aurea* reached almost the same value as the unstressed control (Fig. 7C).

Under water deficit stress, activities of APX in the two species showed a similar

trend, but the activity of *L. radiata* was significantly greater than that of *L. aurea* at each treatment time point. APX activities of the two species simultaneously achieved the maximum at day 12. Subsequently, APX activities of both species decreased and they were 153% and 109% of their unstressed controls at day 16, independently. It is obvious that APX activity of *L. aurea* declined more than that of *L. radiata* (Fig. 7D).

Discussion

High RWC is associated with water deficit stress resistance, and it also has been proposed as a more valuable indicator of plant status (Keles and Oncel, 2002). In this study, we showed that *L. radiata* maintained a relatively higher leaf RWC as compared with *L. aurea* (Fig. 1). This difference could be the cause of the higher tolerance to water deficit stress of *L. radiata* than *L. aurea*.

Decreased P_n is due to stomatal and non-stomatal limitations (Flexas et al., 2006). Plants react to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration, resulting in limitation of ambient CO_2 diffusion to the mesophyll and thus reduction of photosynthesis (Flexas et al., 2006). It is suggested that the photosynthetic capacity of a plant was determined to large extent by its natural habitat and itself, and previous study has showed that *L. radiata* var. *pumila* and *L. aurea* were more adapted to a relatively high irradiance among four *Lycoris* species (Liu et al., 2012). Our results revealed a progressive reduction in the rate of P_n and g_s in the two species. However, *L. aurea* showed a higher decrease in P_n than *L. radiata* (Fig. 2A). Transpiration rate (E) reduction

under water deficit stress is one of the plant responses for water maintenance. It has also been suggested that a major physiological reason for susceptibility differing between plant drought-susceptible and drought-resistant genotypes was the more efficient protection of tissue water status in resistant genotypes through greater decreases in C_i , and thus in E , compared with susceptible genotypes (Grzesiak et al., 2006). Our results revealed higher decrease of gas exchange parameters C_i and E on duration of water deficit stress for *L. radiata* in comparison with *L. aurea* (Fig. 2B and D). Maintaining P_n under reduced g_s and E in *L. radiata* during water deficit stress might lead to the increase in WUE (Fig. 2E). It has been stated that the WUE is critical to plant survival and crop yield. Higher WUE under drought stress is probably a consequence of gas exchange regulation. The E parameter is more influenced by water deficit than P_n in *L. radiata* and it had higher WUE during the water deficit stress period, which showed its ability to save water and uphold physiological activities under water deficit conditions. The present results are in agreement with findings reported previously (Bota et al., 2001; Gordon et al., 1999; Karimi et al., 2015; Zhang et al., 2004). In addition, correlations between P_n and g_s , and between g_s and E were significantly linear and they were enhanced by water deficit stress (Fig. 3). Similar results were also observed previously (Sun et al., 2013). The relatively high AQY in low light indicates that shaded leaves have higher light-use efficiency, which is important for seedling establishment and growth (Zhang et al., 2007). In the current study, the values of LSP and AQY were higher in the leaves of *L. radiata* than those in *L. aurea* under water deficit stress (Table 1). In addition, P_n increased with the increasing of PPFD in both species (Fig. 4).

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Water deficit stress can also alter the tissue concentrations of Chls and Cars (Farooq et al., 2009). Reduction of Chl contents under water deficit stress has been considered as a typical symptom of oxidative stress and may be the result of pigments of photooxidation and Chl degradation (Moran et al., 1994; Zhang and Kirkham, 1996). In addition, as photosynthetic pigments, Cars have the capacity to scavenge singlet oxygen and lipid peroxy radicals, as well as have the capacity to inhibit lipid peroxidation and superoxide generation under dehydrative forces (Havaux, 1998). The total Chl contents significantly decreased in both species under water deficit stress, but the reduction in *L. radiata* was not as great in *L. aurea* (Fig. 5A and B). In addition, the ratio of Car to Chl increased first and then decreased in the two species under water deficit stress while *L. radiata* accumulated more Cars than *L. aurea* (Fig. 5C). These results suggested the presence of genetic variability for pigments retention between the two species under water stress deficit conditions.

Fig. 6. (A) Thiobarbituric acid reactive substances (TBARS) content and (B) membrane stability index (MSI) changes in *Lycoris radiata* and *Lycoris aurea* as influenced by water deficit stress. Data are the means of three replicates with sds shown by vertical bars. Data of each parameter within each species followed by the same letter are not significantly different ($P < 0.05$).

Fig. 7. (A) Superoxide dismutase (SOD), (B) guaiacol peroxidase (POD), (C) catalase (CAT), and (D) ascorbate peroxidase (APX) activity changes in *Lycoris radiata* and *Lycoris aurea* as influenced by water deficit stress. Data are the means of three replicates with sds shown by vertical bars. Data of each parameter within each species followed by the same letter are not significantly different ($P < 0.05$).

Increasing of proline amount due to water deficit stress has been reported previously (Mattioni et al., 1997). It is well known that proline, which accumulates in plants under dehydration and excessive osmotic pressure, contributes toward osmotic adjustment as key osmolyte (Yamada et al., 2005). This molecule acts not only as a protectant of the cell membrane and enzymes but also could provide energy for growth and survival under stress (Kavi Kishor et al., 2005). It was also shown that proline possesses antioxidant capacities (Szabados and Savouré, 2010). As we observed, proline accumulation in *L. radiata* during increasing water deficit was twice the amount found in *L. aurea* (Table 2), suggesting the better osmotic adjustment and better protection against reduced oxidative damage in *L. radiata*. As another organic osmolyte, soluble sugars (such as glucose and sucrose) can quench ROS, thus contributing to stress tolerance (Couée et al., 2006). Former study has showed that the soluble sugars increased significantly with water deficit stress (Khalid et al., 2010). In this study, both the *Lycoris* species accumulated soluble sugar under water deficit stress, whereas at the later stage of stress, the accumulating rate in *L. radiata* was faster than that in *L. aurea* (Table 2).

Water deficit stress also induces oxidative stress in plants by generation of ROS (Farooq et al., 2009). Drought-induced overproduction of ROS increases the content of TBARS. TBARS is the product of lipid peroxidation and its content has been considered an indicator of general oxidative damage (Møller et al., 2007). Low concentration of TBARS has been associated with water deficit stress tolerance in some plant species (Moran et al., 1994; Sharma and Dubey, 2005). Our results showed that the levels of TBARS in *L. radiata* were lower than *L. aurea* after water deficit stress, indicating the better protective mechanism of reduced oxidative damage in *L. radiata*. Moreover, a decrease in membrane stability also reflects the extent of lipid peroxidation. It is also suggested that the membrane disruption may alter water, ion, and organic solutes movement across the plant membrane, which interferes with photosynthesis and transpiration (Almeselmani et al., 2006). We noticed that *L. radiata* had a higher MSI than *L. aurea* under control as well as stress conditions at each treatment time (Fig. 6). This result indicated that lower MSI in *L. aurea* at water deficit stress stage was probably related to higher susceptibility to water loss.

In addition, the degree of damage caused by water deficit stress should be strongly dependent on the efficiency of activated protection and repair mechanisms, such as the antioxidant defenses, which have both enzyme and nonenzyme constituents (Møller et al., 2007). In this study, water deficit stress led to the increased production of ROS in two *Lycoris* species, which is reflected by the increase in the activities of antioxidant enzymes such as SOD, POD, CAT, and APX (Fig. 7). Within a cell, SOD constitutes the

first line of defense against ROS (Ruth et al., 2002). It reacts with O_2^- and converts them to O_2 and H_2O_2 . Water deficit stress also led to a relatively higher increase of SOD activity in *L. radiata* than in *L. aurea*, which meant that SOD activity also associates with the better protection against oxidative damage in *L. radiata* (Fig. 7A). CAT and POD play essential roles as scavengers in H_2O_2 toxicity (Foyer and Noctor, 2005). Several studies have provided evidence of an effective protector role of the POD–CAT–SOD system against oxidative stress in diverse plant species (Miller et al., 2010). APX is one of the components of the AsA–GSH cycle, which provides efficient protection against lethal ROS in all the subcellular organelles of plant cells (Møller et al., 2007). It was primarily located both in chloroplasts and cytosol acted as a key enzyme of the Asada–Halliwell pathway, which also eliminated toxic H_2O_2 (Foyer et al., 1994). In this study, the APX activities in *L. radiata* and *L. aurea* leaves were induced by water deficit stress and the activity of APX is much higher in *L. radiata* than in *L. aurea* (Fig. 7D). These results showing the variability in increasing the activities of these antioxidant enzymes between the two species indicates their differential ability to acquire water deficit stress tolerance.

In conclusion, water deficit stress affects many physiological and biochemical processes in the two *Lycoris* species, and we showed the varying responses and sensitivity to water deficit stress of them. Compared with *L. aurea*, *L. radiata* was more tolerant to water deficit stress as evidenced by its relatively higher water status, higher levels of proline, soluble sugar and pigments, and stronger photoprotection. Moreover, relatively higher antioxidant enzyme activities and lower levels of TBARS in *L. radiata* were also associated with its better protection against water deficit stress-induced oxidative damage.

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