Resurrection Characteristics of Two Gesneriaceae Species from Different Habitats and Their Photosynthetic and Physiological Responses to Water Postprint

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Abstract

Resurrection plants can tolerate extremely arid environments and remain viable after dehydration to 10% RH. Gesneriaceae encompasses numerous resurrection plants, with potentially varying resurrection mechanisms among different taxa. This study examined two Gesneriaceae species distributed in subtropical and temperate limestone regions—Paraboea rufescens and Oreocharis cordatula—to investigate their resurrection capabilities and compare their physiological drought response mechanisms. Leaves were dehydrated for 1, 2, and 3 days, followed by 1 day of rehydration. Changes in leaf morphology, relative water content, photosynthetic activity, membrane integrity, and osmotic adjustment substances were monitored throughout the dehydration-rehydration cycles. The results demonstrated that leaf discs of Paraboea rufescens folded inward upon dehydration, completely enveloping the adaxial surface by day 2, while chlorophyll fluorescence Fv/Fm, an indicator of maximum photosynthetic potential, was inhibited. Conversely, leaves of Oreocharis cordatula exhibited only mild wrinkling, maintained Fv/Fm at control levels, and displayed higher photoprotective capacity [Y(NPQ)]. Following rehydration, leaves of both species unfolded and Fv/Fm recovered. At 2 days of dehydration, both species reduced relative water content to approximately 5%, with electrical conductivity increasing to 51.8% and 56.2%, respectively, while soluble sugar content, an osmotic adjustment substance, rose significantly. These parameters returned to control levels upon rehydration. After 3 days of dehydration, relative water content in both species decreased to approximately 1.5%, post-rehydration electrical conductivity increased to approximately 95%, and chlorophyll fluorescence Fv/Fm was abolished. Chlorophyll a+b content decreased by 50% in Paraboea rufescens during the recoverable dehydration-rehydration cycle, whereas it remained stable in Oreocharis cordatula, indicating that both are chlorophyll-retaining res-

urrection plants capable of rapidly restoring photosynthesis upon rehydration. Membrane lipid peroxidation products remained unchanged and at minimal levels throughout the dehydration-rehydration process in both species, suggesting effective protection against membrane oxidation under extreme drought. In conclusion, both species tolerate dehydration to 5% RH and qualify as resurrection plants. Under severe dehydration, Paraboea rufescens protects itself by curling leaves to avoid excessive light absorption, while Oreocharis cordatula dissipates absorbed excess energy as heat via the PSII photoprotection mechanism, thereby preserving photosynthetic apparatus integrity.

Full Text

Preamble

Resurrection Characteristics and Physiological Responses to Desiccation and Rehydration in Two Gesneriaceae Species from Different Habitats

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Abstract: Resurrection plants can tolerate extremely arid environments and revive after dehydration to 10% relative humidity (RH). The Gesneriaceae family contains numerous resurrection plants, though the mechanisms underlying desiccation tolerance may vary among different taxa. This study examined two Gesneriaceae species—*Paraboea rufescens* from subtropical limestone regions and *Oreocharis cordatula* from temperate limestone regions—to investigate their resurrection capabilities and compare their physiological responses to drought. Leaf discs were dehydrated for 1, 2, or 3 days, then rehydrated for 1 day. Changes in leaf morphology, relative water content (RWC), photosynthetic activity, membrane integrity, and osmotic adjustment substances were measured throughout the dehydration-rehydration cycles. The results showed that *P. rufescens* leaf discs folded inward during dehydration, with the upper epidermis completely enveloped after 2 days of dehydration, accompanied by inhibition of chlorophyll fluorescence Fv/Fm (maximum photosynthetic potential). In contrast, *O. cordatula* leaves only showed slight wrinkling, maintained Fv/Fm at control levels, and exhibited higher photoprotective capacity [Y(NPQ)]. Upon rehydration, leaves of both species unfolded and Fv/Fm recovered. After 2 days of dehydration, RWC in both species decreased to approximately 5%, electrolyte conductivity increased to 51.8% and 56.2% respectively, and soluble sugar content (an osmotic adjustment substance) rose significantly. These parameters

all returned to control levels after rehydration. After 3 days of dehydration, RWC in both species reached approximately 1.5%; upon rehydration, conductivity increased to about 95% and chlorophyll fluorescence Fv/Fm disappeared. Chlorophyll a+b content in *P. rufescens* decreased by 50% during the recoverable dehydration-rehydration cycle, while remaining essentially unchanged in *O. cordatula*, indicating that both are homoiochlorophyllous resurrection plants capable of rapidly resuming photosynthesis after rehydration. Membrane lipid peroxidation product content showed no significant changes and remained at extremely low levels throughout the dehydration-rehydration process in both species, suggesting they can protect their membrane lipids from oxidation under extreme drought conditions. In summary, both species can tolerate dehydration to 5% RH and are therefore resurrection plants. Under severe dehydration, *P. rufescens* avoids damage from excessive light absorption by curling its leaves, whereas *O. cordatula* dissipates absorbed excess energy as heat through PSII photoprotection mechanisms, thereby protecting its photosynthetic apparatus.

Keywords: *Paraboea rufescens*, *Oreocharis cordatula*, desiccation, resurrection plant, chlorophyll fluorescence, osmotic adjustment substance, malondialdehyde, photoprotection

Resurrection plants typically inhabit extremely harsh environments, often appearing in habitats with sporadic rainy seasons, including desert regions in tropical and subtropical zones (Rascio et al., 2005) or karst habitats with relatively abundant rainfall but karst drought phenomena (Zeng et al., 2007; Liu, 2016). Resurrection plants are generally small in stature (Moore et al., 2007) and are relatively rare among higher plants, with approximately 350 confirmed species (Lüttge et al., 2011), and new desiccation-tolerant species continue to be discovered. Resurrection plants serve as excellent models for exploring the physiological, biochemical, and molecular basis of desiccation tolerance in plants. Understanding their unique characteristics will help improve crop yields under water-deficient conditions. The Gesneriaceae family contains many resurrection plants, with reports of twenty to thirty species (Porembski, 2011). *Paraboea rufescens* and *Oreocharis cordatula* are both Gesneriaceae species; the former is distributed in southwestern Guangxi, southern Guizhou, and Yunnan Province in China, growing on limestone rocks and crevices in karst habitats at 700–1,500 m elevation, while the latter occurs in Shangri-La County, Yunnan and Muli County, Sichuan, inhabiting limestone surfaces and crevices on mountaintops and valleys at 2,100–2,700 m elevation. Both habitats are considered centers of diversity for resurrection plants (Rascio et al., 2005). *P. rufescens* is a perennial herb with lignified rhizomes, rarely subshrubs; the upper epidermis is covered with cobweb-like wool that becomes nearly glabrous later, while the lower epidermis is typically densely covered with interwoven felt-like hairs that are tufted, stellate, or dendritically branched. *O. cordatula* is a perennial acaulescent herb with thick, short rhizomes; all leaves are basal and petiolate, with blades that are oblong-lanceolate or oblong-ovate, margins with irregular rounded teeth, upper epidermis densely appressed-pubescent, and lower epidermis densely covered with light brown silky wool (Wang et al., 1990). To investigate whether

P. rufescens and *O. cordatula* possess resurrection characteristics and to compare their response mechanisms, we studied their physiological, biochemical, and photosynthetic changes during dehydration and rehydration.

Plants undergo a series of physiological and biochemical changes in response to drought. Many plants accumulate non-aqueous substances such as amino acids and soluble sugars to replace water and maintain their original cell volume during dehydration (Farrant, 2000). The most common change in resurrection plants after water loss is massive accumulation of soluble sugars and rapid conversion of starch to glucose (Bianchi et al., 1993; Muller et al., 1997). Studies have also found that proline content, an osmotic adjustment substance, typically increases significantly in resurrection plants during dehydration (Tymms et al., 1979; Pandey et al., 2010). Drought-induced peroxides oxidize membrane lipids to produce malondialdehyde (MDA), destroying membrane integrity. In the resurrection plant *Selaginella bryopteris*, MDA content increases by 30% after dehydration to 10% relative water content (Pandey et al., 2010) and even doubles in some cases (Jovanovic et al., 2011), but can recover to control levels after rehydration.

Chloroplast thylakoid membranes are the primary organelles for photosynthesis in plants. Chloroplasts in resurrection plants undergo changes during dehydration and rehydration. Homoiochlorophyllous desiccation-tolerant (HDT) species can maintain most of their chlorophyll content and thylakoid structure during dehydration, with only minor damage to thylakoid membranes, allowing rapid recovery of photosynthesis after rehydration (Strasser et al., 2010; Tuba et al., 1998), making them suitable for short-term intermittent water-deficient environments. In contrast, poikilochlorophyllous desiccation-tolerant (PDT) species completely degrade chlorophyll during dehydration and resynthesize it during rehydration, with membrane structures being repaired (Ingle et al., 2008). Overall, PDT species require more time to revive than HDT species because they need to resynthesize chlorophyll and reconstruct thylakoid structures (Sherwin et al., 1996). Therefore, measuring chlorophyll content during dehydration-rehydration cycles can reveal strategies of photosynthetic organ responses to drought environments. Chlorophyll fluorescence is an important probe for measuring photosynthesis, particularly the light energy conversion of Photosystem II (PSII), and can detect changes in light energy absorption, transfer, dissipation, and allocation, making it an ideal tool for studying the relationship between photosynthetic physiology and stress (Roháček et al., 2008). Photosynthesis in resurrection plants changes during dehydration, with minimal changes in photosynthetic activity under mild dehydration but complete loss under severe dehydration, followed by recovery after rehydration (Farrant et al., 2003; Georgieva et al., 2005).

Based on this background, we selected *P. rufescens* and *O. cordatula*, distributed in subtropical and temperate limestone regions respectively, subjected them to different degrees of dehydration and post-dehydration rehydration, and measured changes in leaf morphology, photosynthetic activity indices, photo-

synthetic pigment content, membrane integrity indicators, and osmotic adjustment substance content during the dehydration-rehydration process. This study aims to investigate the desiccation tolerance characteristics and compare the dehydration-rehydration mechanisms of the two Gesneriaceae species, providing theoretical background for plant drought resistance physiology and biochemical research, and offering applied scientific guidance for crop genetic improvement based on plant physiological and biochemical characteristics.

Materials and Methods

Plant Materials

Paraboea rufescens plants were collected from limestone crevices in the Naigu Stone Forest, Shilin County, Yunnan Province, while *Oreocharis cordatula* plants were collected from limestone surfaces and crevices in Shangri-La County, Yunnan Province. Soil surrounding the plants was collected together with the specimens. After introduction, plants were cultivated in the greenhouse of the Introduction and Domestication Center at the Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences. Greenhouse conditions were maintained at 20–23 °C, with light intensity of 120 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$, a 12 h light/12 h dark photoperiod, and 60% relative humidity.

Experimental Treatments

Mature, fully expanded leaves were selected from the plants, and leaf discs of 1.5 cm diameter were prepared using a hole punch. Leaf discs were subjected to rapid dehydration at 15 °C and 15% relative humidity (RH) for 1 day (Deh1), 2 days (Deh2), or 3 days (Deh3). Dehydrated leaf discs from each treatment were then rehydrated by placing them on filter paper saturated with water in petri dishes and incubating at 20 °C in darkness for 24 h, designated as Reh1, Reh2, and Reh3 respectively.

Samples from different dehydration and rehydration treatments were collected for measurement of relative water content (RWC), photosynthetic pigment content, chlorophyll fluorescence parameters, electrical conductivity, MDA, soluble sugar, and proline content, with five replicates per treatment.

Measurement Methods

Relative Water Content Following Barrs et al. (1962), RWC was determined gravimetrically using the formula:

$$RWC(\%) = 100 \times \frac{(Fresh\ Weight - Dry\ Weight)}{(Saturated\ Fresh\ Weight - Dry\ Weight)}$$

where fresh weight refers to the weight of normally growing leaf discs; dry weight refers to leaf disc weight after oven-drying at 80 °C for 48 h; and saturated fresh

weight refers to the weight when leaf discs were placed in water until no further weight increase occurred.

Chlorophyll Fluorescence Chlorophyll fluorescence of leaf discs was measured using a MAXI-Imaging Pulse-Amplitude (PAM) Instrument (Walz, Germany) and analyzed with ImagingWin Software. Leaf discs were dark-adapted for 20 min, after which fluorescence parameters were measured. Fv/Fm, Y(II), and Y(NPQ) were calculated as follows:

$$Fv/Fm = (Fm - F0)/Fm$$
$$Y(II) = (Fm' - Fs)/Fm'$$
$$Y(NPQ) = (Fm - Fm')/Fm'$$

where Fv/Fm is the maximum quantum yield of PSII; Y(II) is the actual quantum yield; Y(NPQ) is the energy dissipated via non-photochemical quenching; F0 is the minimum fluorescence yield after 20 min dark adaptation; Fm is the maximum fluorescence yield measured after a saturation pulse following dark adaptation; Fm' is the maximum fluorescence yield measured after a saturation pulse under illumination when photosynthesis is stable; and Fs is the steady-state fluorescence yield.

Photosynthetic Pigment Content Leaf discs were gently washed with deionized water and soaked in 3 mL N,N-dimethylformamide, then shaken overnight at 25 °C and 80 $r \cdot min^{-1}$. After the leaves turned white, absorbance was measured at 480, 647, and 664 nm using a spectrophotometer. Calculations were performed as follows:

$$Ca = 12 \times A_{664} - 3.11 \times A_{647}$$
$$Cb = 20.78 \times A_{647} - 4.88 \times A_{664}$$
$$Chla(mg \cdot g^{-1}) = Ca \times V/W$$
$$Chlb(mg \cdot g^{-1}) = Cb \times V/W$$
$$Caro(mg \cdot g^{-1}) = [(1000 \times A_{480} - 1.12 \times Ca - 34.07 \times Cb)]/245 \times V/W$$

where Ca and Cb are the concentrations of chlorophyll a and b in the extract; V is the extraction solution volume; W is the dry weight of leaf discs; and Chla, Chlb, and Caro represent the contents of chlorophyll a, chlorophyll b, and carotenoids in leaf discs, respectively.

Relative Electrical Conductivity Leaf discs were placed in clean test tubes with 2 mL room-temperature deionized water to immerse the plant material. Tubes were incubated on a shaker at 25 °C and 80 r · min^{-1} for 2 h, after which electrical conductivity C1 was measured using a Leici DSS-II conductivity meter. Tubes were then boiled in a water bath for 30 min, cooled to room temperature, and conductivity C2 was measured. Relative electrical conductivity (REC) was calculated as:

$$REC(\%) = (C1/C2) \times 100\%$$

Malondialdehyde (MDA) Content MDA content was determined using the thiobarbituric acid method. Leaf discs were ground in a mortar with 2 mL 10% trichloroacetic acid (TCA) for 2 min. The extract was transferred to a centrifuge tube, the mortar was rinsed with 3 mL 10% TCA, and the rinse was combined with the extract. After centrifugation at 9,500 r · min^{-1} for 15 min, the supernatant was collected and brought to 5 mL with 10% TCA solution. One milliliter of supernatant was mixed with 1 mL 0.6% thiobarbituric acid (TBA), heated in a 100 °C water bath for 20 min, and rapidly cooled. After centrifugation at 9,500 r · min^{-1} at 4 °C for 10 min, the supernatant absorbance was measured at 532, 600, and 450 nm. MDA content was calculated according to Heath and Packer (1968):

$$MDA\ content\ (\mu mol \cdot g^{-1}) = [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times V/W$$

where V is the extraction volume (0.005 L in this study) and W is the tissue dry weight (g).

Soluble Sugar and Proline Extraction and Detection Extraction and detection of soluble sugars and proline followed Li et al. (2004). Samples were ground in liquid nitrogen, mixed with 4 mL 75% ethanol, transferred to centrifuge tubes, and extracted overnight on a shaker. After centrifugation at 4,500 r · min^{-1} for 15 min, the supernatant was collected for soluble sugar and proline determination.

Soluble sugar content was measured using the anthrone colorimetric method: 40 L of extract supernatant was mixed with 2 mL anthrone reagent, heated in a 100 °C water bath for 1 h, and absorbance was measured at 625 nm. Proline content was determined using the sulfosalicylic acid method: 600 L of extract supernatant was mixed with 900 L ninhydrin, heated in a 100 °C water bath for 1 h, then 4.5 mL toluene was added at a 1:3 sample:toluene ratio, oscillated, and incubated at 23 °C for 24 h before measuring the supernatant absorbance at 520 nm.

Standard curves were prepared using different concentrations of soluble sugar and proline standard solutions with the above methods, and sample concentrations were calculated from the standard curves ($g \cdot mg^{-1}$ DW).

Data Processing and Analysis

Outlier detection was performed using the Q-test method. A set of data to be tested was arranged in descending order, and the Q-value was calculated as:

$$Q = \frac{X_{max} - X_{outlier}}{X_{max} - X_{min}}$$

where Xmax is the maximum measured value; Xmin is the minimum measured value; Xoutlier is the value being tested; and Xadjacent is the adjacent value. In this study, with five replicates, outliers were rejected at the 0.05 level if Qcalculated $>$ 0.73 (Welti et al., 2002); otherwise, they were retained.

Significant differences among data were analyzed using ANOVA with SPSS 16.0 software.

Results

Leaf Morphology and Relative Water Content Changes During Dehydration-Rehydration

As shown in [Figure 1: see original paper]A, *P. rufescens* leaves exhibited severe curling after 1 day of dehydration, with the rust-colored woolly lower epidermis fully exposed and chlorophyll fluorescence Fv/Fm nearly disappearing. After rehydration (Reh1), leaves gradually unfolded and Fv/Fm recovered to control levels. After 2 days of dehydration, leaves curled further, but could still unfold after rehydration (Reh2) with Fv/Fm recovery. However, leaves dehydrated for 3 days and then rehydrated could not fully unfold, turned brown, and chlorophyll fluorescence Fv/Fm disappeared completely. [Figure 1: see original paper]B shows that *O. cordatula* leaves remained flat without curling after 1 day of dehydration, with little change in chlorophyll fluorescence, and after rehydration (Reh1), leaf color and Fv/Fm were similar to controls. After 2 days of dehydration, leaves became wrinkled but Fv/Fm remained at control-like levels, and after rehydration (Reh2), leaves unfolded with Fv/Fm recovering to control levels. After 3 days of dehydration, leaves wrinkled further, Fv/Fm disappeared, and rehydrated leaves (Reh3) could not unfold with Fv/Fm becoming zero.

RWC results during dehydration-rehydration ([Figure 1: see original paper]C) showed that fresh leaf RWC was similar between *P. rufescens* (81.7%) and *O. cordatula* (79.9%), with similar water loss rates. RWC decreased to about half the initial value on day 1, then dropped rapidly to 4.2% and 5.6% respectively on day 2, and reached approximately 1.3% and 1.5% on day 3. After rehydration following 2 days of dehydration, RWC recovered to 78% and 85% respectively.

In summary, both species reached approximately 5% RWC after 2 days of dehydration and could revive upon rehydration; after 3 days of dehydration reaching approximately 1.5% RWC, they lost viability.

Chlorophyll Fluorescence Parameter Changes During Dehydration-Rehydration

Fv/Fm reflects the maximum light energy conversion efficiency, i.e., maximum photosynthetic capacity. Results for Fv/Fm during different dehydration-rehydration treatments ([Figure 2: see original paper]) showed that in *P. rufescens*, Fv/Fm values dropped sharply after 1 and 2 days of dehydration, even reaching zero in the Deh2 treatment, but recovered to control levels after corresponding rehydration (Reh1, Reh2). After 3 days of dehydration, Fv/Fm dropped to zero and remained zero after rehydration, indicating loss of potential maximum photosynthetic capacity. In *O. cordatula*, Fv/Fm values remained similar to controls in Deh1, Deh2, Reh1, and Reh2 treatments; after 3 days of dehydration, Fv/Fm dropped sharply and disappeared completely after rehydration.

Y(II) represents actual light energy conversion efficiency under current conditions. As shown in [Figure 2: see original paper], after light adaptation, Y(II) in both species dropped to very low values. After 1 day of dehydration, Y(II) in *P. rufescens* increased slightly while continuing to decrease in *O. cordatula*. After 2 days of dehydration, Y(II) could not be detected in either species. During rehydration treatments, Y(II) showed a slight increasing trend in Reh2 for both species, but could not be detected in other treatments.

Y(NPQ) represents the portion of energy absorbed by PSII that is dissipated as heat through photoprotective mechanisms, reflecting photoprotective capacity. As shown in [Figure 2: see original paper], Y(NPQ) in both species showed similar overall trends to Fv/Fm. However, in *O. cordatula*, Y(NPQ) showed an increasing trend after 1 day of dehydration compared to controls, indicating enhanced photoprotective capacity. After 2 days of dehydration, Y(NPQ) remained at control levels, while *P. rufescens* Y(NPQ) could not be detected. In the Reh3 treatment, Y(NPQ) dropped to zero while Fv/Fm was 0.07, indicating that after 3 days of dehydration, *O. cordatula* PSII retained potential photosynthetic capacity but had lost photoprotective capacity. This demonstrates that *O. cordatula* had stronger photoprotective capacity during 1 and 2 days of dehydration.

Photosynthetic Pigment Content Changes During Dehydration-Rehydration

Photosynthetic pigment content in leaf discs during dehydration-rehydration is shown in . In *P. rufescens*, chlorophyll a content decreased significantly compared to controls after 1 and 2 days of dehydration, and increased after rehydration of 2-day dehydrated samples (Reh2) but not significantly. Chloro-

phyll b content, chlorophyll a+b content, and carotenoids showed similar trends, with chlorophyll a+b content decreasing by 50% after 2 days of dehydration and carotenoid content decreasing by 28%. The chlorophyll a/b ratio showed the opposite trend, with significant increases in Deh1, Deh2, and Reh2 treatments. In *O. cordatula*, chlorophyll a content decreased significantly compared to controls after 1 and 2 days of dehydration, but increased in Reh2 treatment to control-like levels. Chlorophyll b and chlorophyll a+b contents decreased significantly in Deh1 treatment but showed no significant differences from controls in Deh2 and Reh2 treatments. Carotenoid content decreased during treatments but reached significant levels only in Deh2 treatment. The chlorophyll a/b ratio remained unchanged throughout the dehydration-rehydration process. These results indicate that photosynthetic pigment content decreased during dehydration-rehydration, with partial pigment degradation occurring, and *P. rufescens* experienced more degradation than *O. cordatula*.

Membrane Damage Indicator Changes During Dehydration-Rehydration

The plasma membrane is the first organelle to respond to stress. Membrane permeability in dehydrated leaf cells may change, with damage degree indicated by electrical conductivity. As shown in [Figure 3: see original paper], electrical conductivity in *P. rufescens* gradually increased after 1, 2, and 3 days of dehydration, showing no significant change after 1 day but rising significantly to 51.8% after 2 days and reaching 95% after lethal dehydration of 3 days. Conductivity in Reh1 and Reh2 leaf discs showed no significant differences from corresponding dehydration treatments (Deh1 and Deh2), while Reh3 showed significantly decreased conductivity compared to Deh3, likely because after 3 days of dehydration, leaf cells lost viability, cell membranes ruptured, and large amounts of electrolytes leaked out of the leaves, causing a sharp decrease in electrolytes. In *O. cordatula*, conductivity was similar to controls after 1 day of dehydration and rehydration, but increased significantly to 56.2% after 2 days of dehydration (Deh2) and continued rising to 95.7% after lethal dehydration of 3 days. No significant difference in conductivity was observed between Reh2 and Deh2 treatments, while Reh3 showed decreased conductivity compared to Deh3. These results indicate that both species experienced membrane damage after 1 and 2 days of dehydration that was not lethal and could be recovered, but suffered lethal membrane damage after 3 days of dehydration that could not be recovered.

Water stress causes cells to produce peroxidation products that oxidize membrane lipids to generate the lipid peroxidation product MDA. As shown in [Figure 3: see original paper], MDA content in both *P. rufescens* and *O. cordatula* showed changes during different dehydration-rehydration treatments, but these changes were not significant and remained at very low levels compared to the non-resurrection plant *Arabidopsis* (Li et al., 2014). This indicates that membrane lipid peroxidation levels did not change significantly during

dehydration-rehydration, even in the lethal dehydration-rehydration treatment (Reh3), where MDA content did not increase substantially.

Osmotic Adjustment Substance Changes During Dehydration-Rehydration

Soluble sugar content during dehydration-rehydration in both species ([Figure 4: see original paper]) showed significant increases compared to controls after 1, 2, and 3 days of dehydration, with the greatest increase in Deh3 treatment. After rehydration, soluble sugar content in *P. rufescens* decreased significantly compared to corresponding dehydration treatments, while in *O. cordatula*, no significant difference was observed between Deh1 and Reh1, but significant decreases occurred after rehydration following 2 and 3 days of dehydration. Proline content in *P. rufescens* showed no significant changes during dehydration-rehydration, while in *O. cordatula*, proline content increased after 2 and 3 days of dehydration compared to controls. However, proline content in both species remained at the order of magnitude of 1 $\mathrm{g \cdot mg^{-1}}$, whereas in the non-resurrection plant *Arabidopsis*, proline content under control conditions is at the order of magnitude of 10 $\mathrm{g \cdot mg^{-1}}$ (Li et al., 2014).

Discussion and Conclusion

Most resurrection plants grow in arid and semi-arid regions with annual rainfall of only 160–570 mm (Hickel, 1967). Although the native habitats of *P. rufescens* and *O. cordatula* in Shilin and Shangri-La counties, Yunnan, do not have low annual rainfall compared to desert regions (Zhang et al., 2015; Liu et al., 2016), karst drought phenomena occur due to high bedrock exposure, shallow soil, and poor water retention in karst landforms (Zeng et al., 2007; Liu, 2016). Drought affects plant physiological and biochemical characteristics, and during severe water loss, protoplasmic water is completely lost, leaving only a small amount of bound water in cells (Bartels & Salamini, 2001). In this study, leaf discs of both *P. rufescens* and *O. cordatula* decreased to approximately 5% RWC after 2 days of dehydration, and RWC recovered to control levels after 1 day of rehydration, which is far below the 10% recoverable relative water content threshold for resurrection plants (Alpert, 2006), indicating that both species are resurrection plants.

The proline and soluble sugar contents, along with membrane lipid peroxidation and ion leakage results during dehydration and subsequent rehydration, further demonstrated the resurrection characteristics of both species. Soluble sugars accumulated slightly during dehydration, while proline content remained low and essentially unchanged during dehydration and rehydration, similar to other resurrection plants (Bianchi, 1993; Georgieva et al., 2005; Li et al., 2014). This suggests that proline may not be involved in the osmotic adjustment process for extreme dehydration tolerance in these resurrection plants (Li et al., 2014). Membrane integrity, indicated by MDA and ion leakage, remained low in both species after 1 and 2 days of dehydration and corresponding rehydration, similar

to *Paraisometrum mileense* (Li et al., 2014). This suggests that these resurrection plants have unique protective mechanisms for membrane lipids that can maintain membrane lipid composition under extreme drought conditions, indicating that resurrection plants can maintain good membrane integrity during recoverable dehydration. However, as dehydration intensified to 3 days, RWC in both species decreased to approximately 1.5%, photosynthetic activity dropped to zero, and electrolytes leaked almost completely; upon rehydration, RWC and photosynthetic activity could not recover to control levels. This indicates that the leaf discs were over-dehydrated, bound water in leaf cells was lost (Bartels & Salamini, 2001), and leaves lost their recovery ability.

During dehydration-rehydration cycles, HDT species can maintain their photosynthetic pigment levels and photosynthetic organ structure integrity (Drazic et al., 1999). Under dark drying conditions, chlorophyll a+b content in *O. cordatula* showed no significant changes during dehydration to 5% RWC and rehydration, indicating that this species is a homoiochlorophyllous resurrection plant. In contrast, chlorophyll a+b content in *P. rufescens* decreased by approximately 50% when dehydrated to 5% RWC, but showed an increasing trend shortly after rehydration. Unlike PDT species that completely degrade chlorophyll after dehydration and require longer time for repair after rehydration (Lüttge et al., 2011), *P. rufescens* remains an HDT that degrades some chlorophyll after dehydration and rapidly restores its level after rehydration, a pattern also observed in resurrection plants such as *Paraisometrum mileense* (Li et al., 2014), *Myrothamnus flabellifolia* (Farrant et al., 1999), *Ramonda nathaliae* (Drazic et al., 1999), and *Craterostigma wilmsii* (Farrant et al., 2000). This type of resurrection plant can rapidly restore chlorophyll levels and repair thylakoid structures after rehydration (Hallam & Luff, 1980), facilitating rapid recovery of photosynthesis for material synthesis, which is an adaptive strategy for plants growing in intermittent rainfall environments (Sherwin & Farrant, 1996).

Although chlorophyll content tends to be maintained during dehydration, photosynthetic activity in resurrection plants typically disappears completely during dehydration and recovers after rehydration (Georgieva et al., 2005; Evelin et al., 2012). After 1 and 2 days of dehydration, the maximum photosynthetic potential parameter Fv/Fm in *P. rufescens* disappeared, but recovered to control-like levels after corresponding rehydration, which may be related to previously reported protective mechanisms that maintain photosynthetic apparatus integrity during drying (Augusti et al., 2001; Bartels & Salamini, 2001). In contrast, *O. cordatula* leaf discs maintained Fv/Fm at control levels after 1 and 2 days of dehydration, differing from *P. rufescens*. The ability of *O. cordatula* to maintain Fv/Fm during dehydration may be due to its capacity to dissipate excess light as heat through photoprotective mechanisms [Y(NPQ)], indicating stronger photoprotective capacity. After 3 days of dehydration, Fv/Fm disappeared in both species and could not be recovered after rehydration, because excessive dehydration damaged chlorophyll and thylakoid structures (Tuba et al., 1996), and chloroplasts and internal structures disintegrated rapidly after rehydration.

Leaf folding and unfolding are common morphological adaptations in resurrection plants during dehydration and rehydration (Gaff, 1989). This study found that *P. rufescens* leaf discs folded inward during dehydration, exposing the densely white felt-covered lower epidermis to prevent reactive oxygen species damage from excessive light (Dalla Vecchia et al., 1998; Farrant et al., 2003). In contrast, *O. cordatula* showed completely different morphological changes during dehydration-rehydration: leaves remained relatively flat with slight wrinkling after 1 and 2 days of dehydration, and only curled slightly after 3 days of dehydration, much less severely than *P. rufescens*. This demonstrates that the two species have different adaptive mechanisms in leaf morphological structure in response to drought.

In summary, leaf discs of both *P. rufescens* and *O. cordatula* can tolerate dehydration to 5% RWC, and after rehydration, their external morphology, chlorophyll content, photosynthetic activity, and ion leakage indicators can all recover to control levels. However, further dehydration to 1.5% RWC renders these indicators unrecoverable, indicating that both species possess resurrection characteristics. During dehydration-rehydration treatments, the two species share some common physiological and biochemical changes. Both can relatively maintain chlorophyll levels, enabling rapid recovery of photosynthesis when the rainy season arrives. Soluble sugars play an osmotic adjustment role during dehydration stress in both species, while proline may not be involved in the osmotic adjustment process for extreme dehydration tolerance. Both species may have unique protective mechanisms for membrane lipids that prevent membrane lipid oxidation under extreme drought conditions, though these mechanisms require further investigation. However, the two species also exhibit different response mechanisms: *P. rufescens* avoids damage from excessive light during dehydration by severely curling its leaves, while *O. cordatula* has stronger photoprotective capacity and can dissipate excess energy absorbed by PSII as heat through photoprotective mechanisms, thereby protecting its photosynthetic apparatus from damage. Whether the differences in dehydration-rehydration responses between these two species are related to differences in their natural habitats requires further study.

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Note: Figure translations are in progress. See original paper for figures.