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EFFECTS OF DROUGHT STRESS AND REHYDRATION ON CHLOROPHYLL FLUORESCENCE CHARACTERISTICS OF *ERYTHRODONTIUM JULACEUM* (SCHWAEGR.) PAR. IN AREAS OF PUDING KARST ROCK DESERTIFICATION

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Abstract

Anshun city is located in the central Guizhou Province, at an average elevation of 1320 m, annual average temperature 15.1ºC and annual average rainfall of 1397 mm. The soil in Anshun is predominately calcareous (accounting for 85%). Its forest coverage is 5 - 15%, the vegetation coverage is 10 - 90%, the bare rock is 30 - 90%, the land reclamation is 10 - 70%, and the average rocky desertification is 36.79%. Puding district in Anshun is taken as an example for ecological restoration and treatment research, which provides the basis for scientific selection of drought-resistant plants for cultivation in karst rocky desertification areas. Chlorophyll-fluorescence was measured in moss *Erythrodontium julaceum* (Schwaegr.) Par. When drought and rehydration affected chlorophyll content and fluorescence under increased drought stress, the total chlorophyll content first dropped and then increased gradually. F_0 and *qN* of *E. julaceum* increased while the F_m, F_v/F_m, *Yield*, *ETR* and *qP* decreased with drought stress. Fluorescence parameters after rehydration were restored to normal levels by mild to moderate stress, and severe stress is more difficult to return to the control level.

Introduction

Bryophyte is the lowest high-grade plant. Any bryophytes have some special physiological and ecological adaptation mechanisms. They can grow and reproduce under conditions of cold, hot, extreme drought, and weak light and other environments wherein terrestrial plants have difficulty surviving (Yi and Liu 2007).

Drought tolerant moss has a set of physiological mechanisms for coordination, which maintain the integrity of the photosynthetic structure, thereby maintaining the activity of chlorophyll and photosynthetic pigments under dehydration (Tuba *et al.* 1998). Foreign scholars have studied the changes in chlorophyll fluorescence of different species of moss (Csintalan *et al.* 1999, Seel *et al.* 1992) and liverwort (Deltoro *et al*. 1998, Marschall.1999) in dehydration and rehydration processes by chlorophyll fluorescence technique. Among moss and liverwort species, the clearest study was conducted on *Tortula ruralis*, which has become a model plant for studying the physiological ecology and drought resistance mechanism of drought tolerant moss (Proctor *et al*. 2000, Oliver *et al*. 2000). The analysis on chlorophyll fluorescence of three species of moss under dry-wet alternate conditions by Csintalan *et al.* (2000) shows that chlorophyll fluorescence parameters of bryophytes are relatively constant under a certain range of relative water content. Under drought conditions, chlorophyll fluorescence parameters, including F_m, *Fv/Fm*, *qN* and ΦPS II, decrease with water loss. After rehydration, *Fv/Fm* and ΦPS II can gradually return to normal levels in a relatively short period (Oliver *et al*. 2000). Yi (2007) reported that *Fv/Fm, ETo/ABS, ETo/ERo, Area*, and *RC/CSo* of *Grimmia pilifera* P. Beauv. have relatively low water content threshold during the dehydration process. The photosynthetic apparatus of *G. pilifera* has a very strong capacity for dehydration tolerance and can reduce the water content in the plant based on the dry environment; hence, the plant can survive in a dormant state (Yi and Liu 2007).

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In China, many drought tolerant mosses are widely distributed in arid and semi-arid areas. In Guizhou where karst is the most typical, no related study on the changes in photosynthetic characteristics of mosses under drought stress and rehydration has been conducted. *E. julaceum* is widely distributed in karst rocky desertification area. Studies on its adaptability to drought and chlorophyll fluorescence changes after rehydration and the photosynthetic physiological response to the changes in karst rocky desertification are important. This paper uses a severe rocky desertification region near Puding of Anshun as an example to investigate the impact of dry stress and rehydration on photosynthetic physiological characteristics of *E. julaceum* to provide a scientific basis for ecological restoration in karst rocky desertification area.

Materials and Methods

The *Erythrodontium julaceum* (Schwaegr.) Par. is dominant in Puding district of Anshun City from where Xian-qiang Zhang collected the experimental materials. The samples were collected on a sunny day with an average temperature of 25 ± 1°C.

For drought stress treatment, *E. julaceum* grown in pots were subjected to normal condition (sufficiently watered) and drought (water-deficient) condition in dishes for 7 days. The mosses were then cultured in 1/10 Hoagland medium for one week. The gametophytes of similar height were inserted in polyethylene sheets, which were placed in dishes filled with 1/10 Hoagland and PEG-6000 (–2.0 Mpa) solution. The lower end of the specimen was immersed in the solution, whereas the upper end was exposed to air. Since protoplast layers of the plant cells have selective permeability and inside and outside solution have concentration difference, the water molecules can diffuse into the high concentration side from the low concentration side through the protoplast layer. The osmotic potential of PEG-6000 was measured according to the methods described by Michel (Miehel 1973). The specimens were divided into two groups: (1) the control (CK) group, which was cultured in 1/10 Hoagland solution; and (2) the treatment group, which was cultured in 1/10 Hoagland and PEG-6000 (–2.0 Mpa) solution. The indexes were determined at 0, 12, 24, 48, and 72 hrs post-treatment, and rehydration of 24 hrs after drought stress. More than three pots from each variety were used in each independent experiment, and all of these potted plants were rotated daily during drought stress treatment to minimize the environment effect. All experiments were repeated five times.

Measure of photosynthetic pigment: According to the method described by Bao (2005), moss sample ends were clipped. Sediments were removed along with impurities. Water on the surface was dried. Samples were cut into small pieces (about 2 mm or so) for mixing. Approximately 0.2 g of each sample was placed into mortars and then a small amount of quartz sand, calcium carbonate, and 2 - 3 ml of 95% ethanol was added to homogenize the sample; this procedure was performed five times. Ethanol (10 ml) was added and the mixture was ground further to make organizes white. The mixture was allowed to stew for 3 - 5 minutes. The homogenate was then filtered into brown 25 ml volumetric flask and rinsed several times with a small amount of ethanol. 95% ethanol was added to achieve constant volume to determine the absorbance at 665, 649, and 470 nm. The entire pigment extraction was performed under weak light and low temperature (4ºC) conditions. The pigment concentrations were calculated according to the following formulas (Bao *et al*. 2005):

Chl *a* content (mg/l) = $13.95A_{665} - 6.88A_{649}$

Chl *b* content (mg/l) = $24.96A_{649} - 7.32A_{665}$

Total chlorophyall (mg/l) = chlorophyall a + chlorophyall b

Pigment content of samples (mg/l) = $\rho \times V \times N/m \times 1000$

ρ: colorimetric liquid pigment content of sample (mg/l); V: extraction volume (ml); m: sample fresh weight or dry weight (g); 1000: coefficient that converts ml into L .

Determination of chlorophyll fluorescence activity: Mosses gametophytes that are being treated differently are placed in a sample room for 30 min dark adaption. PAM-2100 (Walz, Germany) portable pulse modulated chlorophyll fluorescence instrument is used to determine initial fluorescence (F_0), the maximal photochemical quantum yield (F_v/F_m), photochemical quenching (*qP*), non-photochemical quenching (*qN*), *rETR* (electron transport rate), yield (actual photosynthetic efficiency), and other fluorescence parameters of all labeled gametophytes. Among these, $Fv/Fm=(F_m-F_0)/F_m$, $qP=(F_m'- F_t)/(F_m'-F_o')$, $qN=(F_m-F_m')/F_m- F_o'$, $\Phi PS =(F_m'-F)/F_m'$, $ETR = \Phi PS$ (photosystem II) × *PAR* (photosynthetically active radiation intensity) × 0.84 × 0.5 (Song *et al*. 2009). The procedure was repeated six times, and the average value was obtained. The physical meanings of the involved parameters are obtained, as follows: variable fluorescence (F_v), maximum fluorescence (F_m), real-time fluorescence yield (F_t) initial fluorescence yield under light (F_0'), and maximum fluorescence yield (F_m').

All experiments were repeated at least five times. Data were processed using the SPSS13.0 software and were presented as means ± SD of three independent experiments.

Results and Discussion

Table 1 shows that the content of chlorophyll a generally showed a decreasing trend first and then increased with increasing stress. The content of chlorophyll b shows the same trend. Chlorophyll a of *E. julaceum* decreased from 0.479 $mg.g^{-1}$•FW at the early stage to 0.391 $mg.g^{-1}$•FW after 12 hrs. Then, it increased to 0.851 $mg.g^{-1}$•FW. The changing trend of chlorophyll content can be related to temporary drop of chlorophyll content and photosynthetic capacity at the early stage of stress. A high PEG-6000 solution concentration is around the base of moss. Thus, capillary system formed in stems and leaves of moss plants causes PEG-6000 to reach the top of moss, thereby leading to crystal precipitation with water flow and influencing photosynthesis. Survival of many high-grade vascular plants is difficult under high osmotic stress treatment with high concentration PEG-6000(Wu *et al.* 2004.), but *E. julaceum* can still maintain a certain photosynthetic capacity. From the external morphology, plants do not show withered yellow phenomenon. Thus, *E. julaceum* has strong drought resistance capacity.

Table 1. Effect of PEG drought stress and rehydration on chlorophyall content in *Erythrodontium julaceum.*

Stress time (hr)	Chlorophyall a		Chlorophyall b		Chlorophyall a+b	
	Stress	Rehydration	Stress	Rehydration	Stress	Rehydration
0	0.479 ± 015		0.311 ± 0.19		0.790 ± 0.33	
6	0.505 ± 0.22	0.606 ± 0.22	0.239 ± 0.12	0.419 ± 0.21	0.744 ± 0.29	1.025 ± 0.27
12	0.391 ± 0.18	0.528± 0.19	0.191 ± 0.09	0.171 ± 0.08	0.582 ± 0.23	0.699 ± 0.19
24	0.793 ± 0.31	0.873 ± 0.26	0.349 ± 0.21	0.472 ± 0.24	1.142 ± 0.34	1.346 ± 0.32
48	0.654 ± 0.24	0.733 ± 0.25	0.374 ± 0.16	0.473 ± 0.26	1.028 ± 0.27	1.206 ±0.27
72	0.851 ±0.34	0.902 ± 0.29	0.378 ± 0.14	0.410 ± 0.18	1.229 ± 0.31	1.312 ± 0.37

Initial fluorescence F_o refers to full opening of PS II reaction center, namely, fluorescence level in the full oxidation of primary electron acceptor QA. Heat dissipation of PS II antenna pigment usually decreases *F*, and the destruction to PS II reaction center or reversible inactivation will increase F_o (Krall and Edward 1992, Demmig *et al.* 1987, Song *et al*. 2009). Fig. 1 shows that with increasing stress time, F_o of *E. julaceum* will increase continuously. The difference between severe stress and control is very significant ($p < 0.01$). After rehydration, F_o is slightly recovered. However, because of 72 hrs of severe stress, it cannot recover to normal level ($p > 0.05$).

F_m refers to fluorescence yield as PS II reaction center is fully closed. It reflects the plant's electron transfer ability through PS II. With increasing stress time, F_m shows a decreasing trend. After rehydration, the changing trend of F_m is same as that of F_o. With longer stress time, electron transfer is blocked more obviously (Fig. 1).

F_v/F_m refers to maximum light energy conversion efficiency of PS II. The ratio (F_v/F_m) of variable fluorescence (F_v) to maximum fluorescence (F_m) refers to primary light energy conversion efficiency and potential activity of PS II and is a reliable indicator of the degree of light suppression. When plants are not under stress conditions, this parameter is usually in the range of 0.75 - 0.85 (He *et al*. 2005, Gray *et al*. 1997). It decreases significantly with adversity or damage (Xu *et al.* 1992). At the early stage of stress, F_v/F_m of *E. julaceum* will decrease slowly, if stress is increased, F_v/F_m will decrease significantly from 0.753 at the early stage to 0.522 after stress for 72 hrs (Fig. 1). After rehydration, F_v/F_m is greatly recovered, from 0.754 in 72 hrs to 0.572. A super compensation phenomenon is obvious in the rehydration process, that is, F_v/F_m is slightly higher than the corresponding treatment group. The degree of recovery of *E. julaceum* is higher mainly because plants avoid drought under adverse conditions by temporarily reducing the primary reaction of photosynthesis. Although a certain destruction is observed, it can be rapidly recovered after drought relief.

Yield refers to actual light energy conversion efficiency of PS II, showing a positive relation to the activity of PS II (Song *et al*. 2009). It reflects actual primary light energy capture efficiency of PS II reaction center under partially closed conditions, which can be used as a relative indicator to reflect photosynthetic electron transfer velocity (Eldridge and Tozer 1997). As shown in Fig. 1, at the early stage of stress (within 12 hrs), yield of *E. julaceum* increases slightly and then decreases gradually. Adaption will occur in the early stage of drought stress, and its photosynthetic system can be self-repaired. At the late stage of stress, after 12 hrs yield will decrease significantly ($p < 0.05$). At the early stage of rehydration, yield of *E. julaceum* is slightly decreased, but is still lower than that of control group ($p > 0.05$). After 12 hrs, the difference is significant ($p < 0.05$). This finding indicates that recovery is more difficult.

Photochemical quenching coefficient, *qP*, refers to the share of light energy absorbed by PS II antenna pigment for photochemical reaction. It reflects the openness of PS II reaction center. Non-photochemical quenching coefficient, *qN*, refers to the partial light energy absorbed by PS II antenna pigment to be consumed in the form of heat. Heat dissipation is an important mechanism for the plant to protect PS II (Maxwell and Johnson 2000). *qP* of *E. julaceum* was slightly increased at the early stage. This is a kind of response to adapt drought conditions. It decreased gradually in after 6 hrs. After rehydration, *qP* of *E. julaceum* is slightly decreased, but is still lower than that of control group ($p > 0.05$). *qN* is slightly increased at the early stage, after 12 hrs it increased sharply. Severe stress is three times higher than that at the early stage, with significant difference ($p < 0.01$) (Fig. 1).

Plants show adaptation, injury, repair, compensation, and other reactions at different levels of drought stress, along with different physiological changes and photosynthetic characteristics. In this experiment, chlorophyll content decreased initially and increased subsequently with increasing stress. This shows the reaction-adaption mechanism of plants under adverse circumstance. Chlorophyll fluorescence can be used to rapidly determine the real photosynthesis of plant under drought stress, and it is a reliable method to evaluate photosynthetic mechanism and degree of environment stress. Various fluorescence indicators of *E. julaceum* show different changing trends. F_o and *qN* show an increasing trend. F_m, F_v/F_m, *Yield* and *qP* show a decreasing trend. The decrease of PS II photochemical efficiency of leaves restricts the normal photosynthesis of *E. julaceum.* The increasing trend of F_o shows that drought stress restricts photosynthetic process of *E. julaceum*. With increasing stress, the photosynthetic structure of *E. julaceum* is destroyed more seriously.

Therefore, rehydrated chlorophyll fluorescence characteristics after drought stress reflect the adaption ability and protective mechanism of plants to stress. After rehydration of *E. julaceum* in a short stress period (within 24 hrs), chlorophyll fluorescence parameters (F_o, F_m, F_v/F_m, yield, qP, and qN) are basically recovered to control level. With the increase of stress, it cannot be recovered to control level. The improvement of environmental conditions can recover the damaged PS II reaction center to a certain degree. If the damage is too heavy, recovery time will be extended or cannot return to the normal level.

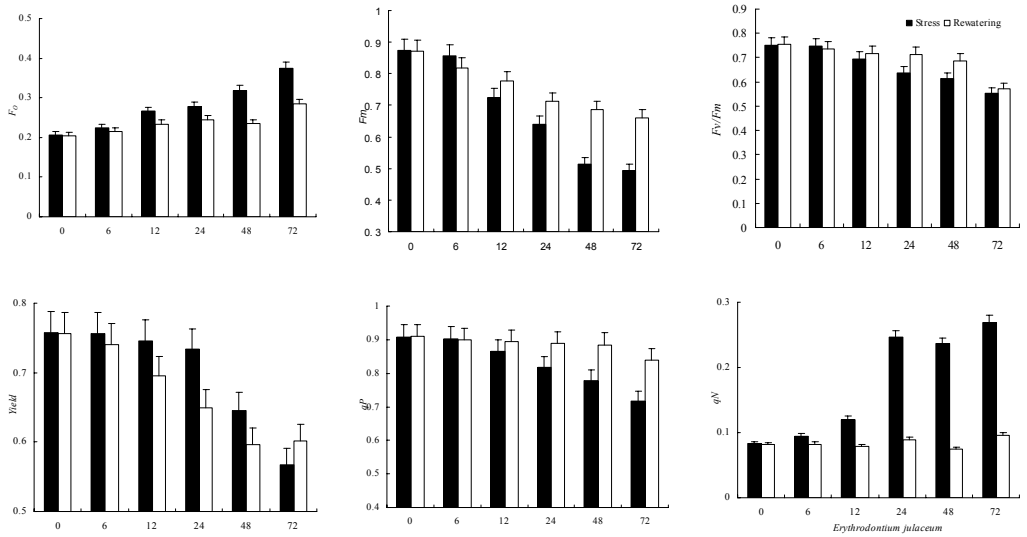

Fig. 1 Effect of PEG drought stress and rehydration on chlorophyll fluorescence parameters in *Erythrodontium julaceum.*

Chlorophyll fluorescence, which can be used to detect the impact of environmental stress on photosynthesis of plants rapidly and sensitively without damage (Schlensog *et al*. 2001, Lazr 2003), reflects rich photosynthesis information. During the dehydration process, the photosynthetic mechanism of *E. julaceum*, which has strong capability of dehydration tolerance, can reduce the water content to very low because of the arid environment to survive in a dormant state. Full dehydration in mild and moderate drought stress period does not cause permanent injury to photosynthetic organ of moss, which is still in a state of recoverable photosynthetic capability. Upon rehydration, physiological metabolic activity can be rapidly recovered. Bryophytes in karst areas have strong capability for physiological drought tolerance. On one hand, the integration of cell and structure is maintained under the arid conditions as far as possible to reduce the damage to cells and metabolic substances. On the other hand, repair mechanism is triggered during rehydration process to rapidly repair the destruction to photosynthetic mechanism caused by dehydration (Bewley 1979).

With increasing drought stress, chlorophyll content of *E. julaceum* generally decreased initially and increased subsequently. This finding is related to the temporary drop of photosynthetic capability.

Chlorophyll fluorescence parameters show regular change with increasing drought stress. When the stress period is short (within 24 hrs), chlorophyll fluorescence parameters basically

return to the control level after rehydration. With longer stress period (more than 24 hrs), stress becomes severe, and parameters cannot return to the control level.

In Guizhou rocky desertification area, many exposed bedrocks are present. The investigation on the relationship between bryophytes growing on the surface of the rock and drought can provide some basic materials for the subsequent screening of drought-tolerant plants during ecosystem recovery.

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