Journal of Plant Physiology 228 (2018) 12–18

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Biochemical responses of the desiccation-tolerant resurrection fern *Pleopeltis polypodioides* to dehydration and rehydration

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ARTICLE INFO

Keywords:
Drought stress
Hydroperoxide
Lipid hydroperoxide
Antioxidative enzymes
Pleopeltis polypodioides
Fatty acids

ABSTRACT

The epiphytic fern *Pleopeltis polypodioides* can tolerate repeated drying and rehydration events without conspicuous damage. To understand the biochemical principles of drought-tolerance, we analyzed the effect of dehydration and rehydration at 25 °C on hydroperoxide and lipid hydroperoxide, the activities of antioxidative (catalase and glutathione-oxidizing) enzymes and evaluated changes in fatty acid composition and saturation levels. Dehydration increased peroxide concentration and the activity of glutathione oxidases, but reduced catalase activity. During dehydration, the biosynthesis of palmitic (C16:0), linoleic (C18:2), linolenic (C18:3) and stearic acid (C18:0) increased 18, 12, 20, and 8-fold, respectively. In contrast, rehydration lowered levels of peroxides, the activity of glutathione-oxidizing enzymes, and fatty acids but increased catalase activity. The coordinated changes during de- and rehydration suggest that lipids and oxidative and antioxidative enzymes are components of the drought-resistance system.

1. Introduction

Among various abiotic stresses, drought is the most common stress encountered by plants and is most responsible for reduced growth and productivity (Osakabe et al., 2014). Typically, plants can sustain moderate drought stress (Jaleel et al., 2009) because they have evolved mechanisms to protect their photosynthetic apparatus and related physiological and biochemical pathways (Scott, 2000; Yordanov et al., 2000). However, extended water shortage (i.e., water potential below the permanent wilting point) induces multiple adjustments such as suppression of cell growth and expansion (Feng et al., 2016), stomatal closure to conserve water (Osakabe et al., 2014), reduction of leaf expansion (Munns et al., 2000), leaf abscission (Patharkar and Walker, 2016; Wolfe et al., 2016), enhanced root growth to improve water uptake (Smith and De Smet, 2012), and increased cell wall elasticity to maintain tissue turgor (Tenhaken, 2015).

Extreme desiccation results in permanent damage for most plants. Cellular activities shut down, photosynthesis is inhibited and susceptible to photo-oxidation, and reactive oxygen species (ROS) are produced, including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydroxyl (OH') ions, and hydrogen peroxide (H_2O_2) (Miller et al., 2010). Under normal, hydrated conditions, ROS molecules are scavenged by various anti-oxidative enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Gill and Tuteja, 2010), and non-enzymatic compounds such as ascorbate (ASA), glutathione (GSH), carotenoids, tocopherols, and phenolics (Sharma et al., 2012). However, extreme desiccation disturbs the balance between the production and scavenging of ROS, resulting in enhanced production of ROS, and uncontrolled oxidative stress that oxidizes proteins, nucleic acids and lipids (Mittler, 2002). Lipid oxidation forms characteristic hydroperoxides that are detrimental to membrane integrity (Gigon et al., 2004; Schmidt and Kunert, 1986) and eventually lead to the death of the affected plant (Sharma et al., 2012).

Certain plants, however, possess vegetative tissues that can sustain water potentials as low as −100 MPa (Gaff, 1997) and are known as desiccation-tolerant or resurrection plants. These plants can lose almost all water and rehydrate without suffering any obvious injury. Desiccation tolerance is a fairly common phenomenon among non-vascular taxa such as mosses, algae and bryophytes (Oliver et al., 2005; Proctor et al., 2007). While several pteridophytes (Cea et al., 2014; Farrant et al., 2009; Layton et al., 2010) and angiosperms (Farrant et al., 2007; Gechev et al., 2012; Ingram and Bartels, 1996) exhibit this phenomenon, gymnosperms are the only group that is not desiccation-tolerant (Alpert, 2000). In recent years, extensive studies have been carried out to understand the tolerance mechanisms of bryophytes (Alpert, 2000; Oliver, 1991; Oliver et al., 2004, 2005; Proctor et al., 2007; Proctor and Tuba, 2002; Vitt et al., 2014), fern allies (Deeba et al., 2016, 2009; Iturriaga et al., 2006; Pampurova and Van Dijck,

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https://doi.org/10.1016/j.jplph.2018.05.006
Received 14 March 2018; Received in revised form 15 May 2018; Accepted 15 May 2018
Available online 18 May 2018
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2014; Pandey et al., 2010; Rafsanjani et al., 2015; X.N. Wang et al., 2010; Yobi et al., 2012), and angiosperms (Bartels, 2005; Bartels and Salamini, 2001; Farrant et al., 1999; Ingle et al., 2007; Müller et al., 1997; Oliver, 1996; Rodriguez et al., 2010; Sherwin and Farrant, 1996; Yang et al., 2003). Fewer studies have focused on the tolerance mechanisms of pteridophytes (Cea et al., 2014; Farrant et al., 2009; Gildner and Larson, 1992; Layton et al., 2010; Voytena et al., 2014). Because pteridophytes represent an important link between non-vascular bryophytes and modern plants (Oliver et al., 2000), investigating their desiccation resilience can provide information on the evolution and mechanism of drought-tolerance among higher plants.

Pleopeltis polypodioides (resurrection fern), can tolerate loss of 95% of cellular water content and regain full metabolic activities within a few hours of rehydration in the presence of liquid water (John and Hasenstein, 2017; Pessin, 1924; Stuart, 1968). In response to drying, the fronds curl inward and expose the dorsal surface. Peltate scales cover the dorsal surface and represent functional surface features (Pessin, 1924). The folding mechanism, which is common in resurrection plants, is thought to be a defense mechanism to protect photosynthetic tissue from light damage (Farrant et al., 1999; Helseth and Fischer, 2005). Additionally, the scales play a vital role in spreading and absorption of water, but also reduce the rate of water loss (John and Hasenstein, 2017) and are thought to inhibit overheating (Manetas, 2003; Watkins et al., 2006). Dehydration induces expression of 31 kDa dehydrin, a type of late embryogenesis abundant (LEA) protein, which plays an important role in supporting membranes and cell walls in desiccated tissue (Layton et al., 2010). Upon rehydration, the levels of dehydrin decrease and the fronds readily expand and turn green (Helseth and Fischer, 2005). Pleopeltis is homoiochlorophyllus, that is, it retains its chloroplasts upon desiccation and has evolved unique mechanisms to protect its photosystems from damage during desiccation (Maslenkova, 2010; Maslenkova and Homann, 2000; Stuart, 1968). Even though morphological adaptions of this fern aid in desiccation-tolerance, there is no information on its physiological response to desiccation.

The goal of this research was to investigate biochemical responses of Pleopeltis to drought and rehydration. Our results suggest that dehydration induces accumulation of peroxides, unsaturated and saturated fatty acids, lowers antioxidative enzyme catalase activity, and increases glutathione-oxidizing enzyme activity. Following rehydration, the levels of stress-related molecules, and fatty acids decline, catalase activity increase, and glutathione-oxidizing enzyme activity decrease. These observations indicate that the oxidative-antioxidative systems, and lipids are an essential part of stress-tolerance system that facilitates Pleopeltis to tolerate and overcome the effects of drought stress.

2. Materials and methods

2.1. Plant materials

Fresh (hydrated) fronds were harvested from live oak (*Quercus virginiana*) trees on the campus of the University of Louisiana at Lafayette (30.21 N, −92.02 W).

2.2. Relative water content

The RWC was measured in fully expanded fronds and were expressed by the equation RWC = [(WDW)/(FW-DW)] × 100. W = weight of the fronds, DW = dry weight, FW = fresh (fully hydrated) weight. Rehydration was initiated by floating fronds on water at 25 °C. RWC of rehydrating fronds was measured every hour for 5 h and then at 24 h intervals. The weight after 24 h rehydration was used as FW.

2.3. Estimation of hydroperoxide

Hydroperoxide (and other water-soluble peroxide) contents were measured with ferrous ammonium sulfate / xylenol orange (FOX 1 assay) (Cheeseman, 2006; Wolff, 1994). Samples were powered in liquid nitrogen and 100 mg were transferred to centrifuge tubes. After addition of two mL 40 mM sodium phosphate buffer (pH 6.8), samples were vortexed, incubated at RT for 45 min, and centrifuged (23,000 *g*, 12 min). The supernatant (100 μL) was mixed with 900 μL of FOX I reagent (250 μM ammonium ferrous sulfate [ammonium iron (II) sulfate hexahydrate, Fluka Analytical, 09719], 100 μM D-sorbitol [Sigma, S1876], 100 μM xylenol orange [Fluka Analytical, 33825] in 25 mM H_2SO_4). After incubation (RT, 30 min in the dark), OD_{560} was measured with a spectrophotometer (Ultraspec 3000, Pharmacia Biotech). Standards were prepared by diluting 30% hydrogen peroxide (H_2O_2, Sigma, H-1009) based on OD_{240} and an extinction coefficient of 43.6 M^{-1} cm^{-1} (Cheeseman, 2006). The calibration curve was based on standards (1–10 nM H_2O_2 per 100 μL) and described OD560 as 0.0763x – 0.004, with x = peroxide content in nM.

2.4. Estimation of lipid peroxide (LOOH)

Lipid hydroperoxide extraction was carried out according to Griffiths et al. (2000) with minor modifications. Samples were powdered in liquid nitrogen using mortar and pestle. Extraction was performed in dim light with chilled reagents. Tissue (100 mg) was extracted in 500 μL of 0.15 M glacial acetic acid and 4 mL chloroform: methanol (1:2 by vol.), quantitatively transferred to 15 mL Falcon tubes. The mortar was rinsed with 1 mL chloroform and 1 mL deionized water, both of which were added to the tubes. The tubes were vortexed and phase separation achieved by centrifugation (11,000 *g*, 15 min, 4 °C). The chloroform extract was transferred into 4 mL amber glass vials, dried and dissolved in 100 μL HPLC-grade methanol. The detection of lipid peroxidation was based on the oxidation of Fe^{2+} to Fe^{3+}, which forms a chromophore with xylenol orange (Gay and Gebicki, 2003; Jiang et al., 1992). The vials were vortexed and 900 μL of FOX-2 reagent (250 μM ammonium ferrous sulfate in 90% methanol and 100 μM xylenol orange dissolved in 25 mM H_2SO_4) added, incubated in the dark (30 min, 25 °C) and OD_{560} determined. The absorbance was based on 1–16 nM tert-butyl hydroperoxide, (Sigma-Aldrich, 458,139) in HPLC grade methanol; OD_{560} = 0.0383x + 0.0048, with x = BHT in nM).

2.5. Catalase assay

Samples were powdered in liquid nitrogen with mortar and pestle and 100 mg was extracted in two mL chilled 50 mM sodium phosphate buffer pH 7, containing 0.01% Triton X. The extract was centrifuged (23,000 *g*, 12 min, 4 °C). Catalase activity was determined in a mixture of 2730 μL of sodium phosphate buffer pH 7.0, 210 μL of plant extract to which 60 μL of 15 mM H_2O_2 were added and mixed. The consumption of H_2O_2 was measured as decrease in OD_{240} for 180 s.

2.6. Consumption of glutathione

The assay was adopted from (Rotruck et al., 1973) as modified by Starlin and Gopalakrishnan (2013). Powdered samples (100 mg) were extracted in chilled sodium phosphate buffer, (40 mM, pH 7, 2 mL), and centrifuged (23,000 *g*, 12 min, 4 °C). 500 μL supernatant was transferred into a fresh centrifuge tube, mixed with 400 μl 40 mM sodium phosphate buffer pH 7.0, 100 μL 10 mM sodium azide (Sigma, S-2002), 200 μL DI water and vortexed. After adding 200 μL (4 mM) GSH (Cayman chemical company, 10007461) and 100 μL 2.5 mM H2O2, the solution was mixed and incubated for one minute. The reaction was terminated by adding 500 μL of 10% trichloroacetic acid, (Aldrich Chemical Company, 76-03-9). The solution was incubated at RT for

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