Research note

Drying Increases Sucrose Contents in the Desiccation-Tolerant Fern *Parahemionitis arifolia* (Burm.f.) Panigrahi

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【 Summary 】

Desiccation-tolerant plants use a variety of strategies to endure water scarcity, among them a rapid accumulation of compatible solutes, especially of non-structural carbohydrates in flowering plants, is well known. However, this strategy is poorly documented in ferns. This study analyzed sugars in the desiccation-tolerant fern *Parahemionitis arifolia*. Sugars were extracted from leaves and rhizomes from fresh plants as well as plants in various stages of desiccation with a deuterium oxide (D_2O)-based phosphate buffer. Water-soluble metabolites were analyzed using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. Results showed that sucrose was the most abundant form of sugar, with its content increasing through the drying process. Our findings suggest that sucrose may play a key role in preventing damage during drought in desiccation-tolerant ferns, similar to what has been documented in other desiccation-tolerant flowering plants.

Key words: desiccation, ^{1}H NMR, *Parahemionitis arifolia*, sucrose.

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研究簡報

乾燥增加耐旱蕨類澤瀉蕨蔗糖含量

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摘 要

耐旱植物具備不同的策略以忍受缺水，其中之一是組織中水溶性溶質的快速積累，尤其是非結構性碳水化合物。此現象在開花植物已有相當了解； 然而，在蕨類植物中仍很少被探討。本研究旨在分析耐旱蕨類植物澤瀉蕨 (*Parahemionitis arifolia* (Burm.f.) Panigrahi) 中的糖分濃度變化，以重水 (D_2O) 配製的磷酸鹽緩衝液萃取新鮮和風乾過程中的葉子和根莖的代謝物後，再以^{1}H核磁共振 (^{1}H NMR) 光譜測定與分析水溶性糖類種類及含量。在所有樣品中，較其他糖類蔗糖含量最為豐富，且乾燥提升蔗糖含量。顯示可能如同耐旱性開花植物，蔗糖對於耐旱蕨類於乾燥的過程中產生保護的功效。

關鍵詞：乾燥、核磁共振氫譜、澤瀉蕨、蔗糖。

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Drought stress slows plant growth and development, and lowers crop yields. Some plants however can withstand dehydration and can recover even after losing 95% of their water content (Gaff 1997, Kessler and Siorak 2007). These special plants are known as desiccation-tolerant plants or resurrection plants (John 2017, Prats and Brodersen 2021). Desiccation tolerance is primarily found in bryophytes and pteridophytes but is rare in angiosperms (Porembski and Barthlott 2000, Alpert and Oliver 2002, Alejo-jacuinde and Herrera-estrella 2022). Desiccation tolerant plants use different strategies to endure water scarcity, among them a rapid accumulation of non-structural carbohydrates in flowering plants is well known (Hoekstra et al. 2001, Vidović et al. 2022); however. this strategy is poorly documented in ferns. This study was undertaken to analyze sugars in the desiccation-tolerant fern *Parahemionitis arifolia* (Burm.f.) Panigrahi, distributed in India (South India to Orissa), Sir Lanka, Bangladesh, Myanmar, Indo-China, southern China, Malaya and eastern Malaysia to the Philippines, and southern Taiwan (Panigrahi 1993, Kuo et al. 2019, Huang et al. 2021). ^{1}H nuclear magnetic resonance (NMR) spectroscopy provides a number of advantages as a routine analytical technique, including sensitivity and precision, and most importantly it is a non-destructive method for quantitative analysis of complex natural samples (Simmler et al. 2014). Our findings, using quantitative 1H NMR spectroscopy, showed that sucrose contents of the fern leaves and rhizomes of *P. arifolia* increased during drying.

Chemicals and solvents

For the ^{1}H NMR analysis, deuterium oxide (D_2O) (99.9 atom % D), 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid (TSP) used as an internal standard for calibration of chemical shifts and quantification, and D-(+) trehalose dihydrate were purchased from Sigma Aldrich (St. Louis, MO, USA). D (+)-Sucrose was bought from Acrose Organics (Fair Lawn, NJ, USA). Deionized water was purified using a Milli-Q® System from Millipore (Molsheim, Bas-Rhin, France). First-grade potassium phosphate, dibasic and potassium phosphate, monobasic were purchased from J.T. Baker (Center Valley, PA, USA).

Plant material and extraction

Parahemionitis arifolia plants were started from spores from Liukuei, Kaohsiung City, Taiwan and grown in a fern greenhouse at the Taiwan Forestry Research Institute for 2 yr. Plants for fresh samples were harvested at about 09:30 and rinsed with water to remove soil particles. The rhizomes and leaves were separated with a sharp blade. Samples were frozen in liquid nitrogen and individually ground into fine powder with a pre-cooled pestle and mortar. The ground plant material was freeze-dried and stored at −80 ℃. Plants for dehydration treatment were harvested and rinsed with water to remove soil particles and put in a transparent box for air-drying (dehydration) at room temperature. After 1 and 3 wk of dehydration, every other plant was removed from the box and cut into samples at around 09:30. Water-soluble metabolites were extracted using a modification of a previously reported method (Kim et al. 2010). Plant samples were plunged into liquid N_2, ground into a fine powder using a pre-cooled mortar and pestle, then placed in a freeze-dryer, and stored at −80 ℃ for a later metabolite analysis. Lyophilized plant material (60 mg) was weighed into a 2-mL Eppendorf tube, then 1.5 mL of a mixture of potassium phosphate buffer (pH 7.4) in D_2O was added, containing 0.01% w/v trimethylsilylproprionic acid sodium salt-d_4 (TSP, w/w). Contents of the Eppendorf tube were boiled in a hot-water bath, thoroughly mixed with a vortex mixer at room temperature, and then sonicated. Tubes were spun in a micro-centrifuge at 12,000 rpm for 10 min. Then, for each sample, 700 µl of the supernatant was transferred to a 5-mm NMR tube.

^{1}H NMR spectrum acquisition

All spectra were recorded on a Bruker AV 500 MHz NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) with a 5-mm dual cryoprobe DCI ^{1}H/^{13}C operating at a proton NMR frequency of 500.13 MHz. For each sample, 16 scans were recorded with the following parameters: spectral width = 19.9947 ppm (10000.000 Hz), 90° pulse, P1 = 15.50 µs, PL1 = −3.9 dB; relaxation delay D1 = 2 s; acquisition time (aq) = 1.6384000 s; type of baseline correction: quad; window function: EM; LB = 0.10 Hz; software used for spectral processing: TopSpin 3.6.5. The sucrose content in plant samples was calculated using the following equation (Chauthe et al. 2012):

$$W_x = (A_x/A_s)(N_s/N_x)(M_x/M_s)W_s;$$

where W_x is the weight of the analyte (per 700 µl of solution), W_s is the weight of the standard (TSP), A_x is the integral value for the analyte, A_s is the integral value for the standard, N_x is the number of protons of analyte integrated, N_s is the number of protons of standard integrated, M_x is the molecular weight of the analyte, and M_s is the molecular weight of the standard.

Water-soluble metabolites of *P. arifolia* extracted and detected in ^{1}H NMR spectra included amino acids, soluble sugars, organic acids, and some aromatic compounds (data not shown). Among these metabolites, sucrose had a higher peak in all samples. To assign peaks of sucrose in ^{1}H NMR spectra of P. arifolia extracts, the ^{1}H NMR spectrum of a sucrose solution was recorded (Fig. 1). Chemical shifts of sucrose were as follow: ^{1}H NMR (500 MHz, D_2O) δ (ppm): 3.48 (t, J=9.5 Hz, 1H, H-4), 3.56 (dd, J=10.1, 3.8 Hz, 1H, H-2), 3.68 (s, 2H, H-1′), 3.77 (t, J=9.65 Hz, 1H, H-3), 3.82 (m, 2H, H-6; 2H, H-6′), 3.85 (m, 1H, H-5), 3.90 (m, 1H, H-5′), 4.05 (t, J=8.55 Hz, 1H, H-4′), 4.22 (d, J=8.85 Hz, 1H, H-3′), 5.42 (d, J=3.85 Hz, 1H, H-1). In Figs. 2A and 3A, the stacked plot of H-1 signals at δ_H 5.42 ppm resonance showed higher peaks for air-dried leaves and rhizomes compared with those of fresh plants. The stacked plot of 3.4~5.5 ppm resonance in ^{1}H NMR spectra of various leaf and rhizome extracts are shown in Figs. 2B and 3B, respectively. Peak areas of fructose and glucose were much lower than that of sucrose. Glucose, fructose, and trehalose were identified by peak assignment using ^{1}H NMR spectra from pure compounds added to extracts and by comparison to published data (Fig. 4). Doublets at 5.24 and 4.65 ppm were due to anomeric protons of α-glucose H-1 and β-glucose H-1, respectively. The combined peak at 4.12 ppm was the only resonance signal clearly assigned to fructose (Cazor et al. 2006, Barclay et al. 2012). The very weak resonance of 1 doublet peak at 5.20 ppm was due to trehalose (Moriwaki et al. 2003).

The spectral region near 5.42 ppm in all of the studied samples had only 1 peak for sucrose H-1 (5.42 ppm, d, J=3.85 Hz, 1H). This peak is usable for integration with the corresponding peak and comparison with the peak

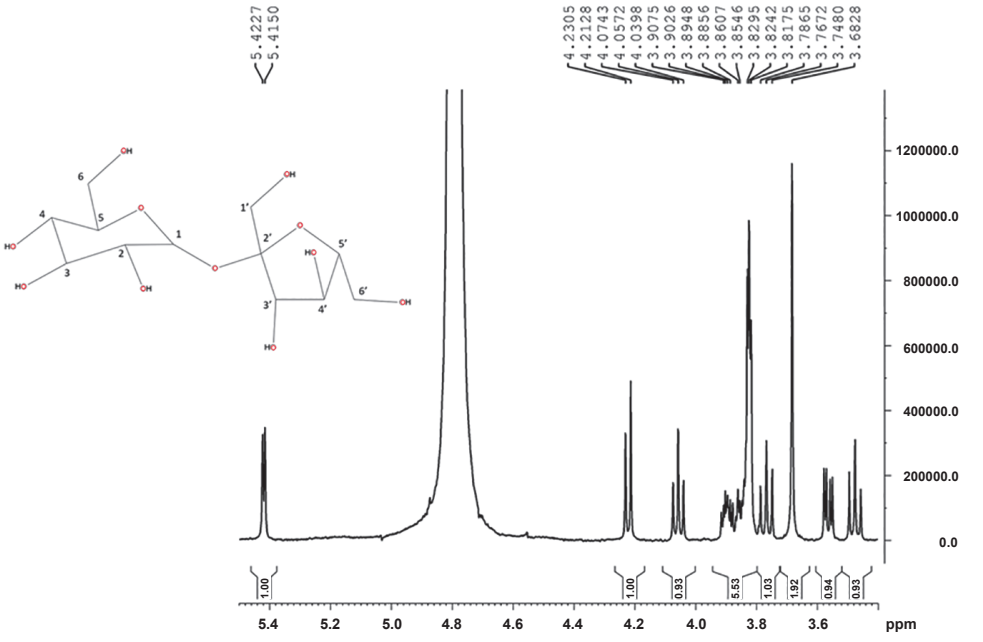

Fig. 1. ^{1}H NMR spectrum of sucrose.

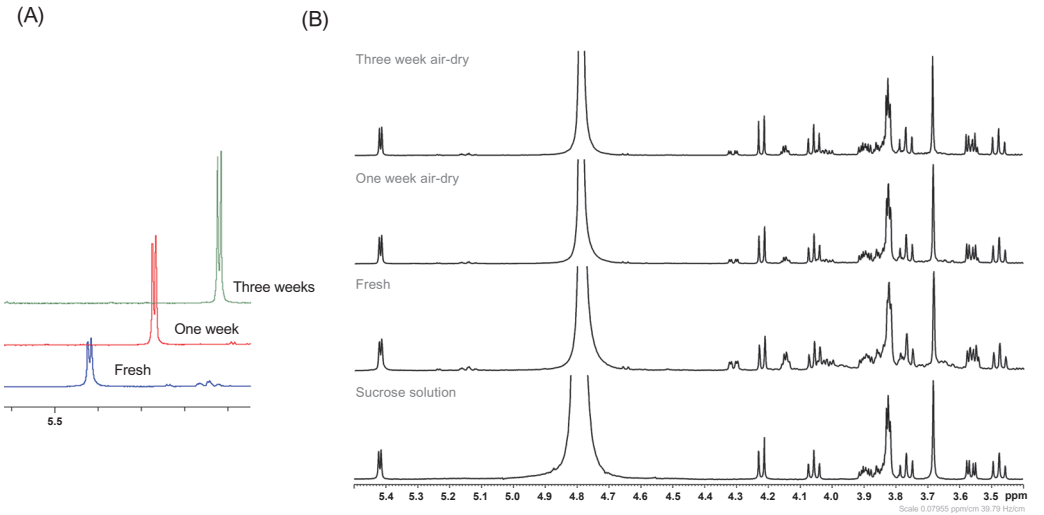

Fig. 2. (A) Stacked plot of ^{1}H NMR spectra of leaf extracts at δ_H 5.42 ppm resonance of sucrose in samples. (B) Stacked plot of ^{1}H NMR spectra δ_H 3.4~5.5 ppm resonance of sucrose in leaf extracts and a sucrose solution.

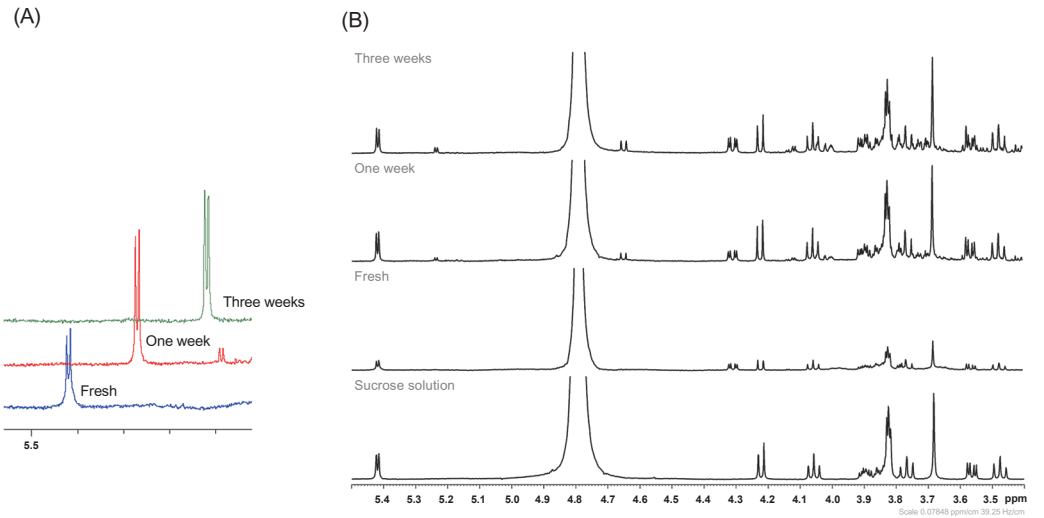

Fig. 3. (A) Stacked plot of ^{1}H NMR spectra of rhizome extracts at δ_H 5.42 ppm resonance of sucrose in samples. (B) Stacked plot of ^{1}H NMR spectra δ_H 3.4~5.5 ppm resonance of sucrose of rhizome extracts and a sucrose solution.

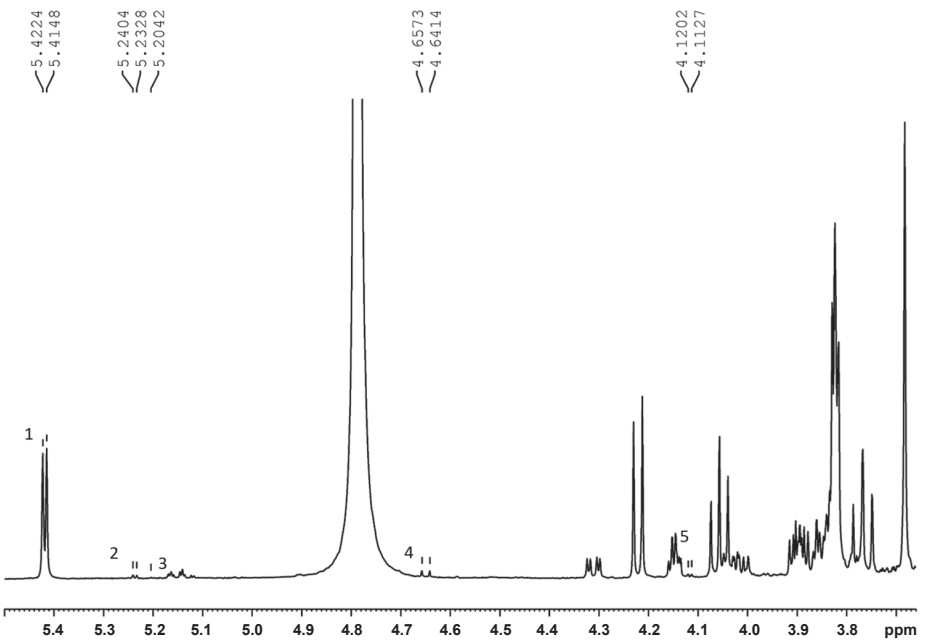

Fig. 4. ^{1}H NMR spectra of leaf extracts of dried plants. 1: sucrose, 2: α-glucose, 3: trehalose, 4: β-glucose, 5: fructose.

of the internal standard. Therefore, sucrose was quantified by integrating the doublet at 5.42 ppm. After calculating the contents of sugars in all samples, the sucrose content was found to be much higher compared to glucose and fructose, and trehalose was only present in a minor amount. Compared to fresh plants, dried plants had higher sucrose contents in leaves and rhizomes. Sucrose contents obviously increased in leaves and rhizomes during drying. α-glucose and fructose contents in leaves were similar in fresh and dried plants, but their contents increased during drying in rhizomes (Table 1). Results show that sucrose

Table 1. Sugar contents (mg/g dry weight) in leaves and rhizomes of fresh and dried plants

	Sucrose	α-glucose	Fructose
Leaves			
Fresh	45.15 (0.3731*)	1.10 (0.0173)	0.50 (0.0238)
1 wk dehydration	66.12 (0.5482)	1.05 (0.0165)	0.55 (0.0261)
3 wk dehydration	69.14 (0.5765)	0.99 (0.0156)	0.59 (0.0281)
Rhizomes			
Fresh	16.88 (0.1083)	0.59 (0.0072)	0.11 (0.0039)
1 wk dehydration	21.66 (0.1394)	1.43 (0.0176)	0.55 (0.0202)
3 wk dehydration	20.58 (0.1279)	2.20 (0.0270)	1.33 (0.0492)

* peak values.

may play a predominant role during drought in desiccation-tolerant ferns, similar to that for flowering plants.

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