NIR monitoring of water status in the resurrection plant *Haberlea rhodopensis* during desiccation and subsequent rehydration processes

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Introduction

Water deficit is the most critical stress for living things. Most higher plants are not able to maintain their vegetative tissue efficiently under a desiccated environment. Nevertheless, some higher plants, known as poikilohydric or resurrection plants, can survive the loss of much of the water from their tissue. During desiccated periods, they stop growth and reproduction, while retaining their life at a greatly reduced rate of metabolism. Surprisingly, even after several years, they can resume their physiological functions fully, within hours after re-watering. Due to this unique and mysterious characteristic, the resurrection plant is regarded as a model plant for studying plant response to drought stress. There are some references that discuss mechanisms of protecting plants from water stress, which include carbohydrate accumulation, protein expression, and generation of abscisic acid.[1] Changes in water and its structure in these plants during the desiccation and subsequent rehydration processes do not appear to have been monitored yet. Near infrared (NIR) spectroscopy enables the gathering of information on water structure.[2–8] Water bands in the NIR spectrum of plant tissue show broad peaks, usually composed of multiple bands. These peaks include information on hydrogen-bonding of water, which is related to the physiological status of the plant tissue. The objective of this study was to examine the dynamic state of water in the resurrection plant during desiccation, and subsequent rehydration processes, using NIR spectroscopy.

Materials and methods

Haberlea rhodopensis cultivated *in vitro*[9] was used as test material. This species is a homoiochlorophyllous, desiccation tolerant shade plant and is regarded as a model plant for studying drought

stress. *Chirita eberhardtii* cultivated *in vitro* was used as a control plant. Desiccation was started by taking the plants out of the cultivation tubes. During desiccation, the plants were left in the dark, but otherwise normal room condition (*ca* 22°C). The subsequent rehydration was applied after seven days of dehydration for *Haberlea rhodopensis* and two days of dehydration for *Chirita eberhardtii*. The roots of the plants were covered by approximately 200 mg of cotton, and they were put into plastic tubes. The rehydration process was started by adding 2 ml of water into the tubes. Transflectance near infrared spectra of the leaves were measured with NIRSystems 5000 (1100 nm to 2500 nm with 2 nm intervals, Foss NIRSystems, MD, USA). Data analysis was carried out using The Unscrambler ver. 9.8 (CAMO, Oslo, Norway). Extended multiple scatter correction (EMSC) and second derivative (Norris Gap, size 3) were applied for data transformation.

Results and discussion

Principal component analysis of the NIR spectra showed that loadings of PC1 were very similar in the both processes (Figure 1), proving that de- and rehydration processes are reversible in the resurrection plant.

The PC1 scores in the desiccation process increased with time after dehydration, while in the rehydration process they decreased with time after watering (Figure 2).

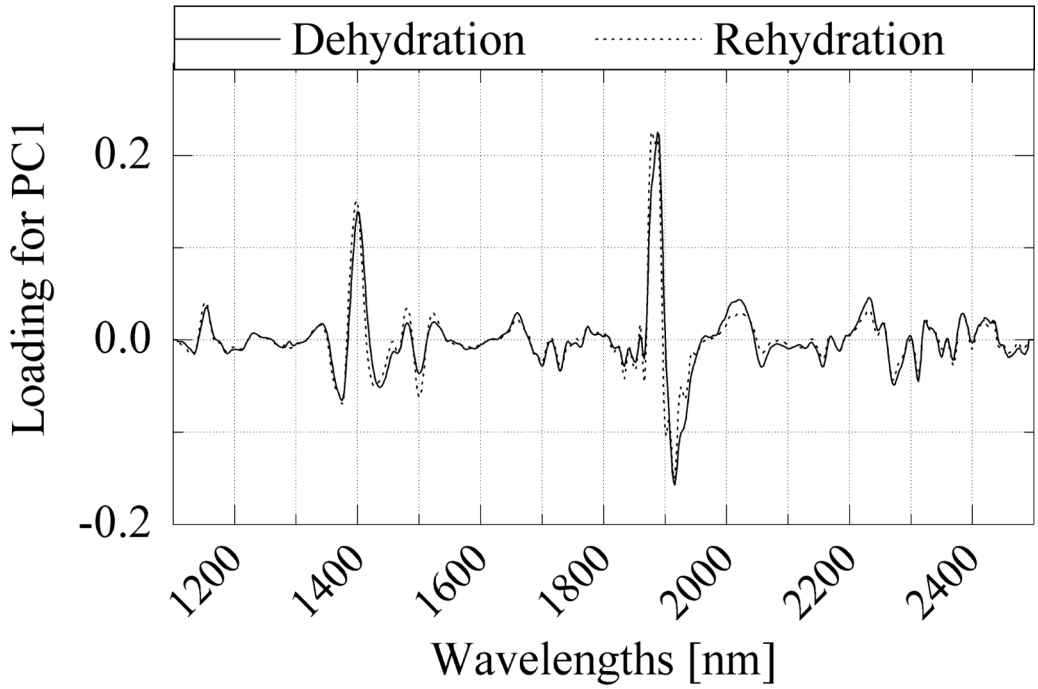

Figure 1. PC1 loading during desiccation and subsequent rehydration processes in *Haberlea rhodopensis* (Explained variances were 95.5% and 72.2%, respectively).

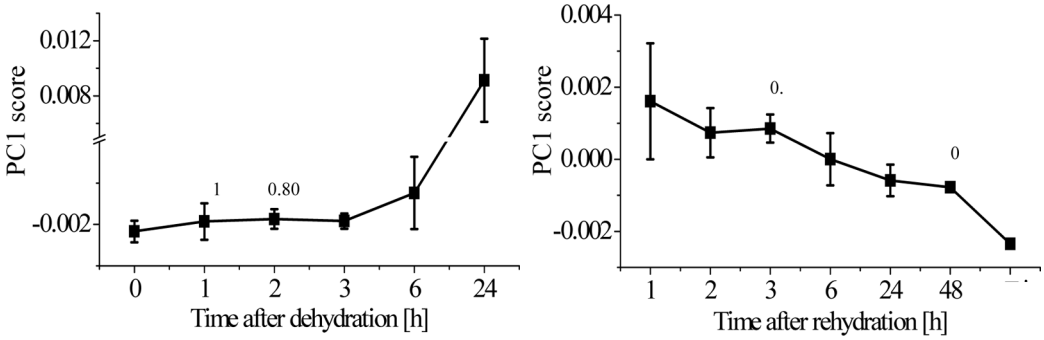

Figure 2. Changes in PC1 score of *Haberlea rhodopensis* during desiccation (left) and subsequent rehydration processes (right). r_w indicates relative weight to fresh weight.

The five most important wavelengths in PC1 loading in both processes were 1888 nm, 1916 nm, 1400 nm, 1374 nm and 1500 nm, which were interpreted as being related, not only to losing and regaining, water but also probably to the special behaviour of resurrection plants during drought stress and subsequent recovery, including generation of ABA, (abscisic acid), metabolic changes in sugar and protein, and structural changes in cell walls. Further investigation to find a relationship between spectral variation and biochemical properties is needed. The spectrum around 1450 nm, which is the first overtone of H_2O, includes information on water species.[2–8] Changes in the 2nd derivative absorbance of a weakly bonded water specie, S_1, are shown in Figure 3 and 4.

As for *Haberlea rhodopensis*, 2nd derivative absorbances at S_r (1346 nm), S_0 (1412 nm) and S_4 (1650 nm) were significantly increased after 24 hours drying, while absorbance at S_1 (1440 nm),

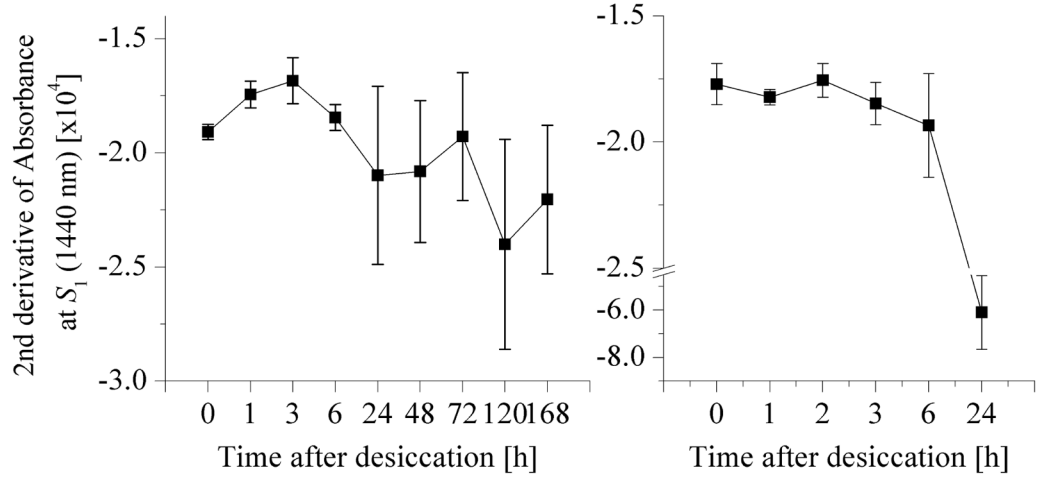

Figure 3. Changes in a weakly bonded water specie, S_1, during the desiccation process (left: *Chirita eberhardtii*; right: *Haberlea rhodopensis*).

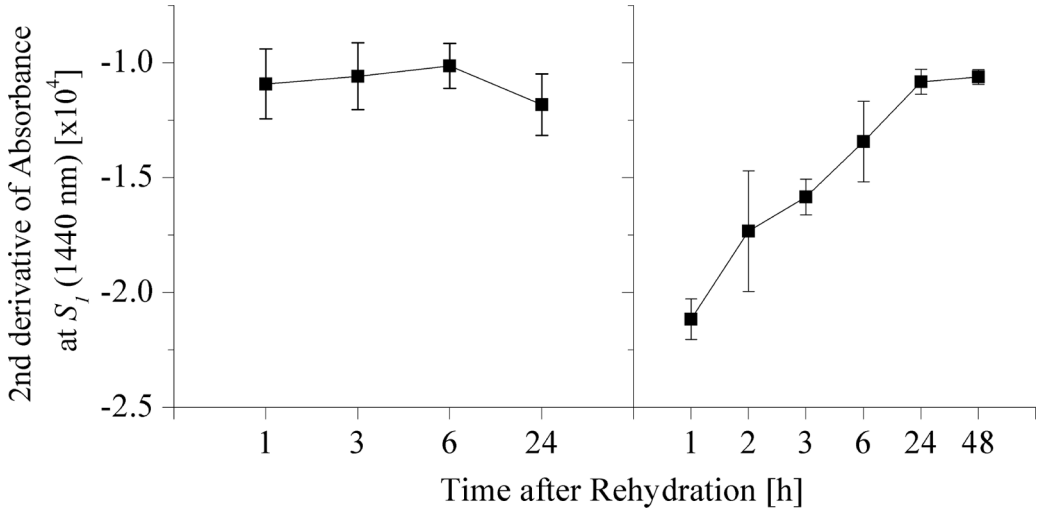

Figure 4. Changes in a weakly bounded water specie, S_1, during the rehydration process (left: *Chirita eberhardtii*; right: *Haberlea rhodopensis*).

S_2 (1462 nm) and S_3 (1490 nm) decreased. In the subsequent rehydration process, absorbance at S_r, S_0, S_3 and S_4 gradually decreased with time after watering, while the S_1 absorbance increased. The S_2 was relatively stable. In the case of *Chirita eberhardtii*, no clear tendency in 2nd derivative absorbance with the progress of desiccation and subsequent rehydration was observed. These results also implied that the actual structure of water in the resurrection plant changed as a result of releasing and regaining water during desiccation and the subsequent rehydration processes.

Conclusion

Changes in the NIR spectral data, using multivariate analysis verified that desiccation and the subsequent rehydration processes are reversible in the resurrection plant. Assignments of important wavelengths found by PCA in the both processes were taken to indicate releasing and regaining, water, as well as other characteristic phenomena in resurrection plant, during drought stress and subsequent recovery. It was also possible that the structure of water molecules in the resurrection plant also changed when water content was decreasing and increasing.

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