Metabolomic assessment of desiccation and rehydration processes of cryptobiotic plants

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INTRODUCTION

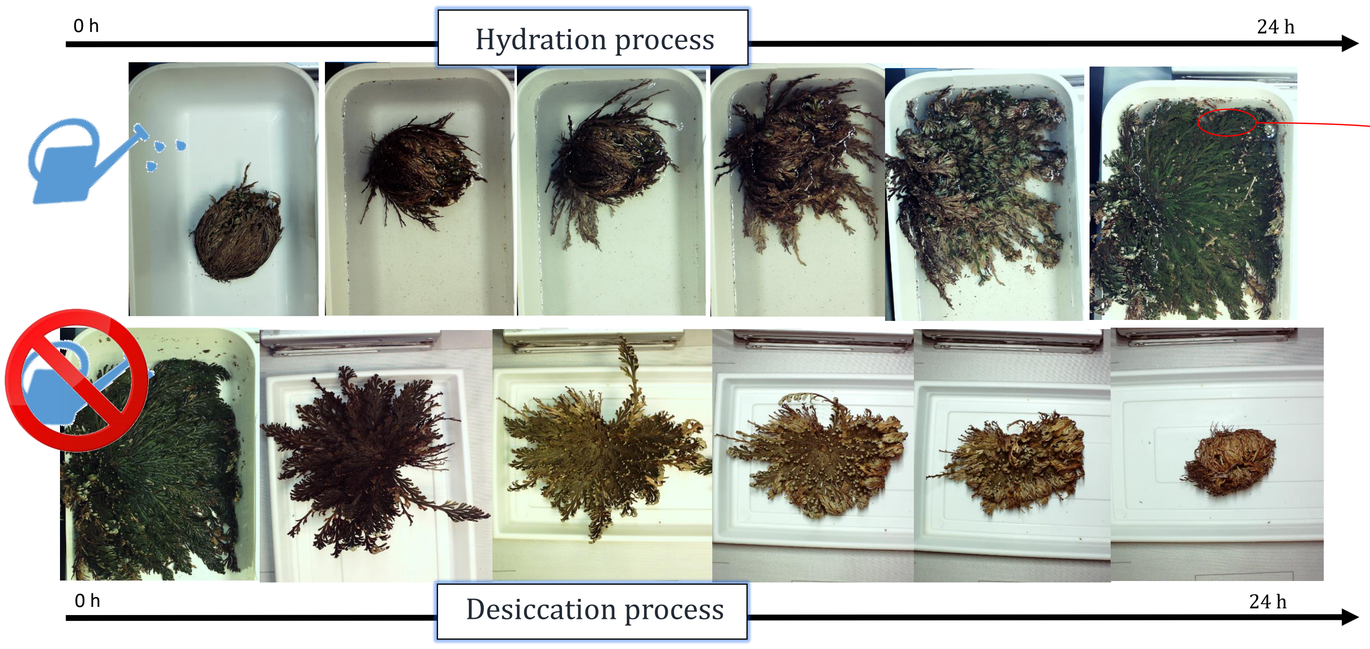
- Water scarcity limits plant distribution and crop production worldwide. Desiccation tolerant species such as *Selaginella lepidophylla* have the ability to survive vegetative tissue drying for prolonged periods of time and 'resurrect' when water is again available (cryptobiosis).
- The study of the plant metabolome changes during the cryptobiotic processes can provide a detailed understanding of the organism's physiological responses to water scarcity stress. [1]

GOALS

- Evaluate changes in *Selaginella lepidophylla* metabolism during the hydration and desiccation processes.
- Identify potential markers for a better understanding of the metabolic pathways affected.

EXPERIMENTAL PROCEDURE

SAMPLE GENERATION

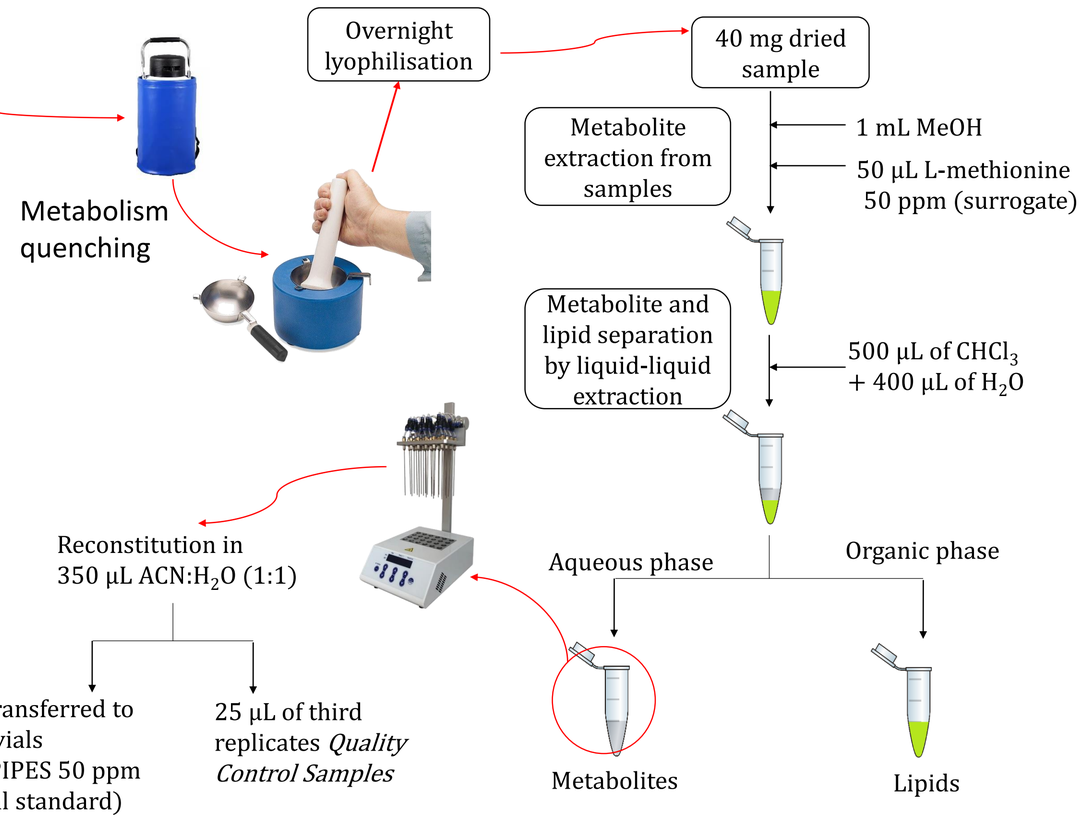


Plants were kept in both processes in an Environmental Test Chamber MLR-352H (Panasonic)

Submersion of plants in abundant water during hydration process. Removal of water during the desiccation process

Samples were stored at -80ºC until extraction

Extraction procedure



LC-MS ANALYSIS

Chromatographic run:
112 samples
26 time points
(12 dessication – 14 hydration) x 4 replicates
8 QCs

Chromatographic conditions

Instrument	Waters Acquity UPLC System
Column	ACQUITY UPLC BEH HILIC (2,1 x 100 mm, 1,7 µm)
Injection volume	10 µL
Flow rate	0,4 mL/min
Mobile Phase A	10mM NH_4COOH + 0.2% HCOOH in 90:10 ACN:H_2O
Mobile Phase B	10mM NH_4COOH + 0.2% HCOOH in 50:50 ACN:H_2O

MS conditions

Mass Spectrometer	Waters LCT Premier oa-TOF
Ionization	ESI (+) and (-)
Mass range	Full scan spectra from 80 to 1.000 Da
Scan cycle	0,3 s

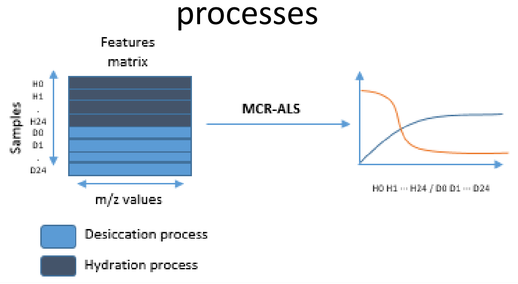
DATA ANALYSIS

LC-MS preprocessing

ROI approach for feature extraction

Multivariate analysis

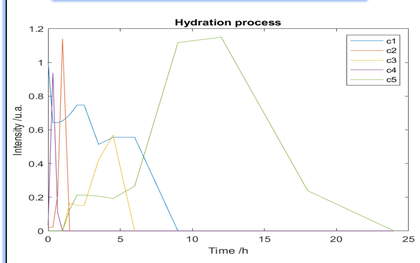
MCR-ALS for unravelling cryptobiotic processes



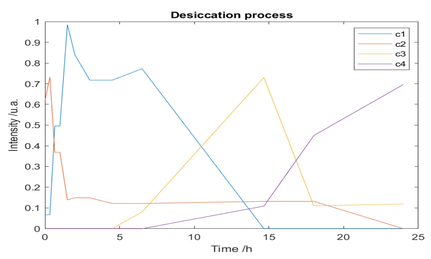
RESULTS

Cryptobiotic profiles

Hydration process



Desiccation process



MCR-ALS resolved concentration profiles (**C**) shows the kinetics from each process. Corresponding spectral profiles (**S**) elucidate the short and long term metabolites responsible for the cryptobiotic adaptation.

Tentatively identified metabolites

Hydration process

Component	ROI m/z value	Adduct	Proposed metabolite	m (Da)	Error (ppm)
1	236,1479	$[M+NH_4]^+$	N-Acetylserotonin	218,1055	36
2	269,0922	$[M+H]^+$	Inosine	268,0808	15
	539,0974	$[M+H]^+$	Amentoflavone	538,0900	0
3	236,2278	$[M+NH_4]^+$	(3E,7E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	218,2035	40
	398,2011	$[M+NH_4]^+$	Prenyl arabinosyl-(1→6)-glucoside or Prenyl apiosyl-(1→6)-glucoside	380,1682	2
4	192,0620	$[M+NH_4]^+$	Brassilexin	174,0252	16
	236,2278	$[M+NH_4]^+$	(3E,7E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	218,2035	40
	539,0974	$[M+H]^+$	Amentoflavone	538,0900	0
5	471,2904	$[M+H]^+$	Ceanothine C	470,2893	13
	552,2295	$[M+NH_4]^+$	Isosyringinoside	534,1949	1
	718,2925	$[M+H]^+$	5-Methyltetrahydropteroyltri-L-glutamic acid	717,3083	19

CONCLUSIONS

- Compounds tentatively identified are mainly antioxidants and osmoprotectants, allowing hypothesizing that global response is towards protecting the plant from oxidative damage. [2, 3] However, biomarkers identification require also confirmation using an MS/MS analysis to reinforce the reliability of the identified metabolites.
- Further work contemplated is performing an untargeted lipidomic analysis to completely determine the metabolic pathways affected when applying the stress.

REFERENCES

[1] Lankadurai, B.P., Environmental metabolomics: An emerging approach to study organism responses to environmental stressors, *Environmental Reviews*. 2013, 21,180-205.
[2] Lee, K., Flavanoids inhibit both rice and sheep serotonin N-acetyltransferases and reduce melatonin levels in plants, *Journal of Pineal Research*. 2018, 65(3).
[3] Zheng, X., Chloroplasti biosynthesis of melatonin and its involvement in protection of plants from salt stress, *Scientific Reports*. 2017, 7, 41236.