Australian Flora Foundation - Progress Report

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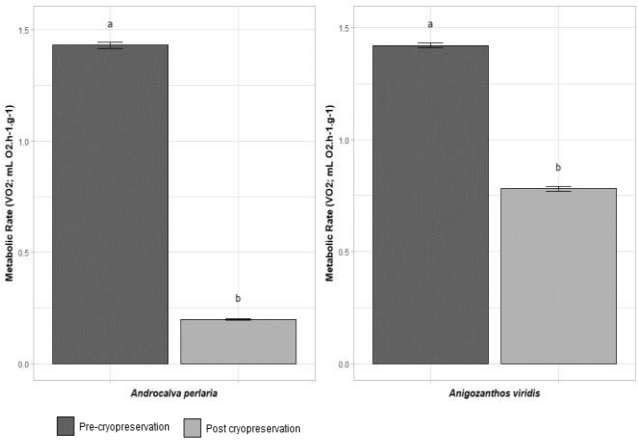
Is mitochondrial function the key to improving the cryopreservation of threatened Australian flora?

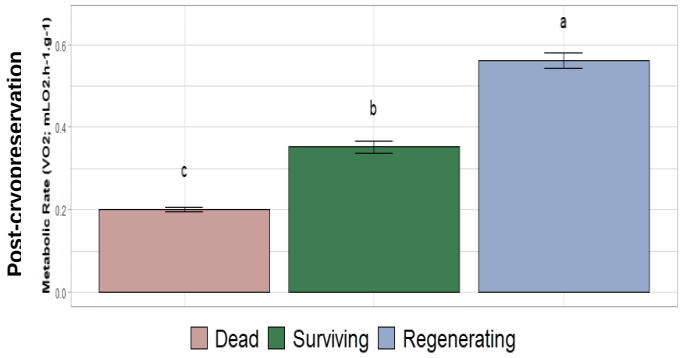
Background: This project aims to advance the fundamental science of metabolic function that impacts the successful cryopreservation of threatened Australian plant species. Cryopreservation is the safest and most effective long-term conservation method of storing valuable species and genotypes, involving storage of living biological material in liquid nitrogen (LN). The integrity of mitochondrial function within cells is essential for the successful recovery of cryopreserved material but there has been very limited investigation in plants. The energy stored in ATP produced by the mitochondria is vital in almost all aspects of cell metabolism, and it is of particular importance for cryopreservation due to its role in providing energy for repairing damaged DNA, the production of new proteins and lipids, and the energy to resume normal cell division and growth after storage. The project will increase our understanding of the stresses experienced by Australian plants during cryopreservation. Specifically, the characterisation of mitochondrial function and integrity in plant tissues will be pioneered as a novel approach to the development of species-specific cryopreservation protocols for some of Australia's endangered and critically threatened rainforest species. This project will pioneer the use new non-invasive techniques to assess mitochondrial function during the cryopreservation process in threatened Australian species. This includes the ASTEC Global Technology Q2 Oxygen Sensing Technology to assess metabolic function after cryopreservation; confocal microscopy with mitochondria-specific fluorescent probes to visualise mitochondrial damage within cells during cryopreservation; and the Seahorse XFe96 Flux analysis, to gain specific insight into the toxic nature of CPAs effect on the various components of mitochondrial function.

Project progress



Q2 Oxygen Sensing study: The initial study utilising the Q2 yielded some interesting and valuable insights, confirming our hypothesis that the cryopreservation process severely affects mitochondrial function. Whilst some difficulties surrounding bacterial contamination in the early trials on the Q2 slowed progress, a robust method has now been developed for analysing shoot tips on this new instrument. The initial study was completed on two Australian species, *Androcalva perlaria* and *Anigozanthos viridis*, both showed significant decreases in metabolic activity after

cryopreservation. Another point of interest this study highlighted was that the shoot tips that visually looked dead still showed considerable metabolic activity during the first couple of days post-cryopreservation, an interesting finding that needs further investigation to see if these shoot tips can be saved. Work is continuing utilising the Q2 to develop cryopreservation protocols that reduce metabolic stress to the plants, and to assess a wider range of species to see the extent of damage that can occur to mitochondrial function during cryopreservation.



Seahorse Flux analysis: Work has only just begun on the Flux analysis trials, limited by the process of starting viable cell cultures, particularly for native species with no established protocols. Currently carrot cell cultures, carrot somatic embryos and *Arabidopsis* shoot tips have been used to test the Seahorse Flux instrument, establishing some base line information and procedures for further testing. Optimisation of the plant tissue material, amount of plant material needed, growth medium, pH and temperature is still essential before further testing can begin on the effect CPAs have on mitochondrial function, aiming to provide some valuable insights into the findings from the Q2 trials.

Fluorescent Probe analysis: The fluorescent probe analysis has not yet begun. Kings Park Science recently procured and upgraded their fluorescent microscope with new filters, camera and operating software from Nikon. For consistency in analysing the data, we have waited until these new additions are completed and calibrated. Work on the fluorescent analysis will take part in 2020 as part of an honours project.

Acknowledgements

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