PLANT TISSUES

PLANT SAMPLE: PHOSPHATE AND SULFATE ASSAY

Molecular Physiology Laboratory

CONTEXT

This protocol is a basic and can be used for different purposes. In our lab we mostly use this type of extraction for phosphate and sulfate assay. Plants can take up only inorganic forms of phosphorus - phosphates (P_i). P_i assay shows metabolically active phosphorus in a plant. Sulfur is available to plants primarily in the form of anionic sulfate (SO_4^{2-}) present in soil. SO_4^{2-} is actively transported into roots and then distributed, mostly unmetabolized, throughout the plant. SO_4^{2-} assay reflects amount of sulfur taken up by plant.

The determination of P_i is based on the colorimetric method in which a blue colour is formed by the reaction of ortho-phosphate molybdate ion and antimony ion followed by the reduction with ascorbic acid at an acidic pH. The phosphomolybdenum complex is read at 620 nm. SO_4^{2-} ion is precipitated in a strongly acid medium with barium chloride. The resulting turbidity is measured photometrically at 420 nm. In both assays photometric results are compared with appropriate calibration standard solutions.

MATERIALS

Instrument: Precellys 24

Precellys Lysing kit: 2 ml hard tissue homogenizing CK28 (Cat. No 03961-1-002.2)

Sample: Snap-frozen plant tissue – leaves, roots, etc. – 30-50 mg

Buffer: 1% acetic acid – 500 µl

PROTOCOL

Extraction

- weight 20-70 mg of frozen tissue in a 2 mL tube and place on ice
- add two 2.8 mm ceramic beads and 500 µl 1% acetic acid
- process in Precellys 24 two times

Speed: 5000 rpm

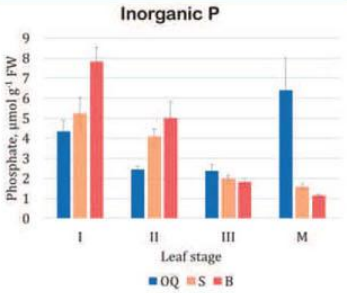
Cycle: 3 x 45 sec (break between runs 5 sec)

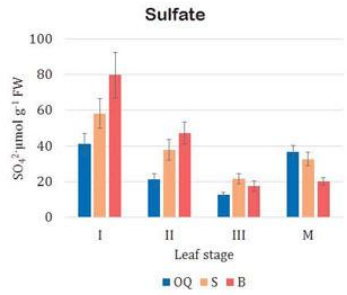
Break: 15 min on ice

- spin at 14 000 rpm for 15 min at 4ºC, transfer supernatant to fresh tube, spin 15 min at high speed again, transfer one more time

Once the extracts are absolutely clear, they are ready for assays.

RESULTS





The graphs show differences in leaf nutrient levels between plants of *Hakea prostrata* from different dune systems. Bassendean (**B**) dunes have the poorest and oldest soils, whereas Old Quindalup (**OQ**) are comparatively younger and richer than B soils, but still quite poor. Spearwood (**S**) dunes are in between these two types.

Leaf developmental stages: **I** – youngest leaves from top of the shoot (0-50% expanded); **II** – 50-75% expanded young leaves; **III** – 50-100% expanded immature leaves; **M** – fully expanded mature leaves from previous flush

CUSTOMER

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CONCLUSION

Precellys 24 is perfect for biological sample preparation. Biological space is entirely separated from the mechanical parts preventing any inside contamination. Up to 24 samples can be homogenized at the same time and in less than five minutes. When the protocol is set up and validated, the sample preparation process remains the same with no bias in analysis, on a time basis, or between operators. Moreover, individual sealed tubes prevents cross contamination between sample preparations.

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