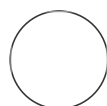


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Spectrophotometric Quantification of Betacyanins in Plant Tissue Produced from the RUBY Reporter Gene

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ABSTRACT

RUBY is a visual reporter gene that produces betanin, a betacyanin that appears red to violet in colour (He et al., 2020). To assay betanin (and other betalains) a spectrophotometric approach has been employed, as seen in Stintzing et al. (2003) and Grützner et al. (2021).

MATERIALS

- 2 ml micro-centrifuge tubes
- Mini pestle
- Methanol
- Ascorbic acid
- Formic acid
- Cuvettes/96 well plates
- Spectrophotometer

SAFETY WARNINGS



[Methanol SDS](#)

OPEN ACCESS

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MANUSCRIPT CITATION:

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Protocol status: In development
We are still developing and optimizing this protocol

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80365

Sample collection

- 1 Punch out leaf discs (as many as possible in the target area) of areas expressing betanin.
- 2 Put leaf discs in 2 ml micro-centrifuge tubes and snap freeze in liquid nitrogen then store in -80 freezer until required.

Sample Preparation

- 3 Take 20-50 mg of frozen tissue and add to 2 ml tubes. Keep on ice.
- 4 Grind down tissue using plastic micro-pestle and add methanol buffer (50% methanol, 1 mM ascorbic acid, 0.5% formic acid) at 10% w/v - i.e. for 35 mg of tissue add 350 ul methanol buffer.
- 5 Dilute samples with mili-Q so that absorption values fall within 0.8 - 1.0 (as previously calculated by Stintzing et al. 2003). Grützner et al. (2021) found that a 12 fold dilution performed well but this could differ.

Spectrophotometric Quantifications

- 6 Put cuvettes or 96 well plates into spectrophotometer and read absorbance at 538 nm (Stintzing et al. 2003).
- 7 Using the formula $BC = (A * DF * MW * 1000) / (\epsilon * L)$ calculate the concentration of betacyanins.

BC = betanin content (mg/L) **A** = absorbance (538 nm) **DF** = dilution factor **MW** = molecular weight (550.47 g/mol) **L** = cuvette path length **ϵ** = molar extinction coefficient (60,000 L/(mol cm))

