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Spectrophotometric Quantification of Betacyanins in Plant Tissue Produced from the RUBY Reporter Gene V.1

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We are still developing and optimizing this protocol

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Abstract

RUBY is a visual reporter gene that produces beatnin, 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

Troubleshooting

Safety warnings

Methanol SDS

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 spectophotometer and read absorbtion at 538 nm (Stintzing et al. 2003).

7 Using the formula BC = (A * DF * MW * 1000) / (ε x 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/(mool cm)

Protocol references

Grützner, R., Schubert, R., Horn, C., Yang, C., Vogt, T., Marillonnet, S., 2021. Engineering Betalain Biosynthesis in Tomato for High Level Betanin Production in Fruits. *Frontiers in Plant Science,* 12. https://doi.org/10.3389/fpls.2021.682443

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