Quantification of Anthocyanin Content

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[Abstract] Anthocyanins are a class of flavonoids and important plant pigments. They attract insects to pollinate flowers, protect plants from UV irradiation, and act as antimicrobial agents against herbivores and pathogens. Biosynthesis of anthocyanin is stimulated by diverse developmental signals and environmental stresses including drought, wounding, pathogen infection and insect attack. Plant hormones such as jasmonates, a stress-related plant hormone, also induce accumulation of anthocyanins. Sensitivity of plants to these stress stimuli can be measured by accumulation of anthocyanins. Here we describe a simple method for measurement of anthocyanins in *Arabidopsis thaliana* seedlings. Amount of anthocyanins are calculated only from absorbances at 530 and 657 nm of crude extract.

Materials and Reagents

1. *Arabidopsis thaliana* seedlings (~10 days after germination)
   Note: Amount of anthocyanin per seedling weight is higher in young seedlings.
2. Bleach solution
3. Sterile dH_2O
4. Methanol
5. Acetic acid
6. Murashige and Skoog medium salt (Wako Pure Chemical Industries, catalog number: 392-00591)
7. Sucrose
8. 2-Morpholinoethanesulfonic acid (MES)
9. Agar (for plant culture)
10. Modified Murashige and Skoog medium (see Recipes)
11. Extraction buffer (see Recipes)

Equipment

1. Paper towel
2. Spectrophotometer
3. Microcentrifuge
4. Microcentrifuge tubes
5. Mortar and pestle
6. Liquid nitrogen
7. Electric balance

Procedure

1. Add bleach solution to *Arabidopsis thaliana* seeds and shake gently for 7 min.
2. Rinse three to five times with sterile dH_2O.
3. Sow surface-sterilized seeds on modified MS medium.
4. Grow the plants under long day (16 h light/8 h dark) or continuous light condition (50~70 $\mu mol/m^2/s$) at 22 °C for 7~10 days.
5. Measure fresh weight of 10~15 seedlings.
   Note: Remove surface moisture with paper towel before measurement.
6. Freeze seedlings with liquid nitrogen and grind in a mortar and pestle.
7. Add 5 volumes (based on fresh weight) of extraction buffer and mix thoroughly.
8. Centrifuge at 12,000 x *g* for 5 min at room temperature.
9. Transfer supernatant to new tube.
10. Centrifuge at 12,000 x *g* for 5 min at room temperature.
11. Transfer supernatant to new tube.
12. Measure absorbances at 530 and 637 nm.
13. Calculate anthocyanin content (Abs_{530}/g F.W.) by [Abs_{530} - (0.25 x Abs_{657})] x 5.
    Note: To correct contribution of chlorophyll to the absorbances at 530 nm, the formula Abs_{530} - (0.25 x Abs_{657}) was used. Multiplication of "5" can be changed depending on volumes of added extraction buffer in step 5. For example, if 10 volumes of extraction buffer was added, multiply "10".

Recipes

1. Modified Murashige and Skoog medium
   1x Murashige and Skoog medium salt
   0.5% Sucrose

0.05% MES

Adjust pH to 5.7 with KOH

0.8% agar

2. Extraction buffer

   45% methanol

   5% acetic acid

Acknowledgments

This protocol is adapted from Nakata *et al.* (2013).

References

1. Nakata, M., Mitsuda, N., Herde, M., Koo, A. J., Moreno, J. E., Suzuki, K., Howe, G. A. and Ohme-Takagi, M. (2013). A bHLH-type transcription factor, ABA-INDUCIBLE BHLH-TYPE TRANSCRIPTION FACTOR/JA-ASSOCIATED MYC2-LIKE1, acts as a repressor to negatively regulate jasmonate signaling in *Arabidopsis*. *Plant Cell* 25(5): 1641-1656.

