Chlorophyll Content Assay to Quantify the Level of Necrosis Induced by Different R Gene/Elicitor Combinations after Transient Expression

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[Abstract] This assay can be used to rapidly and accurately quantify levels of leaf necrosis induced after transient expression of R genes and elicitor combinations (Harris *et al.,* 2013). It is based on the inverse correlation between level of necrosis and chlorophyll content in leaf tissue. It is adapted from the calculations described by (Strain *et al.*, 1971).

Materials and Reagents

1. 1.5 ml tube
2. Leaf discs
   Note: Ensure that the leaf discs are fully submerged in the DMF solution.
3. N, N-Dimethylformamide (DMF) (Sigma-Aldrich, catalog number: D4551-250 ml)

Equipment

1. Spectrophotometer (Spectronic Biomate3) (Thermo Fisher Scientific, catalog number: 335904P)
2. Glass spectrophotometer cuvettes (Sigma-Aldrich, catalog number: Z276898)
   Note: Product Z276898 has been discontinued.

Procedure

1. Place three leaf discs (*e.g.*, 4 mm radius, from Eppendorf lid) into a 1.5 ml tube containing 1 ml of dimethylformamide (DMF). Use a cork borer to excise leaf disk. Include five replicates for each sample type. Ensure that the leaf discs are fully submerged in the DMF solution. Allow the chlorophyll to dissolve into the DMF solution by still incubation overnight at 4 °C.
2. Mix 300 µl of sample solution with 600 µl of DMF in a fresh Eppendorf tube (2 volumes of DMF per volume of sample). Read the absorbance (A) in spectrophotometer at 647 nm and 664.5 nm wavelengths using a glass cuvette.
   Chlorophyll ***a*** content (µg/ml):
   $= (12 \times A_{664.5}) - (2.79 \times A_{647})$
   Chlorophyll ***b*** content (µg/ml):

= (20.78 x A_{647})-(4.88 x $A_{664.5}$)

Total chlorophyll content (µg/ml) = Chl***a*** + Chl***b***

Sample area (for 3 leaf discs at 4 mm radius) (mm^2) = 3 x πr^2

Total chlorophyll content (µg/mm^2) = (Chl***a*** + Chl***b***)/Sample area

Notes

This protocol is most suitable for comparing treatments within an experiment, as the absolute chlorophyll content will vary between different sets of plants and over time. If measuring chlorophyll content after leaf infiltration assays, it is preferable to infiltrate all the treatment types to be compared on a single leaf. This can be repeated, in different configurations, on multiple leaves. To minimize the effects of inter-leaf variability, all the leaf discs from the same treatment type can then be pooled. A minimum of 15 leaf discs (3 per sample, five replicates) for each treatment type is required. While our experience with this protocol has been using leaves of *Nicotiana benthamiana and N. tobacum*, it has also been applied to (at least) *Arabidopsis thaliana* (Pruzinska *et al.*, 2005) - as a proxy for leaf senescence - and could in principle be applied to many other plant species where chlorophyll content is of physiological relevance.

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