Infiltration of tobacco leaf tissue

Introduction

- For transient expression in tobacco (*N. tobacum* and *N. benthamiana*) leaf lower epidermal cells
- Adapted from: Sparkes I, Runions J, Kearnes A and Hawes C., (2006), "Rapid transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants", Nature Protocols, **1**, (4), 2019-2025

Materials

- 4-6 week old *Nicotiana tobacum* or *Nicotiana benthamiana* plants
- Overnight culture (approx. 16h) of *Agrobacterium tumefaciens* (strains GV3101, EHA105 or ABI) containing binary plasmid

Method

1. make infiltration buffer (per sample approx. 3ml)

2. take 1ml of cells into 1.5ml Eppendorf tube and spin down in bench top centrifuge for 5 min at 5000rpm (or 3min at 8000rpm)

3. remove supernatant and add 1ml infiltration buffer, gently re-suspend pellet with pipette and repeat centrifugation to wash sample

4. remove supernatant and add 1ml of infiltration buffer

5. measure optical density of sample with spectrophotometer, take reading at 600nm absorption

6. calculate volume of sample to be used using formula Ci x Vi = Cf x Vf, where Ci is the optical density (OD), Cf is infiltration OD and Vf is 1000µl; infiltration OD varies for each construct, usually between 0.01 and 0.1

7. pierce small hole into plant leaf and draw up resuspended cells into a 1ml syringe

8. place finger over hole, gently turn leaf to infiltrate the lower face of the leaf and gently push liquid sample into leaf

9. incubate for 2-3 days to allow proteins to be expressed before using infiltrated section for EM/ confocal/ protein extraction

Infiltration buffer

- stock buffer:
  0.5M MES (store at 4°C)
  0.02M $Na_3PO_4.12H_2O$ (Sodium Perchlorate) (store at 4°C)
  1M acetosyringone (in DMSO) (store at -20°C)
- 10ml infiltration buffer contain:
  1ml 0.5M MES
  1ml 0.02M $Na_3PO_4.12H_2O$
  1µl 1M acetosyringone
  0.05g glucose
  Deionised water to make up total volume