Extraction of Root Apoplastic Wall Fluid for Apoplastic Peroxidase Activity Assay

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[Abstract] Plant roots secrete a lot of peroxidases to counteract environmental influences. This protocol describes a way to extract root apoplastic wall fluid from Arabidopsis plants and to determine peroxidase activity using guaiacol as substrate.

Materials and Reagents

1. 10 mM Na^+-PO_4 (pH 6.0)
2. Guaiacol
3. Agar
4. MES-K
5. 1/4x modified Hoagland medium (see general growth protocol for recipes)
6. Solid medium (see Recipes)

Equipment

1. UV-vis Spectrometer
2. Arabidopsis growth facility
3. 15 ml Falcon tube

Procedure

1. Plant growth
   Surface-sterilized *Arabidopsis* seeds are planted on ? x modified Hoagland medium (1% agar, 5 mM MES-K, pH 6.0) solidified on 1 ml pipette tip box insert (the part with holes). After germination for 2-3 days under light, the boxes with young seedlings are floated in ? modified Hoagland liquid medium with 5 mM MES-K at pH 6.0, aerated by fish tank water pumps.
2. Extraction of apoplastic wall fluid (AWF)

a. Roots of Col seedlings (2.5 weeks old) are rinsed in de-ionized water and cut into 10 mM Na^{+}-PO_4 (pH 6.0).
b. Vacuum for 5 min on ice, followed by slow release for infiltration.
c. The roots are then dabbed onto filter paper to dry, measured on a balance and recorded as fresh weight.
d. The roots are then put to the plunge barrel of a 5 ml syringe. The syringe barrel is placed in a 15 ml Falcon tube and centrifuged at 3,000 *x g* for 15 min at 4 °C.
e. The AWF is rescued from the tube bottom. Total volume is measured by pipetting. AWF is kept on ice.

3. Total protein quantification

   Protein concentration measurement (Bradford micro assay) is done according to Bio-Rad reagent instruction.

4. Apoplastic peroxidase (APX) activity assay

   To set up a reaction, mix the following:

   Chemical Volume FW Stock conc.

   Guaiacol 880 µl 124.14; D=1.112 g/ml, 20 mM in 10 mM Na-PO_4 pH 6.0

   AWF x µl

   Add 20-x µl 10 mM Na-PO_4 (pH 6.0).

   Start the reaction by adding:

   H_2O_2 100 µl 34.01; 31.4 % 0.3 % (v/v)

   Total volume is adjusted to 1 ml.

   Reactions are followed spectrophotometrically at 470 nm for 2 min at room temperature. Extinction coefficient = 26.6/Mm/cm

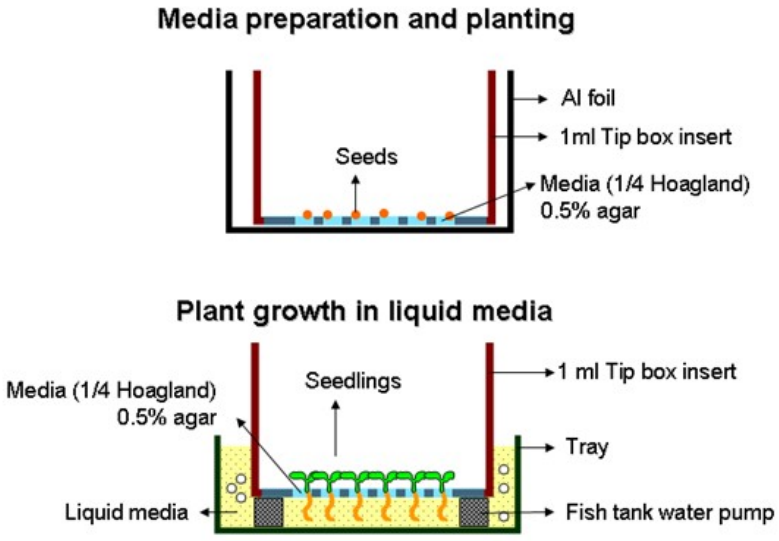

Figure 1.

Recipes

1. Solid medium
   Add 1% agar, maintain the pH with 5 mM MES-K at pH 6.0

References

1. Li, X., Chanroj, S., Wu, Z., Romanowsky, S. M., Harper, J. F. and Sze, H. (2008). A distinct endosomal Ca^{2+}/Mn^{2+} pump affects root growth through the secretory process. *Plant Physiol* 147(4): 1675-1689.

