Staining of *Arabidopsis thaliana* leaves with trypan blue and aniline blue

1) Trypan blue

This staining method is used to reveal hyphal structures and dead plant cells in plant tissues. Fungal structures and dead plant cells are stained blue.

Solution to be prepared:

Trypan blue Solution
Mix 10 g phenol, 10 ml glycerol, 10 ml lactic acid, 10 ml water and 0.02 g of trypan blue together (stock solution).
The working solution is prepared by diluting the stock solution with ethanol (96 %; 1:2 v/v).

Chloral hydrate
Dissolve 1 kg of chloral hydrate in 400ml water (takes several hours). Cover with an appropriate lid.

Staining procedure:

- Infected tissue is transferred into a plastic test tube with a lid and covered with diluted trypan blue solution.
- The tube (lid slightly unscrewed) is placed in a heated water bath and the staining solution is boiled for one minute.
- The tissue is left overnight in the staining solution.
- The next day, distain by replacing the staining solution with chloral hydrate solution. If necessary replace distaining solution several times.
- The samples can be kept in the chloral hydrate solution for several months.

Waste:
The trypan blue solution is discarded into a phenol waste bottle and the chloral hydrate is discard into a chloral hydrate waste bottle.

2) Double coloration, Aniline blue and calcofluor white

The aniline blue staining method is used to reveal callose structures in plant tissue, which appear after infection (papillae, apposition) or during pollen tube formation.
The calcofluor stains chitin present in fungal cell membranes and also binds to cellulose at locations where the cuticle is damaged

Solution to be prepared:

Sodium phosphate buffer (0.07 M, pH=9)
Dissolve 12.46 g of $Na_2HPO_4 \cdot 2H_2O$ in one litre water and adjust the pH to 9 using a solution of $NaH_2PO_4 \cdot 2H_2O$ (0.07 M which is prepared by dissolving 0.966g of $NaH_2PO_4 \cdot 2H_2O$ in 100ml water).

Aniline blue solution
First prepare an aniline blue solution of 0.05 %. Use this solution to prepare an aniline blue solution of 0.005 % using sodium phosphate buffer as the diluting agent.

Calcofluor white solution
Prepare a calcofluor (calcofluor white M2R.S.new) solution of 0.1 % (w/v) in Tris-Hcl (0.1 M pH=8.5).
Cover the bottle with aluminium paper, as calcofluor white is light sensitive.

Staining procedure:

- Distain the samples during a whole night in 96 % ethanol in a Petri dish.
- Incubate the samples in the sodium phosphate buffer (0.07 M, pH=9) for 30 minutes.
- Discard the buffer and add into the dish the appropriate volume of aniline blue solution to cover the leaves (write down the volume).
- Let the tissue impregnate for 60 minutes, discard the aniline blue solution.
- Add 1/10 of the aniline blue volume of 0.1 % calcofluor white into the dish.
- Observe the samples immediately under a microscope with fluorescence and UV filters as calcofluor white is degraded by light.

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