Toluidine-blue staining

- 1. Sterilized seeds were grown on a plate (*1) for 2 weeks.
- 2. TB solution(*2) was poured directly onto the growing plants.
- 3. Plants were submerged in a TB solution for 2 minutes.
- 4. TB solution was removed from the plate for decantation.
- 5. Plate was washed gently in several changes of water in tray to remove excess TB from plants.

(*1) Plate

1/2 Murashige and Skoog's medium containing 0.4% (w/v) gellan gum (Wako Pure Chemicals Industries Ltd., Osaka, Japan)

(*2) Toluidine-blue (TB) solution

0.1% (w/v) TB (Sigma, St Louis, MO, USA) was filtered through a $0.2~\mu m$ filter,

<Reference>

A new method for rapid visualization of defects in leaf cuticle reveals five intrinsic patterns of surface defects in *Arabidopsis*

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