LSOP Title	Transformation of Arabidopsis by Floral Dipping
LSOP No.	LSOP33
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ -waste* *OGTR*
Risk Assessments	
Approved by	*Francois Barbier*
Date Approved	14/09/2021
Date Effective	30/06/2021
Next Review Date	30/06/2022
Contact for Assistance	plantsuccess@uq.edu.au

1.0 Scope

To outline the procedures for floral dipping transformation of Arabidopsis using the Zhang et al., 2006 method (short version)

2.0 Definitions

LB medium – luria Bertani liquid medium

D - days

H – hours

Wt/vol – weight/volume

S – seconds

Inflorescence – a group or cluster of flowers

Siliques – seed capsules

Rosettes – circular arrangement of leaves

3.0 Materials and Equipment

1. Agrobacterium strain
2. Liquid LB medium
3. Appropriate antibiotics
4. Centrifuge
5. Sucrose
6. Stirring bar and plate
7. Pipette and pipette tips
8. Brushwet/Silwet
9. Plastic cover or plastic film
10. Wax Paper

4.0 Prescribed Actions

1. Start preparing the Agrobacterium strain that harbors the gene of interest in a binary vector by inoculating a single Agrobacterium colony into 5 ml liquid LB medium containing the appropriate antibiotics for binary vector selection. Incubate culture at 28 °C for 2 d
2. Use this feeder culture to inoculate a 500 ml liquid LB with the appropriate antibiotics and grow the culture at 28°C for 16–24 h. We exclusively use cells that grow to the stationary phase (OD B1.5–2.0).
3. Collect Agrobacterium cells by centrifugation at 4,000g for 10 min at room temperature, and gently resuspend cells in 1 volume of freshly made 5% (wt/vol) sucrose solution with a stirring bar.
4.  Add Silwet L-77 (toxic) to a concentration of 0.02% (vol/vol) (100 ml per 500 ml of solution) and mix well immediately before dipping. Transfer the Agrobacterium cell suspension to a 500 ml beaker.

   NB in our lab we use Brushwet instead of Silwet.
5. Invert plants and dip aerial parts of the plants into the Agrobacterium cell suspension for 10 s with gentle agitation. We dip not only the inflorescences but also the rosette to soak shorter axillary inflorescences. Remove dipped plants from the solution and drain the treated plants for 3-

5 s. A film of liquid coating the plants should then be visible (Fig. 1b appendix).

6. Cover dipped plants with a plastic cover or wrap them with plastic film. Lay down the treated plants on their sides for 16-24 h to maintain high humidity (Fig. 1c appendix)
7. Remove the cover next day (Fig. 1d appendix). Send the treated plants back to the greenhouse or the growth chamber, and allow them to grow normally for 1 month. Withhold watering when siliques turn brown. Wrap drying plants and loose bolts with a piece of wax paper such as Sample Sak or other means (Fig. 1e appendix).
8. Collect dry seeds

5.0 Appendix

Read the full protocol before starting with this short version (the side notes are useful)

https://www.nature.com/articles/nprot.2006.97

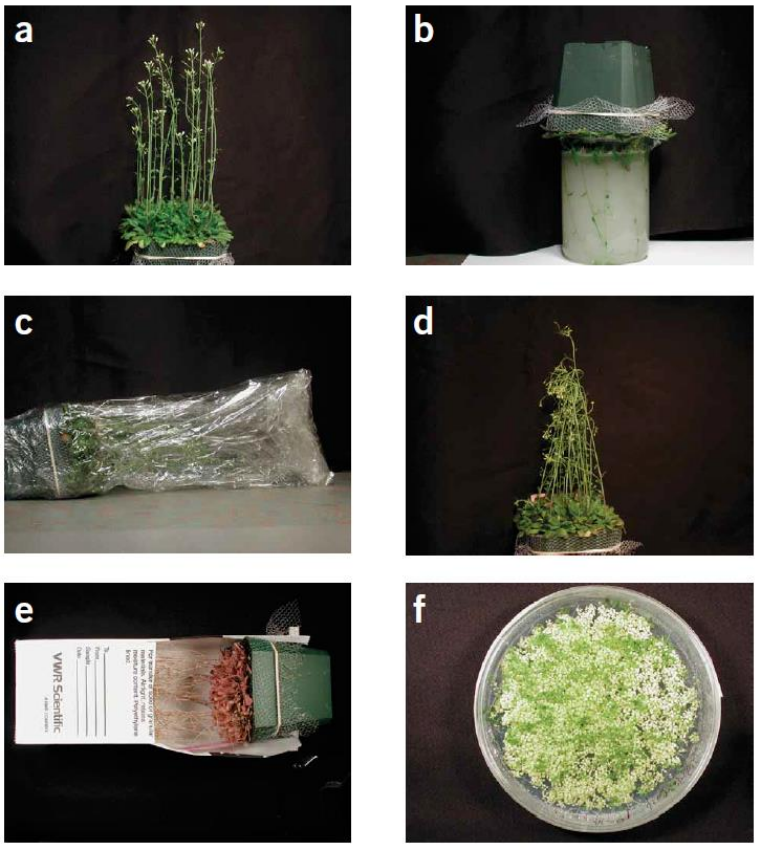

Figure 1 | Stages during the floral dip transformation method.

(a) A good stage for floral dipping is when a pot of healthy plants contain approximately 20–30 inflorescences and some maturing siliques. Siliques should be clipped off

(b) Invert plants and dip their aerial parts in an Agrobacterium cell suspension for 10 s.

(c) Wrap the dipped plants with plastic films to maintain high humidity for 16–24 h.

(d) Remove the plastic covers and grow plants in a growth chamber for 1 month.

(e) Dry and harvest seeds with a sample bag.

(f) Select primary transformants. Transgenic plantlets are readily distinguished from non-transgenic plants by their green true leaves and roots that penetrate into the selection medium. In this experiment, we selected primary transformants using kanamycin (+ carbenicillin) and obtained more than 100 transgenic lines on a single selection plate. Note that non-transformed seedlings germinated as well but their cotyledons became chlorotic and bleached soon after germination, whereas true transformants were very healthy, with green cotyledons and true leaves, and developed roots that penetrated into the medium.