Floral Dip Transformation in *Lepidium campestre*

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[Abstract] Floral dip is a very common technique to stably transform *Arabidopsis thaliana* (Clough and Bent, 1998; Martinez-Trujillo *et al.*, 2004; Zhang *et al.*, 2006) and has also been adapted to some other plant species (Curtis and Nam, 2001; Tague, 2001; Bartholmes *et al.*, 2008). Here, we describe this method optimized for transformation of the Brassicaceae plant *Lepidium campestre (L. campestre)*.

Materials and Reagents

1. *L. campestre* seeds
2. *Agrobacterium* strain GV3101 (carrying a binary vector for plant transformation including resistance gene for bacterial selection and a T-DNA containing a Basta-resistance gene)
3. Seedling substrate (60% white peat; 20% frozen black peat; 20% coconut pulp) (Klasmann-Deilmann GmbH, recipe number: 080)
4. Osmocote mini (The Scotts Company)
5. Triabon (COMPO)
6. Beef extract
7. Yeast extract
8. Peptone
9. Sucrose
10. $MgSO_4$
11. Rifampicin (chromosomal resistance)
12. Gentamycin (helper plasmid resistance)
13. Further antibiotics depending on the transformation vector
14. Silwet L-77 (Lehle Seeds, catalog number: VIS-02)
15. 0.01% basta solution (Bayer CropScience GmbH, catalog number: 79011725)
16. Soil (see Recipes)
17. YEB Medium (see Recipes)
18. Infiltration medium (see Recipes)

Equipment

1. Greenhouse for plant cultivation
2. Cold-room for plant vernalization
3. Vermiculite (1-2 mm)
4. 28 °C incubator with shaking
5. Beaker
6. Centrifuge with temperature control
7. Spectrophotometer
8. Magnetic stirrer
9. Small plastic bags

Procedure

1. Germinate *L. campestre* seeds on soil, keeping the seeds moist and covered until seedlings emerge.
2. Transfer seedlings to individual pots and grow them for 6 weeks with a 16 h photoperiod at 20 °C and 8 h without illumination at 15 °C.
3. Vernalize the plants for 8 weeks at 4 °C with 8 h of illumination.
4. Move the plants back to greenhouse conditions. After 1-2 weeks, inflorescences should emerge.
5. Ideally, the first dipping should take place shortly before the first flowers open.
6. 2 days before dipping, grow a 5 ml over-night culture of GV3101 at 28 °C under constant rotation at 200 rpm in YEB medium.
7. Use this culture to inoculate 500 ml of YEB medium and cultivate as above for 24 h.
8. Pellet *Agrobacterium* cells by centrifugation at 16 °C and 5,500 *x g* for 15 min.
9. Resuspend the cells in approximately 20 ml of infiltration medium by vortexing.
10. Add infiltration medium to reach a final OD_{600} of 2.0 (should result in approximately 400-600 ml).
11. Keep the cells at room temperature in the dark for approximately 2 h.
12. Put the *Agrobacterium* solution in a beaker and stir it constantly using a magnetic stirrer.
13. At least 10-15 plants should be treated per transformation construct, in order to generate several independent transgenic lines (transformation efficiency of ~0.4% results in approximately 1 transformant per plant).
14. Gently spread and open up the plants inflorescence using your finger in order to allow the liquid to reach as much of the surface as possible.
15. Immerse the plant's inflorescence for 5 sec into the *Agrobacterium* solution.

16. Cover inflorescences with a plastic bag to retain humidity.
17. Keep the plants without direct illumination for 24 h (*i.e.* Switch off all lights directly above your plants and close the shades. It does not need to be completely dark, but strong light it known to reduce motility and infectivity of *Agrobacteria*).
18. Repeat the dipping weekly, until no new flowers are produced (3-5 runs).
19. Cultivate the plants until all fruits are yellow and dry (approximately 3-6 weeks).
20. Collect the seeds by manually opening the fruits.
21. Germinate the seeds until cotyledons emerge.
22. Spray seedlings with 0.01% Basta solution in 3-4 day intervals until no further seedlings emerge and Basta-resistant plants can be clearly distinguished from Basta-sensitive plants (approximately 2 weeks).
23. After a few days, Basta-sensitive seedlings stop growing and die while transformed plants continue to grow.
24. Confirm transgene integration by phenotyping PCR or Southern blotting.

Recipes

1. Soil
   Consisting of 8:1:1 seedling substrate, Vermiculite and sand supplemented with fertilizer (1 g/L of each Osmocote mini and Triabon)
2. YEB Medium
   5 g/L beef extract
   1 g/L yeast extract
   5 g/L peptone
   5 g/L sucrose
   0.5 g/L $MgSO_4$
   Supplemented with rifampicin (50 µg/ml), gentamycin (25 µg/ml) and other antibiotics depending on the transformation vector
3. Infiltration medium (always prepare freshly)
   5% sucrose
   0.02% silwet L-77

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References

1. Bartholmes, C., Nutt, P. and Theiβen, G. (2008). Germline transformation of Shepherd's purse *Capsella bursa-pastoris* by the 'floral dip'method as a tool for evolutionary and developmental biology. *Gene* 409(1): 11-19.
2. Clough, S. J. and Bent, A. F. (1998). Floral dip: a simplified method forAgrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16(6): 735-743.
3. Curtis, I. S. and Nam, H. G. (2001). Transgenic radish (*Raphanus sativus* L. *longipinnatus* Bailey) by floral-dip method–plant development and surfactant are important in optimizing transformation efficiency. *Trans Res* 10(4): 363-371.
4. Lenser, T. and Theißen, G. (2013). Conservation of fruit dehiscence pathways between *Lepidium campestre* and *Arabidopsis thaliana* sheds light on the regulation of *INDEHISCENT*. *Plant J* 76(4): 545-556.
5. Martinez-Trujillo, M., Limones-Briones, V., Cabrera-Ponce, J. L. and Herrera-Estrella, L. (2004). Improving transformation efficiency of *Arabidopsis thaliana* by modifying the floral dip method. *Plant Mol Biol Rep* 22(1): 63-70.
6. Tague, B. W. (2001). Germ-line transformation of *Arabidopsis lasiocarpa*. *Trans Res* 10(3): 259-267.
7. Zhang, X., Henriques, R., Lin, S.-S., Niu, Q.-W. and Chua, N.-H. (2006). *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nat protoc* 1(2): 641-646.

