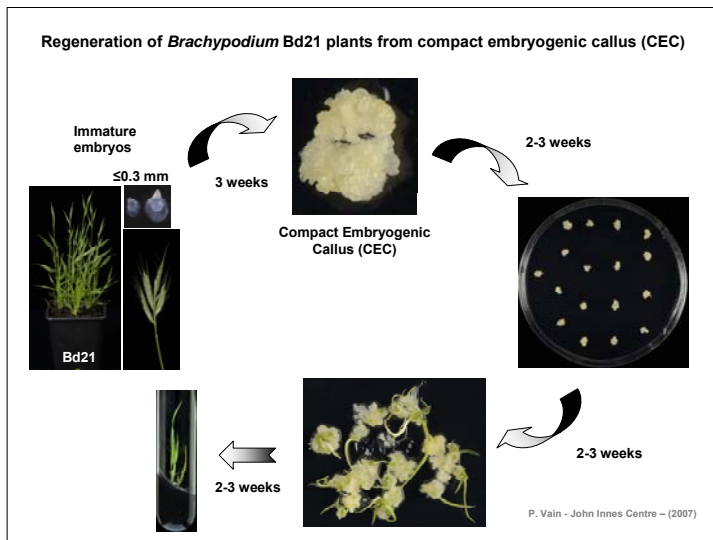




## Protocol for *Brachypodium* tissue culture

[Vain P. et al. \(2008\)](#) Plant Biotechnology Journal, 6:236-245

[Alves S.C. et al. \(2009\)](#) Nature Protocols, 4:638-649.



### Production of compact embryogenic callus from immature embryos

Steps 3 to 9 are conducted under sterile conditions.

1. Collect tillers from 7-9 weeks-old Bd21 plants when the immature seeds are swollen but still green.
2. Select immature seeds with a soft endosperm, remove the top glume (lemma) from seeds. Collect seeds in water. Drain well before sterilisation.
3. Sterilise approximately 20 seeds for 30 sec with 20 ml of 70% ethanol in a sterile Petri dish. Drain ethanol and rinse with sterile deionised water. Add 20 ml of 1.3% sodium hypochlorite solution. Gently shake the seeds for 4 min. Rinse three times with sterile deionised water.
4. Isolate immature embryos up to and including 0.3 mm in length from seeds. Only very small immature translucent embryos will produce homogeneous CEC at high frequency.
5. Culture immature embryos, scutellum facing up, onto MSB3+Cu0.6 solid medium (MS salts, Fe-EDTA, 30 g/l sucrose, 2.5 mg/l 2,4-D, 0.6 mg/l  $\text{CuSO}_4$ , vitamins M5, 2 g/l Phytigel - pH

# BrachyTAG



- 5.8) for 3 weeks at 25°C in the dark.  $\text{CuSO}_4$  significantly promotes the growth and embryogenicity of Bd21 CEC.
6. Excise the shoots under sterile conditions, as they elongate during the first 2-3 days of culture.
  7. After 2.5-3 weeks, fragment CEC with a creamy colour and pearly surface in 2-4 pieces. Transfer pieces of CEC onto fresh MSR3+Cu0.6 solid medium for another 2.5-3 weeks at 25°C in the dark. Discard all non-CEC tissue.

## Regeneration of Bd21 plants

8. Transfer CEC onto the MSR26 regeneration medium (MS salts, Fe-EDTA, 30 g/l sucrose, 0.2 mg/l kinetin, vitamins M5, 2 g/l Phytigel - pH 5.8) for 2-3 weeks at 25°C under 16-h photoperiod.
9. Transfer shoots (rooted or not) to tubes containing MSR63 germination medium (40% MS salts, Fe-EDTA, 10 g/l sucrose, vitamins B5, 7 g/l charcoal, 2 g/l Phytigel, 6 g/l agar - pH 5.8). Culture for 2-3 weeks at 25°C under 16-h photoperiod.

## Seed production from regenerated Bd21 plants

10. Pot regenerated plantlets in 2x2 cm cell-tray containing a wet compost mixture. Grow plants in a Controlled Environment Room (CER) at 22°C with a 20-hour photoperiod. Initially, keep seedlings covered with a propagator lid for 1-2 weeks to ensure adaptation of plantlets to low humidity conditions.
11. Transfer compost and plant (when starting to tiller) into "Roottrainer" pots containing a wet compost mixture. Grow plants in a CER at 22°C with a 20-hour photoperiod for 5-6 weeks.
12. When the seeds are fully mature, stop watering plants and dry plants for 2-4 weeks.
13. Collect spikelets from each plant and store at 1.5°C, 7-10% humidity in the dark. Seeds can be stored for more than 10 years in these conditions.

Reagents, compost and culture media composition, as well as additional practical information is detailed in [Alves S.C. et al. \(2009\)](#) Nature Protocols, 4:638-649.