BrachyTAG

Protocol for growing *Brachypodium* distachyon plants (genotype Bd21)

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

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

Steps 3 and 4 are conducted under sterile conditions.

1. Soak wild-type Bd21 mature seeds in sterile deionised water for 2 h at room temperature in a Petri dish.
2. 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. Place 20 seeds on top of two layers of sterile filter paper soaked with sterile deionised water inside a Petri dish. Incubate for 2 days in the dark at 5ºC to synchronise germination, followed by a week at 25ºC with a 16-h photoperiod.
5. Pot germinated seedlings 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.
6. Transfer compost and plant (when starting to tiller) into “Rootrainer” pots containing a wet compost mixture. Grow plants in a CER at 22°C with a 20-hour photoperiod. The fertiliser present in the compost mixture (50% John Innes Compost no. 2 and 50% peat and grit mix) is sufficient to ensure complete plant development.
7. When the seeds are fully mature (after 2-3 months), stop watering plants and dry plants for 2-4 weeks.
8. 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 and compost composition, as well as additional practical information are detailed in Alves S.C. et al. (2009) Nature Protocols, 4:638-649.