Brachypodium distachyon Cultivation in Growth Chambers or on Light Racks

- Prepare 3 x 3 inch (ca. 7 cm) or smaller pots with moist soil in a holding tray. We use Sunshine Redi-earth soil.

- Sow 3 or 4 seeds in each pot with optimal spacing and cover with a ~1 cm thin layer of soil. Seeds can be placed either upright or laid flat on the soil. We did not observe any difference in germination rates in either position. The pots are covered to maintain high humidity.

- Bd21-3 seeds require stratification for uniform germination. Prior to germination, store seeds in the dark at 4°C for 5-7 days. After cold stratification, plants can be moved to a growth chamber with 14 h light (250-300 μmol/m² s), 21°C; 10 h dark, 18°C. These conditions are optimal for vegetative growth and suitable for viral infections. Bd21-3 typically germinates within 3-4 days. When the cotyledon leaves emerge completely, remove the humidifying covers.

- Water plants regularly as needed. Bd21-3 does not require heavy watering; water only to keep the soil moist. Plants are fertilized once a week with Peters fertilizer (15-5-15).

- In order to promote flowering, Bd21-3 plants has to be vernalized. For high-quantity seed production, allow plants to grow for 2-3 weeks in a shorter day conditions as stated above for enough vegetative material. Next, perform vernalization treatment at 4°C for 2-3 weeks. After vernalization, move them back to shorter or long day conditions (as described below). Bd21-3 plants take ca. 2 to 3 weeks after vernalization to flower and set seed. Stop watering the plants when the panicle appears to be full, usually by 4 weeks post-vernallization. Also, at this stage many leaves will appear senesced.

- Alternatively, for faster life-cycle and reproductive transition, vernalization can be performed at the time of germination in combination with the cold-stratification. Sow the seeds in pots and leave them in 4°C for 2-3 weeks. Next, transfer the pots into a longer day growth chamber with 20 h light (250-350 μmol/m² s), 21°C; 4h dark, 18°C. These plants take ca. 2 to 3 weeks after vernalization to flower and set seed. So, total time to maturity will be about 6-7 weeks. You will however get less seed material by this process, as there is less vegetative growth to support the seed yield.

- After the plants are completely dry, harvest the panicles and separate the seeds. Low seed moisture content is critical for seed viability; harvested seeds are dried overnight in a vacuum desiccator. For long-term storage, keep seeds at 4°C in airtight containers.

**Brachypodium cultivation.** A) Small pots in trays are seeded and covered until plantlets are established. B) Uncovered trays with 3-4 plants per pot. C) Mature Bd21-3 plants with seed heads at ca. 7 weeks. D) Senesced, completely dry plants, ready for harvest. E) Close-up of panicle from senesced plant. The seeds are separated, desiccated, and stored at 4°C. (Images: K.-B.G. Scholthof)
Materials

The materials in the protocols can be sourced from BWI Companies (www.bwicompanies.com) or other Horticultural or Scientific Suppliers.

Trays, Flat Black, No Holes. 100/pack (11” x 21.375”)
Standard 26-cell insert, 100/pack
Osmocote Plus 15-8-11 Tablets (7.5 gm; 8-9 months)
Peters Excel Fertilizer Cal-Mag Special 15-5-15
Sunshine Redi-earth Plug & Seedling
   Formulated with fine Canadian Sphagnum peat moss, vermiculite, starter nutrient charge (with Gypsum) and dolomitic limestone.

Updated: 6 August 2013

Kranthi Mandadi
Karen-Beth G. Scholthof
Jesse D. Pyle