

Selection of transformants in BASTA (Phosphinothricin, glufosinate ammonium) plates

Protocol from Alonso lab (http://www4.ncsu.edu/~jmalonso/Alonso-Stepanova_JAtY.html)

1. Sterilize seeds for 10-15 minutes in 50 ml tubes using 30-40ml of 50% bleach spiked with 3-4 drops of Triton-100 per liter (to avoid seeds clumping and aggregating together). Make sure the seeds are fully resuspended. Invert/shake the tubes with seeds occasionally during the 10-15 minutes of sterilization.
2. Wash the seeds 3-5 times with ~50 ml sterile di H₂O. After each wash, remove as much water as possible.
3. To the seed suspension in di H₂O, add Basta and Timentin to 20 microgram/ml and 300 microgram/ml final concentration, respectively. For example, if the residual volume of seeds in water is 5 ml, add 5 microliters of 10 mg/ml Basta and 300 mg/ml Timentin. Timentin inhibits Agro growth that survived bleach sterilization under the seed coat.
4. Cold-treat the tubes with seeds at 4°C for 2-3 days to equalize germination.
5. Equilibrate the tubes with seeds to room temperature for 15-30 minutes and add to the seed suspension melted, pre-cooled 0.7% top agarose in water. Be careful not to use hot top agarose, as it will kill the seeds. On the other hand, if the agarose is too cool, it will solidify when mixed with the room temperature seed suspension and make clumps. We typically use 1-3 volumes of top agarose per each seed suspension volume. For example, add 5-15 ml of 0.7% top agarose to 5 ml seed suspension.
6. Use plastic single use 10ml pipettes to uniformly distribute the seed/agarose suspension on the top of AT plates (1x MS salts, 1% sucrose, pH 6.0; 0.6-0.8% agar) supplemented with 20 microgram/liter Basta. Plate up to 8000 seeds (~0.2g dry weight) resuspended in 5-15 ml top agarose per each 150mm Petri plate containing 50-70ml AT/Basta.
7. Incubate plates in the light for 1-2 hours at room temperature to improve germination and place the plates in the dark incubator at 22°C for 3 days.
8. After 3 days in the dark, move the plates to the light for 2-5 days. Check the plates periodically. Basta-resistant plants (transformants) will develop green color upon light exposure. Sensitive plants (untransformed) will remain bleached or will fail to germinate all together.
9. Transplant Basta-resistant plants to soil and propagate.