Supporting Information

Zhang and Turgeon 10.1073/pnas.0904189106

SI Text

Detailed Protocol for *Verbascum phoeniceum* Transformation. Seeds of *Verbascum phoeniceum* cv. "Flush of White" (Garden Makers) were surface sterilized by rinsing in 1% (vol/vol) sodium hypochlorite and 0.1% (vol/vol) Tween 20 for 15 min. Seeds were then rinsed three times in sterile distilled water. Sterilized seeds were germinated on Murashige and Skoog (MS, PhytoTechnology Laboratories) medium supplemented with 3% (wt/vol) sucrose and 1× Gamborg's vitamins (100 mg/L myo-inositol, 1 mg/L nicotinic acid, 1 mg/L pyridoxine hydrochloride, and 10 mg/L thiamine hydrochloride) and solidified with 2.5 g/L Gelrite (Research Product International). Axenically germinated plants were kept in a growth chamber with 14 h day/10 h night cycles at approximately 100 μmol of photons m^{-2} s^{-1}. Three- to four-week-old seedlings were used for *Agrobacterium*-mediated transformation. *Agrobacterium* were grown at 28 °C overnight in 15 mL Luria-Bertani (LB) medium containing 100 mg/L kanamycin, 50 mg/L rifampicin, and 25 mg/L gentamycin with shaking at 250 rpm until OD_{600} value reaches 0.7. The culture was centrifuged for 10 min at 2,500 rpm at 25 °C. The LB was decanted, and the bacterial cells were resuspended in 15 mL liquid MS medium. Roots of aseptically grown *V. phoeniceum* were excised, cut into 1-cm sections and bathed in the bacterial suspension for 10 min. The roots were then removed, blotted dry, and placed on co-cultivation medium containing MS, 1× Gamborg's vitamins, 1 mg/L napthalene acetic acid (NAA, Research Product International), 3 mg/L 6-benzyl-aminopurine (BAP, Research Product International), 200 μM acetosyringone (Sigma), and 2.5 g/L Gelrite. After 3 days, the explants were transferred to selection shooting medium containing MS, 1× Gamborg's vitamins, 1 mg/L NAA, 3 mg/L BAP, 100 mg/L kanamycin, and 400 mg/L carbenicillin. Cultures were kept in a growth chamber with the same conditions as those for seed germination for 2 weeks. Newly formed calli were moved to another selection shooting medium containing chemicals as above except solidifying agent, Gelrite, which was replaced by 8% Difco Agar (Becton Dickinson Co.) to avoid hyperhydricity of the calli. When healthy shoots developed, they were excised and placed on a medium containing MS, 1X Gamborg's vitamins, 2 mg/L indole-3-butyric acid (IBA) and 3% sucrose (no antibiotics) for 2 days and then moved to a similar medium without IBA for root induction. Antibiotics were not used at this stage because we found in preliminary experiments that they inhibited root formation, even from transgenic calli. After the roots just emerged from the shoots, the plantlets were moved to selection medium containing MS, 1× Gamborg's vitamins, and 100 mg/L kanamycin for 2 weeks. Plantlets with actively growing root systems in this selection medium were transferred to a soil mixture of Metromix 360 (Scotts) and acclimated slowly to growth chamber or greenhouse conditions. Finally, the plantlets were repotted in the same soil mixture in pots.