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Tragopogon (Asteraceae) transformation and regeneration

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Abstract

Polyploidy or whole-genome duplication is a significant evolutionary force. *Tragopogon* (Asteraceae) includes an evolutionary model system for studying the immediate consequences of polyploidy. Here, we provide an improved transformation and regeneration protocol for diploid and tetraploid *Tragopogon*.

Materials

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Tragopogon transformation and regeneration protocol

1 Prepare *Tragopogon* material

1.1 Remove the pericarp (fruit wall) of *Tragopogon* achenes to expose the seed; sterilize the seeds in 20% (v/v) bleach (containing a few drops of Tween-20) for 20 min (shake slowly). Rinse the seeds 4 times with autoclaved deionized water to remove excess bleach on the seed surface. Blot the seeds on the autoclaved filter paper.

1.2 Grow the seeds on **seed germination medium** (also known as **1/2 MS medium**) (2.22 g/L MS basal medium with vitamins, 30 g/L sucrose, 5 g/L agargellen, pH 5.8) in a sterile ice cream box in an incubator (25 °C, 14 h light/10 h dark, 52.5 µmol m^{-2} s^{-1}; model CU36L4C8 from Percival 26 Scientific, Perry, IA, USA).

1.3 Cotyledons and true leaves are harvested approximately 2 weeks after seed germination for *Agrobacterium*-mediated transformation (Step 3.1).

2 *Agrobacterium* culture

2.1 Streak the *Agrobacterium tumefaciens* strain EHA 105 onto a **selective LB plate** (40 g/L LB agar, 50 mg/L kanamycin, 50 mg/L rifampicin). Incubate the plate at 28 °C for 2 days.

2.2 Pick *Agrobacterium* colonies and inoculate into 20 mL **selective liquid TY medium** (5 g/L tryptone, 3 g/L yeast extract, 50 mg/L kanamycin, 50 mg/L rifampicin, 0.1 mM acetosyringone, pH 5.5). Shake at 100 rpm, 28 °C for 24 h.

3 *Tragopogon* transformation and regeneration

3.1 Prepare leaf explants. Chop leaf tissues from Step 1.3 into 1-cm segments. To promote transformation efficiency, the surfaces of the leaf segments are slightly scored to create wounds using a sterile blade. The *Tragopogon* leaf explants are placed on the **callus induction medium** [4.44 g/L MS basal medium with vitamins, 30 g/L sucrose, 0.5 mg/L naphthaleneacetic acid (NAA), 1 mg/L 6-benzylaminopurine (BAP), 6 g/L gelrite, pH 5.8] for 2 days (in the dark at room temperature, at 23 °C) before transformation (a process referred to as “pre-culture”).

3.2 Prepare *Agrobacterium* suspension. Centrifuge TY medium from Step 2.2 at 2000 rpm for 10 min; remove the TY medium and re-suspend the *Agrobacterium* pellet with **AAM infection medium** (2.95 g/L KCl, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 0.15 g/L $CaCl_2 \cdot 2H_2O$, 0.15 g/L

$NaH_2PO_4 \cdot H_2O$, 10 mg/L $MnSO_4 \cdot 4H_2O$, 3 mg/L H_3BO_3, 2 mg/L $ZnSO_4 \cdot 7H_2O$, 0.75 mg/L KI, 0.25 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.025 mg/L $CuSO_4 \cdot 5H_2O$, 0.025 mg/L $CoCl_2 \cdot 6H_2O$, 0.876 g/L L-glutamine, 0.266 g/L L-aspartic acid, 0.174 g/L L-arginine, 7.52 mg/L glycine, 0.1 g/L myo-inositol, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine hydrochloride, 0.1 mg/L thiamine hydrochloride, 27.8 mg/L $FeSO_4 \cdot 7H_2O$, 37.26 mg/L Na_2EDTA, 0.5 g/L casamino acids, 68.5 g/L sucrose, 36 g/L glucose, 0.1 mM acetosyringone, pH 5.2). For each transformation experiment, prepare 20 mL AAM infection medium with OD_{600} = 0.1-0.2.

3.3 Co-culture of leaf explants and *Agrobacterium*. Immerse explants in 20 mL AAM infection medium for 30 min; explants are thoroughly blotted dry on autoclaved filter paper and transferred to **co-cultivation medium** (4.44 g/L MS basal medium with vitamins, 30 g/L sucrose, 0.5 mg/L NAA, 1 mg/L BAP, 6 g/L gelrite, 0.1 mM acetosyringone, pH 5.2). Place the plates in the dark for 3 days at room temperature (23 °C).

3.4 Transfer the explants to **selective callus induction medium** (4.44 g/L MS basal medium with vitamins, 30 g/L sucrose, 0.5 mg/L NAA, 1 mg/L BAP, 6 g/L gelrite, 300 mg/L timentin, 15 mg/L hygromycin, pH 5.8), and place the plates (covered by foil) inside the incubator (25 °C) for approximately 24 days. Callus will emerge from the cut edge of the explants. Successful transformants will survive on the selection medium and emit green fluorescence under blue light.

3.5 Shoot initiation. Calli of *Tragopogon porrifolius* (2*x*) and *T. mirus* (4*x*) are transferred to the **selective COCO-1** [2.41 g/L Woody plant basal medium with vitamins, 30 g/L sucrose, 2.5 mg/L BAP, 8.5 g/L agargellan, 10% (v/v) coconut water, 300 mg/L timentin, 15 mg/L hygromycin, pH 5.7] and **COCO-3** (identical to COCO-1, but with 15 g/L agargellan instead of 8.5 g/L)) shooting media, respectively. Place the plates inside the same incubator for approximately 5 weeks. The medium is renewed every 2-3 weeks.

3.6 Shoot elongation. Calli with tiny shoots are transferred to **selective WPM-3 medium** (2.41 g/L Woody plant basal medium with vitamins, 30 g/L sucrose, 8.5 g/L agargellan, 300 mg/L timentin, 15 mg/L hygromycin, pH 5.7) for approximately 2 weeks.

3.7 Rooting. The elongated shoots are gradually transferred (up to approximately 4 weeks on selective WPM-3 medium) to **selective COCO-R medium** [2.41 g/L Woody plant basal medium with vitamins, 30 g/L sucrose, 1.5 mg/L indole-3-butyric acid (IBA), 8.5 g/L agargellan, 300 mg/L timentin, 15 mg/L hygromycin, pH 5.7] for rooting.

3.8 After approximately 6 weeks, rooted shoots are gradually transferred to soil and continue their growth inside a growth chamber (14 h light/10 h dark, 25 °C; model AR-1110 from Percival Scientific). After approximately three months of growth in soil, the regenerated plants are subjected to a three-month cold treatment (8 h light/16 h dark, 7 °C). The plants are then moved back to the growth chamber (14 h light/10 h dark, 25 °C), and after approximately 3-4 weeks, most plants begin to bolt and flower.