Micropropagation of Curry Tree from Seed and Nodal Explants

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Curry tree (*Bergera koenigii*) is a relative of citrus that is in demand for culinary, medicinal, and ornamental purposes. Propagation of curry tree from seed is problematic because seeds are typically only produced once per year, have low seed viability when stored for even a short period of time, and exhibit high seedling mortality. In order to meet research and consumer demand for this plant, we have developed a method for micropropagating Curry tree from seeds and lateral buds harvested from mature trees. A step-by-step guide can be found in this publication.

Surface Sterilization

From Seed –Remove the seed coat, which is facilitated by soaking the seeds in water for a few minutes. Place the de-coated seeds in a tea strainer and submerge the strainer into a 50 ml beaker containing 70% ethanol for 3 minutes. Transfer the strainer to a 400 ml beaker containing a 10% bleach solution plus one drop of Tween® 20 (~5µL) and place the beaker on a stir plate containing a magnetic stir bar. Gently stir the solution for 30 minutes. After 30 minutes, move the beaker with the tea strainer to a laminar flow hood and rinse the sterilized seed by transferring the tea strainer to a container of sterile distilled water. (Figure 1.)

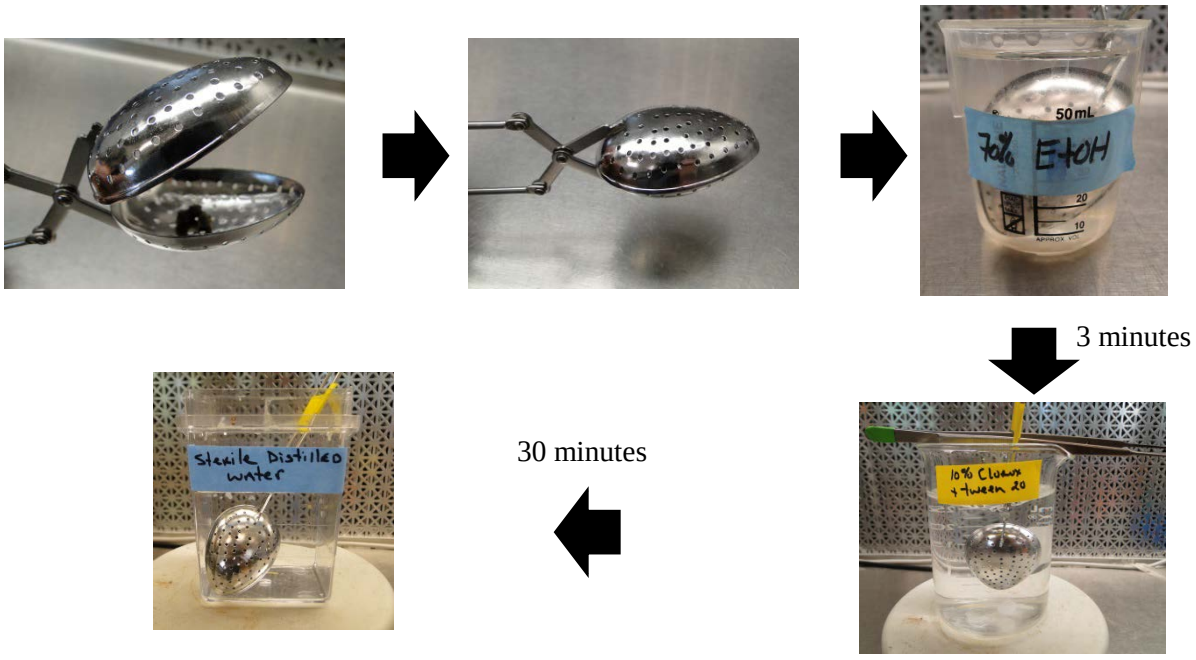

Figure 1. Surface sterilization of curry seeds using ethanol and bleach

From Lateral Cuttings –Remove shoots 4-6-inches in length from branches or from suckers at the base of the plant. Cut the stem sections into approximately 3-4 cm sections between the nodes including at least one node per section. Place cuttings in a 50 mL centrifuge tube and soak with 50 ml of 70% ethanol for 3 minutes. Replace the ethanol with a 20% bleach solution and one drop of Tween® 20, and place the centrifuge tube on a shaker at 250 rpms for 20 minutes. In a laminar flow hood, rinse the tissue three times with sterile distilled water and with sterile instruments, cut off 1 to 2 mm of the end of each stem section to remove tissue damaged by the bleach.

Shoot Production

Seed Germination – In a laminar flow hood, place surface sterilized seeds onto Murashige and Skoog's minimal organics medium supplemented with 30 g/l sucrose, 5.0 mg/l benzylaminopurine (BAP), 0.4mg/l gibberillic acid (GA_3) and 2 ml/l plant preservative mixture (PPM, Caisson Labs http://www.caissonlabs.com/ catalog number PPL33). Despite surface sterilization of the seed, many of the seeds will become contaminated once plated onto tissue culture medium. Therefore no more than 1-2 seeds are placed on each plate. Once multiple shoots begin to develop, subculture the seed along with its developing shoots onto Driver and Kuniyuki (DKW) plant medium supplemented with 30 g/l sucrose, 1.0 mg/l BAP and 0.1 mg/l GA_3, 0.1 mg/l indole-butyric acid (IBA) and 2 ml/ l PPM. (Figure 2.)

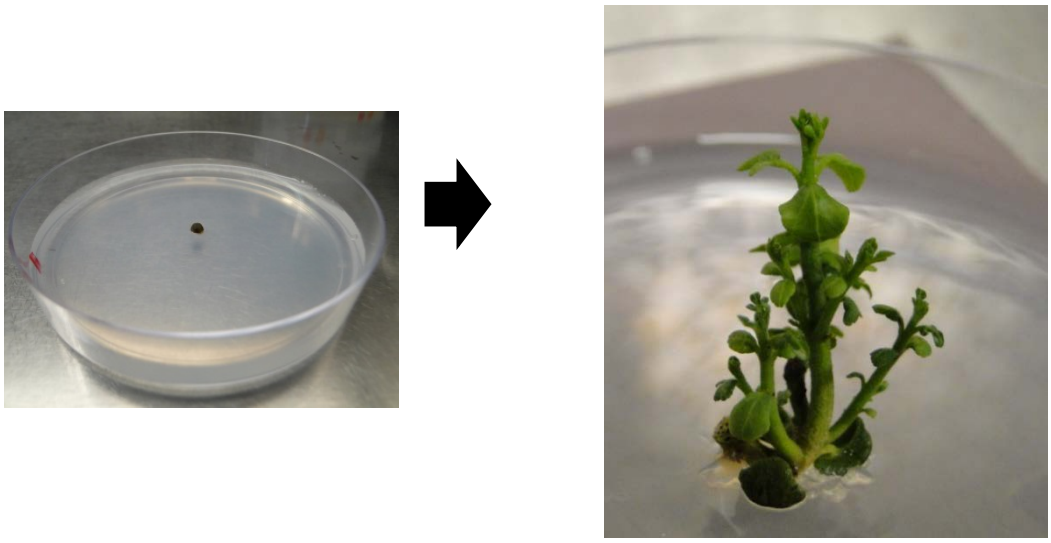

Figure 2. Multiple shoot formation from a curry seed plated on Murashige and Skoog's minimal organics medium supplemented with 30 g/l sucrose, 5.0mg/l benzylaminopurine (BAP), 0.4mg/l gibberillic acid (GA3) and 2 ml/l plant preservative mixture (PPM).

From Lateral Cuttings –In a laminar flow hood, aseptically place the surface sterilized lateral stems section with one to two nodes upright onto DKW medium supplemented with 30 g/l sucrose, 1.0 mg/l BA, 0.1mg/l GA_3, 0.1mg/l IBA and 2 ml/l PPM. After several weeks, shoots begin forming from the nodal meristems. Once the shoots elongate to 2-4 cm they can be removed from the stem section and transferred to DKW supplemented with 30 g/l sucrose, 1.0 mg/l BAP, 0.1 mg/l GA_3 and 0.1mg/l IBA (Figure 3).

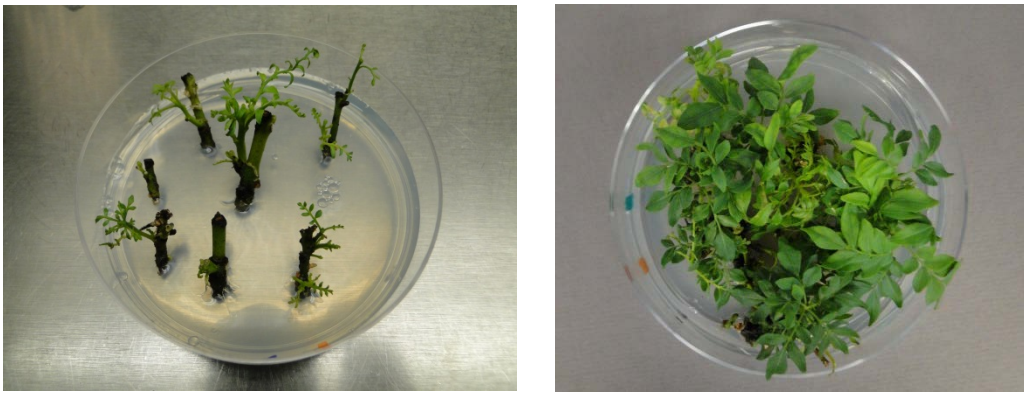

Figure 3. Bud elongation from lateral nodes of curry cultured on supplemented DKW medium (left) and multiple shoot formation after several passages on supplemented DKW medium supplemented (right).

Propagation

Regardless of the explant source, after several subcultures, a basal cluster of tissue forms from which multiple shoots arise. This nurse tissue serves as the source for the production of additional shoots. To subculture the shoots, divide each large basal nurse tissue with attached shoots in half and place each half back onto DKW medium supplemented with 30 g/l sucrose, 1.0 mg/l BAP, 0.1 mg/l GA_3 and 0.1 mg/l IBA (Figure 4). The sub-cultured basal nurse tissue will enlarge and generate additional shoots. This basal nurse tissue provides a continuous supply of new shoots and large shoots can be harvested during each subculture and transferred to rooting.

Figure 4. The propagation of curry is achieved by dividing the basal nurse tissue from which additional shoots arise into two pieces and plating each half onto fresh supplemented DKW medium.

Rooting

To induce rooting, during the propagation phase, harvest individual shoots approximately 2-3 cm in length, and place 7-10 shoots in an 8 ounce solo cup with a dome lid (The Webstaurant Store catalog numbers 760SD8 [cups] and 760SDL8 [Dome lids] http://www.webstaurantstore.com/) containing DKW medium supplemented with 30 g/l sucrose and 10mg/l naphthaleneacetic acid (NAA). After the 7-8 days, transfer shoots into solo cups containing DKW medium supplemented with 30 g/l sucrose. Shoots begin rooting after 3 weeks (Figure 5). When harvesting shoots for rooting, retain 1cm of shoot attached to the basal nurse tissue to encourage additional shoot formation.

Figure 5. Root induction on curry tree shoots after a 7-8 day pulse on DKW medium supplemented with 30 g/l sucrose and 10mg/l naphthalene acetic acid (NAA) followed by 2-3 weeks culture on DKW medium supplemented with 30 g/l sucrose.

Transferring Rooted Shoot To Soil

Once shoots develop roots, they can be acclimatized to soil by transferring them to small pots containing Sunshine Mix 1® or equivalent soil. They should be held at 78° F with high humidity for 3-5 days and then gradually reduce the humidity over 7-10 days (Figure 6). Once the plants have begun to establish in soil, they can be slowly acclimatized to greenhouse conditions.

Figure 6. Rooted micropropagated curry trees acclimated to soil

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