In Vitro Propagation of Lilies

Hussey studied the effect of the cytokinin 6-benzylaminopurine (BA) on the release of axillary buds of *L. pyrenacium* Govan, and an Asiatic hybrid, using adventitious buds from stems as the explant. Optimal production of buds (i.e., 1 to 5 laterals) was promoted by BA concentrations of 2.0-8.0 mg/liter on a Murashige and Skoog (MS) medium. Hussey later found that shoot proliferation in *L. longiflorum* and *L. pyrenacium* was promoted by the use of a cytokinin plus an auxin when pieces of leaves were used as the explant source. Stem sections cultured on a MS medium supplemented with 2.0 mg/liter Indole-3-acetic acid (IAA) and 0.5 mg/liter BA also produced approximately 10 bulblets from –mm sections.

Steinberg *et al.* obtained optimal bulblet formation from leaf blades of *L. longiflorum* 'Nellie White' when explants were cultured on a MS medium supplemented with 10.0 mg/liter Naphthaleneacetic acid (NAA) and 1.0 mg/liter kinetin. This combination, however, was the least effective in promoting shoot formation. Initiation of the bulbs occurred at the base of the explanted leaves, with one to three bulbs forming per explant.

Materials Required

1. 40 culture vessels containing 25 ml of a Murashige Lily Multiplication medium (M513).
2. 10 sterile petri plates, either glass or plastic (100 mm in diameter) (Product No. D940)
3. 250-ml beaker and 500-ml beaker
4. 3 pairs of forceps and 2 scalpels with new blades
5. 3 plastic racks for holding vessels (Product Number C908)
6. Waterproof pen and labels
7. Glass Bead Sterilizer (Product Number S636)
8. Nylon or wire mesh screen (15 x 15 cm)
9. 250 ml of a 20% Clorox solution supplemented with a few drops of Tween-20
10. 300 ml of sterile distilled water
11. 150 ml of 95% ethanol
12. 2 large healthy *Lilium* bulbs

Procedures

1. Wipe down all surfaces in the transfer hood. Allow the hood to run for 15 min before beginning any transfers. Place all items listed above, except for bulb and 250-ml beaker, in hood.
2. Remove outer layer of damaged scales from the lily bulbs and discard. Under running water, begin removing the rest of the scales making certain to remove all soil; then place the scales in a 250-ml beaker containing tap water. Remove the scales down to the point where they become quite narrow and small, then discard the rest of the bulb. Place the nylon mesh over the beaker and place under running tap water so that the water gently agitates the scales. Adding a small amount of detergent may be helpful in the cleaning process. Allow the scales to rinse in this manner for 5-10 min. Once the scales have been

washed thoroughly, pour off all water, remove the nylon mesh, and transfer the beaker of scales to the laminar flow hood. Pour enough 95% ethanol over the scales to cover them, swirl for 3-4 min, and decant the ethanol. Next pour the Clorox solution over the scales and sterilize for 15 min before decanting. Once the sterilant has been removed, rinse the scales three times with sterile distilled water.

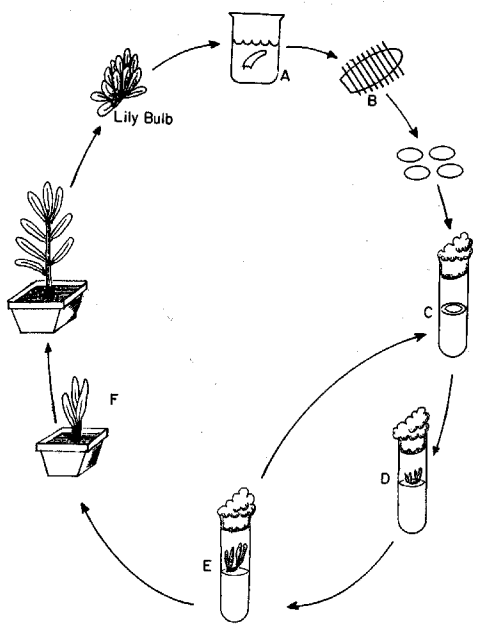
3. Transfer several sterilized scales to a petri dish and section them into 2- to 4-mm thick sections. Note whether each section comes from the base or tip of the scale. After sectioning, weigh each isolated explant and transfer it to one of the culture tubes. Each culture vessel should be inoculated with some sections from the base and tip regions. Once all culture tubes have been inoculated, place them in racks and incubate in low light at 25°C.

Scheduling

Event	**Timing**
Isolation of fresh explants	Day 0
First appearance of nodules	ca. Day 7-14
Noticeable bulblet formation	ca. Day 30
First subculture	ca. Day 60-90

Recording Results

1. Record all details of setting up the experiment.
2. Make visual observations at 14-day intervals.
3. Determine fresh weight and bulblet number after ca. 60 days.



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