In Vitro Establishment and Epiphyllous Plantlet Regeneration of Nymphaea ‘Daubeniana’

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Water lilies (Nymphaea spp.) are the most popular water garden plants but are expensive to propagate. Traditional vegetative propagation techniques are inefficient, and losses to disease also contribute to the high costs. In vitro propagation of disease-free stock may be an effective method for combating disease losses, reducing propagation costs, and facilitating long-term germplasm storage. We found no published reports of establishment and multiplication of Nymphaea species in vitro. However, in vitro establishment of Nelumbo lutea, another member of the Nymphaeaceae, suggests that Nymphaea species may respond to in vitro propagation techniques (Kane et al., 1988). We found this to be the case for the tropical water lily Nymphaea ‘Daubeniana’ Hort. ex O. Thomas, which is described herein.

Nymphaea ‘Daubeniana’, a sterile hybrid, reproduces asexually in situ via formation of epiphyllous plantlets from the adaxial surface of floating leaves above the point of petiole insertion (Masters, 1974). Leaves bearing immature plantlets 3 to 4 mm long were collected from plants maintained in tanks in a greenhouse. Epiphyllous plantlets were excised from donor leaves leaving a 3-mm-diameter ring of leaf tissue surrounding the base of each plantlet. Explants were rinsed in flowing tap water for 30 min. The trichomes were removed and the explants were again rinsed for 30 min in tap water followed by immersion in 50% (v/v) ethanol for 90 sec and a 5-min rinse in sterile deionized water. Explants were agitated in 1.31% (v/v) aqueous sodium hypochlorite containing two drops of Tween-20 per 100 ml for 12 min followed by three successive 5-min rinses in sterile deionized water. Explants were individually transferred into 150 × 25-mm culture tubes and cultured submerged in 12 ml liquid basal medium (BM) consisting of Murashige and Skoog (1962) inorganic salts with 87.6 mM sucrose, 0.56 mM myo-inositol, and 1.2 µM thiamine-HCI supplemented with 10 µM isopentenyladenine (2iP) and 3 µM indole-3-acetic acid (IAA). The medium was adjusted to pH 5.7 with 0.1 N KOH before being autoclaved at 1.2 kg·cm⁻² for 20 min at 121°C. All cultures were maintained at 25 ± 2°C under a 16-hr photoperiod provided by cool-white fluorescent tubes at 50 µmol·s⁻¹·m⁻². Histological sectioning and scanning electron microscopy procedures followed those outlined by Broschat et al. (1989) and Marousky and West (1990), respectively.

Sixteen cultures were established using this surface sterilization procedure. The established explants, cultured for 5 weeks in liquid BM with 10 µM 2iP and 3 µM IAA, remained unbranched and produced nonculturized, highly vitrified leaves without epiphyllous plantlet formation. Plantlets subsequently transferred to the same medium solidified with 0.8% (w/v) TC Agar (Hazleton Research Products, Lenexa, Kan.) for 4 weeks grew slowly, remained unbranched, and produced cutinized nonvitrified leaves without epiphyllous plantlets. Plantlets were subcultured for 5 weeks on 53 × 53-mm polypropylene membrane rafts (Sigma, St. Louis) floated in 7.5 × 7.5 × 10-cm Magenta GA-7 Vessels (Magenta Corp., Chicago) containing 80 ml of BM with the exception that 3 µM thidiazuron (N’-phenyl-N’-1,2,3-thiadiazol-5-ylurea; 98% a.i., NOR-AM Chem. Co., Wilmington, Del.) was substituted for 2iP. Of 16 plantlets subcultured on membrane rafts for 5 weeks, 12 plants produced an average of eight nonvitrified cutinized aerial leaves (Fig. 1A) each bearing a single epiphyllous plantlet in the same position and adaxial orientation (Fig. 1B, C) as those produced in situ (Masters, 1974).

Presently, the physiological mechanism(s) regulating epiphyllous plantlet development in tropical water lilies is unknown. In vitro culture provides an ideal system to further optimize the environmental and hormonal factors regulating plantlet regeneration and ultimately should serve as a method for clonal propagation.

Literature Cited


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