In Vitro Morphogenesis of Five Orchids

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Abstract

In vitro morphogenesis including somatic embryogenesis was studied in five orchids, *Cymbidium, Epidendrum, Oncidium, Paphiopedilum* and *Phalaenopsis*. 1) Sections of pseudobulbs, rhizomes and roots of *Cymbidium ensifolium* var. misericors were induced to formed totipotent calli on a modified MS medium supplemented with 2,4-D and thidiazuron (TDZ). Subculturable calli formed embryoids on a modified MS medium supplemented with BA or TDZ. The embryoids developed into rhizomes and, eventually, rhizomes produced normal plantlets. 2) Small transparent tissues formed from surface and cut ends of root, stem, leaf and flower-stalk internodes of *Epidendrum radicans* on a modified 1/2-MS basal medium with or without thidiazuron (TDZ) after 1-2 weeks of culture. In light, the transparent tissues enlarged and turned into green organized masses on most of the explants. The organized masses with young protocorm-like bodies (PLBs) proliferated on a hormone-free basal medium, and TDZ promoted the proliferation rate. Normal plantlets converted from PLBs on the same TDZ-containing medium after 1 months of culture. 3) In *Oncidium*, embryogenic calli were induced in vitro by using root, stem, leaf and flower stalk internode as explants, and healthy plantlets were successfully obtained from the callus cultures. In addition, direct somatic embryogenesis was established by using leaf segments as explants on a modified 1/2-strength MS medium. 4) In *Paphiopedilum*, totipotent callus was induced from seed-derived protocorms. The calli were induced to form “globular granules”, and then these granules transformed into PLBs. Eventually, well-developed plantlets were obtained from these callus-derived PLBs. 5) For *Phalaenopsis* propagation, seed-derived protocorms were induced to formed callus. The “proembryo-like structures” were found on the surface of the callus before formation of PLBs, and these PLBs also could convert into well-developed plantlets.

INTRODUCTION

In vitro morphogenesis and efficient micropagation protocols of several orchids have been studied. These orchids are usually derived from dividing potted plants or seeds. Propagation of this genus, either by seed or by in vitro culture, is still considered difficult. We obtained three kinds of morphogenesis from callus cultures of *Cymbidium ensifolium* var misericors: rhizomes, shoot buds and granular embryoids. Healthy plantlets were obtained following regeneration via callus-derived rhizomes and granular embryoid-like structures. Similar morphogenesis was also found in *Paphiopedilum, Oncidium*, and *Phalaenopsis*. Practical protocols for mass propagation of these orchids have been developed. Mass suspension cultures of these orchids and plant regeneration from these cells are currently being attempted.

Plant Regeneration from Callus Culture of *Cymbidium ensifolium* var. misericors

Explants from rhizomes, pseudobulbs and roots formed callus on 1/2 MS medium supplemented with 10 mg/l 2,4-D and 0.1 mg/l TDZ after 2-3 passages of subculture with a 6-months interval. Callus was maintained well in the presence of 3.3 mg/l 2,4-D combined with 0.1 mg/l TDZ. Three distinct morphogenetic events, including rhizome formation, shoot bud formation and production of globular granules were observed on basal medium either without growth regulator supplements, with 1 mg/l TDZ, or with 5 mg/l BA after 1-2 months of culture. About 40.2 % of callus-derived granules developed
into rhizomes when transferred onto a modified 1/10 MS medium for 50 days. Eventually, plantlets were obtained from the shoot buds of the callus-derived rhizomes (Chang and Chang, 1998).

**Effect of TDZ on Bud Development of *Cymbidium sinense* Wild**

Seed-derived rhizomes were able to initiate shoot buds in the presence of 0.01-1 mg/l TDZ. However, the further proliferation of rhizomes was retarded by TDZ. Plantlets (about 10 cm in height) were obtained from rhizome-derived shoot buds on medium supplemented with 0.5 mg/l NAA and 1 g/l after 4 months of culture. About 91% and 73% of regenerated plants flowered normally in the 2nd year and the 3rd year, respectively. This result indicated that the treatment of TDZ during bud development may shorten the juvenile phase of this orchid species (Chang and Chang, 2000a).

**In Vitro Flowering of *Cymbidium ensifolium* var. *misericors***

Callus-derived rhizomes produced flowers precociously on a modified 1/2 MS medium containing NAA with 2iP, BA and TDZ after 100 days of culture. TDZ at 3.3-10 µM or 2iP at 10-33 µM combined with 1.5 µM NAA gave the best response on flower induction. Although the in vitro flowers were smaller in size, their morphology is normal. These flowers bloomed for about two weeks in vitro. The pollens of these in vitro flowers are viable, and germinated on an agar medium after 12 hours of culture (Chang and Chang, 2000b).

**Efficient Production of Protocorm-like Bodies of *Epidendrum***

Flower stalk internodes formed a large number of PLBs on 1/2 MS medium supplemented of TDZ. These PLBs grew well and proliferated more on the same medium. The best response on PLB production was found at a modified 1/2 MS medium containing 825 mg/l NH₄NO₃, 950 mg/l KNO₃ and 40 g/l sucrose. BA, kinetin and zeatin-riboside promoted PLB proliferation and subsequent shoot formation. The optimized procedure required about 12-13 weeks from the initiation of PLBs to plantlet formation.

**Somatic Embryogenesis and Plant Regeneration of * Oncidium***

Protocols for in vitro plant regeneration via direct or indirect somatic embryogenesis of *Oncidium* spp. have been established. 1) Leaf segments of the cultivar ‘Gower Ramsey’ were induced to directly form somatic embryos on 1/2-MS medium supplemented with TDZ. Plantlet formation from these somatic embryos was achieved on the same basal medium devoid of plant growth regulators (Chen et al., 1999). 2) Yellowish embryogenic calluses derived from leaf, root and stem explants of the cultivar ‘Gower Ramsey’ were induced and maintained well on 1/2-MS medium supplemented with 2,4-D and TDZ. Somatic embryos formed from these calluses on the same basal medium supplemented with NAA and TDZ. Plantlet conversion was made on the same basal medium devoid of plant growth regulators (Chen and Chang, 2000b; Wu et al., 2004). 3) Flower stalk explants of the cultivar ‘Sweet Sugar’ formed somatic embryos on TDZ-containing medium, and plantlets derived from these embryos were obtained on the same medium (Chen and Chang, 2000a). Leaf segments taken from regenerated plantlets of cv. Sweet Sugar were induced to obtain repetitive somatic embryogenesis (Su et al., in press).

The leaf culture system has been further used to study the effects of tissue culture conditions (Chen and Chang, 2002), explant characteristics (Chen and Chang, 2002), plant growth regulators (auxins, auxin inhibitors, cytokinins, GA₃, growth retardants, ACC and ethylene inhibitors) (Chen and Chang, 2001; Chang and Chang, 2003) on direct somatic embryogenesis.

**Plant Regeneration from Protocorm-derived Callus of *Paphiopedilum***

Explants from seed-derived protocorms were induced to form yellowish callus on a 1/2 MS medium supplemented with 1-10 mg/l 2,4-D and 0.1-1 mg/l TDZ. The callus
maintained well on the same medium, and proliferated more in the presence of 5 mg/l 2,4-D and 1 mg/l TDZ. Shoot buds/PLBs formed from the subculture callus on 1/2 MS medium containing 0.1 mg/l NAA with 0.5 mg/l TDZ or 0.5 mg/l NAA with 3 mg/l 2,4-D. Plantlets derived from the shoot buds/PLBs grew well, and flower normally after three years of culture in greenhouse (Lin et al., 2000).

**Multiple Shoot Formation from Stem Nodal Explants of Paphiopedilum**

Stem nodal explants formed shoots when cultured on a modified 1/2 MS medium supplemented with combinations of 2,4-D (4.52 and 45.25 µM) and TDZ (0.45 and 4.54 µM) for 6 months. These shoots formed roots after transferring onto hormone-free medium for 3 months. The regenerated plantlets grew well in greenhouse with a high survival rate.

**Direct Shoot Bud Formation from Leaf Explants of Paphiopedilum**

Leaf explants directly formed adventitious shoots from wound regions within one month, when cultured on a modified 1/2 MS medium devoid of growth regulators in darkness. The combinations of 2,4-D and TDZ enhanced mean numbers of shoots per responding leaf explant. Plantlets with several roots were obtained from these shoots on hormone-free medium after 22 months of culture.

**Plant Regeneration from Protocorm-derived Callus of Phalaenopsis**

Yellowish callus was induced from seed-derived protocorms on 1/2 MS medium supplemented with low dosages of 2,4-D combined with TDZ. Proembryo-like structures formed on the surface of callus before formation of PLBs, and these PLBs could also convert into well-developed plantlets (Chen et al., 2000).

**Repetitive Embryogenesis from Seed-derived Protocorms of Phalaenopsis**

Seed-derived protocorms formed embryos from their posterior regions on 1/2 MS medium devoid of growth regulators. TDZ at 0.45, 4.54 and 13.62 µM promoted the frequency of direct embryo formation. Repetitive embryogenesis was achieved on TDZ-containing medium. These regenerated embryos converted into normal plantlets on hormone-free medium after 4-6 weeks of culture.

**Literature Cited**

Chen J.T. and Chang, W.C. 2002. Effects of tissue culture conditions and explant characteristics on direct somatic embryogenesis in *Oncidium* ‘Grower Ramsey’. Plant