Celery :-Somatic Embryo Production and Regeneration: Improved Protocols

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Somatic embryogenesis has been proposed as a method for in vitro propagation because of its potential for high proliferation as compared to shoot tip culture (Janick et al., 1989). Recently, celery has been used as a model system for synthetic seed production via encapsulation (Redenbaugh et al., 1984) or desiccation of somatic embryos (Kim and Janick, 1989, 1990, 1991).

For celery, protocols for somatic embryogenesis usually include a callus-induction phase on semisolid medium and an embryo-production phase in liquid medium. In this study, we examined the influence of semisolid vs. liquid callus-induction medium on embryo production and the effect of medium with or without sucrose and with or without CO₂ enrichment on the conversion of embryos to seedlings.

Callus was induced from leaf explants of in vitro-grown celery (*Apium graveolens* L. cv. SB 12) and-explanted to semisolid or liquid medium in 100 × 15-mm petri dishes or 125-ml Erlenmeyer flasks (25 ml each). Semisolid callus induction medium (SCIM) consisted of Murashige and Skoog basal medium (salts plus vitamins) supplemented with 2.3 μM 2,4-D, 2.3 μM kinetin, 0.5 g casein hydrolysate/liter, and 30 g sucrose/liter (Nadel et al., 1989), solidified with 3 g Gelrite/liter (a gellan gum; Scott Labs, Fiskeville, R.I.), and adjusted to pH 5.8 before autoclaving. Liquid callus induction medium (LCIM) had the same composition as SCIM, excluding Gelrite. Seven to nine leaf explants (10 to 15 mm long, 0.2 g total) were cultured per petri dish or flask. The semisolid cultures were maintained for 28 days at 25C under dim fluorescent light, 1 μmol·m⁻²·s⁻¹ photosynthetically active radiation (PAR), with a 16-h photoperiod. Liquid cultures were maintained on a gyratory shaker (100 rpm) under the same regime.

Callus from semisolid medium was transferred into liquid embryo-production medium (EPM) consisting of MS basal medium supplemented with 30 g sucrose/liter. Callus from liquid medium was filtered through a stainless steel sieve (1-mm pore size) and subcultured into EPM. All cultures were placed on a gyratory shaker (100 rpm) under 50 μmol·m⁻²·s⁻¹ of PAR (16-h photoper-

iod) for 3 days. Cultures from SCIM were filtered. Aliquots (0.5 ml packed volume) of proembryos from both cultures were subcultured in fresh EPM under the same conditions for another 6 days. This subculturing usually is not necessary for cultures originated from LCIM, but it was carried out to treat both cultures identically. Embryos from three flasks of each treatment were counted.

Germinating embryos, with 1- to 2-mm cotyledons, and 8- to 10-mm radicles, were harvested after 9 days in EPM. Ten embryos from each flask were subcultured into 140-ml glass jars covered with plastic lids (Magenta, Chicago) and containing 5 g of autoclaved vermiculite saturated with 20 ml of one of four regeneration media: MS basal medium with or without 3% sucrose (denoted hereafter as MS and MS + 3% sucrose, respectively), or half-concentration MS basal medium with or without 1.5% sucrose (denoted as ½MS and ½MS + 1.5% sucrose, respectively). The cultures were kept under 50 μmol·m⁻²·s⁻¹ of PAR (16-h photoperiod) in a CO₂-enriched chamber (20,000 ppm CO₂) or at culture-room atmosphere (800 ppm CO₂). The experimental design was a factorial in a randomized complete block. Each of these 16 treatments (two callus-induction media × four regeneration media × two CO₂ environments) was replicated three times. Regenerated plantlets were counted after 28 days, oven-dried (80C, 48 h), and weighed.

Mean number of celery embryos produced per flask was 262 for SCIM and 622 for LCIM. Based on packed volume of embryogenic structures, production per gram of explant was 888 for SCIM and 3357 for LCIM. LCIM also induced a significant increase in percentage conversion and seedling dry weight (Table 1). These results indicated that LCIM had higher productivity and produced more vigorous embryos than SCIM.

Supplementation of regeneration media with sucrose (1.5% or 3%) significantly increased the conversion of embryos to seedlings on vermiculite substrate (Table 1). We believe that regeneration would be improved by using a germination matrix that would allow more extensive contact with the embryo radicle than does vermiculite. Differences due to media were not statistically significant, but sucrose consistently increased seedling dry weight. The results indicate that seedling regeneration can be achieved autotrophically, which reduces the necessity for sterile conditions.

Regeneration in a CO₂-enriched environment significantly increased seedling dry

weight (Table 1). The use of a CO₂-enriched atmosphere apparently partially-compensated for the reduction in seedling growth caused by sugarless media. For example, at 800 ppm CO₂, seedling dry weights were 0.67 mg with MS and 1.03 mg with MS + 3% sucrose, whereas at 20,000 ppm CO₂, these media produced seedlings of 2.09 and 2.27 mg, respectively.

Embryo production with LCIM was simplified relative to SCIM in two respects: 1) it removes the necessity to harvest individual callus clumps to inoculate the EPM, and 2) it reduces the necessity of third-day sieving to remove explant pieces or callus clumps and subculturing. Moreover, LCIM is applicable to bioreactors. Our results suggest that the combination of LCIM with autotrophic regeneration in a CO₂-enriched atmosphere improves the productivity and in vitro production efficiency of seedlings from celery somatic embryos.

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Table 1. Effects of semisolid (SCIM) vs. liquid (LCIM) callus-induction media, regeneration medium, and CO₂ enrichment on conversion of somatic embryos to seedlings and dry weight of celery seedlings.

Main effects	Conversion (%)	Seedling dry wt (mg)
Callus-induction medium		
SCIM	41.2	1.11
LCIM	71.7 ***	1.70 ***
Seedling-regeneration medium		
MS	43.3 b	1.38
MS + 3% sucrose	62.5 a	1.65
½MS	48.3 b	1.21
½MS + 1.5% sucrose	71.7 a ***	1.36 NS
CO ₂ (ppm) enrichment		
800	56.7	0.80
20,000	56.2	2.00 ***
	NS	

Means separation by Student-Newman-Keuls' multiple range test, *P* = 0.05.

***Significance of main effect at *P* = 0.01 (interactions were not significant).

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