Teaching Methods

Somatic Embryogenesis versus Organogenesis in Cucumber: Developing a Laboratory Exercise

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Summary. Fourteen commercial cucumber (*Cucumis sativus* L.) cultivars were screened on media designed to induce somatic embryogenesis and shoot organogenesis. Cotyledon explants from mature dry seeds and 7-day-old seedlings also were compared for responses. Seven

cultivars (Burpless F₁ Hybrid, Burpee Hybrid II, Cross Country, Picklebush, Sweet Delight, Tasty Green, and Yard Long) responded best for individual regeneration ability. Within the somatic embryogenesis regeneration protocol, 'Picklebush' responded better than most other cultivars, while 'Burpless F₁ Hybrid' responded best in shoot organogenesis. Also, within each type of regeneration, the developmental stage of the cotyledon explants appeared to influence regeneration ability.

In tissue culture there are two basic types of regeneration: somatic embryogenesis and organogenesis (Brown and Thorpe, 1986; Williams and Maheswaran, 1986). A laboratory exercise that compares both types of regeneration using the same cultivars and explants should aid undergraduate college students in understanding the fundamental differences between the two types of regeneration. Cucumber can be regenerated via somatic embryogenesis (Cade et al., 1990; Chee 1990; Ladyman and Girard, 1992; Lou and Kako, 1994; Trulson and Shahin, 1986) and organogenesis (Ali et al., 1991; Al-Juboory et al., 1991; Kim et al., 1988; Msikita et al., 1990; Wehner and Locy, 1981) using the same explant source—cotyledons. However, few comparisons have been made between the two types of regeneration within individual cultivars (Bergervoet et al., 1989; Kim and Janick, 1989) and none utilizing cotyledon explants.

A major factor in determining ability of cucumber cotyledons to regenerate via somatic embryogenesis and/or organogenesis is genotype (Ali et al., 1991; Cade et al., 1990; Kim et al., 1988; Lou and Kako, 1994; Trulson

and Shahin, 1986; Wehner and Locy, 1981).

Explant (organ) type, along with its developmental stage, also contributes to regeneration potential of plants in vitro (Brown and Thorpe, 1986; Williams and Maheswaran, 1986). Comparisons have not been made between different developmental stages of the same explant to determine their contribution to successful regeneration in cucumber.

In developing this laboratory exercise, two objectives were defined: 1) to determine which commercially available cucumber cultivars were best able to respond via one or both types of regeneration and 2) to determine the role of the explant's developmental stage on this regeneration ability. Fourteen commercial cucumber cultivars, including eight fresh market and six pickling types (Table 1), were compared for somatic embryogenic and organogenic responses following the combining of established protocols (Cade et al., 1990; Msikita et al., 1990).

Laboratory exercise

This experiment can be completed in 10 weeks. Directions for conducting this laboratory exercise are given below. Prepare a basal medium, which is composed of Murashige and Skoog (MS) basal salts (Murashige and Skoog, 1962), vitamins (1.0 mg·liter⁻¹ thiamine-HCl, 0.5 mg·liter⁻¹ pyridoxine-HCl, 0.5 mg·liter⁻¹ nicotinic acid, 100 mg·liter⁻¹ myo-inositol), 3% sucrose, 0.8% phytagar, pH 5.8, and autoclave at 121°C for 25 min. Add filter-sterilized plant growth regulators to the basal medium (MS-O) in the following concentrations for somatic embryogenesis: 4.5 µM 2,4-dichlorophenoxyacetic acid (2,4-D) plus 2.3 µM kinetin (Cade et al., 1990) for the induction medium (EMB1) and 5.4 µM NAA plus 2.3 µM kinetin (Cade et al., 1990; Chee, 1990) in the maturation medium (EMB2). The shoot organogenesis (ORG) medium contains 1.6 µM 1-naphthaleneacetic acid (NAA) plus 8.9 µM 6-benzyladenine (BA; Ali et al., 1991; Msikita et al., 1990). Pour all media into 100 × 25-mm petri plates, about 40 ml per plate.

Prepare cotyledons from 7-day-old germinated seedlings following a modified protocol of Cade et al. (1990). Surface-disinfect 10 seeds from each cultivar in 2.5% sodium hypochlo-

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Table 1. *Fourteen commercial cucumber cultivars tested for shoot organogenic and somatic embryogenic responses in vitro.*

Cultivar	Type ^z	Flowering type	Company ^y
Armenian	Fresh	Monoecious	Ferry-Morse Seed
Burpee Hybrid II	Fresh	Gynoecious	W. Atlee Burpee & Co.
Burpless F ₁ Hybrid	Pickling	Monoecious	GROSouth, Inc.
Cross Country, hybrid	Pickling	Gynoecious	Ferry-Morse Seed
Marketmore 76	Fresh	Monoecious	Ferry-Morse Seed
Picklebush	Pickling	Monoecious	W. Atlee Burpee & Co.
Poinsett 76	Fresh	Monoecious	NK Lawn and Garden
Spacemaster	Fresh	Monoecious	Ferry-Morse Seed
Straight Eight	Pickling	Monoecious	NK Lawn and Garden
Sumter	Pickling	Monoecious	Ferry-Morse Seed
Sweet Delight, hybrid	Fresh	Monoecious	Service Seed Division
Tasty Green Hybrid	Fresh	Gynoecious	W. Atlee Burpee & Co.
Tendergreen	Pickling	Monoecious	Ferry-Morse Seed
Yard Long	Fresh	Monoecious	Weeks Seed Co.

^zCucumber type: fresh = fresh market.

^yAddresses: Ferry-Morse Seed, c/o Advanced Seed Co., Fulton, KY; GROSouth, Inc. (formerly Montgomery Seed and Supply Company), Montgomery, AL; NK Lawn and Garden, Minneapolis, MN; Service Seed Division, Amory, MS; W. Atlee Burpee, Warminster, PA; Weeks Seed Company, Greenville, NC.

rite (50% bleach) + 0.5% SDS (sodium dodecyl sulfate) for 30 min then rinse with sterile water. Place seeds on water agar (0.8%) and germinate at 24C (room temperature) in darkness for 7 days, at which time the etiolated hypocotyls should be about 8 to 12 cm in length and the cotyledons should be slightly expanded and partially pushed out of their seedcoats. Excise cotyledons from the seedlings, cut away all margins, then cut the remainder of the explant into four pieces, each about 2 mm². Place explants onto treatment media (EMB1 and ORG), five explants per plate.

Prepare mature dry seed cotyledon explants following a modified protocol of Msikita et al. (1990). On day 7 of the experiment, soak mature dry seeds in sterile water for 5 to 10 min to soften the seedcoats. Once soft, remove seedcoats with forceps. Surface-disinfect decoated seeds in 0.5% sodium hypochlorite (10% bleach) + 0.5% SDS for 10 min. Discard the basal one-third of the decoated seed (contains the embryo) and separate the two cotyledons. Place each cotyledon vertically into treatment media with the cut end submerged, five cotyledons per plate.

Culture all explants at 24C under two different growth conditions (Cade et al., 1990; Msikita et al., 1990). Incubate EMB1 plates in darkness and ORG plates under a 16-h photoperiod under cool-white fluorescent lights (about 52 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF). After 3 weeks, transfer explants from EMB1 to EMB2 and place back in darkness.

After 3 additional weeks (6 weeks after initiation), cut all explants transversely into two pieces and transfer those from ORG onto fresh ORG and those from EMB2 onto MS-O. Incubate all plates under a 16-h photoperiod as described above.

Nine weeks after culture initiation, collect data for each medium and explant type. Calculate percentages of explants that responded to the media (ability to produce somatic embryos or shoots following the individual protocols) and mean numbers of somatic embryos or shoots per explant. Data presented below represent results obtained with 14 commercially available cucumber cultivars that were tested via

both regeneration protocols. Statistical analyses were performed to help determine which of those cultivars would be best suited for use in this laboratory exercise.

Cultivar and explant responses

Fourteen common commercial cucumber cultivars (Table 1) were compared initially for ability to regenerate following protocols for somatic embryogenesis and shoot organogenesis described previously. Nine weeks after culture initiation, data were collected from all explant cultures and analyzed using the General Linear Model procedure of the Statistical Analysis System for Personal Computers, version 6.04. As expected, explants placed on hormone-free medium (MS-O; negative control) never produced somatic embryos or shoots. Cultivars in which >30% of a given explant type (germinated seed cotyledon or mature seed cotyledon) produced either somatic embryos or shoots were retested. Seven cultivars were retested in two separate experiments with two replicate plates (five explants per plate) per experiment (Table 2).

Both types of cucumber (fresh market and pickling) and both flowering types were represented in the seven cultivars chosen for further testing (Table 2). In addition, all five hybrids were among those selected (Tables 1 and 2). These data suggest that hybrid cucumbers may respond better in culture than nonhybrids. The greatest

Table 2. *Mean media effects within seven cucumber cultivars: somatic embryogenesis (EMB) versus organogenesis (ORG) after 9 weeks in culture. Numbers represent means of two independent experiments each containing two replications (five explants per replication).*

Cultivar	Regeneration protocol			
	EMB	ORG	EMB	ORG
	Explants with SEs ^z or shoots (%)		Mean no. SEs or shoots/explant	
Burpee Hybrid II	13.3 a ^y C ^x	1.5 a B	1.5 a	0.0 b
Burpless F ₁ Hybrid	20.0 a ABC	12.5 a A	2.0 a	0.5 b
Cross Country	33.8 a AB	1.3 b B	4.1 a	0.3 b
Picklebush	36.3 a A	0.0 b B	4.6 a	0.0 b
Sweet Delight	15.5 a BC	1.3 a B	1.5 a	0.0 a
Tasty Green Hybrid	7.5 a C	3.8 a B	0.9 a	0.2 a
Yard Long	12.8 a C	0.0 a B	1.9 a	0.0 a

^zSomatic embryos.

^yMeans within rows for each individual response (explant percentages or mean numbers) not followed by the same lower case letter differ significantly ($P \leq 0.05$) according to the F test.

^xMeans within columns for percent explant response not followed by the same upper case letter differ significantly ($P \leq 0.05$) according to the F test.

explant responses were observed in 'Picklebush' for somatic embryos ('Burpless F₁ Hybrid' and 'Cross Country' also responded comparable to 'Picklebush') and 'Burpless F₁ Hybrid' for shoots (Table 2). Due to statistical interactions, the same analysis could not be conducted for numbers of somatic embryos, but all cultivars responded similarly for numbers of shoots produced (data not shown). 'Cross Country' and 'Picklebush' explants were more capable of undergoing regeneration via somatic embryogenesis than shoot organogenesis. 'Burpless F₁ Hybrid' gave best explant responses for both types of regeneration (Table 2).

In addition to cultivar responses, it was noted that explant type also influenced some of the responses that were obtained. Within each type of regeneration across all cultivars, a greater percentage of germinated seed cotyledon explants responded via somatic embryogenesis than mature seed cotyledon explants (28.8% and 10.9%, respectively; significant at $P \leq 0.05$). This was expected, since somatic embryogenesis has been very successful in cucurbits when explants from seedlings were used (Debeaujon and Branchard, 1993). However, the opposite correlation of mature seed cotyledon explants responding better via organogenesis did not prove to be significant at $P \leq 0.05$.

As stated previously, this laboratory experiment can be completed in 10 weeks. Cucumber cultivars have been identified that should give optimal responses via somatic embryogenesis and/or shoot organogenesis. This laboratory exercise should aid undergraduate plant science students in understanding the fundamental differences between the two types of regeneration in vitro and introduce them to the role of an explant's developmental stage in that regeneration success.

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