Embryogenesis from Cotyledon-Derived Callus of *Cucumis sativus* L.

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Early research on tissue culture of cucurbits dealt with the formation of somatic embryos in culture (2, 4). However, only recently have plants been regenerated through embryogenesis (1, 3, 5). Researchers have had the most success with cucumber cotyledon tissue. The objective of this study was to determine the best medium to use for embryo induction and subsequent plant regeneration from cotyledons.

Seeds from 2 cucumber cultivars (Straight Eight and Gy 14) were sterilized in 50% clorox for 30 minutes and rinsed 5 times in sterile distilled water before germinating at 30°C on water agar in a dark chamber. Five-day-old cotyledon explants were cut into six 2 x 2 mm pieces after the cotyledon margins were removed. Five explants were placed on 100 x 15 mm petri plates of Murashige-Skoog (MS) medium containing 0.8% tissue culture agar, 3% sucrose, and one of 25 combinations of 2,4-D and kinetin concentrations in a 5 x 5 factorial design. Growth regulator concentrations were 0.0, 0.25, 0.50, 1.0, and 2.0 mg/l. The plates were kept in the dark at 22°C for 6 weeks. Each piece of 6-week-old callus was then cut in half and transferred to MS medium containing either 0 or 0.5 mg/l of kinetin. Cultures were maintained in the light for 3 weeks and then transferred to MS medium with no growth regulators and 1% agar at 3 week intervals until plants were fully developed.

In this experiment, globular and heart-shaped structures began forming around the edges of the cotyledon tissue after approximately 3 weeks. Embryogenic callus was smooth, yellow to yellow-orange in color, and was easily removed from the surface of the explant. Callus that was hard and nodular, or white and friable did not form embryos. When the embryogenic tissue was removed from 2,4-D and placed in the light, the embryos differentiated into normal bipolar embryos as well as abnormal embryonic structures. Some of the abnormal structures included multiple or fused embryos, embryos without underdeveloped or callused roots, and embryos with callused or malformed leaves.

Embryos developed at varying frequencies on all media containing 2,4-D (Table 1). The highest number of embryos was obtained on a medium with 2.0 mg/l 2,4-D and 0.5 mg/l kinetin. A high frequency was also obtained on a medium containing 1 mg/l 2,4-D and 0.5 mg/l kinetin. Embryos regenerated on the latter medium produced a higher percentage of plants than the former medium. There were no significant differences for the number of embryos and plantlets produced on secondary media with or without kinetin.

The problem of abnormal embryo production and embryo dedifferentiation remains to be solved. A possible solution includes using charcoal in the secondary medium, which may absorb some of the excess 2,4-D that remains when the embryos are transferred from the primary medium. Another solution may be removing embryogenic tissue from the primary media sooner than 6 weeks.

Table 1. Embryo and plantlet regeneration from cotyledon explants of 2 cucumber cultivars cultured on combinations of 2,4-D and kinetin and subcultured onto a medium with or without kinetin[z].

2,4-D conc. mg/l	Kinetin conc. mg/l	Secondary medium with kinetin		Secondary medium without kinetin	
		No. embryos per plate	No. plants per embryo	No. embryos per plate	No. plants per embryo
0.25	0.00	0.6	0.1	0.0	-
	0.25	1.0	0.0	1.6	0.0
	0.50	0.8	0.0	0.3	1.0
	1.00	1.0	0.1	2.3	0.0
	2.00	2.1	0.0	1.1	0.0
0.50	0.00	0.4	0.0	2.4	0.1
	0.25	2.5	0.0	5.3	0.2
	0.50	0.2	0.0	2.6	0.0
	1.00	1.6	0.0	3.2	0.0
	2.00	3.3	-	0.7	-
1.00	0.00	0.0	-	0.0	-
	0.25	3.3	0.2	4.8	0.1
	0.50	5.1	0.2	10.9	0.3
	1.00	2.9	0.1	2.3	0.0
	2.00	3.7	0.1	1.6	0.0
2.00	0.00	5.4	0.0	1.9	0.0
	0.25	1.6	0.1	1.4	0.1
	0.50	10.8	0.2	15.3	0.1
	1.00	9.6	0.1	6.7	0.0
	2.00	3.1	0.1	3.8	0.2
LSD (5%)		8.5	0.3	10.2	0.2

[z]Data for treatments 0.0 mg/l 2,4-D not shown. No embryos were produced on those media. Data are means over 2 lines and 4 replications with 2 petri plates per replication.

Literature Cited

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