Development of Callus and Somatic Embryos from Zygotic Embryos of Cucumber (*Cucumis sativus* L.)

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In the cross *Cucumis sativus* x *C. melo* seeded fruits often develop, but the embryos cease growth at the globular-shaped stage (2). Embryo rescue procedures, suitable for embryos from self pollinations, failed for these hybrid embryos (3). This may in part be caused by the difference in the base chromosome number, i.e. x=7 in *C. sativus* and x=12 in *C. melo*. To overcome this barrier, we are planning to induce callus formation from the hybrid embryos in order to induce chromosome elimination and rearrangements. This might result in cells with an adapted karyotype, from which plants might be regenerated. The present study was undertaken to establish procedures of callus formation and plant regeneration especially from young embryos. Embryos from selfed *C. sativus* were used as a model system.

For supply of the embryos, we used *C. sativus* var. *hardwickii* IVT Gene bank no. (Gbn) 1811A, which was grown in an insect-proof glasshouse with temperature set at 25°C day / 18°C night. Immature seeds were taken out of fruits 7 to 24 days after pollination, and embryos 0.07 to 4.8 mm long from globular-shaped to cotyledonary stages (Table 1) were isolated and incubated on nutrient medium. For each embryo stage at least four different fruits were used. We cultured the globular and heart-shaped embryos enveloped in the embryo sac. A modified Murashige-Skoog medium was used containing as macro salts (in mg/l) NH4NO3 330, KNO3 950, Ca(NO3)2·4H20 350, MgSO4·7H20 165, KH2PO4 85, normal micro salts and organic components, 250 mg/l Edamin, 3% (w/v) sucrose, and 0.55% (w/v) Oxoid agarose. For growth regulators, 4µM BA and 4µM 2,4-D were added, since that combination was reported to induce callus formation and embryogenesis on cucumber leaf explants (1). The medium was filter-sterilized, except for the agarose which was autoclaved. The pH was adjusted to 5.8. Six to nine embryos were incubated per 60 mm petri plate. The cultures were kept in the dark at 27°C. Calli obtained from the embryos were transferred to fresh medium after 6 weeks and thereafter subcultured every 4 weeks. Somatic embryos were transferred to a plant development medium, Murashige-Skoog with 0.5µM K and 0.7% (w/v) Difco Bacto agar.

Table 1 presents the results after three weeks of culture. More than 90% of the globular-shaped embryos did not respond at all (NR) during culture. With older embryos four types of development have been identified: secretion of an exudate (EX), regeneration of somatic embryos directly from the cotyledons (SE), formation of beige callus (BC), and normal growth of the embryos (NG). A drop of a kind of translucent exudate, 5 to 8 mm in diameter, was generally secreted by the heart-shaped embryos, if they were responsive. It contained numerous single cells, which divided and regularly produced cell aggregates and embryo-like structures, 1 to 4 per drop. Direct somatic embryo formation, 1 to 3 embryos per cotyledon, occurred in the heart-shaped and early cotyledonary stage, but the frequencies were rather low. More commonly, in these stages, a watery, beige callus was formed which mostly was accompanied by a spongy, white callus. The two types of calli markedly differed in regeneration capacity. The white callus failed to grow when subcultured, whereas the beige callus, after several subcultures, occasionally formed protuberances of a bright-yellow callus which was embryogenic. Normal development of the embryos *in vitro* was observed in

cotyledonary stage embryos. With prolonged culture, however, these embryos also started to form callus, but this callus was greyer and hardly formed an embryogenic, yellow callus upon subculture.

Results of plant development from the somatic embryos obtained, were rather disappointing so far. Frequency of shoot growth was only 3% and most plants grew abnormally. Since only a few plants were successfully transplanted in soil, more research is required to improve the frequency of recovery of whole plants from somatic embryos.

Notwithstanding the low frequency of plant development from the somatic embryos, the study showed the ability of cucumber zygotic embryos to form undifferentiated tissue and to regenerate plantlets from it. The procedure established might be useful for callus formation and regeneration from hybrid embryos of the cross *C. sativus* x *C. melo*. These embryos will be cultured in further experiments.

Table 1. Response of cucumber embryos from self-pollinated *C. sativus* var. *hardwickii* Gbn 1811A of various stages on Murashige-Skoog medium with 4μM BA and 4μM 2,4-D[z].

Embryos			Response in culture (%)				
Stage[y]	Size (mm)	No.	NR	EX	SE	BC	NG
Globular	0.07-0.15	76	91	8	0	1	0
Early heart-shaped	0.15-0.3	102	25	53	9	13	0
Late heart-shaped	0.3-0.6	63	2	21	8	63	6
Early cotyledonary	0.6-1.2	84	5	7	15	43	30
Mid cotylendonary	1.2-2.4	77	0	0	0	18	82
Late cotylendonary	2.4-4.8	42	0	0	0	7	93

[z]Data were taken after 3 weeks of culture. NR: no response, EX: production of a drop of exudate with embryogenic cells, SE: regeneration of somatic embryos directly from the cotyledons, BC: production of beige callus, NG: normal embryo growth.
[y]Globular and heart-shaped embryos were incubated along with the surrounding embryo sac

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