

In Vitro Regeneration and Flowering of Cucumber Cultivars and Lines
Cultured from Excised Seed

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The ability to obtain whole plants from a single seed may be important when there is a limited number of seeds. Recently, Cade, et al. (1) reported shoot regeneration from cotyledons of Cucumis sativus L. after subculturing and/or transferring to another type of medium and keeping the explants in the dark. Lange and Juvik (2) have also reported regeneration from matured seed cotyledons of several Cucurbita species. In this paper we report preliminary observations regarding shoot regeneration from excised cucumber (Cucumis sativus L.) seed tissues.

Seeds of 18 cucumber cultivars and breeding lines (Table 1) from four various seed companies were decoated and excised into pieces consisting of an embryonic axis and 2 cotyledons. The pieces from an individual seed were tied separately in a cheesecloth bag and surface-sterilized for 10 min in 10% (v/v) Clorox bleach (0.525% sodium hypochlorite) to which a pinch of Alconox® powder had been added as a surfactant. The pieces were later rinsed 5 times with sterilized water. The individual bags were untied and the seed pieces were aseptically transferred to 25 x 150 mm culture tubes (one seed piece per tube) containing 10 ml of modified Murashige and Skoog (MS) (4) high-salt medium supplemented with 6-benzylaminopurine (BAP) (2 mg/l) and alpha-naphthaleneacetic acid (NAA) (0.1 mg/l). The pH of the medium was adjusted to 5.7 prior to autoclaving (15 psi, 15 min) and 7% (w/v) Difco Bacto-agar was added for solidification. The experimental treatment consisted of 15 seeds divided into 2 cotyledons and an embryonic axis for each cultivar. The explants were cultured for 6 weeks under 16/8 hour light/dark photoperiod ($40 \text{ Em}^{-2}\text{s}^{-1}$) and approximately 25°C. The cultures were examined regularly.

Within 3 to 5 days after culturing, the embryonic axes germinated in vitro. When the radicle touched the medium, it developed into a thickened root-like callus covered structure. Subsequent roots were normal in size. Shoots developed 3 to 4 weeks later. The most shoots (100%) were obtained from 'Burpless Hybrid', the least from 'West Indian Gherkin' (Table 1). No flowers were observed.

The pattern of development of cotyledon explants was generally similar in all cultivars. Within 3 days of culturing, the cotyledons turned green and expanded rapidly. By the third week of culturing, cotyledons began to form callus near, but usually below, the cut surface. About 5 weeks after culturing, 7 cultivars developed into embryoid-like structures in the callus, some of which developed into plantlets (Table 1). Shoots were tiny and rosette-like in cultivars 'Spacemaster' and 'Marketmore 76'. Male flowers developed on shoots of 'Burpless Hybrid', VGP 5058, 'Spacemaster' and 'Marketmore 76'. Some of the shoots roots spontaneously. 'Burpless Hybrid' and VGP 5058 shoots were transplanted into pots and later transferred to the greenhouse where flowering and fruiting continued.

Cucumber regeneration from excised seeds appear to differ among cultivars and type of explant used. 'Burpless Hybrid' regenerated better than other

cultivars from both cotyledon and embryonic axis explants. 'West Indian Gherkin' (Cucumis anguria) was worst. The ability to regenerate in vitro may be governed by several factors, especially genotype. Our tissue culture medium did not support plant regeneration for all cucumber cultivars. Thus, it might be essential to develop a suitable type of medium for each cultivar. Lange and Juvik (2) made similar observations for several Cucubita species.

The embryonic axis regenerated shoots faster and better than the cotyledons, but did not flower. Cotyledons are storage organs. Embryoid-like structures consistently formed proximal to the cut surface of isolated cotyledons. This suggests a gradient in growth-promoting factors within the cotyledons, and/or translocation of the factors towards the embryonic axis. The absence of flowering on embryonic axis-derived shoots suggests the stimulus for flower formation on preformed embryonic axes is different than that for adventitious shoots (3).

Rajasekeran et al. (5) reported both male and female flowers in vitro on cultured hypocotyl segments of cucumber cultivar 'Superpickle'. They used MS medium supplemented with benzyladenine (BA) (0.5 or 1.0 mM) and 2,4-d (1.5 or 5.0 mM) and 20 weeks of subculturing, transferring to a medium without growth regulators. In this study we observed no female flowers.

In order to verify the factors regulating flowering, a further study is required. The efficacy of the system as an alternative to conventional cucumber regeneration techniques should also be exploited.

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Literature Cited

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Table 1. In vitro regeneration from embryonic axes and cotyledons of cucumber (Cucumis sativus L.) breeding lines and cultivars².

Cultivar or breeding line	Seed Source	No. of cultures with shoots		No. of cultures with flowers
		Embryonic axis	Cotyle- dons	
Burpee Hyb. II	Burpee	9	0	0
Burpee Pickler	Burpee	10	3	0
Burpless Hyb.	T. Sakata	15	13	8
Flurry (VGY 5922)	Asgrow	7	0	0
High Mark II (WTR 615)	Asgrow	9	0	0
Marketmore 76	Asgrow	10	2	1
MS 613 (VGN 211)	Asgrow	12	0	1
MS 617 (VGD 252)	Asgrow	13	0	0
Poinsett 76 (VGS 160)	Asgrow	12	0	0
Spacemaster	Asgrow	14	5	3
Sprint 40	Asgrow	9	0	0
Straight 8	Burpee	12	3	0
Sumter (VGH 807)	Asgrow	9	0	0
VGH 7073	Asgrow	8	0	0
VGP 240	Asgrow	8	0	0
VGP 5049	Asgrow	13	6	0
VGP 5058	Asgrow	13	6	2
West Indian Gherkin	Hollar	4	0	0

²Numbers are from a total of 30 cultures evaluated, except for embryonic axis which had 15 cultures.