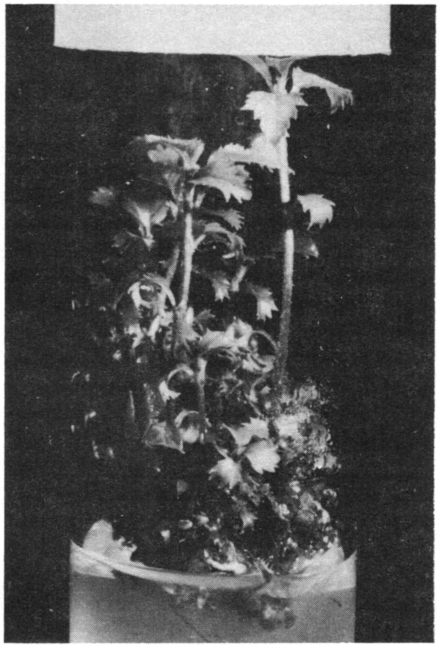

Fig. 1. Shoot proliferation in the first subculture from a lateral bud of *Alnus glutinosa*.

light at 100 $\mu Em^{-2}s^{-1}$ measured at the top of the culture vials.

Thirty days after explanting, when shoot growth had occurred from most of the lateral buds and callus tissue had developed on the basal portion of the explants, the plant materials were transferred to a new 15 ml aliquot of a similar medium and grown under the same conditions. Four weeks after the second transfer, multiple shoot development was observed in about 50% of the culture vessels (Fig. 1). These shoots appeared to originate from the base of the primary lateral bud on the original explant. An average of 10 shoots (1.5 cm long) per vessel were cut from the proliferating tissue. The remaining mass of shoot producing tissue was divided into 2 equal pieces which were placed into 110 ml culture jars containing 40 ml of similar medium on which additional shoots developed.

Microshoots were successfully rooted in the conventional potting medium in a warm, humid environment. They were inserted into Jiffy Mix in a 12 × 19 cm plastic flat which was placed in a wood and plexiglass propagation case filled with moist autoclaved sand and maintained at 24° ± 1°C. The relative humidity was maintained between 75–85% using a humidistatically controlled vaporizer. Four Cool White fluorescent tubes mounted on the plexiglass lid of the box 40 cm above the surface of the sand provided continuous illumination at 92 $\mu Em^{-2}s^{-1}$ at the height of the microcuttings. Microshoots rooted within 3 weeks, after which plantlets were transplanted into 7.5 cm pots containing a modified Cornell peat-lite mix (5) and placed on a greenhouse bench under 50% saran shade cloth. About 95% of the plantlets survived the transfer from the culture vessel to greenhouse, where they soon resembled a typical vigorous 'Saaksmaki' or 'Tuusla' seedling.

This research has demonstrated that rapid vegetative propagation of *Alnus* can be achieved by a system of lateral bud culture. The microshoots, when periodically removed from the culture vessels, can be rooted or used as a clean source of explant material for subsequent propagation. In addition, the proliferating mass of tissue from which the shoots arise can be divided into 3 or 4 pieces, each of which can produce propagules after culture on a similar medium. It has been estimated that a billion plants per year can be obtained from the culture of 1 meristem where a 10 fold multiplication of shoots occurs each month (1). Using the methods detailed above, we have estimated that it is possible to produce over 10 million plants per year from 1 lateral bud of *Alnus glutinosa*.

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In Vitro Adventitious Shoot and Root Formation of Cultivars and Lines of *Cucumis sativus* L.[1]

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Additional index words. tissue culture, cucumber, cotyledon hypocotyl, callus regeneration

Abstract. **Hypocotyl and cotyledon explants of 85 cultivars and lines of cucumber were screened for adventitious shoot and root formation in tissue culture. Tissue was cut from 7-day-old seedlings and grown on a medium consisting of Murashige-Skoog salts and vitamins with 1 mg/liter each of 6-benzylamino purine (BA) and naphthaleneacetic acid (NAA), and 3% sucrose added. No shoots were formed from hypocotyl pieces, while 28 of the 85 lines formed shoots from cotyledon tissue. Thirty-two lines formed at least one root in culture, and there was no difference in the frequency of root formation between cotyledon and hypocotyl tissue. There was no correlation between root and shoot formation. The best 2 lines, PI 279463 and PI 401732, had 53% and 40% of the cotyledon pieces forming shoots, respectively.**

A major problem in applying tissue culture techniques to crop improvement (6) has been the lack of success in regenerating plants from somatic cell culture (5). This is especially true in cucumbers (1, 7). However, shoots have been produced in culture from axillary buds of cucumber (8) and watermelon, *Citrullus lanatus* (Thunb.) Matsum, and Nakai (2, 3), and from hypocotyl tissue of pumpkin, *Cucurbita pepo* L. (9, 10).

Two approaches have been used in the search for a method of regenerating shoots from non-meristematic tissue of cucurbits cultured *in vitro*: testing different concentrations of hormones in the medium (1, 7), and testing different genetic materials (10). The failure of the former approach and the partial success of the latter suggests that the best approach for cucumbers might be to screen lines for shoot production in tissue culture. Bingham et al. (4) screened alfalfa clones for shoot production in tissue culture. They identified clones with 12% shoot production frequency, and were able to increase that to 67% after 2 cycles of selection.

If cucumber lines with shoot regeneration ability were obtained, it would then be possible to identify better regeneration media and, using the best system, to test the value of tissue culture for cucumber breeding. Our objective was to screen cucumber lines and cultivars for shoot production from non-meristematic tissue in *in vitro* culture.

A completely randomized experimental design was used with 85 lines, 2 tissues (cotyledon and hypocotyl), 3 replications (Petri plates), and 5 pieces of tissue per replication. Of the 85 lines used, 45 were *Cucumis sativus* plant introduction (PI) lines, and 40 were cultivars and lines of pickling and fresh-market cucumbers.

Seeds were first rinsed in 95% ethanol and then shaken for 15 min in 2.6% NaClO to surface sterilize them. Ten seeds from each line were then placed on 2 layers of moist filter paper in sterile Petri plates to germinate. After 7 days, 3 cotyledon and 3 hypocotyl explants were taken from each of 5 seedlings. Then, 5 cotyledon or 5 hypocotyl explants were placed in each Petri plate containing a medium consisting of Murashige-Skoog salts

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and vitamins (12) with 1 mg/liter each of BA and NAA, and 3% sucrose added. After 45 days, the number of roots and shoots formed from each piece of callus and visible to the unaided eye was recorded.

Both cotyledon and hypocotyl explants produced callus, although the latter tended to form callus sooner. No shoots were formed from hypocotyl tissue, but 28 (33%) of the 85 lines and cultivars formed shoots from cotyledon tissue (Table 1). An average of 5.5% of the cotyledon explants formed shoots. Usually, each explant produced no more than 1 shoot (64 explants produced 1 shoot each and 6 explants, 12%, produced 2 shoots each). The highest levels of shoot formation were from PI 279463 ('Chojitsu Ochiai' from Japan) and PI 401732 ('L2' from Puerto Rico). The formation of shoots by 53% of the cotyledonary explants of PI 279463 was comparable to the frequency of 85% obtained by Handley and Chambliss (8) on their best propagation medium for axillary buds of 'Carolina' pickling cucumber. Maciejewska-Potapczykowa et al. (11) obtained some shoots from callus produced by cucumber stem pieces in culture, but they did not present data on methods for obtaining shoots since that was not the objective of their experiment.

Table 1. Percent of cucumber cotyledon or hypocotyl pieces forming shoots or roots in tissue culture.[z]

Line or cultivar	Seed origin	Cotyledon pieces forming		Hypocotyl pieces forming roots[y]
		Shoots (%)	Roots (%)	(%)
PI 279463	Japan	53	0	0
PI 401732	Puerto Rico	40	0	0
PI 171612	Turkey	33	0	13
PI 177363	Syria	27	20	0
PI 172846	Turkey	27	0	0
PI 211984	Iran	27	0	0
490	NC State Univ.	22	0	0
PI 103049	China	20	13	0
PI 321011	Taiwan	20	0	0
PI 401734	Puerto Rico	20	0	0
Sprint 440	Asgrow Seed Co.	20	0	0
PI 267746	India	13	20	0
PI 205996	Sweden	13	13	0
PI 181874	Syria	13	7	0
Score	Asgrow Seed Co.	13	7	7
G4U4	Harris Seed Co.	13	0	0
Lucky Strike	PetoSeed Co.	13	0	0
Victory	PetoSeed Co.	13	0	7
Poinmarket	Clemson Univ.	7	27	0
SMR 18	Univ. Wisconsin	7	20	0
Centurion	Northrup-King Co.	7	7	0
HySlice	Castle Seed Co.	7	7	0
Chipper	Clemson Univ.	7	0	0
GUM	Harris Seed Co.	7	0	0
PI 135345	Afghanistan	7	0	0
PI 226510	Iran	7	0	0
PI 344442	Iran	7	0	0
PI 357846	Yugoslavia	7	0	0
Pacer	Harris Seed Co.	0	27	93
PI 249562	Thailand	0	20	0
PI 264228	France	0	13	0
PI 390252	Japan	0	13	7
Tablegreen 72F	Cornell Univ.	0	8	7
Medalist	Harris Seed Co.	0	7	0
Gemini	Clemson Univ.	0	7	0
Marketsett	Clemson Univ.	0	7	0
PI 105263	Turkey	0	7	13
PI 137851	Iran	0	7	13
PI 173892	India	0	7	0
PI 222244	Iran	0	7	7
PI 251028	Afghanistan	0	7	0
PI 293923	South Carolina	0	7	7
Slicemaster	PetoSeed Co.	0	7	0
Carolina	Clemson Univ.	0	0	7
Compact	NC State Univ.	0	0	0
Explorer	Clemson Univ.	0	0	0
EXP 810	Northrup-King Co.	0	0	0
Regal	NC State Univ.	0	0	0
G30	NC State Univ.	0	0	0
G62	NC State Univ.	0	0	0
Lemon	Northrup-King Co.	0	0	0
M41	NC State Univ.	0	0	13
Marketmore 80	Cornell Univ.	0	0	0
NCX 5504	Niagara Seed Co.	0	0	0
PI 109481	Turkey	0	0	0
PI 137839	Iran	0	0	0
PI 169403	Turkey	0	0	0
PI 173889	India	0	0	0
PI 176523	Turkey	0	0	0
PI 188749	Egypt	0	0	7
PI 209066	Ohio	0	0	0
PI 218036	Iran	0	0	0
PI 222720	Iran	0	0	13
PI 227207	Japan	0	0	0
PI 269480	Pakistan	0	0	0
PI 285609	Poland	0	0	0
PI 288994	Hungary	0	0	0
PI 308915	U.S.S.R.	0	0	0
PI 338236	Turkey	0	0	0
PI 342951	Denmark	0	0	0
PI 358813	Malaysia	0	0	0
PI 372906	Netherlands	0	0	7
PI 376063	Israel	0	0	0
PI 385967	Kenya	0	0	0
Raider	Harris Seed Co.	0	0	0
Slicerite	Ferry-Morse Seed Co.	0	0	0
Southern Belle	NC State Univ.	0	0	0
Sumter	Clemson Univ.	0	0	0
Super Slice	Northrup-King Co.	0	0	0
TX 79-1	Texas A&M Univ.	0	0	0
XP 1097	Asgrow Seed Co.	0	0	0
XP 1187	Asgrow Seed Co.	0	0	0
XP 1190S	Asgrow Seed Co.	0	0	0
4902	NC State Univ.	0	0	0
M27	NC State Univ.	0	0	0
LSD 5%		12	11	6

[z]Data are means over 3 replications.
[y]No shoots were formed from hypocotyl pieces from any of the 85 cultivars and lines.

No differences were found between cotyledon and hypocotyl tissue for root formation (3.4 vs. 2.6% of the pieces forming roots, respectively). Thirty-two (38%) of the 85 cultivars and lines formed at least 1 root, and 12 (14%) of the lines formed both roots and shoots, though not necessarily on the same tissue pieces. There was no correlation of the ability to form shoots with the ability to form roots in tissue culture ($r = 0.04$). 'Pacer' cucumber may be useful for root studies in tissue culture because of its exceptionally high rate of root formation, especially from hypocotyl pieces (Table 1).

The lines with the highest frequency of shoot formation in tissue culture, especially PI 279463 and PI 401732, now make it possible to further refine the nutrient and hormone levels of the regeneration medium, and hopefully, to apply tissue culture techniques in a cucumber breeding program.

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