

Multiple Shoot Induction from the Shoot Tip Explants of Cucumber (*Cucumis sativus L.*)

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Introduction: Cucumber is an important horticultural crop in India, cultivated primarily for its fruits for slicing and pickling, juice extraction, and for the preparation of traditional Indian medicines. However, the crop is known to be susceptible to abiotic as well as biotic stress (5). Applications of conventional techniques for its genetic improvement are limited because of interspecific incompatibility. A tissue culture method of propagation that could produce numerous plants would be desirable to produce a sufficient number of plants for inbreeding (2). A system suitable for transformation should ideally produce multiple shoots with minimal peripheral callus production so that the transformation of many of these individual buds is possible (1). This paper describes a technique which allows the production of multiple shoots from adventitious buds in cucumber shoot tip explants.

Materials and methods: Seeds of 'Poinsett 76' cucumber (Petoseed Co., Inc., USA) were soaked in tap water for 15 min. The seeds were surface sterilized with 70% alcohol for 1 min and then 25 % (v/v) commercial bleach " Teepol " (5.25% sodium hypochlorite) (Reckit & Colman of India Ltd., India) for 15 min. The seeds were then thoroughly washed three times with sterile distilled water and kept in 0.1% mercuric chloride solution (w/v) for 10 min. Finally the seeds were rinsed four times in sterile distilled water to remove the sterilant. The seeds were germinated in darkness for 48 hrs on sterile moist cotton.

Shoot tips (5mm length) from 5-7 day old in vitro grown seedlings were excised and used as explants. The explants were inoculated on MS medium (3) containing 3% sucrose (Himedia Co., Mumbai, India) with different auxin (2,4-D, NAA, and IAA) and cytokinin (BAP and Kin) compositions and concentrations. The pH of all the media was adjusted to 5.8 before autoclaving at 121°C for 20 min. The cultures were kept at 25±2°C with a 16 h photoperiod under diffused cool-white fluorescent lamps (80 μ mol m⁻².s⁻¹). Individual shoots were carefully excised

from the shoot clusters arising from the explants and subcultured on media containing the same hormone composition.

Results and Discussion: *Effect of cytokinins on multiple shoot induction:* Shoot tips from 5-7 day old seedlings were used as explants. Adventitious buds were induced from the meristematic regions of shoot tip explants planted on media containing BAP (0.5 – 1.0 mg/l) or Kin (1.0-2.0 mg/l) after 10 days of inoculation. A maximum number of shoots was obtained from adventitious buds after 4 weeks of culture on the medium supplemented with 1.0 mg/l BAP. Lower concentrations of BAP and Kin (below 0.5 mg/l), however, did not promote bud/shoot induction. Between these two hormones, the effects of BAP was greater in producing a higher number of shoots (Table.1). Individual shoots were isolated from the shoot clusters and subcultured in the medium containing the same composition.

Effects of combinations of cytokinins with auxins: Shoot tips of 7 day old seedlings were used as explants. Adventitious buds were initiated from the meristematic regions of the shoot tip explants after 2 weeks inoculation in the treatments containing BAP and NAA at concentrations of 1.0 mg/l and .2 mg/l respectively. In other concentrations of BAP and NAA calluses were produced preventing shoot bud induction. At all concentrations and combinations of 2,4-D/BAP and IAA/BAP, only calluses and subsequent rhizogenesis were noticed (data not shown).

The explants tended to produce callus on media supplemented with BAP and 2,4-D or NAA or IAA at higher concentrations. As many as 22 plant were produced from a single shoot tip explant planted on media containing BAP (1.0 mg/l) and NAA (0.2 mg/l) in successive subcultures in the medium with the same composition. The present study revealed that individual treatments of either BAP or kinetin produced shoots from shoot tip explants, but the number of shoots per explant was low. A low

Table 1. Effect of BAP on multiple shoot induction from the shoot tip explants of *Cucumis sativus*

MS medium + PGR (mg/l)	Number of shoots/explant	Shoot length (cm)
BAP		
0.2	n.d	n.d
0.4	1.0 c	4.8 cd
0.6	3.0 bc	5.2 c
0.8	5.0 b	5.9 ab
1.0	8.0 a	6.1 a

n.d: not determined due to nil response.

The experiment was repeated three time with 20 replicates.

Means followed by same letters within a column are not significantly using Duncan's Multiple Range Test (DMRT)

Table 2. Effect of kinetin on multiple shoot induction from the shoot tip explants of *Cucumis sativus*

MS medium + PGR (mg/l)	Number of shoots/explant	Shoot length (cm)
Kinetin		
0.2	n.d	n.d
0.4	n.d	n.d
0.6	1.0 bc	4.1 b
0.8	2.0 b	4.3 ab
1.0	4.0 a	4.9 a

n.d: not determined due to nil response.

The experiment was repeated three time with 20 replicates.

Means followed by same letters within a column are not significantly using Duncan's Multiple Range Test (DMRT)

Table 3. Effects of combinations of BAP and kinetin with NAA on multiple shoot induction.

MS medium + PGR (mg/l)		Number of shoots/explant	Shoot length (cm)
<u>BAP</u>	<u>NAA</u>		
1.0	0.1	16.0 b	2.2 ef
	0.2	22 a	8.3 a
	0.3	11 c	7.0 b
	0.4	n.d	n.d
	0.5	n.d	n.d
<u>Kin</u>	<u>NAA</u>		
1.0	0.1	5.0 de	1.1 f
	0.2	9.0 cd	2.3 c
	0.3	6.0 d	4.1 d
	0.4	2.0 e	5.4 c
	0.5	n.d	n.d

n.d: not determined due to nil response.

The experiment was repeated three time with 20 replicates.

Means followed by same letters within a column are not significantly using Duncan's Multiple Range Test (DMRT)

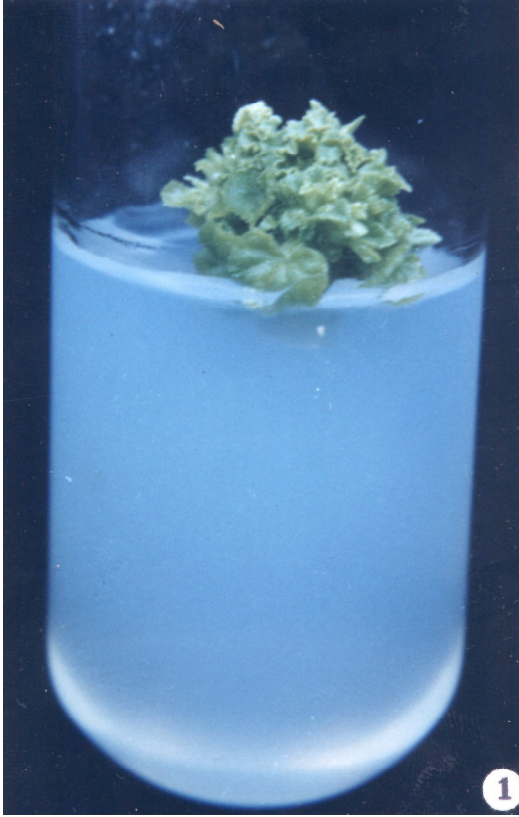


Figure 1. Emergence of multiple shoots (shoot cluster) from the shoot tip explants of 7 day old seedlings of 'Poinsett 76' cucumber.

Figure 2. Elongated shoot excised from the shoot cluster.

concentration of NAA (0.1 mg/l) with optimal concentrations of BAP (1.0 mg/l) was found essential in the induction of adventitious buds and subsequent multiple shoots from the shoot tip explants. At least three subcultures were required to produce the maximum number of shoots per explant. No phenotypic changes were observed in control plants and plants derived from the shoot tip cultures. The system described would be applied for transgenic recovery and in breeding programs. Our results are in agreement with the observation of Plader et al. (4).

Literature cited

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