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In Vitro Mass Propagation of *Cucumis sativus* L. from Nodal Segments

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Abstract: An efficient reproducible protocol for the in vitro multiplication of cucumber (*Cucumis sativus* L.), an important vegetable crop, was developed from nodal explants. Addition of casein hydrolysate to the shoot induction medium (MS + BA) significantly enhanced the number of multiple shoots or growth of the regenerants. Optimum shoot regeneration was observed on Murashige and Skoog (MS) medium containing 1.0 μ M 6-benzyladenine (BA) and (200 mg/l) casein hydrolysate. Rooting of isolated in vitro raised microshoots was readily achieved with (1.0 μ M) α -naphthalene-acetic acid (NAA) in $1/2$ MS. The plantlets thus obtained were successfully established in a greenhouse.

Key Words: *Cucumis sativus*, plant growth regulators, nodal segments, multiple shoot, vegetable crop, casein hydrolysate.

Abbreviations: BA, 6-benzyladenine; CH, casein hydrolysate; KIN, 6-furfurylaminopurine; NAA, α -naphthalene acetic acid; MS, Murashige and Skoog.

Introduction

Cucumber (*Cucumis sativus* L.), a popular vegetable crop of the family *Cucurbitaceae*, is rich in phosphorus, potassium and oxalic acid and is popularly used in salads. Its seeds are diuretic, tonic and refrigerant. The odorless principle of *Cucumis* L. is extractable with alcohol and is used in certain bouquet perfumes (Pandey, 2000).

A good micropropagation protocol could reduce the cost of hybrid seed production, which can account for 30% of the total seedling cost. The commercial application of in vitro techniques in cucurbitaceous taxa has been well demonstrated and the regeneration of plants has been reported from excised cotyledons (Halder & Gadgil, 1982; Gambley & Dodd, 1990, 1991; Singh et al., 1990, 1996; Stipp et al., 2001), leaf explants (Kathal et al., 1988; Mishra & Bhatnagar, 1995; Stipp et al., 2001) and anther culture (Kumar et al., 2003). The present communication describes in vitro multiple shoot regeneration from nodal segment explants, and the rooting and successful greenhouse establishment of cucumber.

Materials and Methods

The seeds of cucumber var. Barsati obtained from the Indian Agriculture Research Institute (IARI), New Delhi were surface sterilised in 0.1% (w/v) HgCl_2 solution for 4 min, subsequently washed 5 times in sterile distilled water and implanted on MS medium (Murashige & Skoog, 1962) supplemented with 3% sucrose and 0.8% bacteriological grade agar (Qualigens Fine Chemicals, Mumbai, India) in culture tubes (Borosil Glass Works Ltd., Mumbai, India) for germination. The in vitro cultures were initiated using nodal segment explants (1.0-1.5 cm in length) from 15 - day-old aseptic seedlings on MS medium containing various concentrations of BA and KIN in the primary set of experiments. In the second set, the effect of BA and a growth adjuvant, casein hydrolysate, in different combinations and concentrations was tested (Table 1).

The pH of all culture media was adjusted to 5.8 before autoclaving at 121 °C for 15 min. All cultures were incubated at 25 ± 2 °C under a 16-h photoperiod provided by cool white fluorescent tubes (Crompton India Ltd.) with light intensity of 2000 lux.

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Table 1. Effect of BA and CH on multiple shoot induction from nodal explant in MS medium.

Treatments	Frequency (%) of shoot regeneration	Mean no. of shoots	Mean shoot length (cm)
BA (0.5 μ M) + CH 200 mg/l	73.3 \pm 3.3 ^{bc}	4.00 \pm 0.31 ^{bc}	6.20 \pm 0.25 ^c
BA (1.0 μ M) + CH 200 mg/l	100.0 \pm 0.0 ^a	8.80 \pm 0.27 ^a	14.20 \pm 0.30 ^b
BA (2.0 μ M) + CH 200 mg/l	60.0 \pm 5.7 ^{cd}	3.40 \pm 0.50 ^{bc}	5.10 \pm 0.90 ^d
BA (3.0 μ M) + CH 200 mg/l	53.3 \pm 3.33 ^d	3.20 \pm 0.50 ^c	4.70 \pm 0.25 ^d
BA (5.0 μ M) + CH 200 mg/l	0.00 \pm 0.00 ^e	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^e
BA (1.0 μ M) + CH 50 mg/l	90.0 \pm 5.7 ^{ab}	4.20 \pm 0.37 ^{bc}	13.70 \pm 0.24 ^b
BA (1.0 μ M) + CH 100 mg/l	96.6 \pm 3.33 ^a	5.00 \pm 0.31 ^b	13.70 \pm 0.25 ^b
BA (1.0 μ M) + CH 300 mg/l	93.3 \pm 3.33 ^a	3.80 \pm 0.37 ^{bc}	15.50 \pm 0.22 ^a

Values represent means \pm standard error of 10 replicates per treatment in 2 repeated experiments. Means followed by the same letter are not significantly different by the Tukey test at 0.05% probability level.

The regenerated shoots from nodal segments were subcultured onto the same shoot induction medium after 3 weeks of culture. In vitro raised microshoots with 5-6 nodes and 4-6 leaves were rooted on MS and $\frac{1}{2}$ MS medium containing various concentrations of NAA (Table 2); 7-9-week-old regenerated microshoots with well developed roots were washed with tap water and transplanted into plastic pots containing Soilrite and irrigated with tap water regularly. The plantlets were covered with polythene bags to maintain high humidity and acclimatised at 25 ± 2 °C under a 16-h photoperiod. After 20 days, the polythene bags were removed and established plantlets were transferred to a greenhouse.

In all the experiments each single treatment consisted of 10 replications and all experiments were repeated twice. Data on multiple shoot regeneration, elongation and rooting were statistically analysed using one-way analysis of variance (SPSS, Version 10) and comparison of means was performed at the $P < 0.05$ level of significance using Tukey's test.

Results

Nodal segments cultured on MS basal medium did not show any response. A varying degree of shoot bud differentiation was observed at lower concentrations of BA (0.5, 1.0, 2.0 μ M) whereas a higher concentration (5.0 μ M) led to the formation of callus. BA proved a more

effective cytokinin than KIN for multiple shoot induction (data not shown).

Out of various concentrations of BA tested, MS medium containing 1.0 μ M BA and 200 mg/l CH proved the best with a maximum of 8.8 ± 0.27 shoots per nodal explant (Table 1) (Figure 1 a,b). At a higher concentration of BA (5.0 μ M) and with the same concentration of CH, callus formation with ill-defined buds was observed after 3 weeks of culture.

In another set of experiments, the response of shoot induction was studied on medium containing BA (1.0 μ M) and different concentrations of CH (50, 100, 200, 300 mg/l). This resulted in multiple shoot proliferation but with varying frequencies (Table 1). The concentration of CH above 200 mg/l resulted in basal callusing and a decrease in shoot induction; however, shoots continued to grow.

For root induction, individual microshoots (8.00 cm) were placed on MS and $\frac{1}{2}$ MS medium supplemented with various concentrations of NAA (Table 2). Rooting occurred in all concentrations but with different rooting percentages, and the optimal response was observed on $\frac{1}{2}$ MS + NAA (1.0 μ M) in terms of average number of roots (8.0 ± 0.31) with mean root length of 3.86 ± 0.30 (cm) per shoot (Figure 1c).

The in vitro produced plantlets showed about 60% survival in Soilrite. After 4-5 weeks, the regenerated

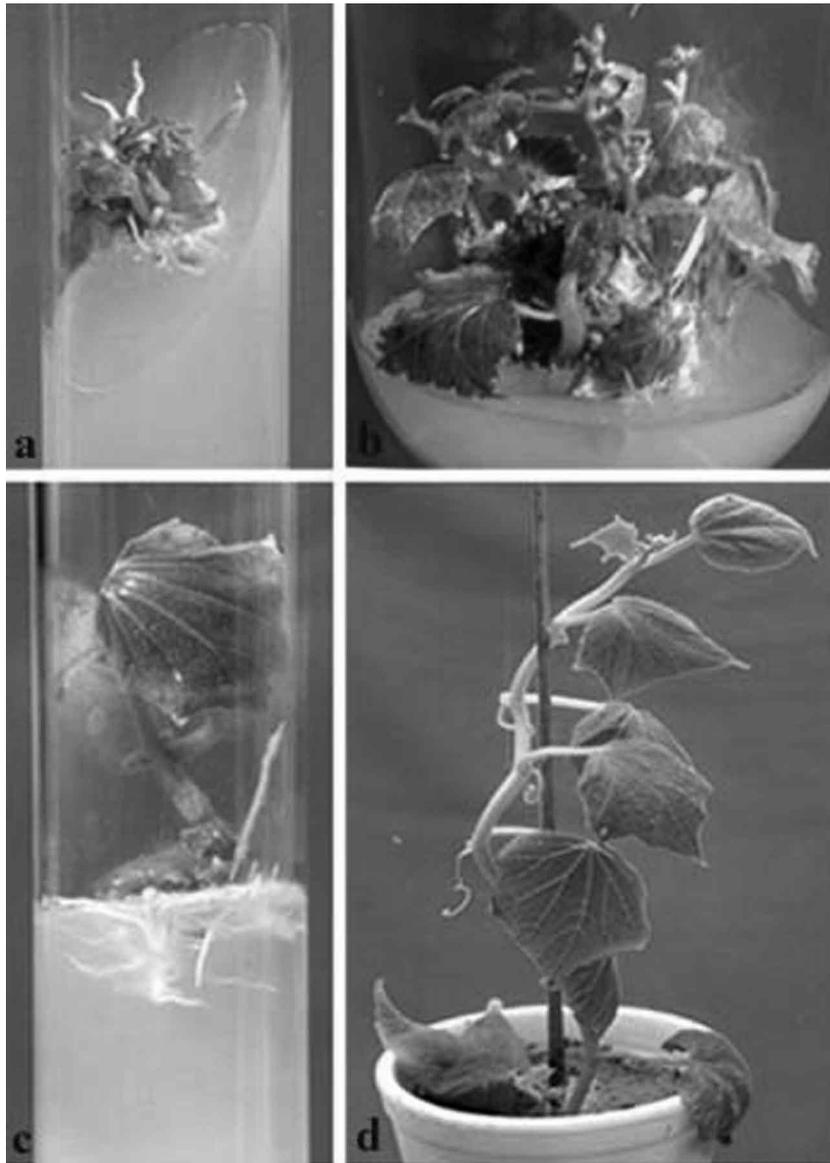


Figure 1. Micropropagation of *Cucumis sativus*. a. Multiple shoots on MS + BA (1.0 μ M) + CH 200 mg/l after 15 days; b. Shoot elongation on MS + BA (1.0 μ M) + CH 200 mg/l after 35 days; c. Root development on $1/2$ MS + NAA (1 μ M); e. An established plant in soil.

plantlets were transferred to soil under greenhouse conditions, where they have been growing well (Figure 1d). The regenerated plants did not show any morphological change.

Discussion

The present findings from *C. sativus* demonstrate the possibility of the mass propagation of cucurbits through

nodal explants. For successful micropropagation, axillary buds or shoot tip cultures are preferred as pre-existing meristems easily developed into shoots while maintaining fidelity. To obtain plantlets with uniform growth characteristics of the mother plant, direct regeneration is essential.

Literature on cucurbits indicates a low rate of regeneration and survival of plants with abnormalities

Table 2. Effect of MS strength and NAA concentration on root induction from in vitro raised microshoots of *Cucumis sativus* L.

Treatments	Frequency (%) of rooting	Mean no. of roots/shoot	Mean root length (cm)
MS + NAA (0.5 μ M)	66.6 \pm 3.33 ^{cd}	5.20 \pm 0.37 ^{cd}	2.42 \pm 0.18 ^c
MS + NAA (1.0 μ M)	90.0 \pm 5.7 ^{ab}	7.00 \pm 0.31 ^{ab}	2.46 \pm 0.20 ^c
MS + NAA (1.5 μ M)	73.3 \pm 3.3 ^{bc}	4.60 \pm 0.40 ^{cd}	2.76 \pm 0.25 ^{bc}
MS + NAA (2.0 μ M)	50.0 \pm 5.7 ^d	2.80 \pm 0.37 ^e	3.12 \pm 0.15 ^{ab}
1/2 MS + NAA (0.5 μ M)	83.3 \pm 6.6 ^{abc}	6.00 \pm 0.44 ^{bc}	2.52 \pm 0.17 ^{bc}
1/2 MS + NAA (1.0 μ M)	100.0 \pm 0.0 ^a	8.00 \pm 0.31 ^a	3.86 \pm 0.30 ^a
1/2 MS + NAA (1.5 μ M)	96.6 \pm 3.3 ^a	5.20 \pm 0.37 ^{cd}	3.42 \pm 0.18 ^{ab}
1/2 MS + NAA (2.0 μ M)	80.0 \pm 5.7 ^{abc}	4.00 \pm 0.31 ^{de}	2.82 \pm 0.12 ^{bc}

Values represent means \pm standard error of 10 replicates per treatment in 2 repeated experiments. Means followed by the same letter are not significantly different by the Tukey test at 0.05% probability level.

such as premature flowering (Gambley & Dodd, 1990). Regeneration from cotyledon, sections of hypocotyls and apical buds with varying regeneration frequency has been reported by Gambley & Dodd (1991).

Our results show enhanced shoot formation by proliferation of axillary buds on a medium fortified with cytokinin and CH. The fortification of cytokinin for

multiple shoot induction at lower concentrations has also been reported (Kathal et al., 1988; Singh et al., 1996).

It is concluded that the manipulation of culture conditions using various combinations and concentrations of growth hormones and other adjuvants can provide a reproducible protocol and reduce the high costs of hybrid seed production.

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