

Full Length Research Paper

Direct regeneration of the medicinal herb *Cucumis anguria* L from shoot tip explants

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A protocol was developed by *in vitro* regeneration by shoot induction of *Cucumis anguria*, a plant used as medicine and vegetable belonging to the family Cucurbitaceae. High frequencies of multiple shoot regeneration were achieved from nodal explants on MS fortified with 2 mg/l 6-benzyl amino purine (BAP) and 0.5 mg/l indole acetic acid (IAA). Five to ten shoots per explant were obtained. The elongated shoots were sub cultured for rooting on MS supplemented with 2 mg/l naphthalin acetic acid (NAA). The *in vitro* raised plantlets were acclimated in green house and successfully transplanted to natural condition with 75% survival.

Key words: *In vitro* regeneration, *Cucumis anguria*, shoot tip explants, MS medium.

INTRODUCTION

Tissue culture techniques are being increasingly explanted for clonal multiplication and *in vitro* conservation of valuable indigenous germplasm threatened with extinction. Greater demands of these plants especially for the purpose of food and medicine are the causes of their rapid depletion from primary habitats. Plant tissue culture is a tool which ensures mass production of plants for their improvement.

Cucumis anguria, a climber belongs to the family Cucurbitaceae. It is distributed in Manapparai and Dindigul area. In Tamil, the plant is called as *Vizavellari* and its fruits are traditionally used for reducing the body heat and stomach pain. The fruits are rich in carbohydrates. This plant is dioecious in nature, limiting pollination and fertilization. Hence, the present study was undertaken to develop a suitable micropropagation technique to contract the natural in their population. There has been progress in tissue culture studies in many Cucurbitaceae members such as *Momordica dioica* (Shiragave and Chavan, 2001), *Coccinia indica* (Venkateswaralu,

2001), *Cetrullus vulgaris* (Dong and Ja, 1991), *Cucumis melo* (Mackay et al., 1989), *Cucumis sativus* (Cade et al., 1987) and *Mormordica charantia* (Mala Agarwal and Reka Kamal, 2004). The present investigation elucidates *in vitro* multiple shoot regeneration through nodal explants of *C. anguria* for better exploitation and also preservation of this valuable germplasm. The objective of the research was to find out the suitable protocol for tissue culture.

MATERIALS AND METHODS

C. anguria (L) seeds were collected from IAP Farms (P) Ltd., Nilakkottai, Dindigul District, Tamil Nadu. The collected seeds were planted at College Garden. The nodal segments were excised from the garden grown plants and washed thoroughly in running tap water for 10-15 min, 1% teepol solution for 5 min and washed five times with distilled water followed by sterile distilled water under aseptic condition. For surface sterilization, explants were sensed by 0.1% HgCl₂ solution for 3-5 min and rinsed with sterile distilled

Table 1. Production of plantlets from shoot tip explants through direct organogenesis.

Hormone concentration (mg/l)		Number of explants cultured	Number of explants Responded	Percentage of Response	Number of Shoot/ Explants mean \pm SD
BAP	IAA				
0.5	0.5	20	8	40	3.50 \pm 0.53
1.0	0.5	20	12	60	4.00 \pm 0.85
1.5	0.5	20	15	75	4.40 \pm 0.99
2.0	0.5	20	17	85	5.71 \pm 0.77
2.5	0.5	20	11	55	4.45 \pm 0.69

water five times to remove the traces of HgCl₂.

Nodal segments were further trimmed to remove excess tissues. The explants were cultured on MS medium with different concentration of plant growth hormones along with 3% sucrose and 0.8% of agar. The pH of the medium was adjusted to 5.7 using 0.1 N NaOH or 0.1 N HCl before autoclaving and adding agar. About 10 ml of the medium were dispensed in each culture tube and sealed with non-absorbent cotton plugs prior to autoclaving at 121°C for 20 min. All cultures were maintained at 16 h photoperiod with 3000 Lux light intensity at 25 \pm 2° C.

Results were observed at regular intervals and tabulated for each treatment; 20 replicates were used and all experiments were conducted thrice.

RESULTS AND DISCUSSION

Direct organogenesis is the development of an organ from the explants itself without intermediate tissue culture development. Production of plantlets by direct organogenesis either from shoot apex where apical meristem is present or from the nodal explants where lateral meristem is present is called micropropagation.

Studies were carried out to produce plantlets from shoot tip explants through direct organogenesis without intervention of callus a significant development, and it was observed that multiple shoot buds originated from Shoot tip explants, when MS was supplemented with various concentrations of 6-benzyl amino purine (BAP) (1.0-10 mg/l) along with indole acetic acid (IAA) (0.5 mg/l). The nodal explants showed slight swelling prior to the emergence of short buds development from the pre-existing material 20 days after inoculation; initially two to five shoot buds per explant emerged 35 days after inoculation and gradually the number of shoot buds per explant increased upto eight to ten (Table 1) on MS fortified with 2.0 mg/l BAP along with combination of 0.5 mg/l IAA. Similar results were observed with BA at a concentration of 2.5 mg/l.

It can be concluded that 1.0 mg/l BAP and 0.5 mg/l IAA are suitable phytohormones for suitable phytohormones for shoot proliferation and shoot elongation from shoot explants of *C. anguria*. This is accordance with the results as reported earlier (Anand and Jeyachandran, 2004; Kulkarni et al., 2002; Yokoya and Handro, 2002;

John Britto et al., 2001).

Following multiple shoot elongation, the healthy shoots (4-5 cm long) were transferred on MS supplemented with different concentrations of NAA. Shoot elongation was simultaneously observed along with root induction in 2.0 mg/l NAA (Table 2). Anitha and Pulliaiah (2002) in *Decalipis hamiltoni* demonstrated similar results.

After 35 days, well developed shoots and roots were observed. Subsequently, cultures were removed from agar medium washed thoroughly and placed in pots containing a mixture of sterilized vermiculate and sterilized soil (1:1). *In vitro* raised plants were acclimated in greenhouse and successfully transplanted into the field with 60% survival.

In the present investigation, high frequency of multiple shoot induction was achieved in *C. anguria* through shoot explants with BAP 2.0 mg/l and combination of IAA (0.5 mg/l). Further, an increase or decrease of this hormone level showed negative trend in multiple shoot formation. NAA (2 mg/l) was found to be an ideal growth regulator for root induction as well as shoot elongation.

Multiple shoots from shoot tip explants on MS medium fortified with BAP (1.0 mg l⁻¹) in combination with abscisic acid (2.0 mg l⁻¹) showed maximum percentage of response (74.4%) with 24.6 shoots per explants. Varisai Mohamed et al. (1999) demonstrated high frequency of shoot multiplication from shoot tip explants of *Macrotyloma uniflorum* on MS medium supplemented with BAP (1.5 mg l⁻¹) and GA₃ (0.5 mg l⁻¹). The above study contradicted the results reported in *Cucurbita pepo* (Pink and Walkey, 1984) where multiple shoot induction from shoot tip explants occurred on modified MS medium with IBA (1.7 μ M), KIN (0.5 μ M) and GA₃ (0.3 μ M). Plate 1 and Figure 1.

In *C. anguria* for shoot-tip culture, BAP (2.0 mg/l) with IAA (0.5 mg/l) was a suitable hormone concentration. When concentration increased or decreased the rate of shoot initiation and shoot proliferation also decreased.

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Table 2. Rooting response of excised shoots of *Cucumis anguria*.

NAA (mg/l)	Response (%)	Root length / shoot (cm)
0.5	-	-
1.0	24	1.6 ± 0.20
1.5	52	1.9 ± 0.08
2.0	64	2.7 ± 0.08
2.5	51	1.8 ± 0.06

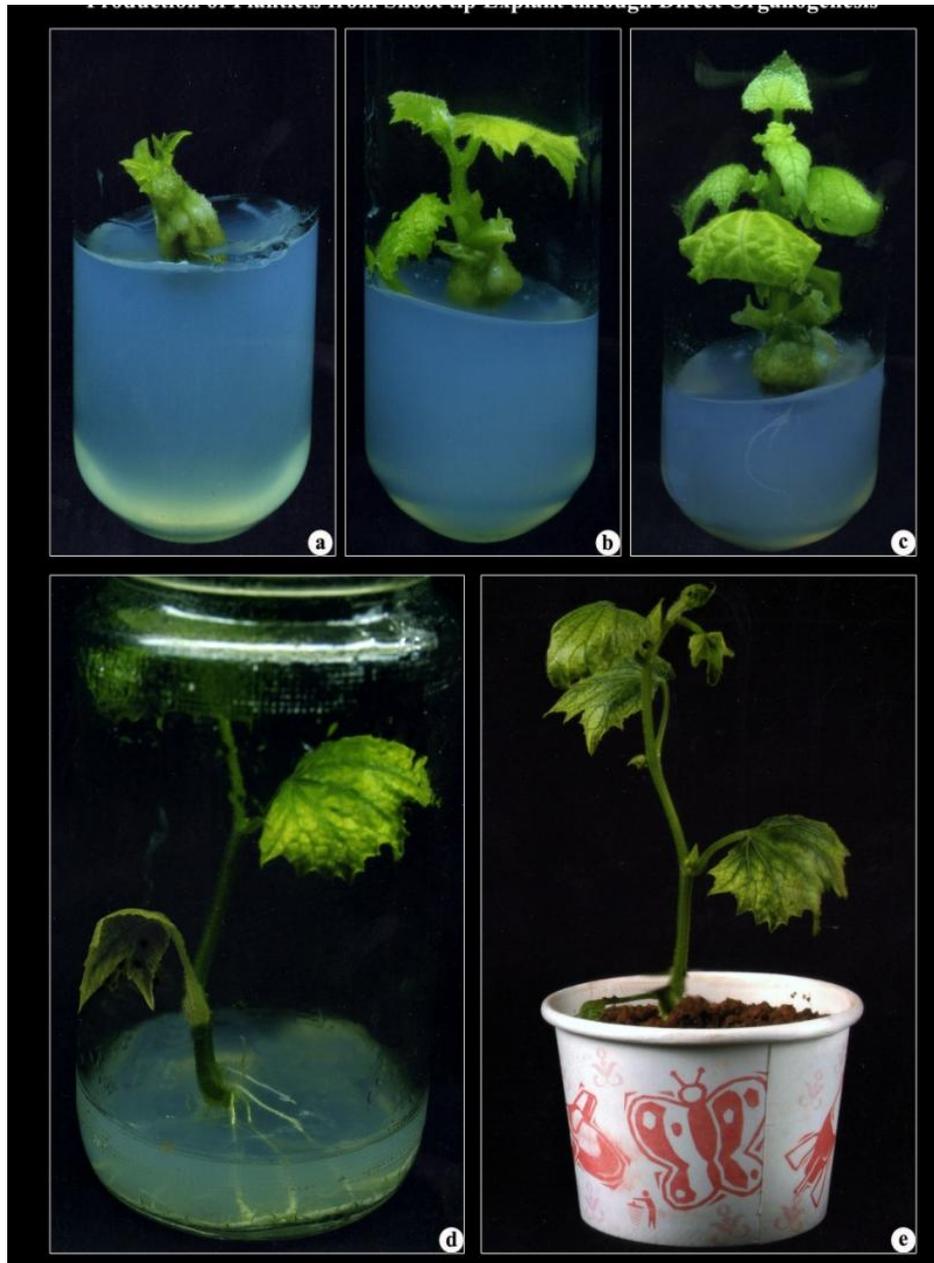


Plate 1. Production of plantlets from shoot tip explants through direct organogenesis A and b, Shoot initiation from shoot tip explants on MS + BAP (2.0 mg/l) + IAA (0.5 mg/l); c, shoot elongation on MS+BAP (2.0 mg/l) + IAA (0.5 mg/l); d, rooting on MS with 1.5 mg/l of NAA; e, hardened plantlet.

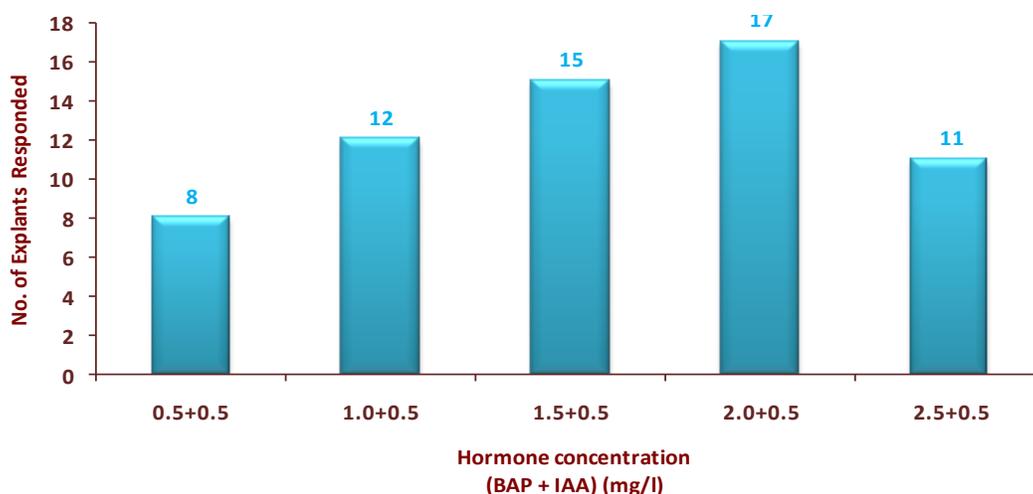


Figure 1. Production of plantlets from shoot tip explants through direct organogenesis.

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