In Vitro plant regeneration of Cucumber (Cucumis sativum (L.) from cotyledon and hypocotyl explants

T Ugandhar[1], M Venkateshwarrlu[2], Gousia Begum[2], T Srilatha[2] and K Jaganmohanreddy[2]
[1]Department of Botany, C.V. Raman P.G. College, Mancherial, Dist-Adilabad.
[2]Department of Botany, Kakatiya University, Warangal, Dist-Warangal.
tugandharbiotech@gmailcom.in

ABSTRACT

In vitro plantlet regeneration has been obtained from 15-20days old seedling cotyledon and hypocotyl segments of *Cucumis sativus* (L.) were examined using various phytoharmons individually and in combination on Murashige and Skoog (MS) semi solid medium supplemented with BAP (1.0-5.0 mg/L) Kn (1.0-5.0 mg/L),IAA (0.5 mg/L)+ BAP (1.0-5.0 mg/L) and IAA (0.5 mg/L)+Kn (1.0-5.0 mg/L) for shoot proliferation IAA (0.5 mg/L)+BAP (3.0 mg/L) was proved to be best for induction of shoots for cotyledon and hypocotyl explants. All regenerated plantlets were rooted on MS medium supplemented with (1.0 mg/L) IAA the regenerated plants grew normally in the green house.

***Key words**: Cucumis sativus* (L.), Hypocotyl, *In vitro* Regeneration, Cotyledon.

Abbreviations: BAP, 6-Benzyl amino purine;; Kin, Kinetin IAA, Indole 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 odorous principle of *Cucumis sativus* 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 and Gadgil 1982, Gambley and Dodd 1990, 1991, Singh *et al.* 1990, 1996, Stipp *et al*. 2001), leaf explants (Kathal *et al.* 1988, Mishra and Bhatnagar 1995; Stipp *et al*. 2001) and anther culture (Kumar *et al*. 2003). The present communication describes *in vitro* multiple shoot regeneration from cotyledon and hypocotyl explants, and the rooting and successful greenhouse establishment of cucumber.

MATERIALS AND METHODS

Aseptic seed germination explants preparation and culture conditions.

The seeds of Cucumber (*Cucumis sativus* (L.), were obtained from Agriculture Research Institution Warangal (A.P) India. These seeds were washed in running tap water for three minutes and then washed repeatedly in double distilled water. Now under aseptic conditions the seeds were surface sterilized with 70% ethanol for one minute followed by a twenty minute treatment with 2% sodium hypochloride and washed with sterilized triple distilled water five times followed by 0.1% Mercuric chloride ($HgCl_2$) for five minutes and rinsed five times in sterile distilled water. The sterilized seeds were then placed on MS basal medium (Murashige and Skoog 1962) solidified with 0.8% bacto agar for germination in 250 ml culture bottles,20 seeds were cultured per bottle containing 30 ml of medium. This was incubated in dark at 26^{o}C till it germinated and then transferred to cool-white-fluorescent light room and incubated at 24$\pm$2^{o}C and allowed to grow. The plant after reaching a height of 6 centimeters was taken in an aseptic condition and cotyledon and hypocotyle were excised using a sterile scalpel and cut into 6-8 mm sections.

Plant Regeneration

The seedling excised (cotyledon and hypocotyls) explants (Fig –I a) were then placed on MS medium containing 3% w/v sucrose with various concentrations of cytokinin BAP(1.0-5.0 mg/L), Kn (1.0-5.0 mg/L) alone and also in combination with auxin IAA (0.5mg/L) + BAP (1.0-5.0 mg/L), and IAA (0.5mg/L) + Kn (1.0-5.0 mg/L) (Tables- 1) the pH of the media was adjusted to 5.8 $\pm$ 1 with I N HCl or 1N NaOH solidified with 0.8% difco –bacto agar and autoclaved at 121^{o}C at psi for 15-20 minutes single explants was inoculated in each culture tube and incubated at 25 $\pm$ 2^{o}C under white fluorescent light of 40-60 µ mol m^{-2} s^{-1} intensity for 16 hrs light /8 hrs dark period. Every two week the explants were transferred to fresh medium. The number of shoots produced was counted 6 weeks after culture. Isolated single shoots after reaching 5 centimeters in size were transferred to MS medium (Murashige and Skoog 1962) supplemented with (0.2 mg/L) IAA for rooting. Plantlets were transferred to the greenhouse for acclimatization and growth.

RESULTS AND DISCUSSION

Multiple shoot buds proliferation was observed within 15-20 days of culture form the cut ends of cotyledon and hypocotyls. The data on *in vitro* regeneration was presented in (Table 1).

Table-1 Effect of BAP, Kn, IAA+BAP and IAA+Kn on direct shoot induction from cotyledon and hypocotyl explants in MS medium

	Cotyledon		Hypocotyl	
Hormone conc. (mg/L)	% of cultures responding	Average number of shoots/ explants (S.E)*	% of cultures responding	Average number of shoots/ explants (S.E)*
BAP				
1.0	55.0	2.0 ± 0.35	53.0	1.8 ± 0.32
2.0	60.0	2.2 ± 0.25	57.0	2.0 ± 0.43
3.0	65.0	3.0 ± 0.27	62.0	2.8 ± 0.32
4.0	50.0	2.8 ± 0.38	52.0	2.4 ± 0.36
5.0	48.0	2.6 ± 0.32	47.0	2.3 ± 0.32
Kn				
1.0	50.0	1.7 ± 0.43	48.0	1.3 ± 0.34
2.0	56.0	2.1 ± 0.34	50.0	1.8 ± 0.32
3.0	58.0	2.8 ± 0.35	53.0	2.2 ± 0.36
4.0	52.0	2.4 ± 0.22	47.0	2.0 ± 0.32
5.0	49.0	2.0 ± 0.45	42.0	1.6 ± 0.42
IAA + BAP				
0.5 + 1.0	60.0	2.6 ± 0.48	58.0	2.3 ± 0.43
0.5 + 2.0	68.0	3.0 ± 0.75	65.0	2.8 ± 0.22
0.5 + 3.0	70.0	3.8 ± 0.36	68.0	3.0 ± 0.23
0.5 + 4.0	65.0	3.2 ± 0.32	62.0	2.6 ± 0.33
0.5 + 5.0	58.0	2.8 ± 0.42	56.0	2.0 ± 0.63
IAA + Kn				
0.5 + 1.0	58.0	2.2 ± 0.42	56.0	1.2 ± 0.32
0.5 + 2.0	62.0	2.0 ± 0.32	59.0	1.4 ± 0.36
0.5 + 3.0	68.0	2.3 ± 0.42	65.0	1.8 ± 0.23
0.5 +4.0	60.0	1.8 ± 0.32	62.0	1.3 ± 0.33
0.5 + 5.0	50.0	1.6 ± 0.32	59.0	1.0 ± 0.33

* **Mean $\pm$ Standard Error**

Effect of BAP and KN

Table 1 represents, direct regeneration of seedling cotyledon and hypocotyl explants to various concentration of cytokinins such as BAP and Kn alone in BAP (1.0 -5.0 mg /L) and Kn (1.0-5.0 mg/L) was studied on direct multiple shoot bud induction. Direct adventitious shoot regeneration on MS medium containing various results. Highest responding cultures with maximum frequency of multiple shoot bud induction was observed at (3.0 mg/L) BAP (3.0± 0.27 shoots/explant) (Fig –I b) followed by 4.0 and 5.0 mg/L BAP, produced (2.8±0.38 and 2.6±0.32 shoots/explants)with 50 and 48% cultures responding. The numbers of shoots were considerably reduced, when BAP concentration was increased. Kn was less responsive compared to BAP in inducing shoot buds from the explant with 1.0 mg/L Kn the cotyledon explant produced (1.7±0.43 shoots/explants) and 50% culture responded 3.0 mg/L Kn was more responsive in inducing maximum number of shoots (2.8±0.35 shoots/explants) with greater frequency (58%) KN at 4.0 and 5.0 mg/L Produced (2.4±0.22 and 2.0± 0.45 shoots/explants) with 52 and 49% cultures responded.

Hypocotyl explants were cultured on MS medium supplemented with various levels of BAP (1.0-5.0 mg/L) Maximum frequency of shoot buds induction (2.8±0.32 shoots/explant) was noted at 3.0 mg/L BAP compared to all other concentration tested shoot capacity was gradually decreased at high concentration of BAP (Table-1).

Similarly hypocotyls explants were cultured on MS medium supplemented with various levels of Kn (1.0 -5.0 mg/L). Highest percentage (53%) of responding cultures were observed at (3.0 mg/L) followed by (4.0 mg/L) and gradually reduced as the level of KN was increased. Maximum number of shoots regeneration (2.0± 0.32 shoots/explant) was found at (4.0 mg/L) Kn. whereas the shoot bud induction was decreased at high level of Kn.

Effect of IAA + BAP and IAA + KN

When the auxin was taken in combination with IAA (0.5 mg/L) + BAP (1.0 -5.0 mg/L) and IAA 0.5 mg/L + Kn (1.0-5.0 mg/L) (Table-1) in combination produced shoots from the explants. At 0.5 mg/L IAA with 1.0 mg/L BAP 60% cultures responded with (2.6 ± 0.48 shoots / explants) maximum number of shoots (3.8± 0.36 shoots/explant) (Fig –I c) with greater frequency 70% were produced at (3.0 mg/L) BAP + IAA (0.5 mg/L) (Fig –I c) As the concentration of BAP was increase from 4.0 mg/L to 5.0 mg/L the number of shoots were considerably reduced (Table -1).

IAA + Kn was less responsive compared to IAA + BAP in including shoot buds from the explants (Table -1) with (1.0 mg/L) Kn and (0.5 mg/L) IAA produced (2.2±0.42 shoots/explants) with 58 % cultures responded. At 3.0 mg/L KN was more responsive in inducing maximum number of shoots (2.3± 0.42 shoots) with greater frequency (68%) KN at 4.0 and 5.0 mg/L produced (1.8± 0.32 and 1.6± 0.32 shoots/explants) with 60% and 50% cultures responding. To find out the efficiency of auxin – cytokinin combination the hypocotyls explants were cultured on MS medium supplemented with IAA (0.5 mg/L) in combination with various concentration of BAP /Kn (1.0 -5.0 mg/L). Direct shoot bud proliferation was found in all the concentrations and combinations of phytohormones used.

Hypocotyls culture on MS medium containing (0.5 mg/L)IAA in combination with (1.0-5.0 mg/L) BAP showed maximum responding culture and more number of shoots /explants (3.0± 0.23 shoots /explant) (Fig –I d) at 3.0mg/L BAP. Average number of shoots production has been gradually decreased at high concentration of BAP (Table -1). Hypocotyls explants were cultured on (0.5 mg/L) IAA in combination with various concentration (1.0,2.0,3.0,4.0 and 5.0 mg/L) of Kn showed (1.2± 0.32,1.4± 0.36, 1.8± 0.23,1.3± 0.33 and 1.0± 0.33 shoot/explant) with 56, 59, 65,62 and 59% responded. For root induction, individual microshoots (8.00 cm) were placed on MS medium supplemented with various concentrations of IAA (1.0mg/L). The *in vitro* produced plantlets showed about 60% survival in Soil rite. After 4-5 weeks, the regenerated

Figure1: Direct shoot induction of cotyledon and hypocotyls culture of *Cucumis sativus* (L.)
a) *in vitro* seedling after 30 days of seed culture b)Direct shoots on (3.0mg/L) BAP from cotyledon culture c) Multiple shoots on IAA(0.5mg/L)+(3.0mg/L) BAP from cotyledon culture d)Direct shoots formation on IAA(0.5mg/L)+BAP(3.0mg/L) from hypocotyls explants after six weeks

DISCUSSION

We were successful in regeneration plants from, cotyledon and hyocotyl culture on MS medium fortified with different concentration of cytokinin ie BAP /KN individually and also in combination on with (0.5 mg/L) IAA.

Maximum number of shoot buds were induced (3.0 mg/L) BAP in comparison to Kn as a role growth regulators with low levels of auxin (0.5 mg/L) were added to the medium containing BAP/Kn it was interesting to find that the shoot induction was enhanced in all the concentrations of cytokinin tested. However the shoot bud proliferation was found to be more on (0.5 mg/L) IAA in combination with BAP/Kn to probably IAA might have triggered the action of BAP/Kn in a proper way for inducing more number of shoots per explant but the combination of IAA + BAP induced higher number of plantlet regeneration among all hormonal combinations and concentrations used.

The present findings from *Cucumis sativus* L demonstrate the possibility of the *in vitro* propagation of cucurbits through cotyledon and hypocotyls explants 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 such as premature flowering (Gambley and Dodd 1990). Regeneration from cotyledon, sections of hypocotyls and apical buds with varying regeneration frequency has been reported by Gambley and Dodd (1991). Similarly Hoque *et al.* (2005) have reported the high frequency of plant regeneration on MS medium containing (2.0 mg/L) BAP in combination with (0.5 mg/L) IAA from cotyledon derived callus in *Memordica dioica.* They have also found the maximum number of shoots per explants on BAP compared to Kn. The essentially to both auxin cytokinin combination for inducing shoot organogenesis has been reported in leaf culture of *Cicer ariutum* (Arockia swamy *et al.* 2000) of the cytokinin used BAP proved as most effective than Kn in inducing shoots, the same finding were recorded in *Capsicum* spp (Phillips and Hubsten berger 1985).

Our results show enhanced shoot formation by proliferation of cotyledon and hypocotyls on a medium fortified with cytokinin and auxins. 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.

Acknowledgment: We are grateful to Professor N.Rama Swamy BOS in Biotechnology, Department of Biotechnology and Principal University College Kakatiya University Warangal (A.P), we thank Mr. K. Madhusudan Rao Principal, C.V. Raman PG College, Mancherial Dist Adilabad (A.P) for their constant encouragement and for providing necessary facilities.

LITERATURE CITED

Arokiaswamy S Varghese G and Ignacimuthu S. 2000. High frequency regeneration of chickpea (*Cicer arietium L.)* Plantlets from leaf callus. *Phytomorphology,* **50**: 297-302

Gambley RL and Dodd WA. 1990. An *in vitro* technique for the production of de novo multiple shoots in cotyledon explants of cucumber (*Cucumis sativus* L.). *Plant Cell Tissue Organ Cultue,* **20**: 177-183.

Gambley RL and Dodd WA. 1991. The influence of cotyledon in axillary and adventitiousshoot production from cotyledonary nodes of *Cucumis sativus* L. (cucumber). *J Exp Bot,* **42**: 1131-1135

Halder T and Gadgil VN 1982. Morphogenesis in some species of the family cucurbitaceae. In:
Rao AN (ed.). *Tissue Culture of Economically Important Plants.* pp 98-103 Singapore: Natl. University

Hoque MD and JW Mansfield. 2004. Effect of genotype and explant age on callus induction and subsequent plant regeneration from root-derived callus of Indica rice genotypes. *Plant Cell Tiss. Organ Cult.***78**: 217- 223.

Kathal R Bhatnagar SP and Bhojwani SS. 1988. Regeneration of plants from leaf explant of *Cucumis melo* cv. pusa sharbati. *Plant Cell Reports,* **7**: 449-451.

Kumar HGA Murthy HN and Paek KY. 2003. Embryogenesis and plant regeneration from anther cultures of *Cucumis sativus* L. *Scientia Hort* **98**: 213-222

Mishra AK and Bhatnagar SP. 1995. Direct shoot regeneration from the leaf explant of cucumber (*Cucumis sativus* L.). *Phytomorphology,* **45**: 47-55.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant,* **15**: 473-497

Pandey BP 2000. *Economic Botany,* pp 76-77. New Delhi: S. Chand and Co. Ltd

Phillips GC and Hubstenberger JF. 1985. Organogenesis in pepper tissue culture. *Plant cell Tissue*. Org. Cult. **4**: 262-269.

Singh MN Kathal R and Bhatnagar SP. 1990. Regeneration of plants from hypocotyl and cotyledon cultures of *Cucumis melo* cv pusa Maduras. *Phytomorphology,* **40**: 401-405.

Singh MN Mishra AK and Bhatnagar SP. 1996. *In vitro* production of plants from cotyledon explant of *Cucumis melo* L. and their successful transfer to field. *Phytomorphology,* **46**: 395-402.

Stipp LCL Mendes BMJ Piedade SMDS and Rodriguez APM. 2001. *In vitro* morphogenesis of *Cucumis melo* var. inodorus. *Plant Cell Tissue and Organ Culture,* **65**: 81-89.