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# EFFECT OF HORMONE CONCENTRATIONS AND AGE OF EXPLANT ON MULTIPLE SHOOT REGENERATION FROM COTYLEDON OF CUCUMBER

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## ABSTRACT

The surface sterilized seeds of cucumber cv. Poinsett 76 were germinated on MS (Murashige and Skoog, 1962) basal medium supplemented with 3% sucrose and solidified with 0.7% (w/v) agar at 26± 2º C in light. Cotyledon explants excised from 5-day- old *in vitro* raised seedlings of cucumber (*cucumissativus* L.) were cultured on MS medium containing different concentrations of BAP(0-10 µM) . When cotyledon explants were cultured on MS medium containing 1.0 µM BAP showed maximum response in terms of multiple shoot formation. The effect of different age of explants from 3-, 5-, 7-, 9- and 11days was also studied. The 5-d old *in vitro*raised seedling showed maximum frequency of shoot regeneration. All the regenerated shoots were transferred to rooting medium containing 2.0 µM IBA. At this concentration of IBA, well developed roots were observed. Well established green plantlets were transferred to pots containing soil and grown up to maturity in the green house.

**Keywords**: Cotyledon, Benzylaminopurine, Shoots formation and MS Medium

## INTRODUCTION

Cucumber (*cucumissativus* L.) is a member of family Cucurbitaceae or gourd family consisting of110 genera's. These are grown in tropical and subtropical regions. It is world's fourth most cultivated crop. China is world's largest producer of cucumber. Cucumber is economically important because its fruits are edible. It is popularly used in salads, relishes and pickles. Its juice is used in treatment of various ulcers. It is good source of vitamins and minerals. Cucumber is most important summer vegetable crop in India grown on an area of about 45000 ha and with an annual production of about 698000MT during 2012–2013.Development of plant from a single cell of the callus is very effective step towards crop improvement. *In vitro*

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regeneration system offers large no. of healthy cucumber seedlings in a short period of time. *In vitro* plant regeneration of cucumber has been reported from various explants type involving cotyledons hypocotyls, petiole, embryonal axis anthers, and leaves (Mohinddinet al., 2005; Songet al., 2007; Vasudevanet al., 2008; Ugandharet al., 2013). Cytokinins in combination with auxins stimulate organogenesis and multiple shoot formation (Selvarajet al., 2007; Ugandharet al., 2011). Plant tissue culture provides an essential tool for establishment of new genetic variation and unique recombination (Plader et al., 1998; Rodevaet al., 2006; Todorovaet al., 2013). The present study describes the *in vitro* development of multiple shoots from cotyledon explant of Cucumber.

## MATERIALS AND METHODS

The seeds of Cucumber were obtained from Indian Agriculture Research Institute, New Delhi, India. These seeds were soaked in water for 5 minutes and seed coat was removed manually. Seeds were washed with alcohol for 30 seconds and surface sterilized with 0.2% mercuric chloride for 5 minutes. Then, seeds were washed with distilled water 3-4 times. Seeds were blotted dry on filter paper and germinated on MS (Murashige and Skoog, 1962) Basal medium supplemented with 3% sucrose and solidified with 0.7% (w/v) agar at 26± 2º C under 16 h photoperiod of cool-white fluorescent light of intensity 80µEm$^{-2}$s$^{-1}$.. All these steps were performed under aseptic conditions. After 5-d of culture, cotyledons were excised from the seedling with the help of sterilized scalpel.

The cotyledon explants were cultured on MS medium supplemented with different concentrations of BAP (0-10 µM). The effect of age of seedling was also studied by culturing the different (3-11) day's old explants on the medium. The explants were subculture on to fresh medium after every two weeks. The healthy green shoots were transferred to rooting medium. The data on the shoot regeneration response was recorded after one month of culture. Well-developed green plantlets were transferred to pots for further growth.

## RESULTS

The cotyledon explants showed efficient shoot formation on different concentrations of BAP (table 1). Highest multiple shoot regeneration was obtained from explants cultured on MS medium containing 1.0µMBAP (fig. 1). The shoot regeneration frequency increases with increase in BAP concentration up to1.0µM and then decreases with further increase in BAPconcentration. 5-d old seedling showed the highest frequency shoot regeneration on medium (table 2.).

**Figure1**. Plant formation from cotyledon explant of *Cucumis sativus L.*

A. seeds of cucumber B.Seeds cultured on MS Basal medium C. Seeds germinated on MS Basal medium

D. 5-d old seedling E. Explant excised from seedling F. Explants cultured on SRM (MS Basal+ 1µM BAP

G. Bud initiation from explant H. Shoots after 2 weeks of culture I. Shoots after 4 weeks of culture J.

Shoot on rooting medium (MS Basal + 2µM IBA) K Rooted shoot L. plantlet in the pot.

**Table 1**.Regeneration response of 5-d old cotyledon explants of *Cucumis sativus*l. on medium supplemented with

Different concentrations of 6-benzylaminopurine

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| Concentration of BAP (µM) | Percentage response (%) | Av. No. of shoots/explant | Av length of shoots (cm)±SE |
|---|---|---|---|
| 0.0 | 0 | 0 | 0 |

**Table 2**.Effect of age of the seedling on regeneration from cotyledon explant of *Cucumis sativus* L.

| Age of explant ( in days) | % regeneration response | Average no. of shoots/explant (Mean± S. E.) | Average shoots length (cm) (Mean± S. E.) |
|---|---|---|---|
| 3 | 69.4 | 4.1± 0.46 | 2.1 ±0.40 |
| 5 | 87.5 | 4.5 ±0.36 | 2.8 ±0.06 |
| 7 | 65.2 | 2.9 ±0.10 | 1.8±0.03 |
| 9 | 45.8 | 2.1±0.04 | 1.0 ±0.02 |
| 11 | 30.0 | 1.0 ±0.56 | 1.0 ±0.56 |

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| | | | |
|---|---|---|---|
| 0.5 | 58.3 | 2.4±0.18 | 1.8±0.08 |
| 1.0 | 87.5 | 4.5±0.13 | 2.8±0.07 |
| 2.0 | 55.5 | 2.0±0.52 | 2.1±0.08 |
| 5.0 | 41.6 | 1.3±0.11 | 1.2±0.03 |
| 10.0 | 0 | callus | - |

### Rooting of shoots

The green well developed shoots were excised from the explants and transferred to MS Basal medium containing IBA. The best rooting was obtained on medium containing 2.0µM IBA (table 3).

**Table 3.** Effect of IBA on rooting from shoots

| Concentration of IBA (µM) | Percentage of response | Average no. of roots (Mean ±S.E.) |
|---|---|---|
| 0 | 25 | 1.0 ± 0.11 |
| 0.5 | 40 | 1.6 ± 0.27 |
| 1.0 | 55 | 2.6 ± 0.33 |
| 2.0 | 70 | 3.0 ± 0.28 |

## ESTABLISHMENT OF PLANTLETS IN THE POTS

Most of shoots had produced roots within 2 weeks of culture. The green healthy plants were taken out from test tubes and washed in running tap water to remove the agar and transferred to pots containing soil. Each pot was enclosed in polybag and maintained in the plant growth chamber at 25± 2º C under 16 h photoperiod of cool-whitefluorescent light of intensity

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80μEm$^{-2}$s$^{-1}$. Polybags were punctured weekly. About 80% plants survived. Then, these plants were transferred to greenhouse till maturity.For each treatment, 24 explants were cultured and each experiment was repeated thrice. Visual observations of the cultures were taken every week. The data on the percentage of explants showing their respective results was recorded after one month of culture.

## DISCUSSION

In the present study, high frequency of multiple shoot regeneration was successfully achieved on MS medium supplemented with BAP. When the explants were cultured on MS basal medium no shoot formation occurs whereas explants on MS medium with hormone induce shoot formation within a week. The frequency of multiple shoot formation and number of explants showing shoots significantly increases with increase in BAP levels. The shoot regeneration response starts to decline with levels above 1.0 μMBAP. The length of shoots also gets decreased with increase in BAP concentration. Similar results were also obtained on multiple shoot regeneration from cotyledon explants using BAP in the medium (Gambley and Dodd 1990). BAP in combination with various other hormones is widely used to induce shoots from different explant types.The regeneration frequency and number of shoots per explant was also affected by different day old explant of cucumber. The explants cultured from the 5-d old *in vitro* grown seedlings showed the maximum shoot regeneration response. The shoot regeneration frequency and the number of regenerants decreased when the seedlings older than 5 days were used.This response was perhaps due to storage of reserve food to the embryonic axis in the cotyledons.The present study on *cucumis sativus* L. provides us an efficient *in vitro* regeneration protocol for multiple shoot regeneration from cotyledon explant of cucumber.Nearly 85% of cotyledon explants regenerated shoots which is much higher response. It is concluded that the manipulation of culture conditions using different concentrations of growth hormones and other parameters can provide a cost effective reproducible protocol.

## REFERENCES

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- PladerW., S.Malepszy, W.Burza, Z.Rusinowski (1998): The relationship between the regeneration system and genetic variability in the cucumber (Cucumis sativusL.). Euphytica 103, 9-15.
- Rodeva V.,S.Grozeva,V.Todorova(2006): In vitroanswer of Bulgarian pepper (Capsicum annuumL.) varieties. Genetika, Serbia 38, 129-136.
- Todorova V.,S.Grozeva,V.Rodeva,S.Masheva(2013): Breeding evaluation of pepper lines obtained by in vitroanther culture. Genetika, Serbia 45,601-610.

- Gambley R. L. and Dodd W. A.1990. An in vitrotechnique for the production of de novo multiple shoots in cotyledon explants of cucumber (Cucumis sativusL.). Plant Cell Tissue Organ Cultue, 20: 177-183.
- Mohinddin A.K.M.,Z.C.Abdullah,M.K.U.Chowdhury,S.Napis(2005): Enhancement of dventitious shoot regeneration in Cucumis sativusL. using AgNO3. Plant Tissue Culture 15, 15-23.
- SongH.,Q.F.Lou,X.D.Luo,J.N.Wolukau,W.P.Diao,C.T.Qian,J.F.Chen(2007):Regeneration of doubled haploid plants by androgenesis of cucumber (Cucumis sativusL.). Plant Cell, Tissue and Organ Culture 90, 245-254.
- Gandhar T.,T.Srilatha,M.A.Imran(2013): Callus induction and somatic embryogenesisfrom leaf explants of Cucumber(Cucumis sativumL.). International Journal of Integrative sciences, Innovation and Technology2, 29-33.
- Vasudevana,N.Selvaraj,A.Ganapathi,S.Kasthurirengan,R.V.Anbazhagan,M.Manickavasagam,C.W.Choi(2008): Leucine and spermidine enhance shoot differentiation in cucumber (Cucumis sativusL.). In Vitro Cellular & Developmental Biology - Plant 44, 300-306.
- Selvaraj N.,A.Vasudevan,M.Manickavasagam,S.Kasthurirengan,A.Ganapathi (2007): High frequency shoot regeneration from cotyledon explants of cucumber via organogenesis. ScientiaHorticulturae 112, 2-8.
- Gandhar T.,M.Venkateshwarrlu,G.Begum,T.Srilatha,K.Jaganmohanreddy(2011): In Vitro plant regeneration of Cucumber(Cucumis sativum(L.)from cotyledon and hypocotyl explants. Science Research Reporter 1, 164-169.

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 05 Issue 12
April 2018

क Murashige, T. and F. Skoog, 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture. Physiol. Plant., 15: 473-479.