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ORIGINAL RESEARCH ARTICLE

In vitro Multiplication of Vegetable Crop in Brinjal (*Solanum melongena* L.)

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ABSTRACT

The present investigation was undertaken *Solanum melongena* L. is an important medicinal plant *in vitro* culture techniques may be used for the biotechnological advancement of this plant. Commercially prepare MS medium was used as comparison medium for regeneration of shoot index of *Solanum melongena* L. The observations were record after five weeks of inoculation showed callus induction of MS medium. After sub culturing of callus on fresh MS medium supplemented with 2.4-D callus induction was increased in size. Explants were placed an MS media under perfectly sterilized conditions and incubated at 25±2ºCfor specific time period. Callus initiation time callus growth period were found to vary in different explants as well as in different culture media of the same explants. Plants having garden soil with organic compost and irrigated with half strength MS solution and water desirable to achieve high survival percentage.

Key words: Eggplant, Foodcrap, *Solanum melongena*, Solanaceae. Vegetable crop, Brinjal.

INTRODUCTION

Among the immature vegetables, brinjal is not only occupies major area in cultivation but also by consumption in Tamilnadu. Egg plant, *Solanum melongena* L. also known as Aubergine in Europe, Brinjal in India, is one of the non tuberous species of the night shade family Solanaceae (kantharaja and Golegaonkar, 2004). The varieties of *Solanum melongena* L. show a wide range of fruit shapes and colours, ranging from white, yellow, green through degrees of purple pigmentation to almost black (Maral moranditochae *et al*., 2011). It is an economically important crop in Asia, Africa and the Subtropics (India and Central America) and it is also cultivated in some warm temperate regions of the Mediterranean and South America (Sichahkr *et al*., 1993). The fruits are known for being low in calories and having a mineral composition beneficial for human health. They are also rich source of Potassium, Magnesium, Calcium and Iron (Zenia *et al*., 2008). Unripe fruit of egg plant is primarily used as cooking vegetable (Gargi chakravarty *et al*., 2011). Egg plant is a perennial but grown commercially as an annual crop. Asia has the largest egg plant production which comprises more than 90% of the world production and 299,770 ha in area of cultivation. It has many medicinal values and its fruit helps to lower the blood cholesterol levels, and is suitable as a part of a diet to help regulate high blood pressure (Lawande *et al*., 1998).

MATERIALS AND METHODS

The present study (*Solanum melongena* L.) was collected from Faculty of agriculture, Annamalai University, annamalainagar, Tamilnadu.

Culture media

Major and minor nutrients were used according to the description of supplemented with only sucrose (20 g/ L).CaCL2 (166.2 mg/ L) and 0.8 agar. The pH of the medium was adjusted between 5.6 – 5.8 before gelling with agar (0.6% W/V) and autoclaved at 1210 C under 15 Ib pressure for 15 – 20 min.

Preparation of stock solution

1. The macronutrients are dissolved in 500ml double distilled water.
2. The micronutrients are dissolved in 250 ml of double distilled water.
3. I was dissolved in 250 ml of double distilled water.
4. The vitamins were dissolved in 100 ml of double distilled water. Agar and sucrose were weighted as when added. But stock solutions were prepared and stored in refrigerator, stock solution

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of vitamins were prepared and stored in a deep freezer chamber.

Precautions in the preparation of media

1. All the stock solution was stored in glass bottles under refrigeration.
2. Iron stock was stored only in an amber colored bottle.
3. The bottle must thoroughly washed by double distilled water.
4. Details about the date and concentration of all stock solution were labeled with glass marking Pen.

Growth regulators

The following growth hormones were supplemented to the media individual as well as combination at different concentrations as maintained below:

1. NAA and BAP at the concentration of 0.5 to 3.0 mg/L.
2. NAA and BAP combination at the concentration of 0.5 to 0.3 mg/L.
3. NAA + GA3 and BAP + GA3 combination at the concentration of each 0.5 mg/L.

Sterilization of explants

The shoot tip explants were washed thoroughly in running tap water for 30 minutes and placed in detergent solutions. After that the explants were washed thoroughly with double distilled water. Then the explants were transferred in front of laminar airflow and disinfected with 0.1 percent v/v aqueous mercuric chloride ($HgCl_2$) for a period of 8 minutes. Finally, they were rinsed with several changes of sterilized distilled water. The disinfected plantlets were taken for inoculation.

Inoculation

Solidified MS basal medium containing B5 vitamins, 0.8 % agar, 3 % sucrose and different combination of growth regulators as previously specified (0.5 to 3.0 mg/L) were used .The shoot tip were inoculated vertically on the MS medium containing different concentration of BAP (0.5 to 3.0 mg/L). Before, starting inoculation all the laboratory instrument such as media containing were transferred to laminar airflow chamber and the platform surface of the chamber was swapped with 70% alcohol after swapping the UV light switched on for 30 minutes. After that the UV light became switched “off” and “on” the ordinary light. Before inoculation hands were rinsed with 70 percent alcohol. Then the explants were inoculated on the medium. The instruments used aseptic manipulations were sterilized by dipping in 70 percent alcohol followed by flaming and cooling. The inoculations were carried out in vicinity of flame. The explants has been put in it and immediately covered with cotton plug. Each treatment represented three cultures tube. The cultures were kept under 16 hrs light/ day (2400 Lax) photoperiod at 25± 20C. The shoot regeneration was assessed after 4 weeks in culture length of 2.0 and above. The subsequent sub culture was made on the medium, which shared maximum shoot regeneration.

RESULTS AND DISCUSSION

Among two incubation conditions treated for hardening, plantlets maintained in unsterilized soil with ordinary water and open shade conditions showed maximum success which could be due to the synergistic effect of efficient microbes available in the soil (Selvaraj *et al*., 2001; Shahzad and Siddiqui, 2000; Sharp *et al*., 1980). Propagules with more number of roots exhibited low survivability as previously noticed by (Durnad *et al*., 1982).

Thus, from the above discussion, it can be concluded that leaf explants are suitable for clonally propagation, leaf explants may be used for higher rate of shoot multiplication. The protocol standardized in the present study is reproducible and be used in future biotechnological improvement programme of this medicinal plant.

Shoot explants cultured on MS-B5 basal medium without growth regulator thrived only for few days and did not show any morphogenetic response. Among four auxin (2,4-D, NAA,IBA and IAA) tested to find out the effect on the induction somatic embryos from shoot explants, 1.5 mg/l, 2,4-D was found to be more effective in inducing embryogenic cultures. After the inoculation, slight colour change in explants was followed by shrinkage. Minute nodule like structures appeared on the upper surface of shoot veins and from cut margins within 7 days of inoculation. Later these nodules like pre-embryogenic structures differentiated into different stages of embryo development like globular, heart, torpedo and cotyledonary and ultimately grew into plantlets. Maximum number of embryos were induced in the 1.5 mg/l, 2,4-D and minimum was observed in both lower (1.0 mg/l) and hight (2.0mg/l) concentrations of 2,4-D. Maturation and development of embryo into plantlets needed same subculture medium containing same concentration of 2, 4-D complete plantlets were recovered from 50^{th} day onwards (**Table 1**).

Medium containing NAA was equally good for embryogenesis. But the rate and percentage of differentiation was comparatively very less. Moreover, the differentiated embryos had a tendency to form callus. Among different concentrations of NAA (0.5-3 mg/l), NAA at 2 mg/l favoured the induction of somatic embryos from shoot explants. All other concentrations failed, to develop somatic embryos. Further maturation and development of develop were found difficult on NAA supplemented medium. Plantlets could not be recovered even in subculture on different concentration of NAA supplemented media (**Table 2**). The media containing different concentration of IBA or IAA (0.5-3 mg/l) inhibited differentiation and suppressed the somatic embryogenesis.

Solid media viz., MS, B5 and MS-B5 containing 2,4-D (1.5 mg/l) and NAA (2 mg/l) were tested for their efficiency on the induction and development of somatic embryos from the shoot. Among the two different conditions, 2,4-D showed maximum percentage of response, whereas NAA had lesser effect on the induction of somatic embryogenesis. Induction of embryogenesis was higher in the MS-B5 solid medium. Somatic embryogenesiswas not accomplished in B5 medium (**Table 3**).

Four different carbohydrates (Sugar, maltose, fructose and glucose) were tested at different levels to study their effect on embryogenesis. Among the four carbohydrates, sucrose was most promising for embryogenesis. Glucose and fructose reduced the frequency of embryogenesis. Maltose showed very less response on embryogenesis. Of the different concentrations of sucrose tested, 3 percent was most effective. Comparison of sucrose with commercial cane sugar on somatic embryogenesis showed similar result and the cane sugar was not found inferior to sucrose (**Table 4**).

Shoot explants cultured on MS-B5 basal medium without growth regulators did not thrive and shrivelled off. Combination of auxins and cytokinins promoted either callus or shoot but formation. Explant had a tendency of producing callus when inoculated horizontally on the surface of the medium. Both NAA and BAP each at a level of 2.0 mg/1 improved the ability of shoot explants and produced direct regenerations of adventitious buds from the margins. After three weeks of primary culture, large number of adventitious shoot buds appeared on the surface of the explants. Nearly 94 percent culture showed positive response and maximum number of shoot buds were noticed on the medium with NAA and BAP, each at 2.0 mg/1 (**Table 5**). Any increase or decrease in the concentration of NAA and BAP reduced the shoot bud initiation and enhanced the callus formation.

The shoot explants exposed to various concentration and combinations of NAA and Kn exhibited positive response for callus mediated organogenesis. Explants when cultured on the medium containing NAA and Kn each at 1.5 mg/1 produced maximum number of callus mediated adventitious shoot buds (**Table 6**).

The medium containing NAA and BAP was observed to induce shoot and root simultaneously whereas in the medium containing NAA and Kn, shoot formation occurred first and it was followed by root formation. The roots are lesser in number and thicker than the plants of medium containing NAA + BAP medium.

Different media viz., MS, B5, MS-B5 containing NAA and BAP (each 2.0 mg/1) Kn (each 1.5 mg/1) were tested for the influence of shoot regeneration. Among the two different conditions NAA and BAP showed maximum percentage of response, whereas NAA and Kn regenerated maximum number of adventitious shoot along with MS-B5 medium (**Table 7**). The same medium also supported the growth of healthy shoots with maximum mean shoot length while the other media produced lesser number of regenerated shoots in the order of MS and B5.

Among four different sugars tested, sucrose showed maximum response of shoot induction. It was followed by glucose and fructose, while maltose was inefficient. To find out the optimum level of sucrose requirement, different levels of sucrose were (1.5%) tested in MS-B5 media. Three percent level was found to be optimum. Comparison of sucrose with commercial cane sugar on shoot regeneration showed similar results and the cane sugar was not found inferior to sucrose.

In vitro raised plantlets via somatic embryogenesis were directly taken for hardening. Shoots developed through organogenesis (2.0 cm and above in length) were excised from the culture tube and sub cultured into medium containing two different concentration of MS salts (half and full strength) supplemented with 0.5 to 2.0 mg/1 IBA. Rooted shoots acclimatized very well following their transfer directly to plastic

pots containing mixture 1:1:1 sterilized or unsterilized garden soil, Red soil and compost. The plants were watered using ½ strength MS nutrient solution or water as and when required. Nearly 50 percent of the plantlets were maintained under A/C room after covering with plastic bags. The remaining plantlets were placed under shade with a short exposure (2.3 hrs) to sunlight, every day. The plants grown from both conditions were transferred to larger pots containing 2 litre of the soil mixture (Garden- 1, Red- 1, Compost- 1) and placed in green house. Then, the plants were transplanted to field. The plants growing in field were observed for their growth. Around 60-70 percent of success was achieved following transplantation of the regenerated plant from shade incubation to the field condition. Plants maintained under A/C showed lesser success (35-45%) (**Table 8**).

Table1: Effect of different concentrations of 2,4-D on induction of somatic embryogenesis from shoot in MS-B5 solid medium in the primary culture

Growth regulator 2,4-D	Globular		Heart		Torpedo		Tiny Plantlet
	Culture showing response (%)	Mean No. of somatic embryo / explants	Culture showing response (%)	Mean No. of somatic embryo / explants	Culture showing response (%)	Mean No. Of somatic embryo / explants	
0.5	50	3	44	2	38	1	0.5
1.0	67	8	63	5	59	4	1.3
1.5	90	22	81	18	69	12	9.2
2.0	65	7	58	5	52	3	1.1
2.5	45	2	42	1	35	n.d	n.d
3.0	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.d. = not determined due to nil response

Table 2: Effect of different concentrations of NAA on induction of somatic embryogenesis from shoot in MS-B5 solid medium in the primary culture

Growth regulator 2,4-D	Globular		Heart		Torpedo		Tiny Plantlet
	Culture showing response (%)	Mean No. of somatic embryo / explants	Culture showing response (%)	Mean No. of somatic embryo / explants	Culture showing response (%)	Mean No. Of somatic embryo / explants	
0.5	45	4	41	2	n.d	n.d	n.d
1.0	49	5	44	4	n.d	n.d	n.d
1.5	63	8	59	6	n.d	n.d	n.d
2.0	82	18	73	15	52	3	n.d
2.5	43	4	38	2	35	n.d	n.d
3.0	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.d = not determined due to nil response

Table 3: Effect of different types of medium containing 2,4-D (1.5 mg/l) on the induction of somatic embryogenesis in the primary culture

Medium	Globular		Heart		Torpedo		Tiny Plantlet
	Culture showing response (%)	Mean No. of somatic embryo / explants	Culture showing response (%)	Mean No. of somatic embryo / explants	Culture showing response (%)	Mean No. Of somatic embryo / explants	
MS	70	8	61	5	52	3	1.1
B5	48	5	40	2	n.d	n.d	n.d
MS-B5	90	22	81	18	69	12	9.2

n. d = not determined due to nil response

Table 4: Effect of different carbohydrates on somatic embryogenesis of shoot in MS-B5 solid medium containing 2,4-D (1.5 mg/l) in the primary culture of after five weeks interval

Carbohydrates	Globular		Heart		Torpedo		Tiny Plantlet
	Culture showing response (%)	Mean No. of somatic embryo / explants	Culture showing response (%)	Mean No. of matic embryo / explants	Culture showing response (%)	MeanNo. of somatic embryo / explants	
Glucose 3%	48	3	43	2	37	1	0.5
Fructose 3%	42	2	35	1	29	n.d.	n.d.
Maltose 3%	13	1	n.d.	n.d.	n.d.	n.d.	n.d.
Sucrose 1%	68	5	64	3	3	1	0.5
Sucrose 2%	83	15	77	11	11	7	4.0
Sucrose 3%	90	22	81	18	18	12	9.2
Sucrose 4%	87	17	79	13	13	10	8.1
Sucrose 5%	71	4	66	2	2	1	0.5
Cane sugar 3%	84	18	71	12	12	8	4.7

n.d = not determined due to nil response

Table 5: Mean number of countable shoots (average length of 2.0 cm or longer) produced per shoot explants on multiplication media with various combination and concentration of NAA and BAP after five weeks of culture

Growth regulators (mg/l)		Culture shooting response (%)	Mean number of shoot buds/ explants	Average shoot length (2.0 cm <)
NAA	BAP			

0.5	0.5	n.d.	n.d.	n.d.
1.0	1.0	32	08	1.5
1.5	1.5	79	21	2.4
2.0	2.0	94	40	2.0
2.5	2.5	75	18	1.6
3.0	3.0	n.d.	n.d.	n.d.

n.d = not determined due to nil response.

Table 6: Effect of media containing NAA and BAP/Kn on shoot regeneration after six weeks interval

Explants	Media	Culture shooting response (%)	Average number of shoot buds/ explants	Average shoot length (2 cm <)
Shoot (1)	MS	82	18	1.8
	B5	75	09	1.2
	MS-B5	94	10	2.0
Shoot (2)	MS	72	17	1.4
	B5	66	08	1.1
	MS-B5	81	24	2.3

1. NAA and BAP each at 0.2 mg/l
2. NAA and Kn each at 1.5 mg/l

Table 7: Effect of carbohydrate on shoot regeneration after eight weeks interval on MS-B5 medium containing NAA and BAP/Kn

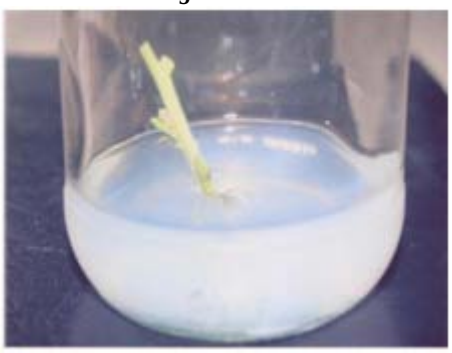
Carbohydrates	Mean number of shoot bud/Shoot explants	
	(1)	(2)
Glucose 3%	11	7
Fructose 3%	8	5
Maltose 3%	1	1
Sucrose 1%	9	11
Sucrose 2%	27	17
Sucrose 3%	39	23
Sucrose 4%	28	16
Sucrose 5%	12	10
Cane sugar 3%	35	20

1. NAA and BAP each at 0.2 mg/l
2. NAA and Kn each at 1.5 mg/l

Table 8: Effect of nutrient concentration of MS-B5 medium and IBA on rooting of shoot lets after two weeks of culture

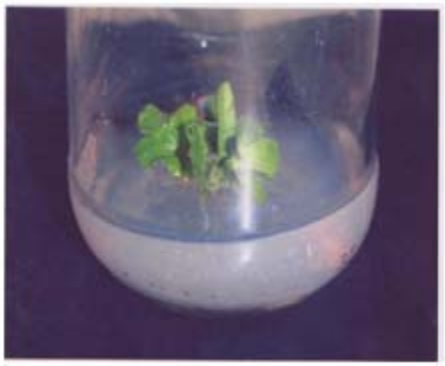
Medium (Strength)	IBA(mg/l)	Culture showing response (%)	Average number of root explants	Average root length (cm)
MS-B5	0.5	82	17	2.3
MS-B5	1.0	90	22	4.8
MS-B5	1.5	77	18	2.5
MS-B5	2.0	69	15	1.8
MS1/2-B5	0.5	71	15	2.7
MS1/2-B5	1.0	87	18	4.2
MS1/2-B5	1.5	68	16	2.5
MS1/2-B5	2.0	54	13	1.6

Plate: *Solanum melongena* L.



Initiation of *Solanum melongena* after two weeks interval

Multiplication of *Solanum melongena* after five weeks interval



Shoot and root period of *Solanum melongena* after seven weeks interval

Mother plant of *Solanum melongena*

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