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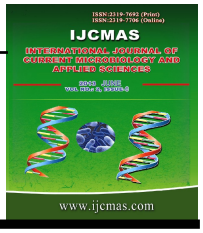

Original Research Article

An improved method for the in vitro propagation of *Solanum melongena* L.

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A B S T R A C T

Brinjal is a tropical vegetable cultivated throughout the tropical regions of India. The varieties of *Solanum melongena* L. display a wide range of fruit shapes and colors. An experiment was conducted for the production of tolerant variety with the native variety of brinjal cv. Valuthalai produce long and white coloured fruits. The callus regeneration efficiency of tender shoot tip, hypocotyls, leaf and stem explant were analysed using MS basal media supplemented with different combinations and concentrations of phytohormones. The frequency of callus induction for shoot tip, hypocotyls, leaves, stem explant were 76.43%, 81.56%, 74.37% and 84.92% respectively and the maximum proliferation found at 0.6mg/l 2, 4-D. The multiple shoot induction was observed with the various concentrations of auxins and cytokinins such as BAP, KIN, IAA, NAA. The effectiveness was observed in their various combining rations for the induction and proliferation. The maximum efficiency of multiple shooting was obtained at 0.2mg/l BAP and 0.6mg/l NAA and o.4 mg/l IAA. The successful rooting was recorded on media supplemented with 0.4mg/l IBA.

Keywords

Shoot tips; co-cultivation; rooting, explants; phyto-hormones.

Introduction

Eggplant can be consumed raw, boiled, cooked stuffed. It can be used in variety of preparations like soups, pickles, etc. (Asaolu *et al*., 2002). The area under brinjal cultivation is estimated as 0.51 million ha with total production of 8,200,00Metric tons (FAO, 2005). During its cultivation the total loss caused by this insect pest is 5 - 20 % in shoot and 10 - 70 % in fruit (Das *et al.*, 2000). Several groups have provided evidence that eggplant extracts have a significant effect in reducing blood and liver cholesterol rates in humans and adult rats (Silva *et al.*, 1999).

MS basal medium was also reported to be effective for root induction and growth for a Malaysian eggplant variety (Taha and Tijan, 2002). It was reported that the hormone free MS medium was sufficient for shoot elongation (Franklin and Sita, 2003)**.** It was reported that biotechnology in eggplant have focused in aspects related to genetic resources and improvement of agronomic traits (Collonier *et al.*, 2001).

It is a good source of vitamins and minerals (Rotino *et al.*,1990). It is low in calories and high in potassium and so could be used to control diabetes, hypertension and obesity (Singh *et al.*, 2006). Superoxide anion radical scavenging and iron chelating activities of nasunin a major component of anthocyanin pigment in eggplant peels were demonstrated by electron spin resonance (Noda *et al*., 2000). Recently it was reported, that purified anthocyanin from eggplant protected mice against cyclophosphamide mutagenicity *in vivo* (Azevedo *et al*., 2007).

The object of the study was to develop disease resistant variety of valuthalai plants which has higher nutritive value after the *Agrobacterium tumefaciens* mediated transformation for the T-DNA insertion.

Materials and Methods

The native brinjal variety Valuthalai was collected from different regions of Kerala. It has great commercial importance. The size of the fruit is considerably long. This variety is disease resistant to bacteria, virus, mycoplasma. It is a drought resistant plant breed. The life cycle of the plant is an annum.

The high of the plant averages about 1.5 metres. The valuthalai brinjal seed variety collected from the regions of Kerala and specimen were maintained in the green house of K.S.Rangasamy college of Technology, Tiruchengode. Explants for *in vitro* studies were collected from the garden plant.

The seeds obtained from the valuthalai brinjal variety was carefully drawn and soaked in water for 2 hours.

The seedlings were washed in running tap water and the unwanted waste materials and microbes were surface sterilized. The seedlings were first washed with running tap water for 15 to 20 minutes followed by tween 20 for 10 minutes. Then it was washed with 1% bavistin and 70% ethanol. The seedlings were washed with sterile distilled water and further with the 0.1% mercuric chloride.

Macronutrients (50x) which was composed of various constituents of nitrogen, phosphorus, potassium which helps for the growth of plant in higher rate. Micronutrients (100x) were prepared with trace elements which prevents necrosis of the plant. Additional constituents such as vitamins, myoinositol, iron source, amino acids were prepared and stored at -20ºC. Carbohydrate source was provided to the media freshly during preparation. Agar as a supporting media of 0.8% was added (Murashige and Skoog's media, 1962). The pH of the medium was adjusted to 5.6 to 5.8. The surface sterilized valuthalai seeds maintained at the proper temperature at 25º±2ºC with a 16 hr photoperiod with the light intensity of 3000 Lux. After 7 days germination of plantlets from the seedlings were observed. The test tube and cotton tube method was used for the maximum proliferation.

For multiple shoot induction the cotyledon, shoot tips explants were chosen. The MS media supplemented with various hormones were utilized. The hormone BAP alone was used in the range from 0.01 to 1.0 g/l in the media or in the combination with the IAA, KIN and NAA was also used. The varying concentration from 0.05 to 1.0 g/l IAA was also used with BAP. The NAA concentration from 0.2 to 1.5 g/l was used in combination with 0.2mg/l BAP for multiple shoot

induction. KIN in the concentration ranging from 0.2 to 1.0 g/l with BAP, IAA and NAA was used to determine the maximum multiple shoot induction as alone and combination of hormones. The rooting was initiated by the hormone IBA provided with the varying concentration ranging from 0.2 to 1.0mg/l. The shoot length, rooting efficiency, induction and proliferation of the explants in the hormones were calculated subsequently. After the maximum germination in the provided media, it was subcultured periodically for every 20 days interval. After the multiple shoot with rooting formation, the callus was induced for the Agrobacterium co-cultivation process. Hence 2, 4-D was used for the callus proliferation. With the concentration from 0.2 to 1.0 g/l 2, 4-D was used. For the callus induction as cut base callus from explants 2, 4-D in combination with BAP was used in the combination of both ranging from 0.2 to 1.0g/l. From each calli regeneration media with BAP was used to regenerate the plant from the transformed plants with Agrobacterium T-DNA insertion. The growth of the plants after transformation was observed in the regeneration media.

The developed plantlets from the callus were transferred into hardening process to determine the growth range of the transplanted plantlets with their disease resistance capability with the control plants. The valuthalai *in vitro* plantlets washed with water to remove agar followed by washed with 1% bavistin were transferred to plastic tray, which composed of vermicompost, red soil, clay in the ratio of 1:2:1. It showed quick response of the plantlets in the green house. The germination efficiency of the plantlets was 80%. The rate of growth of the plants in the green house from the state of in-vitro to field transformation was determined with their varying sort of growth parameters and disease resistant characteristics.

Results and Discussion

In vitro seed germination was achieved on cotton bed method and test tube method on MS basal medium. At the 8^{th} day seeds were germinated and by the 18^{th} day the maximum rate of growth were observed. It was reported that the process optimization is useful in large scale production of *in vitro* shoots through high efficiency automation in future facilitating micropropagation (Padma Mallaya *et al*., 2011). In the micropropagation of shoot tip and cotyledon explants, the shoot induction was good at the concentration of 0.2g/l BAP. At lower concentrations of BAP, the results of shoot induction were not observed (Table 3, Figure 2).

It was reported that the combination of 2.0 mg/l BAP+0.5 mg/l NAA required 8.2 days for callus induction from stem explants (Table 5). On the other hand, the combination of 2.0 mg/l BAP + 0.1 mg/l NAA needed 10.8 days for callus induction from root explants (Table 4; Figure 3). So, callus induction from stem required minimum days (Ray *et al.,* 2011). The results coincide with the results of others were the multiple shoot induction were observed in the combination of BAP and IAA but with the concentration of 0.2mg/l BAP and 0.6 mg/l NAA with higher shoot induction with the formation of cut base callus (Figure 1). So it induced for the multiple clone production. It was reported that at the concentration of 1.0mg/l BAP with 2.5mg/l IAA showed greater response for the shoot regeneration with the height of 2.5 cm (Mason *et al.*, 2002). The length of the plant was also maximum after 5 weeks of photoperiod. In

Table.1 Effect of bap on multiple shoot induction in brinjal

S.No	Concentration of BAP(mg/l)	Total no of explants	No of explants responded	Percentage of response (%)	M±SD
1.	0.01	20	11	55	3.56±2.1
2.	0.02	20	12	60	4.23±1.9
3.	0.03	20	10	45	5.2±2.6
4.	0.04	20	17	85	4.1±3.4
5.	0.05	20	16	80	3.9±1.5
6.	0.06	20	18	90	2.96±2.3
7.	0.07	20	13	65	2.8±1.6
8.	0.08	20	16	80	3.3±2.3
9.	0.10	20	18	90	4.6±3.1
10.	0.15	20	18	90	5.9±2.5
11.	0.20	20	19	95	5.3±1.4
12.	0.50	20	18	90	4.6±2.6
13.	1.0	20	17	85	2.5±1.9

Table.2 Mean and standard deviation for the shoot length formation in the multiple shoots induction with BAP and IAA

S.No	BAP (mg/l)	IAA (mg/l)	Total no of explants	Total no of explants responded	M±SD	Percentage of induction
1.	0.2	0.05	20	15	5.4±2.8	75%
2.	0.2	0.1	20	18	6.1±3.2	90%
3.	0.2	0.2	20	17	6.0±4.1	85%
4.	0.2	0.4	20	19	6.5±1.9	95%
5.	0.2	0.6	20	18	5.9±2.3	90%

Figure.1 Graphical representation for the combination of BAP and IAA

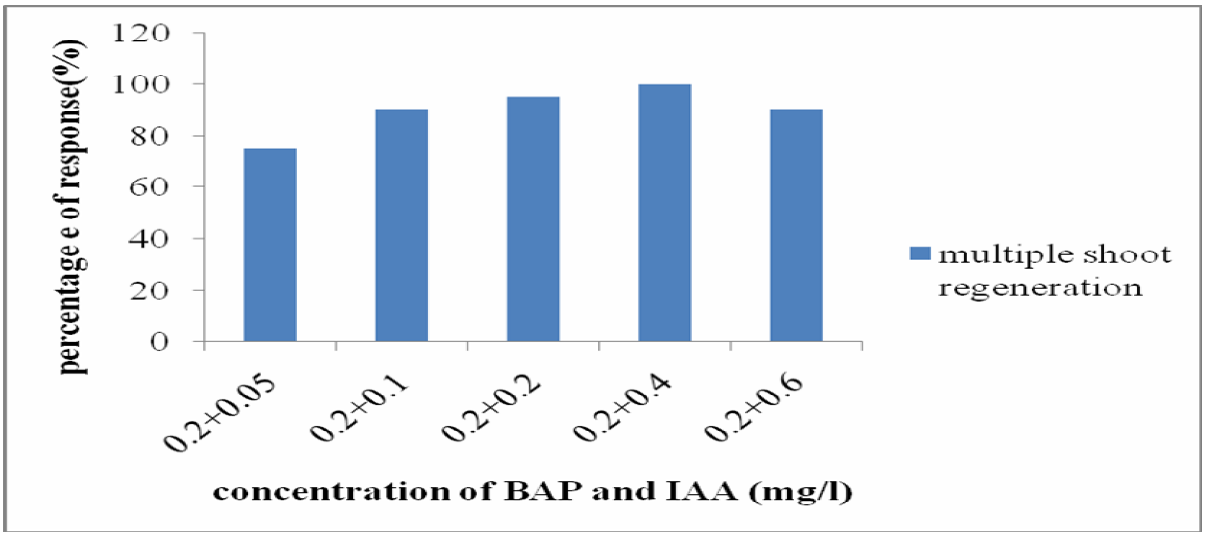

Table.3 Effects of BAP and NAA on shoot regeneration

S.No	BAP (mg/l)	NAA (mg/l)	Total no of explants	Total no of explants responded	M±SD	Percentage of regeneration (%)
1.	0.2	0.2	20	15	4.1±3.2	75
2.	0.5	0.4	20	14	2.4±4.5	70
3.	1.0	0.6	20	17	3.6±1.2	85
4.	1.2	0.8	20	13	3.2±1.5	65
5.	1.4	1.0	20	12	1.9±1.8	60

Figure.2 Graphical representation for the effect of BAP and NAA

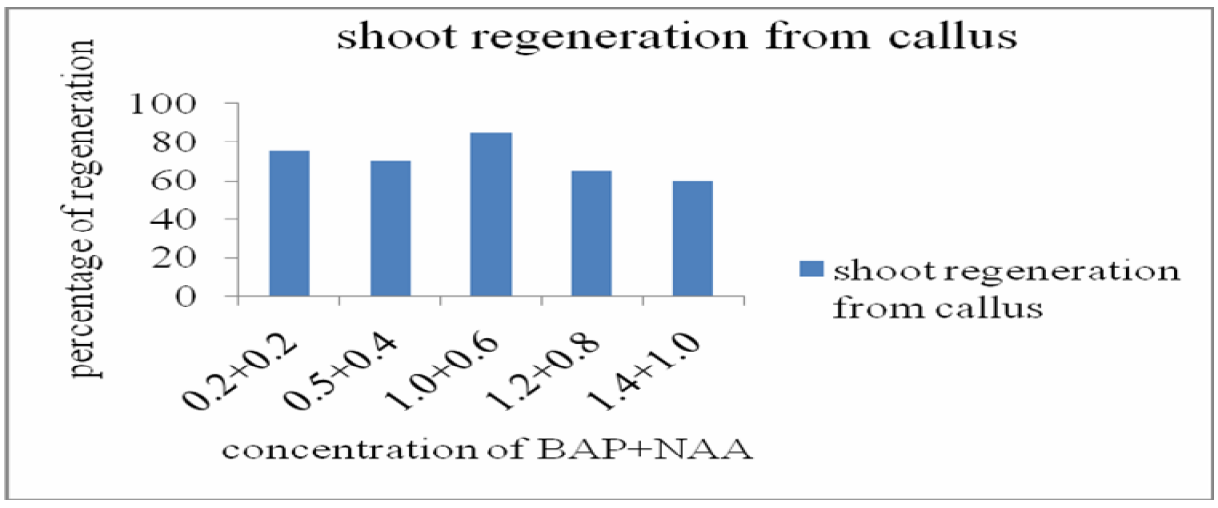

Table.4 Effect of IBA on root induction

S.No	IBA (mg/l)	Total no of explants	Total no of explants responded	M±SD	Percentage of response (%)
1.	0.2	20	9	2.5±2.9	45
2.	0.4	20	15	3.1±2.8	75
3.	0.6	20	11	2.94±2.0	55
4.	0.8	20	13	3.56±2.9	65
5.	1.0	20	10	2.6±2.5	50

Figure.3 Graphical representation for the effect of IBA on root inductions

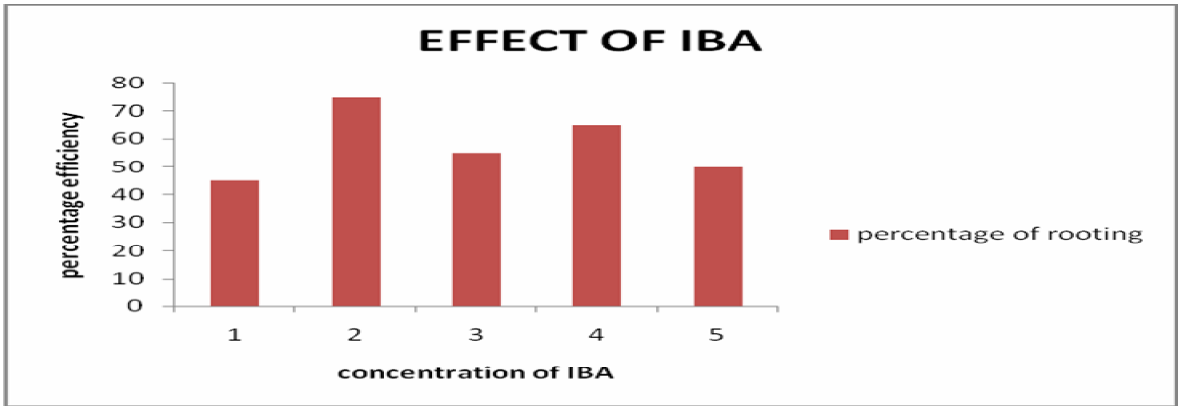

Table.5 Effect of 2, 4-d and bap on callus induction

S.No	BAP (mg/l)	2, 4-D (mg/ml)	Total no of explants	Total no of explants responded	M±SD	Percentage of callusing (%)
1.	0.2	0.2	20	18	3.2±2.1	90
2.	0.4	0.4	20	16	3.9±1.7	80
3.	0.2	0.6	20	19	3.4±1.2	95
4.	0.8	0.8	20	14	2.9±3.2	70
5.	1.0	1.0	20	17	1.8±1.9	85

the valuthalai variety, IAA (0.4 g/l) with BAP (0.2 g/l) induced multiple shoot formation from the callus (Table 1).

In the combinations of BAP, NAA, IAA the multiple shoot formation were effective in the concentration of 0.2+0.6+0.4g/l (Table 2). The maximum rate of proliferation was observed in the above combination. The results of multiple shoot proliferation relates with the embryogenic callus to Embryoid formation (Swamynathan *et al.,* 2010). The rooting was observed effectively in the rooting hormone IBA at the concentration of 0.4g/l efficiently. The rooting was also observed in the MS media without hormones in the subcultured stage of shoot tip and cotyledon explants. The callus induction was observed higher at the combinatorial effect of 0.2g/l BAP and 0.6g/l 2, 4-D. Hard and soft nature of the callus was observed depending on the explants and hormone. Browning of the callus was also observed which showed the synthesis of secondary metabolites production.

The callus developed was co-cultivated (Claudia Magioli and Elisabeth Mansur, 2005). The callus was regenerated with higher degree with the various combinations of BAP, KIN, IBA and IAA. The maximum rate of plant regeneration was observed in the plantlets after transformation. The developed plantlets were transferred into the plastic tray after slow progress into green house and farming. The response of the plant was found to be higher in the soil constituting the clay+red soil+vermicompost in the ratio of 1:2:1 where the observed rate of disease resistance with the control plantlets was higher. Thus the results showed that the disease resistant plant varieties have been induced.

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