

Direct Organogenesis (Shoot and Root) of Egg Plant (*Solanum melongena* L.) Through Tissue Culture

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Abstract: Brinjal (*Solanum melongena* L.) is one of the most popular, nutritional vegetable crops. It plays a vital role in the national economy as a cash crop. Major loss of this crop plant throughout world occurs due to biotic as well as abiotic stresses which cause to loss of maximum yield. Brinjal improvement using conventional approaches have been aimed for improvement of agricultural traits such as size of fruit, weight, shape and resistance to diseases, pest's penetration and stress of brinjal. For these purposes, tissue culture was established as a conventional approach for brinjal. There is a different combination of different hormones used for in vitro regeneration of brinjal. The hormones 2, 4- D, IAA, BAP, NAA and kinetin used in various combinations with different ratio. Explants such as stem and leaves were used for regeneration but stem showed better response. Explants showed direct shoot regeneration after about 4, 5 weeks of inoculation. Moreover regeneration of roots on two different types of media, one is hormone free MS media and other is supplemented with 0.5mg/l IAA occurred but hormone free MS media was most favorable as it has given rooting after about 2 to 3 weeks after shooting.

Key words: Direct Organogenesis • Tissue culture • Eggplant • Brinjal

INTRODUCTION

Eggplant (*Solanum melongena* L.) belongs to the family *Solanaceae* and genus *Solanum*. In all over the world there are about 25 refined species of a genus *Solanum* that includes the potato, tomato and various eggplant species [1]. *Solanum melongena* L. also called an eggplant and have different names in different societies and civilizations as aubergine or brinjal. It is a commercially important vegetable as well as a cash crop. It is found mostly in moderate and tropical parts of the globe. As compare to other crop plants like tomato, it is rich in vitamins and minerals that increase its total nutritional value [2].

It is vegetable crop grown in summer and covers about 8670 hectors of whole area of the Pakistan and its largest share for sowing area is subcontinent Punjab that shares about 4890 hectors and its production is about 60890 tones [3]. Plant tissue culture offers a well-organized method for making materials free from pathogen and preservation of germplasm in order to control upon this situation. For the improvement of crops normally

helpful tool is tissue culture as in plant breeding the probable value of tissue culturing has been extensively predicted. Principle of totipotency is responsible for regeneration of commercially important plants via tissue culture [4].

Tissue culture improved the quantity, yield and quality of crops through regeneration at high frequency and genetic engineering technologies. After culturing, explants produced callus on appropriate media. A lot of research programs have been taken out to examine factors that affect and enhance plant regeneration. After explants formation from seeds both callus induction from explants and regeneration require a suitable concentrations of plant growth hormones supplemented with cultured media. Tissue culture analysis data and protocols for embryo formation, organogenesis and protoplast culturing have been well recognized [5-7].

Plant regeneration from tissue culture is a vital component of biotechnology and it is necessary for change in genetic makeup of plants for making it diseased free [8]. A proficient and reproducible regeneration system *in vitro* is considered as a vital part of successful

transformation. *In vitro* regeneration of eggplant from different explants through organogenesis, there is a numeral reports available by Kamat and Rao [9], Sharma and Rajma [10] and Magioli, *et al.* [11] and for somatic embryogenesis reports available by Yadav, *et al.* [12].

Regeneration of shoot from segments of hypocotyls of eggplant has been documented in the presence of growth hormone IAA [2]. Shortly after this importance of tissue culture was noticed as it was a significant technique which was used for the increase yield of genotypes [13]. Formation of callus and ability of plants to regenerate have been deliberated or studied in eggplant from different explants such as root tip, leaf segments and shoot tip explants [14].

MATERIALS AND METHODS

Seeds of brinjal variety NS-797 were taken from Horticulture Department of National Agriculture Research Center, Faisalabad, Pakistan.

Sterilization: All materials for tissue culture lab experiment such as scissors, forceps, glassware's (Petri dishes, flasks, media jars and test tubes) washed with running tap water and after wrapping in aluminum foil or newspaper, autoclaved at pressure 15 to 20 atm and high temperature 120°C to remove contaminants and made them aseptic. Culture medium was used in the experiment called MS media [15].

Preparation of Stock Solutions: Stocks of growth regulators such as IAA, NAA, BAP and kinetin were also prepared in 1M sodium hydroxide by dissolving 0.5mg in 50ml distilled water. Weight by volume stocks were prepared in 1/1 ratio.

Surface Sterilization and Germination of Seeds: Seeds were washed with running tap water for 2 - 3 minutes in Petri dish. Floating seeds were considered to be empty and they were discarded. Later on, the nutritional seeds were surface sterilized with 20% (w/v) sodium hypochlorate for 3 - 4 minutes inside Laminar flow and finally washed four to five times with sterile distilled water. The seeds were then kept on a sterilized Petri dish containing sterile filter paper to remove excess of water droplets. The surface sterilized seeds were then inoculated into test tube containing agar solidified basic MS medium with sucrose for supporting seed germination and seedling development. 1 - 2 seeds were inoculated in each test tube.

Table 1: Direct regeneration media containing different concentrations of Indole Acetic Acid, Kinetin and coconut milk

IAA	Kinetin		
	0 mg/L	1.0 mg/L	1.5 mg/L
0 mg/L	RM1	RM2	RM3
0.3 mg/L	RM4	RM5	RM6
0.5 mg/L	RM7	RM8	RM9

Table 2: Composition of regeneration media (RM) containing various concentrations of hormones (Kinetin and Indole Acetic Acid) and coconut milk

Type of Regeneration media	Composition of regeneration media
RM ₁	0 mg/l IAA + 0 mg/l Kinetin + MS media
RM ₂	0 mg/l IAA + 1 mg/l Kinetin + MS media
RM ₃	0 mg/l IAA + 1.5 mg/l Kinetin + MS media
RM ₄	0.3 mg/l IAA + 0 mg/l Kinetin + MS media
RM ₅	0.3 mg/l IAA + 1 mg/l Kinetin + MS media
RM ₆	0.3 mg/l IAA + 1.5 mg/l Kinetin + MS media
RM ₇	0.5 mg/l IAA + 0 mg/l Kinetin + MS media
RM ₈	0.5 mg/l IAA + 1 mg/l Kinetin + MS media
RM ₉	0.5 mg/l IAA + 1.5 mg/l Kinetin + MS media
RM ₁₀	0 mg/l NAA + 0 mg/l BAP + MS media
RM ₁₁	0 mg/l NAA + 2 mg/l BAP + MS media
RM ₁₂	0.3 mg/l NAA + 0 mg/l BAP + MS media

Explants Sterilization: Seeds were used as source of explants for tissue culture experiments. Seeds were excised by inoculating them on wet filter paper. Embryos swelled up and then were excised and after surface sterilization these sterilized seeds were inoculated into test tube containing agar solidified MS medium [15], with sucrose for supporting seed germination and seedling development. 1 - 2 seeds were inoculated in each test tube.

Shoot Regeneration Media: Before shifting to regeneration medium, explants were broken down into small pieces, each piece acting like an embryo, so that hormones can reach each cell of the proliferated explant. Later on, explants were transferred to regeneration medium supplemented with different concentrations of growth hormones such as BAP, Kinetin, IAA and NAA. Initially regeneration was tested on medium containing different concentrations of IAA (0, 0.3mg/l and 0.5mg/l) and kinetin (0, 1mg/l, 1.5mg/l) + coconut milk.

Regeneration was also experimented with different concentrations of NAA (0, 0.3mg/l and 0.5mg/l) in combination with different concentrations of BAP (0mg/l, 2mg/l). Various regeneration media containing diff conc. of hormones and their compositions shown in

Table 1, 2. Finally the best concentrations of growth hormones in combination were tested and selected. Cultures were kept at 27°C, with 12 hours light cycle in every 24 hours.

Root Regeneration Media: For root induction, hormone free MS media and MS media supplemented with 0.5mg/l IAA were used.

RESULTS AND DISCUSSION

Brinjal is one of the most popular, delicious and nourishing vegetable. Major loss of this crop plant occurs throughout the world due to abiotic stress that is responsible for decreasing its production.

Seed Germination of Brinjal: The germination of brinjal seeds was initiated after duration of 10 days of inoculation by placing them periodically in dark at 27°C, for 3-4 days and in light for 5-6days. Seeds were germinated and 90% of seeds produced seedlings after about 15 days of inoculation on basic MS medium. Size of seedling formation was 3-4cm long. Germination and seedling formation of brinjal from seed is shown in (Fig. 1), Sammaiah, *et al.* [16] were also allowed to germinate the seeds of brinjal on MS media.

Direct Regeneration of Brinjal Shoots on Kinetin, IAA and Coconut Milk: For further improvement of regeneration of brinjal, different concentrations of Kinetin and IAA (Indole Acetic Acid) in a balanced ratio were used for experiment with the addition of coconut milk in combination. It was found that kinetin could not give regeneration alone but could give regeneration with additional hormone such as IAA (Fig. 2). When the concentration of kinetin was set up as about 1.5mg/l and that of IAA as 0.5mg/l containing coconut milk 25% then it was found that explant induced the regeneration of shoot within test tube. It was established as a new protocol because of addition of coconut milk for the regeneration of shoot.

The result was found after about 26 days of inoculation and regeneration frequency was increased upto 70% Table 3. Brinjal, although responds well to in vitro culture system, shows genotypic differences to somatic organogenesis [18]. Similar findings of using IAA and kinetin for regeneration described in *S. melongena* by several authors [6]. Mukherjee *et al.* [18], used 2.0 mg/l kinetin to induce direct organogenesis without the

Table 3: Analysis Of Variance (ANOVA) of brinjal *in-vitro* shoots regeneration on different regeneration media (RM)

Direct regeneration	df	S.S	M.S	F-value	P-value
Between	5	18152.333	3630.467	382.54	0.0000
Within	18	171.000	9.500		
Total	23	18323.333			



Fig. 1: Seed germination and formation of brinjal seedling from seeds after 2-3 weeks of inoculation on basic MS media

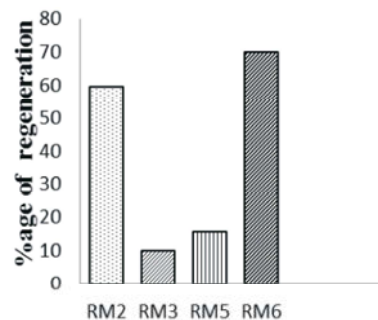


Fig. 2: Regeneration of brinjal shoots on different regeneration media (RM) containing various hormones (BAP+NAA, IAA + Kinetin) and coconut milk



Fig. 3: Shoot induction of brinjal after 25days of inoculation on MS media supplemented with 0.5mg/l NAA and 2mg/l BAP



Fig. 4: Root regeneration from shoots of brinjal after 40 days of inoculation on hormone free MS medium

formation of callus. Percentage of brinjal shoot regeneration on different regeneration media containing 25% coconut milk and various concentrations of kinetin + IAA were observed (Fig. 3), Gloria *et al.* [19], observed good direct shoot formation due to presence of auxins. Magioli *et al.* [11], also found that hypocotyl explants were less responsive towards regeneration using cytokinin in a Brazilian eggplant variety. But earlier reports indicate that in most of the cases *in vitro* regeneration in eggplant occurs from either hypocotyl or leaf explants via organogenesis [9, 10, 18, 20, 26].

Regeneration of Roots of Brinjal (*Solanum melongena* L.):

Roots were regenerated within 2-3 weeks after the inoculation of regenerated shoots on media (0.5mg/l IAA + MS medium) as well as hormone free MS media. Mostly the shoots regenerated their roots on basic MS media and a few on this Media (0.5mg/l IAA + MS medium). Most of the shoots developed roots by days 10 to 13 of inoculation. Higher number of roots was induced on basic MS or hormone free MS media. Similar type of results for induction of roots in plant growth hormone-free basic medium have been described in *S. melongena* by Sarker, *et al.* [22]. Regeneration of brinjal root shown in (Fig. 4) Somatic embryos germinated into plantlets with roots when transferred into MS medium supplemented with auxin, which was found by Bastaki, *et al.* [23] and by Jayasree, *et al.* [24].

MS basal medium was also reported to be effective for root induction and growth by Taha and Tijan [25], for a Malaysian eggplant variety. Moreover, MS supplemented with 0.1 mg/l IBA was also effective for root induction. NAA and IAA were found to be

ineffective for root induction in eggplant varieties used in this study. Magioli *et al.* [11], reported the induction of roots using half strength of MS supplemented with IAA. IBA was used PGR for root induction [26, 27].

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