In vitro Techniques for Propagation of Brinjal (Solanum melongena L.)

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Abstract: Solanum melongena L. is one of the most popular, nutritional vegetable crop. It plays a vital role in the national economy as a cash crop. Biotechnological applications of Brinjal (Solanum melongena L.) for improvement of quality and quantity of crop. Tissue culture techniques are largely used for the increasing value of this crop now days. 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 with various combinations of different ratio. Various explants such as stem and leaves were used for regeneration but stem showed better response. In case of stem, among the different combinations BAP + NAA showed better callus induction formation and also somewhat regeneration. Cytokyanins such as kinetin with combination of Auxin (IAA) and coconut water showed highest percentage of direct shoot regeneration.

Key words: In vitro techniques • Biotechnological applications • Eggplant • Hormones

INTRODUCTION

Solanum melongena L. also called eggplant 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 [1]. Eggplant 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 [2]. There are over 75 genera and total 2000 species of solanum, from which about 150-200 have tuber formation and belong to section Tuberarium while major number about 1800 are not bearing tuber. Most common is round or egg shaped forms of brinjal, placed under variety called esculentum. The elongated and slender types are grouped under var. serpentinum and the dwarf brinjal plants are put under var. depressum [1].

Kingdom: Plantae, Class: Magnoliopsida, Subclass: Asteridae, Order: Solanales, Family: Solanaceae, Genus: Solanum, Species: S.melongena. In South Asia, the weedy and wild relatives of eggplant (S. melongena sensu lato) and in Africa, Middle East and West Asia (S. incanum sensu lato) form brinjal eggplant complex’. In this taxa S.incanum is classified into three species S. lichensteinii Wild, S. campylacanthum A. Rich. and S. incanum L [3]. In Pakistan various kinds of vegetables are refined and cultivated on 225.4,000 hectare while their production is 2879.9,000 tons [4]. 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 [5]. Brinjal was firstly originated in India as it is native of India [6].

In India three crops of brinjal are planted; first is planted during the “kharif” season, second during the November-February and third one is in the month of March. Brinjal primarily grown in India by small and trivial farmers and it is an important source of income for them. Brinjal is grown in about entire India and it adapts and survives well to all agro climacteric circumstances. To liberate agro-climatic changeability, Country was divided into 4 regions, viz. eastern, western, northern and southern based on geographic locations. It is mostly local vegetable crop of India as there the enormous fruited
cultivars of domestication of brinjal occurred. In a book “Origin of cultivated plants” published in (1886), De Candolle also described that the species *S. melongena* has been known in India from very old times and considered to be as a native of Asia. [7], considered that it was firstly originated in the region of Indo-Burma. Different colors, shapes and forms of brinjal were generally found throughout South-East Asia, signifying in this part mostly variation occurs and it was central area of variation. Mostly central part of its diversity is the region Myanmar. As such morphological difference, variation and diversity based on isoenzyme and noticed in huge germplasm collection from India was given by [8].

Brinjal (*Solanum melongena L.*) is one of the most popular, delicious and nourishing vegetable crop in Bangladesh. After potato in reverence of total land and production (3, 70,000 mt) brinjal is at 2nd position as significant vegetable crop in Bangladesh [9]. It is considered to be originated in secondary centre of origin in china and Indian sub-continent (Bangladesh) [10]. Brinjal is an important vegetable crop related to solonaceaus and among these vegetables, the most widespread and accepted vegetable crop in Bangladesh is brinjal that is grown upto 0.51 million hector with total production of 8,200,00mt that is total area under brinjal cultivation. It is also known as aubergine and melongena in Bangladesh and different countries of the world [11].

**Distribution:** The distribution of eggplant also occurred in China and it is present from about 1500 years in China and after that from South Asia it was moved to Africa. Brinjal is cultivated recently in mediterranean region and now in tropical and temperate zones mostly in Southern Unites Stated [10]. Evidence to this was given by [8], based on the isoenzyme and morphological variation noticed in large germplasm collection from India. After India eggplant is found mostly in China, Japan and Indo-burma as 2nd centre of origin [6]. During the 15th century, Arabs have been introduced eggplant to the West [12]. Eggplant has been divided into three main types 1st is of shape of egg (*S. melongena var. esculentum*); elongated (*S. melongena var. serpentum*) and short (*S. melongena var. depressum*) [1].

Brinjal has been used as in many customary medicines [13]. For example, for treatment of bronchitis, diabiatise, asthma, dysentery and cholera its tissue extract has been widely used and similarly its leaves and edible parts are valuable in decreasing level of blood cholesterol. Anthocyanin pigment of *Solanum melangen* contain its major component called nausin reduces preoxidation of lipid [14]. In Sanskrit writing or journalism its commercial and medicinal value is present [1, 12, 13]. In Bangalore at the ‘National Consultations’ about 2, 5000 medically important compounds extracted from brinjal a disgustingly overstated claim unacceptable to those who knew some phytochemistry. For Ayurvedic medicines eggplant is also much important ingredient [15]. Brinjal is a valuable crop and it has a predictable role in global diets. Many purposes of biotechnology mainly use of somaclonal variations, somatic hybridization, haploidy and genetic transformation are using due to eggplant ability of regeneration in cell and tissue culture [16]. Wild species of eggplant carry many economically important genes that show resistance to important pests. By using conservative approaches and conventional breeding eggplants agronomic traits such as size of fruit, weight, shape and resistance to pests and diseases have been improved [1].

**Biotic Stress:** Most severe one biotic stress factor is the shoot and fruit borer (*Leucinodes orbonalis* Guen) among stress biotic factors which occur throughout year at all stages of crop growth. Cultivation of brinjal is suffering from various microorganisms’ diseases and diseases by insects. About thirty to forty percent loss of its total yield has been occurred due to *Leucinodes orbonalis* [17]. Biotic stress has become major risk of diseases for cultivation of brinjal (*Solanum melongena*) [18].

Many problems occur during the production of brinjal which cause massive loss of yield is unpopular among farmers due to lack of communal action, high labor necessities and problems of difficulty involved in their application. A few years ago an insect and pest resistance hybrid of brinjal called “Bt” brinjal has been produced through genetic engineering or biotechnology. This genetically engineered eggplant expressed toxin protein that is just like as to be produced naturally by bacterium known as *Bacillus thuringiensor* “Bt” brought about by insertion of “Bt” gene into genome as by phenomenon called genetic engineering remains switched on and much effective in all parts [19]. In India important variety of brinjal as “Bt” brinjal that can be willingly in use for field farming has been developed. Furthermore, the probability of insect-resistant genetically modified crops in rising crop yield and dropping insecticide use on plants in the upward countries has been established by several studies [20, 21]. The distribution of “bt” brinjal between producers and customers or consumers and its prospective economic advantages have been estimated using the economic superfluous method, it being the
most common technique to assess the impact of commodity-related technical development in farming [22]. The progress towards the improvement of this crop for insect, pest resistance is hampered mainly due to the wide prevalence of sterility in the progeny and occurrence of genetic incompatibility following intergeneric and interspecific crosses respectively [23, 24].

Transgenic Technology (Biotechnology): Transgenic technology called biotechnology has emerged as a choice to chemicals in controlling pests, reducing of herbicides, management of related troubles and also many other benefits are providing. At the same time, application of genetically modified technology has raised some apprehensions like food safety, failure of biodiversity, safety of environment, etc. But GM technologies should be capable to overcome intimidating challenges. For example food and environmental security, etc. that were faced by the globe be sacrificed in the middle of fear and unawareness [25].

From many applications of Mendelian genetics, one major is green revolution and it was useful for improvement of crops and vegetables. Green revolution has resulted in increasing production or yield of many crops by changing conditions of environment. But almost immediately the fact was realized that such increased yields may be decreased or inhibit due to increase of population, thus new strategies were necessary for increasing productivity, so protocols for In vitro regeneration were essential part of such de novo strategies. No longer was it completely dependent on fertilization and pollination but there was only one way to modify genetically to plant [26]. Shortly after this importance of tissue culture was noticed as it was a significant technique which was used for the increase yield of genotypes.

Tissue culture techniques largely used for the increasing value of many crops in these days. Shoot initiation from undifferentiated mass of cells culture can induce phenotypic and genetic changes in the regenerated plants in lab work (conditions). Such genetic changes have been called “Somaclonal variation” [27]. Such somaclonal variations are mostly due to different hormones in different conc. in culturing media. Such somaclonal variations are caused by different growth hormones studied in solanum melangena [28]. Due to absence of mass scaling techniques and successful grassland delivery systems, application of regeneration of plants in practical for separation of somaclonal variation are to move to slowly [29]. Although brinjal is refined or cultured all over the tropical and subtropical regions. It contains minerals such as iron, phosphorus and riboflavin in more quantity as in form of calories. Eggplant is at high the risk of insects, diseases and pests that can damage the crop much and has harmful effect on quality, economy, production rate and storability. 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 [30].

Somatic Embryogenesis: According to species type and explants type study is taken on somatic embryogenesis and its morphological aspects of species and this study has shown that somatic embryos of different species have different origin. For example somatic embryos in tomato formed from epidermal cells of explants of hypocotyls. In peanut meristmatic cells of cali of cotyledon form somatic embryos [31]. While in brinjal somatic embryos formed from perivascular parenchyma cells which can give rise adventitious roots in cultured media containing NAA [32].

Tissue Culture (Embryo Formation, Organogenesis and Protoplast Culturing): Tissue culture analysis data and material (protocols) for embryo formation, organogenesis and protoplast culturing have been well recognized [16, 33-35]. Effectiveness of regeneration described in variable systems of solanum melongena L. [36]. So, specific difference in callus formation can be seen by type and amount of different growth hormones. For example regeneration of shoot from segments of hypocotyls of eggplant has been documented in the presence of growth hormone IAA by [37]. The significance of tissue culture in breeding of plants has been documented extensively and usually it is responsible for improvement of crops as somaclonal variations were seen by [28], within plants via somatic embryogenesis induced by NAA or 2, 4-D in eggplant. As embryogenesis with NAA was better organized and proficient than with 2,4-D. 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 by [38]. Callus induction efficiently observed during culture of leaf segments in MS medium containing 0.5mg/l NAA and 2mg/l BAP by [39], who cultured leaf explants of eggplant on MS media.
supplemented with IAA, BAP, NAA and 2, 4-D. At 2mg/l, 4-D induced early callus formation NAA produced greenish callus while total production of callus was enhanced due to addition of NAA 0.5mg/l and BAP 2mg/l.

**In vitro Plant Culture:** The firstly experiments to culture plant cells under *In vitro* conditions were conducted more than one hundred years ago [40]. Morgan used a term a totipotent cell is one that has ability to regenerate into complete organ and whole plant [41]. He found this property of totipotency is only for plant cells. Meristem or shoot tip culture has been used for three general purposes. The most common use has been for micro propagation of a wide range of ornamental and crop species. Other is production of valuable vegetable as well as crop species in aseptic conditions [42,43], documented about use of eggplant in medicines due to which it is much cultivated and propagated *In vitro*, as it is good treatment and remedy for liver and diabetic patients and also has good anyurvedic properties. Advanced biotechnological method such as genetic transformation can be functional as an optional approach to control over such serious problems of conventional breeding such as the progress of disease and insect resistance for such crop. A well-organized and reproducible *In vitro* renaissance system is well thought-out as an essential part of doing well transformation. Through organogenesis *In vitro* regeneration of brinjal from explants has a number of reports. [37,44]. Larkin reported about tissue culture importance and somaclonal variations. Seed born pathogens can be inserted over many generations in crops with some expression of symptoms. Plant tissue culture or regeneration method acts as a proficient technique for pathogen free materials and preservation of germplasm. Tissue culture techniques are broadly used for the development of a variety of crops. *In vitro* shoot initiation from callus culture can stimulate hereditary and phenotypic changes in the regenerated plants. He called these genetic changes as “Somaclonal variation”. Leaf explants of *L. peruvianum* established higher morphogenic potential than *L. esculentum*, while another wild relative of tomato called *Solanum pennellii* has different response with the type of medium used [45].

**Regeneration of Shoot:** 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. 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 calli changed their color into greenish and at last gave the regeneration of shoot within test tube. It was established as a new protocol for the regeneration of shoot. The result was found after about 26 days of inoculation and regeneration frequency was increased upto 70%. Similar case was reported by [28], who documented that by the addition of another hormone such as NAA with kinetin in MS medium, regeneration
frequency increased in comparison to explants shifted from medium containing individually 2, 4-D or kinetin. According to [34], when Kinetin was supplemented into the MS medium, the size of callus was decreased. Similar case appeared here and callus size in comparison to calli produced by 2.0 mg/l BAP + 0.5 mg/l NAA decreased and most of the energy consumed by shoot induction. Similar findings of using IAA and kinetin for regeneration described in S. melongena by several authors [16,33], who described about protocols of tissue culture for de novo organ formation and protocols for somatic embryogenesis.

Regeneration of Roots: Roots were regenerated finally either directly from callus or shoots. Roots were regenerated within 1-2 weeks after the inoculation of regenerated shoots on media (0.5 IAA + MS medium) as well as hormone free MS media. Mostly the shoots regenerated roots on basic MS media and a few on Media (0.5 IAA + MS medium). Most of the shoots developed roots by days 10 to 13. Higher number of roots was induced on MS 0 or hormone free media. Similar type of results for formation of root in plant growth hormone-free medium have been described in S. melongena by [50, 51].

Growth Hormones: [52], demonstrated that, a control of growth hormones, especially those of auxins and cytokinins, determines the developmental destiny of cultured cell. They described their original study with tobacco tissue cultures. The irresistible success in regenerating a wide variety of plants via organogenesis and somatic embryogenesis by In vitro manipulation of auxin (s) and cytokinin (s) reflects the power and general applicability of the central system of belief developed by these researchers. [53], investigated that cytokinins usually increases cell division and induce shoot formation and auxiliary shoot proliferation and auxins promote root formation. High cytokinin to auxin ratio enhances shoots proliferation while high auxin to cytokinin ratio results in root formation. [54], performed an experiment and noticed that leaf and cotyledon pieces, shoot apices and hypocotyls segments of sunflower (Helianthus annuus L.) were regenerated on Murasiage and Skoog's medium containing different concentrations of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D) or of the cytokinin 6-benzylaminopurine (6-BAP).

Different explants produced different responses and different hormones gave different responses. Growth hormone such as 6-BAP induced callus formation from different explants. 2, 4-D separately formed weak and nodular calli while abundant growth of compactly arranged green calli formed due to 6-BAP. The calli are formed due to 6-BAP developed many shoots and some of which induced flower formation In vitro. [55], explained that external supply of auxin and cytokinin is required for the formation of flower buds on thin-layer tissue explants of Nicotiana tabacum cv Samsun. Speed of flower bud initiation and the number of flower buds formed depends upon interaction between both plant growth regulators during this regenerative process. Separation in time of the hormone application during culture revealed that the cytokinin (benzyl adenine) plays an important role in flower bud initiation whereas auxin (indoleacetic acid) stimulates in particular the differentiation of flower buds. The uptake of each hormone was proportional to the concentration supplied in the medium and the uptake of one hormone appeared independently of the presence of the other. Metabolism studies showed the conversion of IAA by the tissue to at least 13 metabolites after 24 h of culture.

[56], demonstrated that embryogenic calli were obtained by culturing seeds of both varieties (Inqilab-91 and Pavon-76) on agar-solidified Linsmaier and Skoog’s (LS) medium supplemented with 3.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). The callus cultures were sub-cultured by fresh media at 4-5 weeks interval and thus maintained for 1 month. The embryogenic calli carefully cleared of any surrounding non-embryogenic callus and then were grown on the gel-solidified basic Murasiage and Skoog’s (MS) medium with 3% (w/v) sucrose after at least two subcultures. Highest frequency of green spot formation and plant regeneration (84% in Inqilab-91 and 52% in Pavon-76) was obtained at 0.5 and 0.1 mg/L of BAP and IAA, respectively. Concentrations of BAP in large quantity within media proved to be toxic and fatal for callus in some cases.

[57], demonstrated In vitro callus induction and regeneration and contact of pesticides on multiple calluses and shoot induction in response to concentration of pesticides. It was obtained by the adding up of three different pesticides such as Endlosulfan, Rogor and Kitazin and observed at different levels of these three pesticides on Murashige and Skoog’s (MS) medium supplemented with Indole acetic acid and Benzyl amino purine (BAP). Pesticides have opposite effect on callus growth as it was decreased with rising level of pesticides in culture medium. Growth of callus and shoot induction was noticed as abnormal in some concentrations of pesticides in the culture medium. Among the different media hardened by adding pesticides, application of
Rogor at 25 ppm has given maximum assembly of callus induction and shoots that was noticed as 76.0 and 11.0.

**Callus Induction:** Seedling was cut into small pieces with help of scissor and each piece operated like explant. Various explants were used for this purpose and among these explants; stem was comparatively more responsive for callus induction than other explants such as leaf and root. Growth hormone such as 2, 4-D was functional in order to optimize callus induction for undifferentiated splitting up of cells from the explants used. NS-797 cultivar of brinjal showed good comeback and calli started to appear from shoot explants after just 5 to 6 days of inoculation on CIM1 (2mg/l 2, 4-D+MS media). Over 40% of explants showing callus induction on CIM1 (2mg/l, 2, 4-D+MS media).

Percentage of calli induction was increased upto 65.08 % in CIM2 (3mg/l 2, 4-D+MS media) and it has produced non embryogenic calli within 22 days of inoculation of explants. When we increased concentration of same hormone 2, 4-D up to 5mg/l frequency of callus induction again decreased much and only 33.1% of the explants formed calli. Hormone (2, 4-D) used for induction of callus in brinjal and 3mg/l of 2, 4-D was best for callus induction from stem explants in about 15 days. 2, 4-D was the hormone of choice for callus induction in wheat by [50], who reported 2, 4-D was the major ingredient in callus induction. Similarly another combination of hormones such as 2mg/l BAP (Benzyl amino purine) and 0.5mg/l NAA (Naphthalene acetic acid) on MS media formed 50% of calli induction from shoot explants after about 25 days of inoculation. Similar type of result as calli formation by inoculation of explant in BAP and NAA was also documented by [58], who had been performed experiment by taking same conc. of these hormones 2mg/l BAP and 0.5mg/l NAA in MS media.

**CONCLUSION**

It is concluded that 2, 4-D was the hormone for both embryogenic and non embryogenic callus induction. Solanum melongena L. (eggplant) has given maximum regeneration frequency of shoot when Kinetin and IAA were used in combination with coconut milk, NAA (Naphthalene Acetic Acid) and BAP (Benzyl Amino Purine) were found to be better for embryogenic calli induction and shoot regeneration whereas direct shoot regeneration better response achieved by using coconut milk with combination of hormones kinetin and IAA.

**REFERENCES**


