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Eggplant (*Solanum melongena* L.) as a Model Plant for Tissue Culture and Genetic Transformation Studies

R. Gunasekar[1], M. Kannan[2]

[1]Asst. Professor and Head, Department of Vegetable Crops, APHC, G. B. Nagar, Kalavai-632 506, India
[2]Dean, Adhiparasakthi Horticultural College, G. B. Nagar, Kalavai-632 506, India

(Affiliated to Tamil Nadu Agricultural University, India)

Abstract: ***Eggplant (Solanum melongena L.,) is used as both vegetable as well as medicinal plant for the treatment of several diseases, including diabetes, arthritis and liver complaints. Eggplant is susceptible to a number of diseases and pests capable of causing serious crop losses. This problem has been addressed by hybridizing eggplant with wild resistant Solanam species, which are the sources of useful agronomic traits. Non availability of resistance sources in cultivated species, cross-incompatibility with wild relatives and linkage drag of undesirable genes are the problem in conventional breeding methods. The application of in vitro methodologies has resulted in considerable success. Eggplant is highly responsive to various tissue culture techniques. Direct organogenesis and somatic embryogenesis are widely studied protocol in this crop, but potential of regeneration varies with genotype, explant and culture media supplemented with various growth hormones. Among growth regulators, auxin and cytokinin are of more significance as their ratio determines callogenesis, rhizogenesis, embryogenesis and regeneration in eggplant. Eggplant tissue present a high morphogenetic potential that is useful for developmental studies as well as for establishing biotechnological approaches to produce improved varieties, such as embryo rescue, in vitro selection, somatic hybridization and genetic transformation. The potential of this species as a model plant for studying various aspects of plant genetics and morphogenetic potential is also discussed. In the present study, important factors that affect invitro regeneration as well as genetic transformation are analyzed.***

Keywords: *Solanum melongena* L, model plant, bio technology, in vitro regeneration, genetic transformation

1. Introduction

Eggplant (*Solanum melongena* L.) also known as brinjal is an admired vegetable crop of the family *Solanaceae* and grown all over the world. It can be cultivated throughout the year and grown on small family firms and considered to be important source of nutrition and cash income for many resource poor farmers in many of the Asiatic countries. As compared to other crops like tomato, it is rich in vitamins and minerals that increase its total nutritional values (Kallo, 1993). It is used for the treatment of several diseases, including diabetes, arthritis and liver complaints. (Shukla Naik, 1993).

Eggplant is susceptible to a number of diseases and pests. In South and South East Asia, eggplant is extensively damaged by the infestation of *Leucinodes orbonalis* commonly known as shoot and fruit borer. To control this pest, various chemical control measures have been recommended. Consequently, growers use excessive and unrecommended pesticides, which is a matter of concern for food safety, environmental degradation, pest resistance and resurgence and economics of the crop. The non-availability of resistance for diseases such as Bacterial wilt (*Ralstonia solanacearum*), *Fusarium* wilt (*Fusarium oxsysforium*), *Verticillium* wilt (*Verticillium dahliae*) and Cucumber mosaic virus in common cultivar, cross-incompatibility with wild relatives (*Solanummammosum, Solanum incanum and Solanum grandiflorum*)and inadvertent linkage drag of undesirable genes (Baksh and Iqbal, 1979) are problems in developing intrinsic plant resistance through conventional breeding approach. Biotechnological tools like *in vitro* propagation, genetic engineering and molecular biology have helped overcoming constraints of conventional breeding, and identification and introduction of useful genes that confer resistance to pests and diseases, and tolerance to a biotic stresses in eggplant.

The application of *in vitro* methodologies in eggplant has been initiated and resulted in considerable success. Principle of totipotency is responsible for regeneration of commercially important plants via tissue culture (Krikorian and Bequam, 1969). Totipotency is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Eggplantl tissues have been found to have a high morphogenetic potential that is useful for developmental studies as well as for establishing biotechnological approaches such as, embryo rescue, *in vitro* selection, somatic hybridization and genetic transformation to produce improved varieties resistance to biotic and a biotic stresses. The establishment of efficient *in vitro* regeneration systems is also the first step for developing genetic transformation protocols in order to introduce novel traits or to study regulation of gene expression in plants. The availability of efficient protocols for *in vitro* regeneration, both via organogenesis and embryogenesis, as well as for genetic transformation of eggplant, offers an excellent model system to investigate plant physiology *in vitro*. Accordingly, reports published in the last few years provided several examples for the use of eggplant as a model plant and were discussed herein. In this review, an attempt has been made to bring out recent trends in *in vitro* plant regeneration and genetic transformation in eggplant (*Solanum melongena* L.).

2. *In Vitro* Plant Regeneration

Eggplant is highly amenable to cell, tissue and organ culture (Kantharagan and Golegaonkar, 2004). Plant regeneration from tissue culture of eggplant can be achieved via somatic embryogenesis (Ammirato, 1983) and organogenesis (Flick *et al*.1983). It can be done directly from cultured explants or calli of cell suspension (Fassuliotis.*et al*, 1981), anther (Rhatun *et al*.2006), microspore (Lian *et al*.2004) and protoplasts (Borgato *et al*.2007).

Several other protocols for the plant regeneration via direct and indirect organogenesis have been listed in table**1**and **2**. In those protocols, the regeneration efficiency has been reported to be affected by different factors, such as combination of growth regulators, explant type and genotype. Explant age affected regeneration as younger leaves showed better organogenesis than mature one (Zhang, 1999). Most of the organogenic systems reported are based on supplementing culture media such as Murashige and Skoog medium with auxins and cytokinins, either alone or in combination (Sharma Rajam 1995a).

Various type of explants have been used for the induction of organogenesis in eggplant, including cotyledon, hypocotyl, epicotyl, shoot tip and root (Sarker *et al*.2006), leaf and stem (Taha and Tizan 2002).Organogenesis is the morphogenesis of plantlets directly from explant without the intervention of callus. Anatomically and histologically, longitudinal section of leaf explants formed numerous meristematic zones within the tissue that subsequently converted into shoot buds (Mukhergee *et al*.1991). The formation of shoot bud was characterized by the appearance of shoot apex with the developing leaf primordial (Sharker *et al*.2006). Genotype played an important role in organogenesis of the shoot directly from the explant. Different species such as *Solanu aethiopicum* and *Solanummacrocarpon* showed difference of potential in direct plant regeneration, where, 70-100% explants with a mean of two to seven shoots per explant were obtained (Gisbert *et al*.2006).The regeneration efficiencies reported in those systems were relatively low (approximately seven shoot/explant) (Gleodie *et al*.1983), except in the one described by Sharma and Ragam (1995) who achieved the production of 20 shoot/explant. The use of low concentration of thidiazuron (1-phenyl-3- (1, 2, 3-thiadiazol-5-yl) urea)100-200nMwas reported to induce efficient organogenesis (around 20shoot/explant) from leaf and cotyledon explants (Magioli *et al*.1998, 2000). Different growth regulators such as auxins and cytokinins have been used for direct organogenesis. The effect of different sugars has been studied and the highest regeneration rates were observed in media with low sucrose concentration (11 and 22 mM) during shoot development. However, the normal concentration of sucrose used in Murashige and Skoog medium (88 mM) induced more efficient root development (Mukherjee *et al*. 1991).

Protocols for inducing somatic embryogenesis (indirect organogenesis) from eggplant tissues have also been described, using different growth regulators and explant types (Table-2). The explants used for the induction of somatic embryos in eggplant are immature seed embryo, cotyledon, leaf, anther and microspore. The first report was published by Yamada *et al. (*1967), who induced somatic embryogenesis from zygotic embryos cultured on MS medium supplemented with indole-3-acetic acid (IAA).Similar to the organogenesis process, the efficiency of somatic embryogenesis depends on several factors, including genotype, explant type and growth regulators. The genotype is the most important factor affecting somatic embryogenesis and significant quantitative differences in their capacity to form embroid among different species like *Solanummelogena, Solanummelogena*var *insanum, Solanum gilo, Solanum integrifolium* and their F1 hybrids and cultivars (Rao, 1992; Kaur *et al*.2011 and 2013).The molecular investigation using Polymerase Chain Reaction (PCR) of different cultivars for the induction of somatic embryos indicated that the embryogenic response is due to the differences in mRNA expression and consequently gene expression patterns (Afele et al. 1996). Among auxin, naphthalene acetic acid (NAA), 2, 4-D-dichlorophenoxyacetic acid (2, 4-D) and indole-3-acetic acid (IAA) generally favour callogenesis and naphthalene acetic acid (NAA) and indole 3, butric acid (IBA) promotes rhizogenesis (Kamat and Rao 1978). However, conversion of somatic embryo into plantlets is usually limited due to abnormalities such as hyperdricity, lack of apical meristem, cotyledon fusion and in efficient maturation (Saif and Nishimora 1994). Nevertheless, conversion rates can reach up to 92 % by culturing matured embryo on MS media with one % phytogel (Saif and Nishimora 1994; Magioli *et al.* 2000).

The differences in regenerative potential of callus, number of roots and time required for regeneration are also observed (Dobariya and Kachhadiya, 2004). Stem elongation and rooting of plantlets is a crucial process in eggplant regenerative system. Small shoots of eggplant plantlets require *in vitro* condition for shoot elongation. Hormone free MS or half MS medium has been most frequently used for elongation of plantlets (Sarkar *et al.*, 2006). Elongation of eggplant plantlets has found to be effectively induced by MS medium fortified with (GA_3) Gibberellic acid (Shivraj and Srinath, 2011) and GA_3 along with (TIBA) 2, 3, 5-triiodobenzoic acid (Shivraj and Srinath, 2011). Eggplant developed roots in hormone free MS medium (Sarkar *et al*., 2006) and MS medium with (IBA) indole, 3, butric acid (Shivraj and Srinath, 2011). The highest root induction frequency has also been observed in full strength of MS medium with (IAA) indole-3-acetic acid (Hegde *et a*l. 2012).

Studies on the morphogenesis aspects of organogenesis and somatic embryogenesis have been performed in various species of brinjal genotypes, explants and the type and concentration of growth regulators. Brief information related to the type of explants of eggplant genotype, supplementing media along with different growth regulators at different concentrations is given in the table**1** and **2**.

Invitro regeneration system has been used as a strategy to select valuable agronomic traits in eggplant. Several useful traits from wild species such as resistance to nematode and atrazine have been found in somatic hybrids obtained from protoplast fusion (Collonier *et al.*, 2001). Di-haploid plants were produced through another culture of somatic hybrids between *Solanummelongena* and *Solanum aethiopicum* with

the objective of obtaining fusarium resistance (Rizza *et al.,* 2002). Plant regeneration through somatic embrogenesis and somaclonal variants were observed by Rotino *et al., (*1991) and callus lines resistant to culture filtrate of *Verticillium* were also reported by Rotino *et al., (*1987). The somaclonal variation in eggplant is caused by the hormonal concentrations in culture medium. The effect of growth regulators such as naphthalene acetic acid (NAA) and 2, 4-D-dichlorophenoxyacetic acid (2, 4-D) on somaclonal variation were studied in eggplant (Laksman Naik and Ravali 2016).Frequencies of somaclonal variations in leaf shape, plant height, fruit shape and pollen fertility were higher with naphthalene acetic acid (NAA) than that of 2, 4-D-dichlorophenoxyacetic acid (2, 4-D) as endorsed by Hitomi *et al., (*1998). Therefore, the future research would determinate the importance of new somaclonal lines for genetic variability in eggplant. (Zayova *et al.*2010).

3. Genetic Transformation Via Agrobacterium

Genetic transformation of eggplant via agrobacterium was first reported by Guri and Sink (1998) using leaf explants and a cointegrate binary vector, although no success was achieved. Later, Fillipone and Lurquin (1989) reported the transformation of leaf and cotyledon explants using the wild supervirulent strain A281.Genetic engineering of eggplant has been already reported (Fari, 1995; Pal *et al.,* 2009; Rai *et al.,* 2103; Pratap *et al.,* 2011 and Singh *et al.,* 2013). An optimization of factors that influence transformation efficiency, including length of pre and post culture periods, explant type and genotype was performed using an organogenic system (Magioli *et al.,* 2000). Recently, a plant regeneration system through the explants*viz.*, cotyledon and hypocotyl was achieved for the genetic transformation of neomycin phosphotransfersae-11 (npt-11) and â-glucuronidase (gus)genes into the calliof *Solanum melongena*L**.cv.** Pusa Purple Long using binary vector (PBI 121) (Anita kumara *et al.,* 2013).Kumari *et al.* (2012) also standardized a protocol for genetic transformation in eggplant with *npt-II* and *gus*genes.

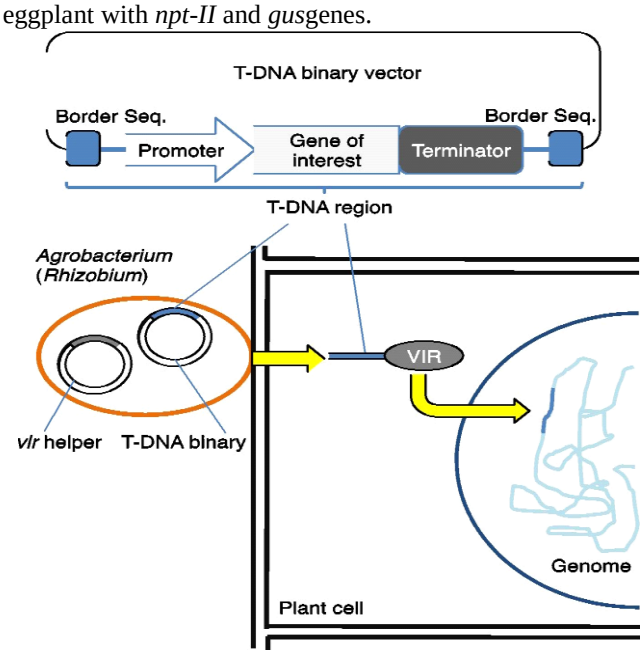

Figure 1: Transformation of Gene of Interest (GOI) from agrobacterium into plant cell

Reports showed that the production of transgenic eggplant through somatic embryogenesis either fails to occur or is achieved with very low efficiency (Fillipone & Lurquin 1989, Fári *et al.* 1995). It has been demonstrated that both co-cultivation with agrobacterium and presence of bacterial antibiotics used in transformation protocol cause a reduction of 80-99% in the number of embryo/explant. The bacterial antibiotics used to control the *Agrobacterium* strains during gene transformation are cefatoxime, rifampicin, ampicillin and venomycin. The inhibitory effect on somatic embryos development may be due to the phytotoxic effect of bacterial antibioticsand the delicate process of gene regulation such as alteration in transcriptional and translational process that are induced in early culture stage (Magioli *et al.,* 2001a).

Following the basic protocols of genetic transformation, successful introduction of agronomic traits into eggplant was achieved. Resistance to Colorado potato beetle (*Leptinotarasa decemlineata)* a pest that developed resistance to synthetic insecticide and become a serious problem in Europe and America. Chen *et al., (*1995) have produced transgenic lines with the introduction of *Bacillus thuringiensis* (Bt) genes. But resistance to Colorado potato beetle was not observed. Later, different groups obtained lines resistant to CPB by using mutagenized version of CryII B (Arpaia *et al.,* 1997) and a synthetic version of CryIII A Bt genes (Jalencovic *et al.,* 1998).

There are other examples of genetic improvement of eggplant via *Agrobacterium tumefaciens*. Resistance to brinjal shoot and fruit borer (*Leucinodes orbanalis*) was obtained in transgenic plants harboring Bt (Cry1Ab) gene (Kumar *et al.,* 1998). Gayathri *et al.,* 2013 carried out a genetic transformation studies using CO-2 variety of eggplant to transfer the Cry2Ax1 a synthetic gene to develop resistance against shoot and fruit borer. Molecular analysis of transformed plants confirmed the presence of transgene Cry2Ax1 in the plant genome. Insecticidal Crystalline Protein (ICP) product of Cry genes, affect the midgut of the insect by binding the midgut receptor when they are ingested by them, which ultimately leads to the death of the insect. A trial showed significant level of resistance against Cucumber Mosaic Virus disease after the transformation of CMV-CP gene (Cucumber Mosaic Virus Coat Protein gene) into eggplant cv Pusa Purple Long (Pratap *et al*2010). Tolerance against osmatic stress induced by salt, drought and chilling stress was achieved in transformants expressing the bacterial mannitol phosphodehygrogenase (mtlD) gene (Prabhavatai *et al*., 2002). The mtlD gene encodes mannitol 1-phosphate dehydrogegase. Transgenic plant carrying mtlD gene converts mannitol1-phosphate to mannitol via nonspecific phosphatases. Overexpression of the mtlD gene involved in the biosynthesis of osmolytes such as, mannitol, sorbitol, trehalose and proline in various transgenic plants showed increased abiotic stress tolerance (Bhauso., 2014). These resistant transgenic eggplant plants can potentially be used for the development of new varieties.

A well-established example of the metabolites that are involved in stress tolerance, is the low-molecular-weight aliphatic polyamines, including putrescine, spermidine, and spermine. The critical role of polyamines in stress tolerance is suggested by several lines of evidence: firstly, the

transcript levels of polyamine biosynthetic genes, as well as the activities of the corresponding enzymes, are induced by stresses; secondly, elevation of endogenous polyamine levels by exogenous supply of polyamines, or over expression of polyamine biosynthetic genes, results in enhanced stress tolerance; and thirdly, a reduction of endogenous polyamines is accompanied by compromised stress tolerance (Liu *et al.*, 2015). Different plant *in vitro* morphogenic systems have been used to study the role of polyamines (PA), which are proposed as a new class of growth regulators involved in differentiation, reproduction, disease resistance and stress (Scoccianti *et al.* 2000). The effect of polyamines in the process of *in vitro* morphogenesis has been studied in eggplant by analyzing cellular levels of free and conjugated polyamines and the activity of enzymes involved in biosynthesis and oxidation of polyamines or by treating explants with exogenous polyamines and inhibitors of synthesis of polyamines. The expression pattern of *Atgrp-5* gene (glycin-rich protein isolated from *Arabidopsis thaliana*) in transgenic eggplants harboring a construct containing an *Atgrp-5* promoter-GUS fusion showed to be highly regulated during developmental processes and to have preferential expression in epidermis and stem phloem to produce polyamines involved in multiple stress tolerant (Magioli *et al.* 2000),

It was reported that genetic transformation with polyamine biosynthetic genes encoding arginine decarboxylase (ADC), ornithine decarboxylase (ODC), *S*-adenosylmethionine decarboxylase (SAMDC) or Spd synthase (SPDS) improved environmental stress tolerance in various plant species (Gill and Tuteja, 2010). It is also interesting to note that transgenic plants over expressing ADC, SPDS or SAMDC could tolerate multiple stresses including salinity, drought, low and high temperature and parquet toxicity. Such multiple abiotic stress tolerance is of practical importance since plants often suffer from several concurrent forms of environmental stress during their life cycle.

4. Conclusion

In conclusion, eggplant provides a unique system to study morophogenesis, somoclonal variation and genetic transformation. An efficient plant regeneration and transformation protocols can serve as a platform for the transfer of important economic traits through genetic engineering, inducing somaclonal variation, *in vitro* mutation and development of somatic hybrids, determining resistance and tolerance to biotic and abiotic stresses. Therefore, remarkable progress can be made in eggplant improvement through the combination of both conventional breeding and biotechnological approaches. Furthermore, the pyramiding of the genes encoding the enzymes of Polyamines biosynthetic pathways will also be helpful for further enhancing the tolerance potentials of any vegetable crop for various stress factors.

Table 1: Direct Organogenesis in eggplant

Explant	Direct Organogenesis	Reference
Leaves and cotyledons	MS + 0.2 μ*M* TDZ	Magioli *et al*., 1998
Cotyledon and hypocotyl	MS + 0.1 mgL-1 IAA	Picoli *et al*., 2000
Leaf and stem	MS + 0.5 mgL-1 NAA	Taha and Tizan, 2002
Cotyledon and leaf	MS + 0.1 or 0.1 μ*M* TDZ	Gisbert *et al*., 2006
Cotyledon, hypocotyl, shoot tip, root	MS + 1.0 mgL-1 BAP + 1.0 mgL-1 Kin	Sarker *et al*., 2006
Meristem	MS (liquid)+ 2.0 mgL-1 BAP, MS (semisolid)+ 2.0 mgL-1 BAP+1 mgL-1 NAA, MS (semisolid)+ 1.0 mgL-1 BAP	Sharmin *et al*., 2008
Cotyledonary nodes	MS + 2.0 mgL-1 BAP + 1.0 mgL-1 2iP	Kanna and Jayabalan, 2010
Cotyledon, hypocotyl and leaf	MS + 2.5 mgL-1 BAP + 1.0 mgL-1 KN	Kaur *et al*., 2011
Cotyledon	MS+ 1.0 mgL-1 Zeatin	Prasad *et al*., 2011
Leaf	MS+ 1.0 mgL-1 TDZ+ 4.02 g/l nitrogen, +2.36% sucrose.	Naveenchandra *et al*., 2011
Cotyledon, hypocotyl and leaf	MS + 2.0 mgL-1 BAP + 0.5 mgL-1 Kn	Shivraj and srinath, 2011
Cotyledon nodal segments and shoot tip.	MS + 2.0 mgL-1 BAP + 1.0 mgL-1 Kn	Bhat *et al*., 2013

Table 2: Somatic embryogenesis in eggplant

Explant	*Somatic embryogenesis*	*Shoot induction*	*Reference*
Immature embryo cultures	MS + IAA	No Media	Yamada *et al*., 1967
Hypocotyl, otyledon, leaf	LS+ 0.4 mgL-1 2, 4-D	Hormone free LS	Alicchio *et al*., 1982
Leaf	MS+10 mgL-1 NAA	Basal MS	Gleddie *et al*., 1986
Leaf	MS +0.5-2.0 mgL-1 NAA	Basal MS	Rao, 1992
Hypocotyl, cotyledon and leaf	MS+ 1 mgL-1 NAA + 2 mgL-1 BAP	No media	Sharma and Rajam, 1995 a
Hypocotyl, cotyledon, leaf, epicotyl	MS +2.5-10.0 mgL-1 NAA	½ MS+1% phytagel	Magioli *et al*., 2001
Hypocotyl, cotyledon and root	MS+ 1.0mgL-1 NAA (hypocotyls), 1.5 mgL-1 NAA (cotyledon) and 2.0 mgL-1 NAA (root)	MS+ 2.5 mgL-1 IAA + 0.5 mgL-1 BAP	Mir *et al*., 2008
Cotyledon and hypocotyl	MS + 2.0 mgL-1 NAA + 0.5 mgL-1 BAP	Hormone free MS	Zayova *et al*., (2008, 2012)
Immature seed embryo, cotyledon.	MS+ 10.5 mgL-1 NAA (cotyledon), MS+ 8.0 mgL-1 NAA+ 0.1 mgL-1 KN (seed embryos	Hormone free MS medium	Swamynathan *et al*., 2010
Hypocotyl, root and leaf.	MS + 2.0 mgL-1 BAP + 0.5 mgL-1 NAA	MS + 2.0 mgL-1 BAP + 0.5 mgL-1 NAA	Ray *et al*., 2010
Hypocotyl, cotyledon and leaf	MS + 1.5 mgL-1 IBA + 1.0 mgL-1 BAP	MS + 2.5 mgL-1 BAP + 1.0 mgL-1 kin + 0.2% activated charcoal	Kaur *et al*., 2013

Abbreviation: **MS,** Murashige and Skoog; **LS**, Linsmier and Skoog; **BAP,** 6-benzylamino purine; **NAA,** naphthalene acetic acid; **IAA,** indole, 3, acetic acid; **IBA,** indole, 3, butyric acid; **ZT,** zeatin; **KN,** kinetin; **NOA,** naphthoxy acetic acid; **TDZ,** thidiazuron. **2, 4-D,** 2, 4-dichlorophenoxyacetic acid; **BA,** 6, benzyladenine; **GA3,** gibberellic acid; **TIBA,** 2, 3, 5-triiodobenzoic acid;

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