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Review article

PLANT REGENERATION IN EGGPLANT (*Solanum melongena* L.) A REVIEW

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ABSTRACT: Eggplant is highly responsive to various tissue culture techniques. Somatic embryogenesis and direct organogenesis are widely studied protocols in this crop, but potential of regeneration varies with genotype, explant and culture media supplemented with different combination and concentration of growth hormones. The genotype is the most important factor affecting somatic embryogenesis and organogenesis. Embryogenic competence occurs even within explant segments. Among growth regulators, auxins and cytokinins are of more significance as their ratio determines callogenesis, rhizogenesis, embryogenesis and regeneration in eggplant. Organogenesis and somatic embryogenesis related gene expression has been studied and transcripts have been analyzed through molecular studies. Efficient plant regeneration protocols would make a platform for exploitation of useful somaclonal variations, mutation breeding, induction of di-haploids, and genetic transformation with economically important genes for the improvement of eggplant.
Key words: Callus, somatic embryogenesis, organogenesis, hypocotyl, cotyledon, leaf.

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Abbreviations: MS, Murashige 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; PCR, polymerase chain reaction; ADC, arginine decarboxylase.

INTRODUCTION

Eggplant (*Solanum melongena* L., 2n = 2x = 24) is a widely adaptive and highly productive vegetable crop of tropical and subtropical regions world, which suffers from various abiotic and biotic stresses particularly insect-pests [1,2]. To control the pests, various biological and biochemical control measures have been recommended, but cryptic nature of the pest is a big hindrance in efficient management. Consequently, growers use excessive and un-recommended pesticides, which is a matter of concern for food safety, environmental degra-dation, pest resistance and economics of the crop. The non-availability of resistance in cultivated, cross-incom-patibility with wild relatives (*Solanum mammosum*, *Solanum incanum* and *Solanum grandiflorum*) and inadvertent linkage drag of undesirable genes [3] are problems in developing intrinsic plant resistance through conventional breeding approach. Thus, use of biotechnological techniques can be an alternative approach to tackle such issues. In eggplant, somatic embryogenesis was first reported from immature seed embryos of two different cultivars by culturing on MS [4] medium with supplementation of indole-3-acetic acid (IAA) [5]. Although, this crop is most amenable to *in vitro* culture, still its genetic make-up, explant and culture media affect its regeneration potential [6]. Genotype and explant are the most important factor affecting somatic embryogenesis and its further regeneration [7, 8, 9, 10, 11, 12]. The response of growth hormones in the culture media is also variable within genotype and explant for somatic embryogenesis and organogenesis [13].

The plant tissue culture methods also provide base for the improvement of crop. To induce somaclonal variations, *in vitro* mutations, herbicide tolerance, di-haploid induction, genetic transformation of economically important genes and development of somatic hybrids, efficient plant regeneration protocol is required. Such advance tech-niques in combination with conventional breeding give a momentum to the improvement of a crop. Thus, realizing the prospects for future research, relevant literature to "Plant regeneration in eggplant (*Solanum melongena* L.)" has been reviewed.

Tissue Culture

Plant tissue culture is recognized as a source to generate useful genetic variability (somaclonal variation) for crop improvement [14, 15]. Tissue culture is the growth of tissues or cells separate from the organism. The literal meaning of tissue culture refers to the culturing of tissue pieces, i.e. explant culture. Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micro propagation.

Morphogenesis

Morphogenesis (from the Greek morphê shape and genesis creation, literally, "beginning of the shape") is the biological process that causes an organism to develop its shape. It is one of three fundamental aspects of developmental biology along with the control of cell growth and cellular differentiation. The process controls the organized spatial distribution of cells during the embryonic development of an organism. Morphogenesis can take place also in a mature organism, in cell culture or inside tumor cell masses. Morphogenesis also describes the development of unicellular life forms that do not have an embryonic stage in their life cycle, or describes the evolution of a body structure within a taxonomic group. Morphogenetic responses may be induced in organisms by hormones, by environmental chemicals ranging from substances produced by other organisms to toxic chemicals or radionuclides released as pollutants, and other plants, or by mechanical stresses induced by spatial patterning of the cells. The capacity of cultured plant tissues and cells to undergo morphogenesis, resulting in the formation of discrete organs or whole plants, has provided opportunities for numerous applications of in vitro plant biology in studies of basic botany, biochemistry, propagation, breeding, and development of transgenic crops.

Importance of Tissue Culture in Brinjal

Brinjal is highly susceptible to different insects, pests and diseases that exert a deleterious effect on yield, market quality, storability and international germplasm distribution. To overcome this situation, plant tissue culture offers an efficient method for pathogen free materials and germplasm preservation of plants. Eggplant tissues 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. Taken together, these characteristics also make eggplant a complete model for studies on different areas of plant science, including control of gene expression and assessment of genetic stability of somaclones derived from different morphogenetic processes. The potential value of tissue culture in plant breeding has been widely recognized, and it is generally used as useful tool for crop improvement. Regeneration of valuable economic plants through tissue culture based on the principle of totipotency, individual plant cell is capable of regenerating new plantlets. Anwar *et al*. (2002) cultured the aborigine leaf explants on MS media containing IAA, BA (benzyl adenine), and IBA, NAA or 2, 4-D at 2 mg /l. NAA produced greenish, fast-growing callus. 2, 4-D induced early callus production from the petiole, while BA induced green callus production from the upper surface of the lamina. The addition of NAA or IBA at 0.5 mg/l in BA supplemented medium increased the mass production of callus and shoot regeneration. The regeneration efficiency of the plant decreased in MS medium supplied with kinetin (2 mg/l) and NAA (0.5 mg/l).

Tissue culture protocols for organogenesis, somatic embryogenesis, another culture and protoplast culture have been well established [16, 17, 18, 19, 20]. However, the regeneration efficiency reported in different systems of *S. melongena* was relatively low [8]. The type and concentration of a given growth regulator can cause significant differences in the callus formation and morphogenic responses of eggplant. For example, Kamat and Rao [21] reported shoot regeneration from hypocotyl segments of S. melongena in presence of indole acetic acid (IAA). In response to different α-naphthalene acetic acid (NAA) and thidiazuron (TDZ) concentrations using the same explant both embryogenesis and organogenesis were reported [22, 23]. Macchia *et al*. [24] induced callus and root formation from leaf explants of F1 hybrids in eggplants on MS medium containing indole-3-butyric acid (IBA). Induction of organogenic calluses from cultured roots and subsequent differentiation into shoot buds were observed on MS medium in the presence of TDZ and 6-benzylaminopurine (BAP) [25].

The seeds of brinjal were collected and the seeds were then washed thoroughly in running tap water. The instruments like scalpels, forceps, needles etc. were sterilized inside the Laminar Air Flow Cabinet. Other requirements like petridishes, distilled water and glassware were sterilized by an autoclave. The surface sterilization of these seeds was carried out by dipping and flaming method under a Laminar Air Flow Cabinet and others were rinsed in 70% ethyl alcohol for one minute, and then thoroughly washed with sterilized distilled water. The alcohol treated seeds were sterilized with 0.1% $HgCl_2$ solution for 8-10 minutes, few drops Tween-20 per 100 ml was also added at that time. The seeds were then washed 5-6 times with sterilized distilled water. The seeds were then ready for placement into the media. Sterilized seeds were placed into seed germination medium in petridish. Six seeds were placed in each petridish. The culture was then incubated in dark till the germination of seeds. These were then transferred to 16 hours light for normal seedling growth. MS [4] basal medium with different concentrations and combinations of BAP (0, 2.0, 3.0 and 4.0 mg/l) and NAA (0, 0.1, 0.5 and 1.0 mg/l) were used. Six pieces (2-3 mm) of stem segments were arranged horizontally on each petridish and gently pressed into the surface of the sterilized culture medium with various concentrations and combinations of hormones like NAA and BAP. The petridish was covered and sealed with para film. Leaf segment from each germinated seedling were cut into small pieces using sterilized scalpel under a laminar air flow cabinet. Six pieces of leaf segments were arranged on each petridish and gently pressed into the surface of the sterilized culture medium. The petri dishes were covered and sealed with para film. Root tip segments (0.5mm) were placed on a sterilized petridish under a laminar air flow cabinet. The petridish was covered and sealed with para film. Cultured on MS media supplemented with different combinations and concentrations of BAP (0, 2.0, 3.0 and 4.0 mg/l) and NAA (0, 0.1, 0.5, and 1.0 mg/l). The highest amount of callus (48.66%) was produced on MS medium containing 2.0 mg/l BAP and 0.5 mg/l NAA from stem and 8.2 days required for callus formation. The growth of callus was faster on MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA from the stem. Maximum number of plant regeneration through callus from stem containing 2.0 mg/l BAP and 0.5 mg/l NAA were 3.4 (23.287%) and from leaf containing 2.0 mg/l BAP and 0.5 mg/l NAA were 1.6 (11.94%). Successful development of morphogenic calli was obtained from isolated microspores, resulting in the production of putative spontaneously doubled haploids.

Organogenesis

Organogenesis has been successfully achieved in cultivated and wild varieties as well as their hybrids. Fassuliotis [26] was the first to report regeneration in *S. sisymbriifolium* Lam., a wild species of eggplant. Stem parenchyma cells were isolated and cultured on Linsmeier-Skoog (LS) medium supplemented with 6-(γ,γ-dimethlallylamino)-purine (2ip) and indole -3-acetic acid. Kamat and Rao [21] reported shoot regeneration from hypocotyl segments of *S. melongena* and the F1 hybrids in the presence of cytokinins, Kinetin and zeatin. The study by Allicho *et al.* [27] has also shown the presence of cytokinin is necessary for the shoot differentiation in eggplant. Adventitious shoots have been formed by BAP, zeatin or kinetin supplemented media [28, 29, 8]. Recently, use of thidiazuron (TDZ) has shown to enhance shoot organogenesis; the leaves and the cotyledons respond best to TDZ [19]. Different sources of explant have been used for the induction of organogenesis in eggplant, including hypocotyl [21, 23, 27, 8, 19], leaf [28, 27, 29, 8, 19], cotyledon [27, 8, 19], epicotyl [22], stem nodes [19] and roots [25]. The use of low concentrations (100-200 nM) of thidiazuron (TDZ) was also reported to induce efficient organogenesis in five cultivars (around 20 shoots/explant) from leaf and cotyledon explants [19]. The effect of different sugars and osmotic conditions has been studied and highest regeneration rates were observed in media with low sucrose concentrations (11 and 22mM) during shoot development. However, the normal concentration of sucrose used Murashige and Skoog (MS) medium (88mM) induced more efficient root development [29]. Table-1.

The somaclonal variations in eggplant are caused by the hormonal concentrations in culture medium. The effect of the growth regulators NAA and 2,4Dichlorophenoxyacetic acid (2, 4-D) on somaclonal variations in eggplant were studies. However, practical application of plant regeneration for isolation of somaclonal variation has laggeddue to the non-availability of mass scaling techniques and effective field delivery systems [6]. Table-2.

Calluses of *S. melongena* could be successfully induced from in vitro germinated seedlings when explants from cotyledons and hypocotyls were cultured on MS media added with NAA and BAP .Levels and NAA/BAP ratio supplemented to MS medium caused dedifferentiation of cells in the used cotyledon- and hypocotyl-derived tissues. Also, callus induction response may be affected by the specificity of explants used for shoot regeneration; thus, explants from 30-days old seedlings revealed better callus induction frequency and subsequent regeneration compared with explants from in vitro developed older plants. Further, the differences in callus and organ induction may be a result of genotype or cultural conditions [10, 19, 12, 8]. Indirect regeneration can lead to *Solanum melongena* improvement and even to incorporation of valuable and desirable traits into eggplant cultivars.

Table-1: *In vitro* regeneration studies of eggplant (*Solanummelogena* L.).

Mechanism	Explant	Growth Regulators (µM)	Reference
Organogenesis	Hypocotyl	5.7 IAA + 4.4 BAP	Kamat &Rao 1978
	Hypocotyl	1 BAP alone or with 0.09 NAA	Matsuoka & Hinata 1979
	Hypocotyl, cotyledon and leaf	1.8 2.4-D	Alicchio *et al*. 1982
	Leaf	44.4 BAP	Gleddie *et al*. 1983
	Leaf	9.3 KIN	Mukherjee *et al.* 1991
	Hypocotyl, cotyledon and leaf	11.1 BAP + 2.9 2.4-D	Sharma & Rajam 1995a
	Epicotyl, hypocotyl, cotyledon, leaf and node	0.2 TDZ	Magioli *et al.* 1998
	Root	0.5 TDZ + 13.3 BAP	Franklin & Sita 2003
Embryogenesis	Zygotic embryos	5.4 NAA	Yamada *et al.* 1967
	Hypocotyl	43 NAA	Matsuoka & Hinata 1979
	Cell suspension culture and leaf	54 NAA	Gleddie *et al*. 1983
	Cotyledon	27 NAA	Fobert& Webb 1988
	Leaf and cotyledon	5.4 NAA	Fillippone & Lurquin 1989
	Hypocotyl	2.7 – 10.8 NAA	Ali *et al*. 1991
	Leaf	43 NAA + 0.5 KIN	Rao& Singh 1991
	Leaf and cotyledon	50 2.4-D	Saito & Nishimura 1994
	Hypocotyl, cotyledon and leaf	5.7 - 54 NAA	Sharma & Rajam 1995a
	Leaf and cotyledon	54 NAA	Magioli *et al.* 2001

IAA - indole-3-acetic acid; BAP - 6-benzylaminopurine; NAA - α-naphthaleneacetic acid; 2.4-D - 2.4-dichlorophenoxyacetic acid; KIN - Kinetin; TDZ - thidiazuron.

Table-2: Cultural conditions and used media for each experimental phase for Indirect Organogenesis in *S. melongena*

Stages	**Culture media**	**Culture conditions**
Callus induction	MS + 2mg/l NAA + 0.5 mg/l BAP	42 days in darkness, 25ºC ± 1ºC
Callus subculture	MS + 0.1mg/l NAA + 1 mg/l BAP + 0.2 mg/l GA3	28 days, 16h light/8h dark, 25ºC ± 1ºC
Shoot regeneration	MS hormone-free	21 days, 16h light/8h dark, 25ºC ± 1ºC
Shoot elongation	MS with 0.4 mg/l GA3	21 days, 16h light/8h dark, 25ºC ± 1ºC
Root formation	MS hormone-free or with 0.1 mg/l IBA	28 days, 16h light/8h dark, 25ºC ± 1ºC
Acclimatization	Peat:Perlite:Sand 2:1:1 (v/v/v)	18-20 days, 16h light/8h

pH =5.8; all types of media were autoclaved at 120 C for 20 min; light intensity – 40 µmol m-2s^{-1}

CONCLUSION

Research work has mainly been focused on the develop-ment of regeneration protocol, somaclonal variations and their physiological as well as morphological aspects in eggplant. An efficient plant regeneration protocol is a pre-requisite for the exploitation of various biotechnological techniques. However, practical utility of the basic protocol is still far away. It can serve as a platform for the transfer of economically important traits through genetic engi-neering, inducing somaclonal variations, *in vitro* muta-tions, double-haploids induction, development and utilize-tion of somatic hybrids, determining herbicide or pesticide tolerance limits in eggplant. Therefore, a remark-able pro-gress can be made in eggplant improvement through the combination of conventional and biotechnological approaches.

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