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Review

Plant regeneration in eggplant (*Solanum melongena* L.): A review

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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.

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 (Singh et al., 2000; Kaur et al., 2004). 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 degradation, pest resistance and economics of the crop. The non-availability of resistance in cultivated, cross-incompatibility with wild relatives (*Solanum mammosum*, *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. 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 (Murashige and Skoog, 1962) medium with supplementation of indole-3-acetic acid (IAA) (Yamada et al., 1967). Although, this crop is most amenable to *in vitro* culture, still its genetic make-up, explant and culture media affect its regeneration potential (Kantharajah and Golegaonkar, 2004). Genotype and explant are the most important factor affecting somatic embryogenesis and its

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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.

further regeneration (Afele et al., 1996; Sharma and Rajam, 1995(a or b?); Dobariya and Kachhadiya, 2004; Franklin et al., 2004; Huda et al., 2007; Mir et al., 2008). The response of growth hormones in the culture media is also variable within genotype and explant for somatic embryogenesis and organogenesis (Slater et al., 2003).

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 techniques 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.

PLANT REGENERATION

Eggplant is highly amenable to cell, tissue and organ culture (Kantharajah and Golegaonkar, 2004). Plant regeneration from tissues of eggplant can be achieved via embryogenesis (Ammirato, 1983) and organogenesis (Flick et al., 1983). It can be done directly from cultured explants or from calli of cell suspension (Fassuliotis et al., 1981), anther (Khatun et al., 2006), microspore (Miyoshi, 1996; Lian et al., 2004) and protoplasts (Saxena et al., 1981, 1987; Kim and Shin, 2005; Oda et al., 2006; Borgato et al., 2007).

Somatic embryogenesis

Somatic embryogenesis is the process of a single cell or a group of cells initiating the developmental pathway. It was first reported in eggplant from immature seed embryos cultured on MS medium supplemented with IAA (Yamada et al., 1967). In general, it is independent or inversely related to organogenesis (Matsuoka and Hinata, 1979). The different factors such as genotype, explant, combination of growth hormones and some other factors affect somatic embryogenesis in eggplant (Kantharajah and Golegaonkar, 2004).

The genotype is the most important factor affecting somatic embryogenesis and significant quantitative differences in their capacity to form embroids among different species like *S. melongena*, *S. melongena* var. *insanum, Solanum gilo*, *Solanum integrifolium* and their F1 hybrids, cultivars, and inbred lines (Alicchio et al., 1982; Gleddie et al., 1983; Ali et al., 1991; Rao, 1992; Anisuzzaman et al., 1993; Huda et al., 2007; Mir et al., 2008, Zayova et al., 2008; Chakravarthi et al., 2010; Kaur et al., 2011a and 2013). The differential responses for regeneration of adventitious shoots and somatic embryos, number of days to shoot initiation and mean number of shoots per callus (Sharma and Rajam, 1995a; Afele et al., 1996; Dobariya and Kachhadiya, 2004) are also there among cultivars. The molecular investigation using polymerase chain reaction (PCR) of different cultivars for the induction of somatic embryos indicated that embryogenic response is due to differences in mRNA expression and consequently gene expression patterns (Afele et al., 1996).

The type of explant is also an important factor for induc-tion of somatic embryos in eggplant (Kantharajah and Golegaonkar, 2004). The use of immature seed embryo (Yamada et al., 1967; Swamynathan et al., 2010), hypo-cotyl (Alicchio et al., 1982; Sharma and Rajam, 1995a; Zayova et al., 2008; Swamynathan et al., 2010, Ray et al., 2010, Kaur et al., 2011a and 2013), cotyledon (Alicchio et al., 1982; Fari et al., 1995b; Zayova et al., 2008; Tarre et al., 2004; Huda et al., 2007; Swamynathan et al., 2010; Kaur et al., 2011a and 2013), leaf (Alicchio et al., 1982; Macchia et al., 1983; Gleddie et al., 1986; Rao and Singh 1991; Ray et al., 2010; Kaur et al., 2011a and 2013), root (Jahan and Syed, 1998; Franklin et al., 2004; Mir et al., 2008; Swamynathan et al., 2010; Ray et al., 2010), anther (Khatun et al., 2006), microspore (Miyoshi, 1996 and Lian et al., 2004) and protoplasts (Saxena et al., 1981 and 1987, Kim and Shin, 2005, Oda et al., 2006; Borgato et al., 2007) have showed different potential for somatic embryogenesis. The differences in regenerative potential of callus, number of shoots and time required for rege-neration in sub-cultures are observed also (Dobariya and Kachhadiya, 2004). The embryogenic competence varies even within hypocotyl and leaf segments (Sharma and Rajam, 1995b; Magioli et al., 2001), which can be due to gradient phytohormones (Ulvskov et al., 1992), develop-mentally regulated genes (Momiyama et al., 1995), distri-bution of polyamine content, arginine decarboxylase (ADC) activity and metabolism correlated with the position in eggplant (Fobert and Webb, 1988; Sharma and Rajam, 1995a, 1995b; Yadav and Rajam, 1997; Yadav and Rajam, 1998). Size and age of explant did not affect callus-initia-tion response, but showed marked influence on shoot regeneration response (Prakash et al., 2012).

Growth hormones like auxins, cytokinins, gibberellins and abscisic acid play role in plant regeneration. However, auxins and cytokinins are of more significance as their ratio determines callogenesis, rhizogenesis, embryogenesis and regeneration. Among auxins, naphthalene acetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D), and IAA generally favour callogenesis and naphthoxy acetic acid (NOA), indole butyric acid (IBA) promotes rhizogenesis (Kamat and Rao, 1978; Fobert and Webb, 1988) in eggplant. However, different concentrations of NAA required for callusing (0.8 mgL^{-1}), rooting (0.016 mgL^{-1}), embryoid formation (8.0 mgL^{-1} NAA) and shooting (no NAA) (Matsuoka and Hinata, 1979; Swamynathan et al., 2010). Growing medium supplemented with IBA resulted in white, friable, and slow growing callus with roots; NAA gave green and fast growing callus; 2, 4-D

induced early callus (Macchia et al., 1983; Anwar et al., 2002). Prolonged and continued callus sub-culture on medium containing 2,4-D progressively lose its ability to regenerate (Reynolds, 1986). Callus induction and somatic embryogenesis on different medium supplemented with different auxins (Alicchio et al., 1982; Gleddie et al., 1986; Saito and Nishimura, 1994; Sharma and Rajam, 1995a; Fari et al., 1995b; Magioli et al., 2001; Picoli et al., 2000; Mir et al., 2008) is listed in Table 1. Among several cytokinins, kinetin (Kin) is effective for shoot bud regeneration (Kamat and Rao, 1978; Alicchio et al., 1982). Other cytokinins 6-bemzylamino purine (BAP) or thidiazuron (TDZ) (Kaparakis and Alderson, 2002), BAP (Picoli et al., 2000), 6-BA (Li et al., 2003) also produced highest percentage of somatic embryos in different explants of eggplant as listed in Table 1. The cytokinins not only inhibit the NAA-induced embryogenic response, but also act synergistically to promote callus growth (Gleddie et al., 1983).

Cytokinin-auxin interactions either promoted or inhibited the development of shoots and roots depending upon their ratio in the medium (Kamat and Rao, 1978). The rege-neration also depends upon the type and concentration of cytokinin. The high concentrations of benzyladenine and all concentrations of kinetin promoted organogenesis, while low concentrations of benzyladenine induced somatic embryogenes as well as organogenesis (Reynolds, 1986). Generally, higher level of auxins and lower of cyto-kinine favours somatic embryogenesis. MS / LS medium supplemented with combination of 10 mgL^{-1} 2, 4-dichloro-phenoxyacetic acid and 1 mgL^{-1} kinetin (Reynolds, 1986), 2ip (γγ-isopentyladenine) and IAA (Fassuliotis, 1975), 8 mgL^{-1} NAA and 0.1 mgL^{-1} Kin (Rao and Singh, 1991, Swamynathan et al., 2010), Zeatin @ 2 mgL^{-1} and NAA @ 0.01 mgL^{-1} (Fari et al., 1995), 1 mgL^{-1} NAA and 2 mgL^{-1} BAP (Salih and Al-Mallah, 2000), NAA or IBA at 0.5 mgL^{-1} (Anwar et al., 2002), 6-BA+ ZT (Zeatin) and 6-BA+IAA or ZT+ IAA (Yu et al., 2003; Li et al., 2003), 2.0 mgL^{-1} NAA + 0.05 mgL^{-1} BAP, 2.0 mgL^{-1} NAA and 0.5 mgL^{-1} BAP, 2 mgL^{-1} 2,4-D + 0.05 mgL^{-1} BAP and 2 mgL^{-1} NAA+2.5 mgL^{-1} BAP (Rahman et al., 2006; Huda et al., 2007; Hossain et al., 2007; Zayova et al., 2008; Chakravarthi et al., 2010) induced the callus in eggplant (Table 1).

Gene expression during initial stage of somatic embryogenesis in eggplant revealed that 2,4-D induces specific alteration in gene expression due to differential display of RNA (Momiyama et al., 1995). In spite of this, an antioncogen homolog and the activation of retro-transposon were described during early stages of somatic embryogenesis (Momiyama et al., 1996). Differential display and restriction fragment length poly-morphism (RFLP) analysis resulted in the identification of one organogenesis and two somatic embryogenesis related transcripts (Bucherna et al., 2001).

The frequency of embryogenesis depended on optimal ratio of NO_3^- : NH_4^+ (2:1) in the medium. The optimal su-crose concentration of the medium was 0.06 M, whereas, elevated or reduced level inhibited the embryo-genesis in eggplant (Gleddie et al., 1983). Sucrose concentrations of 0.2 - 0.5% induced somatic embryo-genesis, 1% led to embryogenesis and shoot regene-ration and 2% provoked maximum shoot regeneration, whereas, increased sucrose levels from 3 to 5% decreased the regenerating ability. The lowered sucrose concen-tration from 2 to 0.2% also caused complete bleaching, which can be used for selection of herbicide-resistant mutants (Farooqui et al., 1997). The pesticides like Endlosulfan, Rogor and Kitazin in relation to their concentrations also affected callus induction and multiple shoot formation in eggplant. The callus growth decreased with increasing level of pesticides in medium. Some concentrations (50 - 500 ppm) of pesticides in the medium also formed abnormal callus growth and shoot induction. Among pesticides, Rogor (25 ppm) induced maximum callus (76.0%) and shoots (11.0), whereas, Kitazin 45% EC showed more inhibitory effect than the Endosulfan and Rogor (Sammaiah et al., 2011a, 2011b).

Plant regeneration from tissue culture of *S. melongena* L. can be achieved via embryogenesis (Ammirato, 1983) and organogenesis (Flick et al., 1983). Light could help the development of adventitious rooted shoots from callus (Macchia et al., 1983; Salih and Al-Mallah, 2000). High concentration of 2ip and low concentration of IAA led to differentiation of leaflets with morphogenetic variation in leaves and cytological studies of plants indicated them genetically aberrant (Fassuliotis, 1975). LS medium without hormones also regenerated plant from callus (Alicchio et al., 1982). Also, MS medium supplemented with different concentrations and combinations of cytokinins and auxins (Table 1) produced more shoot primordial and rooted shoots in calli derived from cotyledon, hypocotyls, leaf and root explants (Macchia et al., 1983; Anwar et al., 2002; Yu et al., 2003; Franklin et al., 2004; Rahman et al., 2006; Chakravarthi et al., 2010). Plants regenerated through somatic embryogenesis had somaclonal varia-tions. Frequencies of somaclonal variations in leaf shape, plant height, fruit shape and pollen fertility was higher with NAA than that of 2, 4-D (Hitomi et al., 1998). There-fore, the future research would determine the importance of new somaclonal lines for genetic variability of eggplant (Zayova et al., 2010, 2012).

Organogenesis

Organogenesis is the morphogenesis of plantlets directly from explants without the intervention of callus in the culture. This omits the callus and embryoid phases, reduces use of auxin from the *in vitro* culture and leads to direct formation of new shoots from the explants. Anatomically and histolosically, longitudinal sections of leaf explants formed numerous meristematic zones within the tissue, that subsequently converted into shoot buds (Mukherjee et al., 1991). The formation of shoot buds was characterized

Table 1. Somatic embryogenesis in eggplant.

Explant	Somatic embryogenesis	Shoot induction	References
Immature embryo cultures	MS + IAA		Yamada et al. (1967)
Hypocotyl	MS + 0.016 mgL^{-1} - 0.8 mgL^{-1} NAA (callus), MS + 8.0 mgL^{-1} NAA(embryogenesis)	Hormone free MS	Matsuoka and Hinata (1979)
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	Kao/ NT (liquid)+ 10 mgL^{-1} NAA or Kao/ NT(liquid)+ 2 mgL^{-1} 2,4-D, Kao/ NT(liquid)+ 1 mgL^{-1} 2,4-D	Kao/ NT (solid)+ 10 mgL^{-1} NAA or Kao/ NT(solid)+ 2 mgL^{-1} 2,4-D, Kao/ NT(solid)+ 1 mgL^{-1} 2,4-D	Gleddie et al. (1986)
Stem segments	MS+ 10mgL^{-1} 2, 4-D +1 mgL^{-1} kin	MS+ +1 mgL^{-1} kin	Reynolds (1986)
Cotyledon	MS+1.0-5.0 mgL^{-1}NAA	Hormone free MS	Fobert and Web (1988)
Hypocotyl	MS+ 0.5-2.0 mgL^{-1} 2,4-D	Hormone free MS	Ali et al. (1991)
Leaf	MS+ 8 mgL^{-1}NAA + 0.1 mgL^{-1} Kin	Basal MS	Rao and Singh (1991)
Leaf	MS +0.5-2.0 mgL^{-1} NAA	Basal MS	Rao (1992)
Cotyledon	50 αM 2,4-D	half-strength MS solid medium without hormones	Saito and Nishimura(1994)
Hypocotyl, cotyledon and leaf	MS+ 32.2 µM (hypocotyls) and, MS + 10.7 µM (cotyledon and leaf)	-	Sharma and Rajam (1995a)
cotyledon	TMG+ 2 mgL^{-1} Zeatin + 0.01 mgL^{-1} NAA(callus), TMG+4 mgL^{-1} NAA(SE)	TMG+ 2 mgL^{-1} Zeatin + 0.01 mgL^{-1} NAA(callus), TMG+4 mgL^{-1} NAA(SE)	Fari et al. (1995)
Leaf	10.73 *mM* NAA+0.5m M putriscine	-	Yadav and Rajam (1997)
Stem and leaf	MS+ 1 mgL^{-1} NAA + 2 mgL^{-1} BAP	MS+ 1 mgL^{-1} NAA + 2 mgL^{-1} BAP	Salih and Al-Mallah (2000)
Hypocotyl, cotyledon, leaf, epicotyl	MS + 54 αM	½ MS+1% phytagel	Magioli et al. (2001)
Hypocotyl and cotyledon	MS +2.5-10.0 mgL^{-1} NAA	-	Picoli et al.(2000)
Leaf	MS +2 mgL^{-1} 6-BA+0.5 mgL^{-1} IBA, MS +2 mgL^{-1} 6-BA+0.5 mgL^{-1} NAA	MS +2 mgL^{-1} 6-BA+0.5 mgL^{-1} IBA, MS +2 mgL^{-1} 6-BA+0.5 mgL^{-1} NAA	Anwar et al. (2002)

Table 1. Contd.

Cotyledon, hypocotyl	MS +1.0-2.5 mgL^{-1} 6-BA	MS +1.0-2.5 mgL^{-1} 6-BA	Yu et al. (2003)
cotyledon	54 mM NAA	MS basal	Tarre et al. (2004)
Root	MS + 0.45 mM TDZ (Thidiazuron) and 13.3 mM BA (6-benzyladenine)	MS + 0.45 mM TDZ (Thidiazuron) and 13.3 mM BA (6-benzyladenine)	Franklin et al. (2004)
Cotyledon and young leaf explant	MS+1 mgL^{-1} BA MS+2 mgL^{-1} KIN MS+1 mgL^{-1} BA+1mgL^{-1} KIN MS+2 mgL^{-1} BA+1mgL^{-1} KIN MS+2 mgL^{-1} KIN+1 mgL^{-1} BA MS+2 mgL^{-1} BA+2 mgL^{-1} KIN	MS+1 mgL^{-1} BA MS+2 mgL^{-1} KIN MS+1 mgL^{-1} BA+1mgL^{-1} KIN MS+2 mgL^{-1} BA+1mgL^{-1} KIN MS+2 mgL^{-1} KIN+1 mgL^{-1} BA MS+2 mgL^{-1} BA+2 mgL^{-1} KIN	Dobariya and Kachhadiya (2004)
Cotyledon and midrib	MS+ 2.0 mgL^{-1} NAA and 0.05 mgL^{-1} BAP	MS+ 2.0 mgL^{-1} Zeatin and 1.0 mgL^{-1} BAP	Rahman et al. (2006)
Cotyledon	MS+ 2.0 mgL^{-1} NAA and 0.05 mgL^{-1} BAP, MS+ 1.0 mgL^{-1} BAP+ 0.5 mgL^{-1} GA3	MS+ 2.0 mgL^{-1} NAA and 0.05 mgL^{-1} BAP, MS+ 1.0 mgL^{-1} BAP+ 0.5 mgL^{-1} GA3	Huda et al. (2007)
Cotyledon	MS+ 2.0 mgL^{-1} NAA + 0.05 mgL^{-1} BAP, MS+ 2.0 mgL^{-1} 2,4-D+ 0.05 mgL^{-1} BAP,	MS+ 0.75 mgL^{-1} NAA+ 1.5 mgL^{-1} BAP, MS+ 2.0 mgL^{-1} NAA+ 0.5 mgL^{-1} IBA	Hossain et al. (2007)
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 hypocotyl	MS + 2.0 mgL^{-1} NAA + 0.5 mgL^{-1} BAP	Hormone free MS	Zayova et al. (2008, 2012)
immature seed embryo, cotyledon, shoot	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, 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
Cotyledon	MS+ 2 mg/ mgL^{-1} l NAA+2.5 mgL^{-1} BAP	MS+2.5 mgL^{-1} each BAP and KN	Chakravarthi et al. (2010)
Cotyledon	MS + 2 mgL^{-1} NAA	MS+0.5 mgL^{-1} IAA +3.0 mgL^{-1} BAP	Sammaiah et al. (2011a& 2011b)
Hypocotyl, cotyledon and root	MS+2.5 mgL^{-1} /l IAA + 0.5 mgL^{-1} BAP	MS+2.5 mgL^{-1} IAA + 0.5 mgL^{-1} BAP	Mir et al. (2011)
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)
shoot tip, hypocotyls, leaves, stem	MS+0.6 mgL^{-1} 2, 4-D	MS+0.2 mgL^{-1} BAP, MS+0.6 mgL^{-1} NAA, MS + 0.4 mgL^{-1} IAA	Robinson and Saranya (2013)

Table 2. Organogenesis in eggplant.

Explant	Direct organogenesis	References
Hypocotyl	MS + 2.8-11.4 µM IAA, MS + 4.7 µM KIN, MS + 2.3-4.6 µM ZT	Kamat and Rao **1978**
Leaf	MS + 2.0 mgL^{-1} Kin+ 88mM sucrose, MS + 2.0 mgL^{-1} Kin+ 5.5 and 11mM glucose	Mukherjee et al., (1991)
Leaf	MS + 1.0 mgL^{-1} BAP +0.5 mgL^{-1} ZT	Perrone et al., 1992
Hypocotyl, cotyledon and leaf	MS + 11.1 µM BA and 2.9 µM IAA	Sharma and Rajam, 1995a
Cotyledon	TMG + 2 mgL^{-1} Kin	Fari et al., 1995
Leaf	MS + 0.1 µM TDZ and MS + 10 or 20 µM2ip	Billings et al., 1997
Leaf	MS + 0.001-1 μgml^{-1}TDZ and MS + 5-20 μgml^{-1} 2ip	Jelenkovic and Billings 1998
Leaves and cotyledons	MS + 0.2 w*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 0.2 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
Hypocotyls, cotyledon 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
Hypocotyl (inverted)	MS + 0.5 mgL^{-1} TDZ	Mallaya and Ravishankar, 2013

by the appearance of shoot apex with the developing leaf primordial (Sarker et al., 2006). Genotype played important role in organogenesis of the shoots directly from the explants. Different varieties and species such as *Solanum aethiopicum, Solanum macrocarpon* showed different 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; Sarker et al., 2006; Shivraj and Srinath, 2011).

The direct regeneration potential also varied with the tissue system used on a well defined medium. Different explants had differential response to regeneration (Sharma and Rajam, 1995a; Magioli et al., 1998; Zhang, 1999; Taha and Tizan, 2002; Sarker et al., 2006; Gisbert et al., 2006; Kanna and Jayabalan, 2010; Shivraj and Srinath, 2011; Kaur et al., 2011) on different media combinations containing cytokinins and auxins. Hypocotyl and cotyledon explants had different morphogenetic potential for numbers of adventitious shoots (Sharma and Rajam, 1995a; Zhang, 1999). Explant age also affected regeneration as younger leaves showed better organogenesis than mature ones (Zhang, 1999).

Different growth regulators such as auxins and cytokinins have been used for direct organogenesis (Table 2). Among these, auxins had influenced the regeneration of shoot buds and roots in eggplant (Kamat and Rao, 1978). Presence of any cytokinin in the media led to shoot organogenesis from leaf explants (Gleddie et al., 1983; Polisetty et al., 1994). However, combinations and concentrations of auxins and cytokinin should be optimum for

having maximum number of regenerated shoots in eggplant (Mukherjee et al., 1991; Fari et al., 1995; Magioli et al., 1998; Zhang, 1999; Picoli et al., 2000; Sarker et al., 2006). Combinations of two cytokinins had shown proficient shoot differentiation (2 to 7 shoots per explant) in eggplant (Iannamico et al., 1993; Billings et al., 1997; Jelenkovic and Billings, 1998; Gisbert et al., 2006; Shivraj and Srinath, 2011; Kanna and Jayabalan, 2010).

Low sugar concentrations enhanced shoot regeneration, where, higher concentration of glucose and lower of sucrose showed better effects (Mukherjee et al., 1991; Polisetty et al., 1994). Shoot regeneration process had also been affected by the gelling agents and agar was found superior to gerlite (Perrone et al., 1992). Peptone had no effect on reducing hyperhydric shoots of *S. melongena* and *S. integrifolium*. Culture vessels with gas-permeability by membrane filter reduce the percentage of hyperhydric shoots and increased survival rate than sealed vessels (Takamura et al., 2006).

Elongation and rooting of plantlets

Small shoots require elongation *in vitro*. Hormone free MS or 1/2MS has been most frequently used for the elongation plantlets in eggplant (Gleddie et al., 1983; Magioli et al., 1998; Franklin and Sita, 2003; Franklin et al., 2004; Gisbert et al., 2006; Sarker et al., 2006; Borgato et al., 2007; Mir et al., 2011). Sometimes, MS fortified with gibberellic acid (GA3) (0.1 to 1.5mgL^{-1}) (Shivraj and Srinath, 2011), 0.5 mg/l 2,3,5-triiodobenzoic acid (TIBA) and 0.1 mg/l GA3 (Naveenchandra et al., 2011) Zeatin and Augmentin (Billings et al., 1997) elongated eggplant shoots also.

Eggplant developed roots upon transfer to medium containing IAA (Fassuliotis, 1975), hormone-free MS medium (Saxena et al., 1981; Gleddie et al., 1983; Taha and Tizan, 2002; Gisbert et al., 2006; Sarker et al., 2006) and MS medium containing 0.1 -1.5 mgL^{-1} 3-indol butyric acid (Jahan and Syed, 1998; Borgato et al., 2007; Sharmin et al., 2008; Chakravarthi et al., 2010; Shivraj and Srinath, 2011; Zayova et al., 2012; Robinson and Saranya, 2013, Bhat et al., 2013). Half strength MS medium containing 0.08 mgL^{-1} NAA also developed roots of 90% shoots (Kanna and Jayabalan, 2010). Half-strength MS medium supplemented with 0.6 w*m* IAA (Magioli et al., 1998) and 5.0 mg sucrose and 2.5 gl^{-1} gellan gum (Kim and Shin, 2005; Oda et al., 2006) induced rooting of plantlets. Quarter strength hormone free MS medium induce roots also (Dobariya and Kachhadiya, 2004), however, MS+ 3.0 mgL^{-1} BAP was used for better root induction with respect to average number (14 - 15) and mean length (12 cm) (Rahman et al., 2006).

Hardening and field establishment

Most of the species grown *in vitro* require acclimatization process in order to ensure that sufficient number of plants survive and grow vigorously on transferring to the soil. It took 3-4 months from initiation to establishment in pots *ex vitro* for 99% survival rate (Polisetty et al., 1994), however, rooted plants can be acclimatized in 14 days with 80% success (Salih and Al-Mallah, 2000; Taha and Tizan, 2002; Chakravarthi et al., 2010; Kanna and Jayabalan, 2010; Shivraj and Srinath, 2011; Kaur 2011a, b). Rooted shoots were transferred for establishment in polythene bags filled with a potting mixture of sand, soil and FYM in 1:2:1 ratio (Dobariya and Kachhadiya, 2004). The plantlets were successfully established in polycarbonated polyhouse with 100% survival rate (Bhat et al., 2013). When root system was developed well, plants were hardened in the glass house and transferred to the field for flowering, fruiting and seeding (Kamat and Rao, 1978; Gleddie et al., 1983; Jahan and Syed, 1998; Magioli et al., 1998; Franklin et al., 2004; Sarker et al., 2006).

CONCLUSIONS

Research work has mainly been focused on the development of regeneration protocol, somaclonal variations and their physiological as well as morphological aspects in eggplant. An efficient plant regeneration protocol is a prerequisite 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 engineering, inducing somaclonal variations, *in vitro* mutations, double-haploids induction, development and utilization of somatic hybrids, determining herbicide or pesticide tolerance limits in eggplant. Therefore, a remark-able progress can be made in eggplant improvement through the combination of conventional and biotechnological approaches.

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