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Eggplant (*Solanum melongena* L.): tissue culture, genetic transformation and use as an alternative model plant

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RESUMO— (Berinjela (*Solanum melongena* L.): cultura de tecidos, transformação genética e uso como planta modelo). A berinjela é uma espécie solanácea não tuberosa de importância agronômica, cultivada principalmente por seus frutos. Na medicina popular, a berinjela é indicada para o tratamento de várias doenças, incluindo diabetes, artrite, asma e bronquite. A berinjela é suscetível a várias doenças e pragas que causam perdas econômicas significativas. Esse problema tem sido abordado com técnicas convencionais de melhoramento, utilizando espécies silvestres resistentes de *Solanum*, que possuem uma grande diversidade genética e são fontes de genes de interesse agronômico. A aplicação de metodologias *in vitro* à berinjela tem resultado em sucesso considerável. Os tecidos de berinjela apresentam um alto potencial morfogenético, sendo úteis para estudos de desenvolvimento e para o estabelecimento de abordagens biotecnológicas para a produção de variedades melhoradas, tais como o resgate de embriões, a seleção *in vitro*, a hibridização somática e a transformação genética. O conjunto dessas características também torna a berinjela um modelo completo para estudos em diferentes áreas de pesquisa, incluindo o controle da expressão gênica e a avaliação da estabilidade de somaclones derivados de diferentes processos morfogenéticos. Neste trabalho, são analisados fatores importantes que afetam a eficiência dos processos de regeneração *in vitro* por meio de organogênese e embriogênese, assim como de transformação genética, explorando ainda o potencial da espécie como planta modelo para o estudo de vários aspectos da genética e fisiologia vegetais.

Palavras-chave: *Solanum melongena* L., biotecnologia, cultura de tecidos, transformação genética, planta modelo

ABSTRACT —(Eggplant (*Solanum melongena* L.): tissue culture, genetic transformation and use as an alternative model plant). Eggplant is an agronomically important non-tuberous solanaceous crop grown primarily for its large oval fruit. In popular medicine, eggplant is indicated for the treatment of several diseases, including diabetes, arthritis, asthma and bronchitis. 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 *Solanum* species, which present a wide genetic diversity and are source of useful agronomic traits. The application of *in vitro* methodologies to eggplant has resulted in considerable success. 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. In the present study, important factors that affect the efficiency of *in vitro* regeneration through organogenesis and embryogenesis as well as genetic transformation are analyzed. The potential of this species as a model plant for studying various aspects of plant genetics and physiology is also discussed.

Key words: *Solanum melongena* L., biotechnology, tissue culture, genetic transformation, model plant

Introduction

Eggplant (*Solanum melongena* L.; Division Anthophyta; Class Dicotyledoneae; Order Solanales) is an agronomically important non-tuberous solanaceous crop grown primarily for its large oval fruit. Eggplant is native to India and China and was probably introduced to Europe by Arabic traders and then brought to North America by early European settlers.

In popular medicine, eggplant is indicated for the treatment of several diseases, including diabetes, arthritis, asthma and bronchitis. In addition, several groups have provided evidence that eggplant extracts have a significant effect in reducing blood and liver cholesterol rates in humans (Khan 1979; Jorge *et al.* 1998) and adult rats (Silva *et al.* 1999). Nasunin, a major component of anthocyanin pigment of eggplant, has been shown to inhibit lipid peroxidation (Igarashi *et al.* 1993). More recently, free radical scavenging and iron chelating activities of nasunin were demonstrated by electron spin resonance (Noda *et al.* 1998; 2000). Furthermore, anti-mutagenic activity of pheophytin components from eggplant fruit extracts

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acting against several chemical mutagens was demonstrated by the *Salmonella*/microsome assay (Yoshikawa *et al.* 1996a; 1996b).

Eggplant is susceptible to several diseases and pests that cause serious crop losses. This problem has been addressed by hybridizing eggplant with wild resistant *Solanum* species, which present a wide genetic diversity and are source of useful agronomic traits. However, this approach is limited by sexual incompatibilities (Collonier *et al.* 2001) and difficulties in obtaining fertile progenies (Gleddie *et al.* 1986). In addition, traditional improvement methods may be hampered by the scarcity of natural resistance sources for some important diseases, impairing the obtention of resistant varieties (O'Brien 1983; Melo & Costa 1985; Lin & Xiao 1995). For example, no natural resistance sources are known for anthracnosis (*Colletotrichum gloeosporioides*), southern wilt (*Ralstonia solanacearum*) and the most common fungal disease of eggplant in Brazil, the *Verticillium* wilt (*Verticillium dahliae*).

The application of *in vitro* methodologies to eggplant has resulted in considerable success. 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.

Recent reviews on applications of biotechnology in eggplant have focused in aspects related to genetic resources and improvement of agronomic traits (Collonier *et al.* 2001; Kashyap *et al.* 2003). In the present review, important factors that affect the efficiency of *in vitro* regeneration through organogenesis and embryogenesis as well as genetic transformation are analyzed. The potential of this species as a model plant for studying various aspects of plant genetics and physiology is also discussed.

Tissue culture

Early studies of *in vitro* regeneration of eggplant were based on culturing cell suspensions (Fassuliotis *et al.* 1981; Gleddie *et al.* 1983), anthers (Isouard *et al.* 1979; Dumas de Vaulx & Chambonnet 1982; Tuberosa *et al.* 1987) and protoplasts (Jia & Potrykus 1981; Saxena *et al.* 1981; Bhatt & Fassuliotis 1981; Guri & Izhare 1984; Gleddie *et al.* 1986; Nishio *et al.* 1987; Li & Zhang 1988; Clark *et al*. 1988). Successful development of morphogenic calli was obtained from isolated microspores, resulting in the production of putative spontaneously doubled haploids (Miyoshi 1996).

Several other protocols for plant regeneration via direct and indirect organogenesis have been developed from different eggplant tissues (Tab. 1). 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. Most of the organogenic systems reported are based on supplementing culture media with auxins and cytokinins, either alone (Kamat & Rao 1978; Matsuoka & Hinata 1979; Alicchio *et al.* 1982; Gleddie *et al.* 1983; Sharma & Rajam 1995a) or in combination (Kamat & Rao 1978; Matsuoka & Hinata 1979; Sharma & Rajam 1995a).

Different sources of explant have been used for the induction of organogenesis in eggplant, including hypocotyl (Kamat & Rao 1978; Matsuoka & Hinata 1979; Alicchio *et al.* 1982; Sharma & Rajam 1995a; Magioli *et al.* 1998), leaf (Gleddie *et al.* 1983; Alicchio *et al.* 1982; Mukherjee *et al.* 1991; Sharma & Rajam 1995a; Magioli *et al.* 1998), cotyledon (Alicchio *et al.* 1982; Sharma & Rajam 1995a; Magioli *et al.* 1998), epicotyl (Magioli *et al.* 1998), stem nodes (Magioli *et al.* 1998) and roots (Franklin & Sita 2003). The regeneration efficiencies reported in those systems were relatively low (approximately 7 shoots/explant) (Gleddie *et al.* 1983; Mukherjee *et al.* 1991; Sharma & Rajam 1995a), except in the one described by Sharma & Rajam (1995a), who achieved the production of 20 shoots/explant in one of the four cultivars studied. The use of low concentrations (100-200nM) of thidiazuron (TDZ) was also reported to induce efficient organogenesis in five cultivars (around 20 shoots/explant) from leaf and cotyledon explants (Magioli *et al.* 1998; 2000). 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 (Mukherjee *et al.* 1991).

Protocols for inducing somatic embryogenesis from eggplant tissues have also been described, using

Table 1. *In vitro* regeneration studies of eggplant (*Solanum melogena* 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.

different growth regulators and explant types (Tab. 1). The first report was published by Yamada *et al.* (1967), who induced somatic embryogenesis from zygotic embryos cultured on MS supplemented with indole-3-acetic acid (IAA). Similarly to the observed in the organogenic process, the efficiency of somatic embryogenesis depends on several factors, including genotype, explant type and growth regulators. In general, high frequencies of somatic embryogenesis induction are obtained in response to the auxins α-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) (Matsuoka & Hinata 1979; Gleddie *et al.* 1983; Ali *et al.* 1991; Saito & Nishimura, 1994; Magioli *et al.* 2001a). However, conversion of somatic embryos into plantlets is usually limited due to abnormalities such as hyperdricity, lack of apical meristem, cotyledon fusion and inefficient maturation (Gleddie *et al.* 1983; Saito & Nishimura 1994; Magioli *et al.* 2001a). Nevertheless, conversion rates can reach up to 92% by culturing mature embryos MS solidified with 1% phytagel (Saito & Nishimura 1994; Magioli *et al.* 2001a). A review on somatic embryogenesis in eggplant focusing on the factors that affect the process as well as its practical applications was recently published by Kantharajah & Golegaonkar (2004).

Studies on the morphological aspects of somatic embryogenesis have been performed in a number of species and showed that somatic embryos can have different origins according to the species and the explant type. For example, in geranium and tomato somatic embryos are formed from the epidermal cells of hypocotyl explants (Hutchinson *et al.* 1996; Newman *et al.* 1996). In peanut, embryos can be formed from meristematic cells of calli derived from cotyledons, from parenchymatic cells of young leaves or from meristematic and submeristematic cells of apical meristems (Sagare *et al.* 1995). In eggplant, somatic embryos induced by NAA are formed from perivascular parenchymatic cells originating indeterminate meristematic masses, which can either give rise to adventitious roots or pro-embryogenic masses (Tarré *et al*. 2004).

The type and concentration of a given growth regulator in association to specific genotypes can cause significant differences in the morphogenetic responses of eggplant. For example, Kamat & Rao (1978) using hypotocyl explants induced only the development of rhizogenic calli in the presence of NAA and a-naphthoxyacetic acid (NOA), while regeneration through organogenesis was obtained in the presence of IAA. Both organogenesis and embryogenesis were observed by Matsuoka & Hinata (1979), in response to different NAA concentrations using the same explant type. Following that study, Matsuoka (1983) screened the effect of different concentrations of NAA

(21.5-108μM) in somatic embryogenesis and obtained best results in the presence of 37.8-48.6μM. Gleddie *et al.* (1983) induced somatic embryogenesis from leaf explants and cell suspension cultures of cultivar Imperial Black Beauty in the presence of 54μM NAA, whereas Fillippone & Lurquin (1989) had obtained best results with leaf and coyledon explants of cultivar Violetta Lunga di Napoli in the presence of a much lower concentration (5.4μM) of the same growth regulator. Mariani (1992) also reported somatic embryogenesis in response to 54μM NAA using seedlings of cultivar Giulietta germinated in the dark. Fobert & Webb (1988) observed a correlation between the development of adventitious roots and somatic embryos in cotyledons from cultivar Imperial Black Beauty, in response to different NAA concentrations. Rhizogenesis was induced in low concentrations (0.5-2.7μM), while intermediate levels favored somatic embryogenesis (5.4-27μM) and higher concentrations (54-270μM) mainly induced callogenesis. Similar results were obtained with the Brazilian variety F-100 (C.S. Magioli, dados não publicados). On the other hand, Rao & Singh (1991) cultivated leaf explants in medium supplemented with 0.5 and 10.8μM NAA and failed to induce embryogenesis with the same concentrations. In their report, embryogenic calli were obtained in the presence of higher NAA concentrations (27 and 64μM), although best results were achieved when NAA was combined with 0.5mM Kinetin (KIN). Higher embryogenesis rates in cotyledons cultured in the presence of 50μM 2,4-D were obtained by Saito & Nishimura (1994), as compared to 54μM NAA. Ali *et al.* (1991) also observed embryogenesis in hypocotyl explants from variety Insanum in the presence of low concentrations of 2,4-D (2.3-9.0μM). In contrast, Alicchio *et al.* (1982) reported the occurrence of organogenesis in response to low concentrations of the same auxin. High induction frequencies of somatic embryogenesis were obtained from leaf and cotyledon explants of the Brazilian variety F-100 in the presence of 54μM of NAA, whereas 2,4-D in different concentrations failed to induce embryo development (Magioli *et al*. 2001a). The occurrence of both somatic embryos and shoot primordia has been reported to occur in the same callus after prolonged culture in the presence of 21.6μM NAA (Fári *et al.* 1995).

In recent years, there has been increasing awareness of bactericidal antibiotics stimulating or inhibiting the regenerative potential of explants, depending on each specific combination between a given antibiotic and plant species. The presence of cefotaxime, ampicillin or vancomycin did not affect embryogenic callus formation and development from leaf and cotyledon explants of eggplant. However, antibiotic treatment reduced the number of embryos produced per callus by 52% (cefotaxime and amplicillin) and 37% (vancomycin) (Magioli *et al.* 2001a). Cefotaxime was found to enhance callus fresh weight, but also causing a decrease on the rate of embryo regeneration, whereas timentin showed no effect on embryo differentiation (Picoli *et al.* 2001).

In vitro regeneration systems have been used as strategies to select valuable agronomic traits in eggplant. Several useful traits from wild species such as resistance to nematodes and atrazin have been found in somatic hybrids obtained from protoplast fusion (Gleddie *et al.* 1985; Guri & Sink 1988a; Collonier *et al.* 2001). Dihaploid plants were produced through anther culture of somatic hybrids between eggplant and *S. awthiopicum gilo*, with the objective of obtaining *Fusarium* resistance (Rizza *et al.* 2002). Specific applications of anther culture and somatic hybridization in breeding programs for eggplant improvement were recently reviewed (Collonier *et al.* 2001; Kashyap *et al.* 2003).

Somaclonal variation is also a possible means of obtaining variation for plant breeding, although studies on somaclonal variants with useful traits are limited. In eggplant, somaclonal variants were observed by Rotino *et al.* (1991) and callus lines resistant to culture filtrate of *Verticillium* were reported by Rotino *et al.* (1987) and Alicchio (1990). Selection of atrazine resistance was achieved by using mutagenized organogenic explants (Farooqui *et al.* 1997). In addition, the frequency and inheritance of somaclonal variations related to morphological characters and pollen fertility among plants derived from somatic embryos induced by 2,4 D or NAA were analysed by Hitomi *et al.* (1998).

Genetic transformation via *Agrobacterium*

Genetic transformation of eggplant via *Agrobacterium* was first reported by Guri & Sink (1988b), using leaf explants and a cointegrate vector, although no success was achieved with a binary vector. Later, Fillipone & Lurquin (1989) reported the transformation of leaf and cotyledon explants using the wild supervirulent strain A281. Transgenic plants were obtained by Rotino & Gleddie (1990) and Fári *et al.* (1995), using organogenic regeneration systems. An optimization of factors that influence transformation

efficiency, including length of pre and post-coculture periods, explant type, and genotype was performed using a TDZ-based organogenic system (Magioli *et al.* 2000). The efficiency of transformation protocols based on organogenesis may be influenced by the antibiotic used to eliminate *A. tumefaciens*. For example, augmentin can cause enhanced shoot proliferation induced by TDZ (Billings *et al.* 1997). Recently, an organogenic system from root explants was applied in a protocol for transformation of variety MEBH 11. These explants demonstrated a high susceptibility to *Agrobacterium* and quick regeneration capacity on selection media, resulting in 82.5% of transgenic calli induction with a means of 24 transgenic shoots per callus (Franklin & Sita 2003).

The production of transgenic eggplant through somatic embryogenesis either fails to occur (Fillipone & Lurquin 1989) or is achieved with very low efficiency (Fári *et al.* 1995). It has been demonstrated that both cocultivation with *Agrobacterium* and the presence of bactericidal antibiotics used in transformation protocols cause a reduction of 80-99% in the number of embryo/explant. The inhibitory effect on somatic embryos development may result from the interaction between the physiological alterations caused by these treatments and the delicate processes of gene regulation that are induced in early culture stages (Magioli *et al.* 2001a).

Following the establishment of basic protocols, successful introduction of agronomic traits into eggplant was achieved (Tab. 2). Resistance to Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) (CPB), a pest that has developed resistance to synthetic insecticides and became a serious problem for agriculture in Europe and America (Arpaia *et al*. 1997), has been pursued by a number of groups. Chen *et al.* (1995) have produced transgenic eggplant lines with the introduction of *Bacillus thuringiensis* (*Bt*) genes, but resistance to CPB was not observed. Later, different groups obtained lines resistant to CPB by using mutagenized versions of *cryIIB* (Arpaia *et al.* 1997; Iannacone *et al.* 1997) and a synthetic version of *cryIIIA Bt* genes (Jelencovic *et al.* 1998). Field trials demonstrated high levels of resistance in transgenic plants produced after the introduction of a mutagenized *Bt cryIIIB* gene, without detrimental effects on nontarget arthropods (Acciarri *et al.* 2000). These resistant transgenic eggplants can potentially be used for the development of new varieties.

Besides CPB resistance, there are other examples of genetic improvement of eggplant via *A. tumefaciens*. Resistance to *Leucinodes orbanalis* was obtained in transgenic plants harboring the *Bt* (*Cry1Ab*) gene. Tolerance against osmotic stress induced by salt drought and chilling stress was achieved in transformants expressing the bacterial mannitol-1-phosphodehydrogenase (*mtlD*) gene (Prabhavathi *et al.* 2002) and transgenic hybrids harboring the parthenocarpic gene *DefH9-iaaM* presented a significant yield increment that resulted in a 10% reduction in cultivation costs (Donzella *et al.* 2000).

Eggplant as a model plant

In addition to providing tools for selection of valuable agronomic traits, *in vitro* culture systems can be used as models to study molecular signals in plant physiology and development. 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 will be discussed herein.

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). Eggplant tissues may favour a clearer picture of these molecular signals in differentiation processes, considering their ability to regenerate plants *in vitro* through different morphogenetic processes. 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 effect of polyamines, polyamine precursors and biosynthetic inhibitors during embryogenesis was reported by Fobert & Webb (1988). A relationship between the spatial distribution of free and conjugated endogenous polyamine and the differential morphogenetic potential within explants have been observed during embryogenesis (Yadav & Rajam 1997) and organogenesis (Scoccianti *et al.* 2000).

Table 2. Genetic transformation of eggplant (*Solanum melogena* L.) via *Agrobacterium.*

Explant	Gene	Observations	Reference
Leaf	*NptII*	Success with a binary vector, no information about transformation efficiency	Guri & Sink 1988
Cotyledon / Leaf	*NptII*	Stable transformation using the wild supervirulent strain A281	Filippone & Lurquin 1989
Leaf	*NptII* and *CAT*	Transformation efficiency of 7,6%	Rotino & Gleddie 1990
Cotyledon	*NptII* and *GUS*	10% of transgenic organogenic calli regenerated plants	Fari *et al.* 1995
Hypocotyl	*Bt (cry IIIB)*	Resistance to CPB was not observed	Chen *et al.* 1995
Hypocotyl	*Bt (cry IIIB)*	Resistance to CPB using a mutagenized version of the *Bt cryIIIB* gene	Arpaia *et al.* 1997
Cotyledon	*Bt (cry IIIB)*	Resistance to CPB using a mutagenized version of the *Bt cryIIIB* gene	Iannacone *et al.* 1997
Leaf	*Bt (cry IIIB)* and *NptII* and *GUS*	Influence of growth regulators and antibiotics on transformation efficiency	Billings *et al.* 1997
Leaf	*Bt (cry IIIA) and GUS*	Resistance to CPB using a synthetic version of the *Bt cryIIIA* gene	Jelenkovic *et al.* 1998
Cotyledon	*Bt (Cry 1Ab)*	Resistance to *Leucinodes orbonalis* using a synthetic *cry1Ab* gene	Kumar *et al.* 1998
Leaf	*Luc*	Evaluation of the stability of luciferase gene expression	Hanyu *et al.* 1999
Cotyledon	*pAtgtrp-5::GUS* and *NptII*	Optimization of factors which influence transformation efficiency	Magioli *et al.* 2000
Cotyledon	*DefH9-iaaM*	Parthenocarpic transgenic plants	Donzella *et al.* 2000
Cotyledon	*MtlD*	Tolerance against osmotic stress	Prabhavathi *et al.* 2002
Root	*NptII* and *GUS-INT*	Stable transformation using root explants	Franklin & Sita 2003

NptII - neomycin phosphotransferase II, *CAT - chloramphenicol acetyltransferase*, *Bt (cry IIIB) - Bacillus thuringiensis cry IIIB*; *Bt (cry IIIA) - Bacillus thuringiensis cry IIIA*; *Bt (Cry 1Ab) - Bacillus thuringiensis cry 1Ab*; *GUS - β-glucuronidase*; *Luc - luciferase*; *pAtgrp-5* - regulatory region of the *Arabidopsis thaliana* glycine rich protein 5; *DefH9-iaaM* - regulatory region of the *DEFICIENS 9* gene from snapdragon and the auxin-synthesizing gene coding region *(IaaM)* from *Pseudomonas syringae* pv *savastanoi*; *MtlD* - bacterial *mannitol-1-phosphodehydrogenase* gene.

Putrescine has been positively correlated to the efficiency of somatic embryogenesis and shown to occur in different levels during the process, while spermine and spermidine showed no significant effect (Sharma & Rajam 1995b; Yadav & Rajam 1997; 1998). In hypocotyl segments, high levels of conjugated spermidine along with high levels of total PA could be correlated with the formation of somatic embryos (Sharma & Rajam 1995b). Studies using root cultures of eggplant demonstrated that PAs, particularly spermidine, are intricately involved in root growth and differentiation of lateral roots (Sharma *et al.* 1997).

The accumulation of BiP (Binding Protein), an endoplasmic reticulum resident, stress-related protein of the Heat shock protein (Hsp) 70 family, was demonstrated in hyperhidric plants originated from an organogenic system of eggplant. This finding supports the assumption that the monitoration of BiP synthesis can be used to detect intracellular stress in plants (Picoli *et al.* 2001). Eggplant was also used as model plant for the study the expression and the incorporation of the luciferase gene (*luc*) and LUC activity in a long-term period. The expression of the *luc* gene was found to be stable and its specific activity was shown to fluctuate in response to environmental conditions (Hanyu *et al.* 1999).

Somatic embryogenesis systems in eggplant are also good models to study the earliest stages of embryo development. Specific alterations in gene expression during early stages of somatic embryogenesis induced by 2,4-D have been described through differential display of RNA species by Momiyama *et al.* (1995) and Afele *et al.* (1996), with the identification of three classes of genes that were newly expressed or showed enhanced expression during the first 10 days of culture. Differential cultivar responses to the induction of somatic embryogenesis were correlated to differences in gene expression patterns (Afele *et al.* 1996). In addition, an antioncogen homolog and the activation of retrotransposon were described during the early phase of somatic embryogenesis (Momiyama *et al.* 1996). Differential display and Restriction Fragment Length Polymorphism (RFLP) analyses were used to study

alterations in DNA methylation and gene expression in cell suspension cultures which produced either somatic embryos or shoots, resulting in the identification of one organogenesis and two somatic embryogenesis related transcripts (Bucherna *et al.* 2001).

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 (Magioli *et al.* 2000), confirming the observations on tobacco and *Arabidopsis* (Sachetto-Martins *et al.* 1995). One important aspect of this work was that embryogenesis in eggplant could be efficiently induced from leaves of *in vitro* transgenic plants. In contrast, induction of somatic embryogenesis in *Arabidopsis* is based on the use of immature zygotic embryos, involving difficult and time-consuming procedures.

Considering that epidermis and stem phloem are sites for pathogen penetration and diffusion, *Atgrp* promotor has potential biotechnological interest and may be used in programs aiming at the genetic improvement of eggplant by introducing genes which confer resistance to diseases or pests. In addition, the analysis of GUS expression during the early stages of somatic embryogenesis using the embryogenic system established by Magioli *et al.* (1998) demonstrated that this promoter is activated simultaneously with the first anatomical events leading to embryo development, indicating that *Atgrp-5* may participate in the early cellular events necessary for entering an embryogenic program (Magioli *et al.* 2001b).

In conclusion, eggplant provides a unique system to study morphogenesis and somaclonal variation, taking into account that *in vitro* regeneration can be induced from different explants, by distinct growth regulators and morphogenetic pathways. In addition, the availability of efficient transformation protocols favors gene regulation studies, especially those related to embryogenesis, with advantages over other species. From this perspective, eggplant can be considered as an alternative model plant to study different aspects of plant biology.

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