Study of genetic transformation efficiency via organogenesis and embryogenesis in eggplant (*Solanum melongena* L. cv. Embú): effects of co-culture, temperature and kanamycin and hygromycin-based selection procedures

Picoli, E. A. de T.[1], Brommonschenkel, S. H. [2], Cecon, P. R.[3], da Silva, D. J. H.[4], Fári, M. G.[5] and Otoni, W. C.[1]

[1]Departamento de Biologia Vegetal and Instituto de Biotecnologia Aplicada à Agropecuária BIOAGRO;
[2]Departamento de Fitopatologia;
[3]Departamento de Informática and
[4]Departamento de Fitotecnia. Universidade Federal de Viçosa, 36571-000, Viçosa, MG, Brasil.
Fax: +55-31-3899-2580 e-mail: wotoni@ufv.br
[5]Department of Vegetable Crops & Horticultural Biotechnology, University of Debrecen, Centre of Agricultural Sciences, H-4032 Debrecen, Böszörményi út 138, Hungary

Summary: The effects of kanamycin and hygromycin-based selection and co-culture temperature ranging from 22 to 28 °C upon eggplant transformation efficiency were evaluated. Both morphogenic pathways, somatic embryogenesis and organogenesis, were adopted using cotiledonary and hypocotyl explants, respectively. Somatic embryos were recovered in the presence of both antibiotics, although lesser escapes were observed in hygromycin-supplemented medium. Indeed, selection provided by this antibiotic was more efficient compared to kanamycin, nevertheless, shoot regeneration was not observed with hygromycin. Significant difference on the frequency of cotiledonary explants displaying callus (FEC) was observed as embryogenesis was concerned, although a higher number of embryos was observed in hygromycin selective media. The frequency of explants presenting callus (FEC), embryos (FEE) and shoots or buds (FERG) did not differ statistically for the tested co-culture temperatures, although higher regenerant number was observed at 24 °C.

Key words: *Solanum melongena*, genetic transformation, antibiotics, co-culture temperature

Introduction

Eggplant (*Solanum melongena* L.) is a solanaceous species which fruits are consumed as food and spice. Its use as medicinal and ornamental plant is also described (*Cruz*, 1965; *Hedrick* 1972). *S. melongena* is a diploid (2n = 24) and autogamous species which displays cross pollination frequencies ranging from 5 to 6.75% (*Sangowawa*, 1988; *Badr* et al., 1997). Among eggplant breeding goals, pathogen resistance is highlighted, although, the lack of resistance characteristics in the genetic pool and genetic incompatibility with resistant wild species are limiting to eggplant breeding programs (*Rotino* et al., 1997; *Collonnier* et al., 2001).

Increasing economic value of the eggplant is observed in the Brazilian market, where Instituto Brasileiro de Geografia e Estatística (IBGE) data registered in 1996 an overall production of 39549 tons. In Brazil, the cultivar Embú (*Filgueira* 1982) and *'F-100'*, *'Super F-100'* and *'Ciça'* hybrids are the most grown genotypes (*Giordano* 1991; *Reifschneider* et al., 1993).

Eggplant is considered a species with pronounced feasibility regarding *in vitro* culture responses, where research on genetic transformation protocols (*Guri & Sink*, 1988; *Rotino & Gleddie*, 1990; *Fári* et al., 1995), insect resistant transgenic plants (*Iannacone* et al., 1995; *Arpaia* et al., 1997; *Jelenkovic* et al., 1997) and virus diseases (*Picoli*, 2000) are mentioned. Reviews on applied biotechnology dealing with eggplant are published (*Rotino* et al., 1997; *Collonnier* et al., 2001). Recent studies explored *Solanum melongena* L. cv. *Embú in vitro* morphogenesis (*Picoli*, 2000; *Picoli* et al., 2000, *Picoli & Otoni*, 2001).

Compared to classical breeding, plant genetic transformation does not depend on sexual compatibility, it demands less time to obtain a variety with the desirable trait and lower number of crosses to recover a genotype. Despite these advantages, its success depends on efficient DNA transfer, transgenic cell selection and regeneration systems

(*Eady & Lister*, 1998). It is well documented that several factors affect genetic transformation of various plant species. *Alt-Möerbe* et al. (1988) observed the influence of temperature on the induction of vir D2, a protein associated with T-DNA transfer, suggesting that it may influence transformation efficiency. The evolutive relation of T-DNA transfer process and conjugation system in bacteria (*Lessi & Lanka*, 1994), allied with the temperature dependence of this system (*Fulner & Nester*, 1996), agrees with this hypothesis. Other features, as bacteria cell concentration (*Lin* et al., 1994) and *vir* genes inducing substances (*De Block*, 1993) may also influence transformation protocols. The antibiotics used for selection are coupled with the regeneration of primary transformants, where only transformed cells should regenerate. Nevertheless, non-transformed plants are observed among the regenerants. Though the need of information on Brazilian eggplant cultivars, this work aimed the optimization of a regeneration and transformation protocol for the cultivar Embú. The co-culture temperature and selection based on kanamycin and hygromycin were evaluated in putative transformants regenerated by somatic embryogenesis or organogenesis.

Material and method

Plant material

Seeds of eggplant (*Solanum melongena* cv. Embú) were purchased from commercial establishments in Viçosa, Brazil. Surface-sterilization was performed by immersion of the seeds in 70% (v:v) ethanol for 1 min, followed by 20 min in a 5% (v:v) sodium hypochlorite solution containing 0.1% (v:v) Tween 20, followed by four rinses in sterile distilled water. Thereafter, seeds were soaked for 24 h, at 26±2°C, in sterile distilled water on a rotatory shaker (100 rpm). Seeds were germinated in a Phytacon (Sigma Chemical Co.,USA) containing 100 ml of germination medium. The latter was composed of MS-based salts (*Murashige & Skoog*, 1962) supplemented with B5 vitamins (Gamborg et al., 1968), 100 mg l^{-1} myo-inositol, 2% (w:v) sucrose, pH 5.7±0.1, and solidified with 0.28% (w:v) Phytagel (Sigma Chemical Co.). After autoclaving (1.2 kg cm^{-2}, 121°C for 15 minutes) and the medium being solidified, 25 to 30 seeds were sown per recipient. *In vitro* grown seedlings (16–20 days after germination) were aseptically manipulated and used as the source explants. Cultures were maintained under 16:8 h light:dark regime, 30 µmol m^{-2} s^{-1} light radiation provided by two fluorescent tubes (Luz do Dia Especial, 20 W, Osram, Brazil).

Agrobacterium strains

Agrobacterium tumefaciens C58C1 pGV 2260 strains, diverging only on the presence of the vectors pRGG neo 5 and pRGG hpt, harboring neomycin phosphotransferase II (*npt*II) and hygromycin phosphotransferase (*hpt*) for plant selection, respectively. Besides the kanamycin resistance gene (pRGG *neo5*) and hygromycin (pRGG *hpt*), these strains also harbored the *gus* marker gene for β-glucuronidase. These constructs, kindly provided by *Dr. István Nagy* (ABC - Agricultural Biotechnology Center, Gödöllõ, Hungary), are shown in *Figure 1*.

Pre-culture, inoculation and Agrobacterium co-culture

Regeneration protocols were described by *Picoli* (2000) and *Picoli* et al. (2000), where somatic embryogenesis were inducted in cotyledonary explants by the use of 5 mg l^{-1} naphtalene acetic acid (NAA), and organogenesis held on hypocotyl segments in medium supplemented with 0.1 mg l^{-1} indol acetic acid (IAA). The explants were kept on non-selective regeneration medium for 24 hours. Next, they were inoculated with an *Agrobacterium* solution, optical density 0.4 (λ = 600 nm), for five minutes. After inoculation, the explants were returned to non-selective medium for additional 24 hours (co-culture period). In the evaluation of antibiotics selection experiment the temperature was set to 28°C. All Petri dishes had a sterile filter paper (80 mm diameter) between the medium and the explants.

Medium and culture conditions

After co-culture, the cotyledon explants were transferred to selective medium with it abaxial face in contact with the medium. Disposable Petri dishes with 9 cm diameter (J. Prolab, Brasil) were used, each containing 25 ml of medium composed of MS salts, B_5 vitamins and 0.8% agar (w/v) (Sigma Chemical Company, EUA). Each cotyledon section was considered one explant. NAA at 5 mg l^{-1} was used for promoting somatic embryogenesis in cotyledonary explants. The same conditions were adopted for hypocotyl explants, where each segment was considered to be one explant, although organogenesis was induced by means of 0.1 mg l^{-1} IAA in the latter type of explant. Either organogenesis and somatic embryogenesis selection was performed with 50 mg l^{-1} kanamycin (Sigma Chemical Company, EUA), or with 7.5 and 10 mg l^{-1} hygromycin (Sigma Chemical Company, EUA), respectively. All selective medium also contained 300 mg l^{-1} timentin (SmithKline Beechan Brasil Ltda).

Antibiotics selection and co-culture temperature

The effects of kanamycin and hygromycin selection over putative transformants were evaluated. *A. tumefaciens* strains C58C1 neo5 and C58C1 hpt were used for genetic transformation of eggplant. Explants submitted to somatic embryogenesis and organogenesis were transformed with both strains. The influence of four co-culture temperatures, 22, 24, 26, and 28°C, on primary regenerants was evaluated. *Agrobacterium* strains C58C1 hpt and C58C1 neo5 were selected for experiments of somatic embryogenesis and organogenesis, respectively. In all experiments of somatic embryogenesis, the frequency of explants regenerating calli (FEC), frequency of explants with embryos (FEE) and the

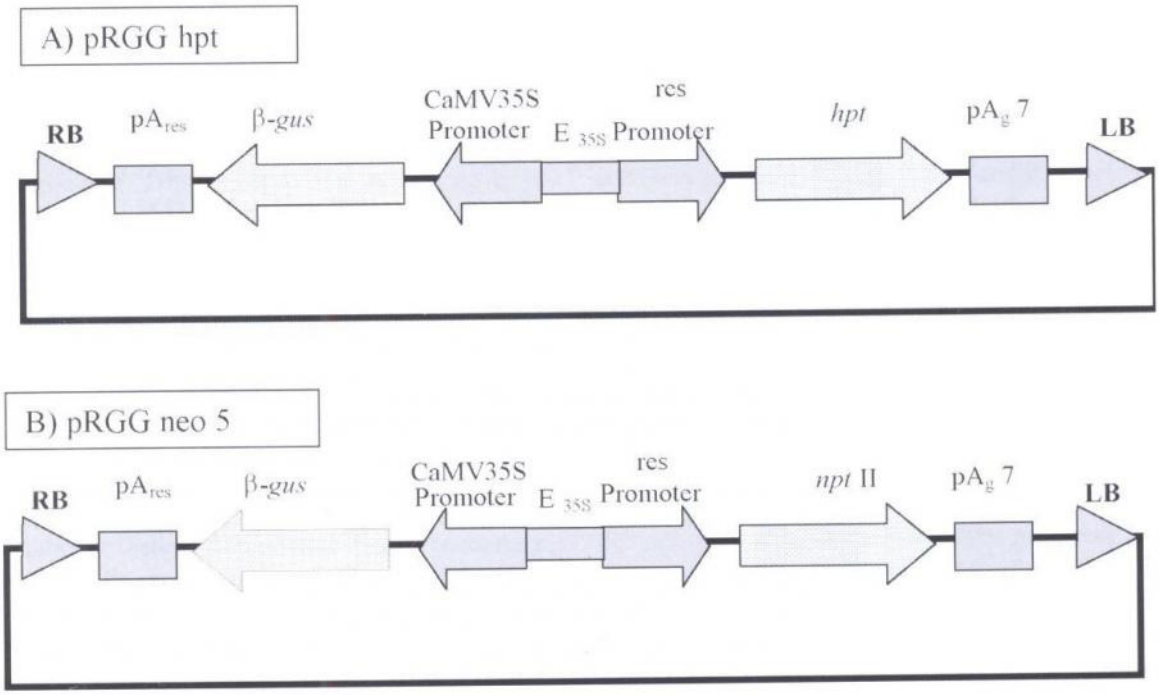

Figure 1 – Vectors used for eggplant 'Embú' transformation. **A** –Vector pRGG hpt and **B** – Vector pRGG neo 5. RB: right border; CaMV 35S: CaMV RNA 35S promoter; β-*gus*: β-glucuronidase gene; *npt* II: neomycin phosphotransferase II; *hpt*: hygromycin phosphotransferase; and LB: left border.

number of developed embryos (NE) were evaluated. As organogenesis was concerned, the frequency of explants regenerating buds or shoots (FEBS) and the number of formed shoots (NS) were evaluated.

Shoots and embryo development

Embryo maturation was performed in MS medium with 150 nM GA_3 (*Magioli* et al., 1996), and normal shoots were transferred to an MS medium containing 0.5 mg l^{-1} IAA (*Fári* et al. 1995a; 1995b). The flasks were sealed with polypropylene plastic films (Goodyear, Brazil). Afterwards, plants had their roots washed in water and were transferred to plastic cups with de-ionized water than covered with plastic bags. Plants were kept at room conditions while holes were cut in the plastic bags every second day. After the end of a week, they were transferred to organic substrate (Plantmax) and kept in greenhouse.

Identification of the transgenics

Tissue samples of the regenerated individuals were cut and submitted to hystochemical detection of the product of the gene *gus*, besides PCR reactions using specific primers to *npt*II and *hpt* genes. Genomic DNA samples used in the PCR were extracted according to *Fulton* et al. (1995). The *gus* hystochemical essay was performed as described by Jefferson et al. (1987).

Aiming amplification reactions of the hygromycin resistance gene the following primers were used: 5′→ 3′, **HPTf** (C G C T T C T G C G G G C G A T T T G T G T A C G) and **HPTr** (T C A G C T T C G A T G T A G G A G G G C G T G G). The primer sequences for the kanamycin resistance gene *npt*II were: **NPTr** (G C G G T C A G C C C A T T C G C C G C C) and **NPTf** (T C A G C G C A G G G G C G C C C G G T T).

Statistical analysis

The Completely randomized design was used in the experiments, which were composed of at least twenty replicates per treatment. Each Petri dish was considered a replicate containing 8 and 12 explants for somatic embryogenesis and organogenesis, respectively. FEC, FEE and FEBS were scored as the explant average per Petri dish. The total number of developed embryos (NE) and the total number of shoots (NS) per treatment were also evaluated. FEC, FEE and FEGR were submitted to Tukey test at 5% and 1% probability as the antibiotic selection and co-culture temperature experiments were performed.

Results and Discussion

In non-selective medium regeneration occurred normally as somatic embryogenesis or organogenesis (*Figures 2A and 2B*), nevertheless, embryo and bud development were not observed when explants were cultured in selective medium. Most of the regenerants were hyperhydric for embryogenesis (*Figure 2C*) and organogenesis. The ones displaying normal phenotypes were acclimatized as described (*Figure 2E*). Hyperhydricity is considered a

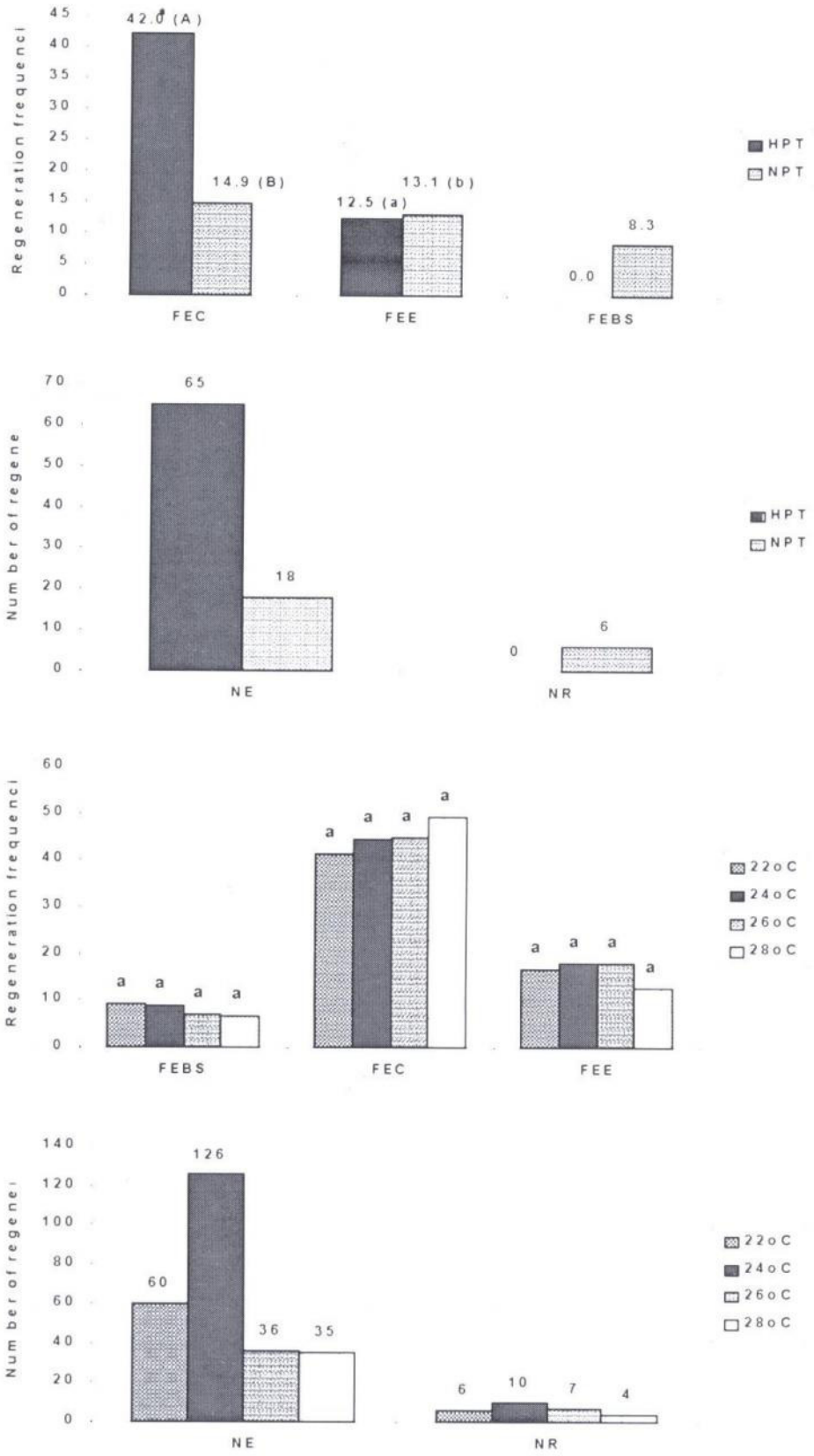

Figure 2 – Hygromycin and kanamycin selection effect, and, co-culture temperature effect on the regeneration of putative transformants and on the total number of total eggplant regenerants. **A** – Antibiotic selection effect (HPT – hygromycin phosphotransferase; NPT – neomycin phosphotransferase II) on the frequency of explants regenerating calli (FEC), embryos (FEE) and buds or shoots (FEBS). **B** – Antibiotic selection effect on the total number of regenerants (NE – total number of embryos; NR – total number of shoots). **C** – Co-culture temperature effect on FEC, FEE and FEBS. **D** – Co-culture temperature effect on NE and NR. The media followed by the same letter did not differ by Tukey test, at 5 and 1% probability.

constraint that requires further studies, as the development of hyperhydric plants is problematicl. Some hypothesis on how is this phenotype induced are presented by *Gribble* et al. (1998) and *Leshem* et al. (1998). Recently, it was observed that hyperhydricity is associated with stresses and with BiP, a protein of Hsp 70 family that is induced by a variety of stress conditions in sweet pepper (*Fontes* et al., 1999) and eggplant (*Picoli* et al., 2001). The frequency of explants with calli (FEC) was statistically significant at 5 and 1% probability with Tukey test (Figure 2A). Somatic embryogenesis was indirect and the higher callus induction favored genetic transformation of sectors or cells which is observed by the higher number of regenerated embryos in hygromycin as compared to kanamycin selective media (*Figure 2B*). Although, the frequency of explants with embryos (FEE) did not differ significantly, which suggested that more embryos were differentiating per cotyledonary explant, agreeing with the above hypothesis. This difference between FEC and FEE may be associated with variation of endogenous phytohormones as observed by *Sharma* & *Rajam* (1995). High variation coefficients of the experiment also suggest great discrepancy in morphogenic responses (*Table 1*).

Interestingly, organogenesis was not observed in hygromycin selective medium (*Figures 2A and 2B*). Similar results were observed for eggplant (*Guri* & *Sink*, 1988) and *Eucalyptus* transformants (*Dias* et al., 2001), both had no success in shoot regeneration. A deleterious effect or a high rate of selection caused by hygromycin are possible causes of these results. In this way, statistical testing was not possible for comparing of the frequencies of explants regenerating buds or shoots (FEBS) (*Table 1 and Figure 2A*).

Greater selection efficiency in medium with hygromycin was observed in the frequency of transformed regenerants, as compared to kanamycin and for the tested concentrations (*Table 1*). Besides that, selection efficiencies of 70 to 90%, and 0.42 to 25% were observed for both antibiotics, respectively. Somatic embryos developed in hygromycin selective medium is noticed in Figure 4D, where necrosis of the explant is also observed. Hygromycin displayed more transformed embryos and better selection as kanamycin did not present the same results in two independent experiments (not presented data). *Picoli* & *Otoni* (2001) detected calli and embryo presence in selective media, although its development was not observed. *Bec* et al. (1998) observed similar results in embryogenic nodular units of rice. These units had some degree of development in inductive selection media, no matter processing either transformed or not transformed sectors. Divergence of antibiotics exerts some selective pressure on the regenerated embryos, giving possibility to non-transformed embryos in selective media, thus contributing to the lower efficiency of kanamycin. All the same, *Picoli* (2000) used kanamycin for selecting transformed shoots and embryos of eggplant resistant to *Tospovirus*. Although 100 mg l^{-1} kanamycin had been used in this work, similar regeneration frequencies were observed (*Table 1*). Options for selective agents are necessary as distinct morphogenic responses of different plants and tissues are observed (*Eady* & *Lister*, 1998). Kanamycin-based selection is frequently used for eggplant transformation (*Rotino* & *Gleddie* 1990, *Fári* et al., 1995b; *Chen* et al., 1995; *Billings* et al., 1997; *Szász* et al., 1998). *Pollock* et al. (1983) noticed different toxicity levels of aminoglicosides in *Nicotiana plumbaginifolia* cells, besides its activity being dependent on pH. Antibiotics such as hygromycin were efficient in selecting *Capsicum annuum* regenerants as compared to kanamycin (*Mihálka* et al., 1998). It was also efficient for *Allium cepa*, the same not being observed for kanamycin (*Eady* & *Lister*, 1998). Kanamycin at 50 mg l^{-1} was not efficient for selecting eggplant cv. *Embú* transformants, similarly to *Mihálka* et al. (1998) results, where, other antibiotics, hygromycin among then, were more effective than kanamycin in selecting *C. annuum* regenerants. *Grant* et al. (1998) affirmed that good selection of pea primary transformants was obtained with 75 mg l^{-1} kanamycin. Even though, higher concentration was used in the multiplication ohase aiming the reduction of non-transformed plants in this system. Although the results of kanamycin lethality curves (*Picoli*, 2000; *Picoli* et al., 2000) and the works of *Peña* et al. (1995) and *Billings* et al. (1997) supporting the use of lower antibiotics concentrations, 50 mg l^{-1} kanamycin was not adequate as an substantial

Table 1 – Total number of explants used in the experiments, frequency of transformed individuals (number of transformed individuals*100/total number of explants) and variation coefficient of regeneration frequencies (CV = 100 * $\sqrt{QMR}/\bar{X}$).

	Organogenesis						Embryogenesis					
	HPT	NPT	22 °C	24 °C	26 °C	28 °C	HPT	NPT	22 °C	24 °C	26 °C	28 °C
Number of explants	324	324	240	240	240	240	198	198	160	160	160	160
Frequencies of transformed regenerants (%)	0	1.23	0.42	0.83	2.5	0.42	90*	25**	70*	90*	90*	80*
Variables												
Coefficient variation (%)	FEBS –		FEBS 110.99				FEC 52.63	FEE 115.72	FEC 46.49		FEE 76.41	

* – Values from a sample of 20 regenerated individuals; ** – average value of two experiments from 20 sampled individuals; FEC – frequency of explants regenerating calli; FEE – frequency of explants regenerating embryos; and FEBS – frequency of explants regenerating buds or shoots.

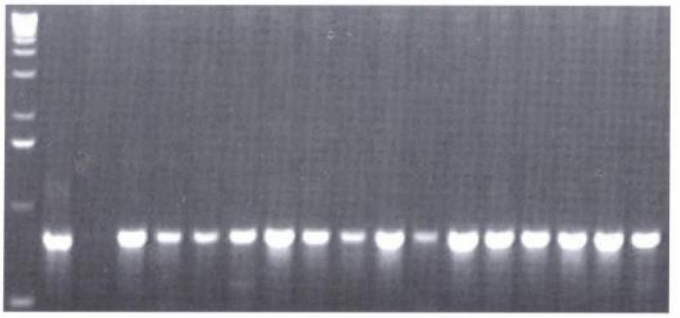

Figure 3 – Polimerase chain reaction of genomic DNA extracted from eggplant embryos transformed with *A. tumefaciens* (C58C1 pRGG hpt) using HPTf and HPTr primers. M: Kb ladder marker; +: pCAMBIA 1301 plasmid (positive control); –: non-transformed eggplant (negative control); and 1–15: transformed embryos.

frequency of non-transformed regenerants and low repeatability of experiments was detected. An alternative is the use of higher concentration of antibiotics even though it may lead to lower regeneration frequencies. *Jong* et al. (1994) used two *Agrobacterium* strains in genetic transformation of chrysanthemums. For each strain, four constructs – p35SGUSINT, pMOG410, pCPO201 and pMOG131 – derived from pBIN19 were tested. All constructs harbored the selection gene Nos *npt*II and 35S*gus* reporter gene. In both strains, pMOG410 plasmid provided higher number of plants expressing *gus* gene (blue points), at the seventh and the thirty-fifth days after transformation. According to the authors, *gus* gene being next to the right border might have promoted higher transient expression of this gene, as sampling effects and detection of the *gus* gene may have contributed to its more stable expression with the pMOG410 plasmid. Although only kanamycin was used for selection in this work, the results suggest that plasmid alteration may also influence transformation process. It is worth note that the constructs used in the present work differed only by the resistance gene to kanamycin (C58C1 neo5) or hygromycin (C58C1 hpt). Nevertheless, considering the positive amplification of the PCR bands (*Figure 4*) and GUS positive reaction (*Figures 2F and 2G*), there were observed some events PCR positives and GUS negatives, and vice-versa. *Jelenkovic* et al. (1998) described similar results in which 300 putative transformants resistant to *Leptinotarsa decemlineata* were obtained. Of these, 185 were GUS positive and 115 GUS negative. Toxicity tests *in plants*, preformed with 146 GUS positive plants revealed four phenotypic characteristics: 87 GUS positive and resistant to *L. decemlineata*, 36 GUS positive and susceptible, 7 GUS negative and resistant and 16 GUS negative and susceptible to the beetle. According to the authors, this may be explained by possible gene silencing. In this context, *Lacorte* (1998) referred to works on some plant and tissue, particularly reproductive organs, presenting GUS-similar activity, which would explain in part the different transformant classes.

Gene inactivation after its insertion or integration of a deficient copy may also explain a lack of expression as suggested by *Van der Krol* et al. (1990) and *Bec* et al. (1998). The presence of repeated copies of transgene sequences, and its correlation with homology-dependent gene silencing (HDGS), was observed by some researchers (*Hamilton* et al., 1998). This effect of the number of copies or the presence of homologue sequences may be related to the low expression of the *gus* gene, as for the lower transformation efficiency, if the antibiotic resistance gene was silenced. In addition to differences in phenolic compounds, sugars and pH of the medium probably would affecting transformation efficiency. The authors did also observe an inhibitory effect of claforam and ciclohexamide on *gus* expression. The stress that led to hyperhydric phenotype of plants may somewhat be related to the absence of *gus* expression in eggplant. *gus* expression variation were also observed after transformation of clones of *Betula pendula* (*Lemmetyinen* et al., 1998).

Co-culture temperatures did not influence callusing (FEC), embryogenesis (FEE) and organogenesis (FES) (*Figure 3C*) significantly. Even though, the number of regenerating embryos observed were 60, 126, 36 and 35, respectively for 22, 24, 26 and 28 °C. Considering the same co-culture temperatures, the number of regenerated shoots were 6, 10, 7 and 4, respectively (*Figure 3D*). Higher number of regenerants was observed for embryogenesis and organogenesis at 24 °C. Despite these results, more escapes were noticed as kanamycin was used for shoot selection (*Figure 3D and Table 1*). Bias on the frequency of explants regenerating calli (FEC) and embryos (FEE) might have occurred as a consequence of the development of non-transformed calli and embryos, which did not differentiate further. The thermo sensitive step of tumor induction *in plants* is the activation of the *vir* region (*Alt-Möerbe* et al., 1988), as for *vir* D2, the authors verified that buffered acidic solution of sugar and acetoseryngone was enough for its induction. The plasmid *vir* region is responsable for T-DNA transference from the bacteria to the plant (*Alt-Möerbe* et al., 1988; *Brasileiro,* 1993). Among the genes present in this region, *vir* A, B, D and G sequences are coupled with tumor induction, and, *vir* C, E, F and H, associated with transformation efficiency and the host range of bacteria (*Brasileiro,* 1993; *Lessi* & *Lanka,* 1994). VIR D1 and VIR D2 proteins are the ones that recognize the borders. VIR D1 is a topoisomerase that allows DNA strand uncoiling and VIR D2, an endonuclease that cuts the inferior strand of the T region. VIR D2 is linked covalently to 5' extremity, protecting and guiding the T-DNA strand to the plant cell nucleus. *Alt-Möerbe* et al. (1988) showed that temperature acts on the induction of *vir* D2 gene, and that it could influence the transformation efficiency. Recent works verified that co-culture temperature influenced the transformation efficiency of tomato (*Costa* et al., 2001) and *Phaseolus acutifolius* (*Dillen* et al., 1997). *Dillen* et al. (1997) observed that the best co-culture temperature for *P. acutifolius* callus was 22 °C, independently of the type of

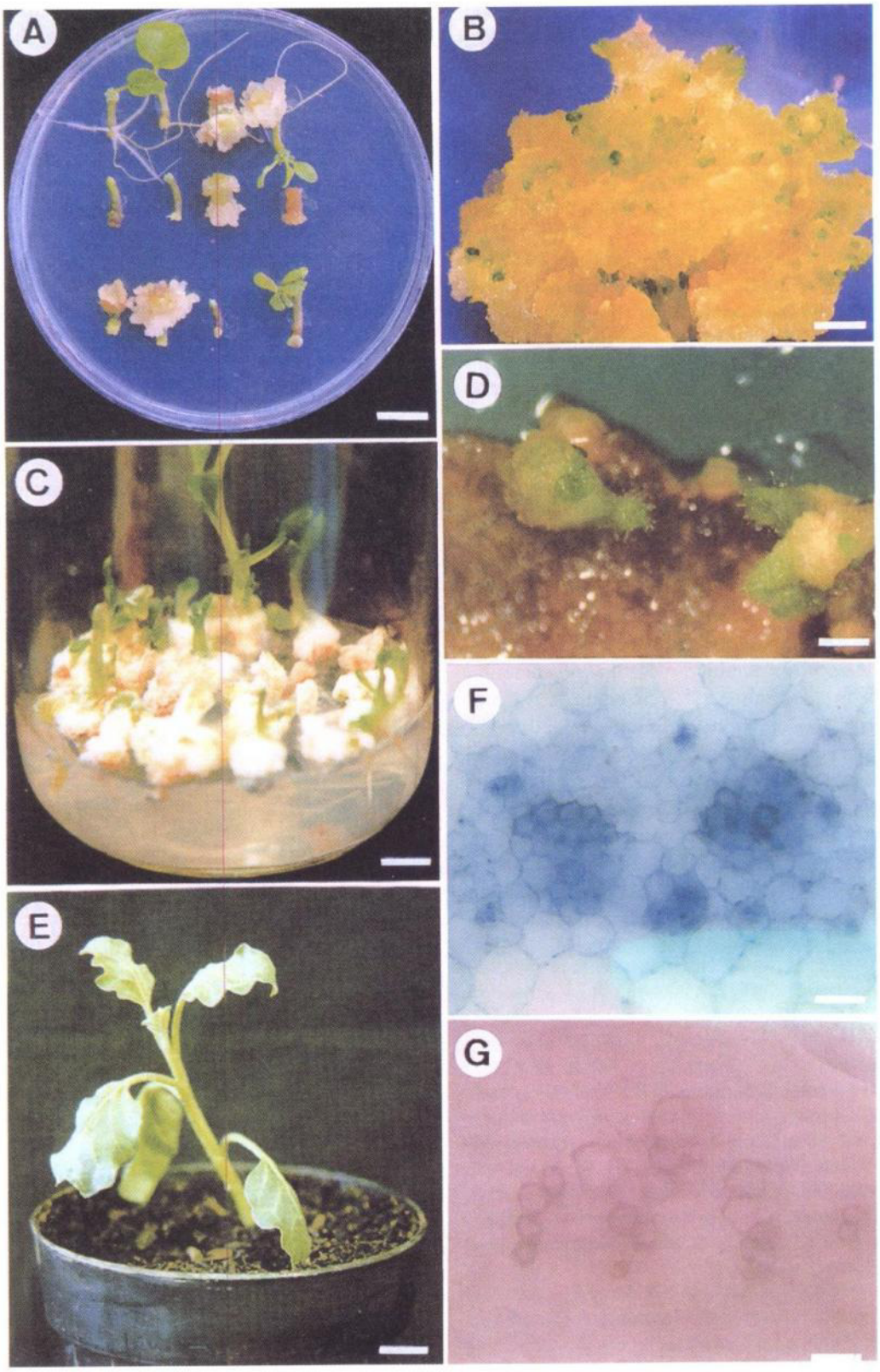

Figure 4 -Regeneration and development of putative transformants, and hystochemical essays of transformed and non-transformed eggplant regenerants. **A** – Organogenesis in hypocotyl segments in non-selective medium (Barr = 0.9 cm). **B** – Somatic embryogenesis in cotyledonary explants in non-selective medium (Barr = 0.25 cm). **C** – Putative transformants in selective maturation medium with hygromycin, hyperhydricity symptoms (Barr = 0,6 cm). **D** – Cotyledonary explant necrosis and somatic embryo developing in selective medium with hygromycin (Barr = 0.1 cm). **E** – Non-hyperhydric plant transformed with *npt*II gene, acclimatization process (Barr = 1,2 cm). **F** – Stem transversal section photomicrography of an event transformed with *npt* II and *gus* genes, GUS positive vascular region detail (Barr = 14 μm). **G** – Stem transversal section photomicrography of a non-transformed event, GUS negative vascular region detail (Barr = 14 μm).

helper plasmid. The *gus* expression at 25 °C was lower than at 22 °C, and not observed at 29 and 15 °C. Similar patterns were observed for *Nicotiana tabacum* leaf explants although the levels of expression were reduced between 22 and 29 °C. In spite of a high variation among treatments, higher expressions occurred, clearly, between 19 and 22 °C. *Costa* et al. (2001) verified a significant effect of co-culture temperature on tomato regeneration. The average number of buds per explant was also higher at 22 °C. The transformation efficiency was superior at 22 than at 24 °C. To enable DNA transference among bacteria *pilli* must occur. *Fullner* et al. (1996) verified that the formation of *pilli* on *A. tumefaciens*, the induction of virulence genes of the Ti plasmid was needed at low temperatures. The *pilli* formation depended on the induction of *vir* genes, as this structure was not observed in plates without acetoseryngone. When bacteria were grown at 28 °C, the cells with *pilli* were rare. This data are consistent with the hypothesis that tumorogenesis processes and T-DNA transference are less efficient at high temperatures (*Alt-Möerbe* et al., 1988; *Fullner & Nester*, 1996; *Dillen* et al., 1997), and that there is a evolutive relationship between T-DNA transference and bacterial conjugation (*Lessi & Lanka*, 1994).

The present data suggest the influence of co-culture temperature and selective antibiotic on the regeneration efficiency and in obtaining a transgenic eggplant. Hygromycin provided a more efficient selection of transformed embryos, although, was deleterious to organogenesis. Besides, hygromycin selection resulted in a statistically superior frequency of explants with calli (FEC) as compared to kanamycin. Co-culture temperatures of 24 °C lead to more regenerants, either for somatic embryos or shoots. Even though GUS reaction is considered to be an indirect proof of transgene insertion, false positive and false negative results are disadvantages of its use for selection of primary transgenics.

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