Influence of antiobiotics on NAA-induced somatic embryogenesis in eggplant (*Solanum melongena* L. cv. Embú)

Picoli, E. A. T.[1], Otoni, W. C.[1✉], Cecon, P. R.[2] and Fári, M.[3]

[1]*Departamento de Biologia Vegetal, Universidade Federal de Viçosa,*
36571-000, Viçosa, MG, Brazil; e-mail: wotoni@mail.ufv.br (✉)
Fax: +55 31 899 2580
[2]*Departamento de Informática, Universidade Federal de Viçosa, MG, Brazil*
[3]*Agricultural Biotechnology Center (ABC), Gödöllő, Szent-Györgyi A. u. 4,*
H-2100, Hungary/ Consultant, Codevasf/Embrapa/Agroinvest,
Embrapa Semi-Árido (CPATSA), Br 428, Km 152, Zona Rural, Petrolina-PE, Brazil

Summary: The influence of increasing concentrations of naphthaleneacetic acid and the antibiotics cefotaxime, timentin, kanamycin, and hygromycin on eggplant (*Solanum melongena* L. cv. Embú) somatic embryogenesis was investigated. Cotyledon explants were excised from 16 to 20 days old *in vitro* grown seedlings. NAA promoted somatic embryogenesis, although its concentrations had no influence on the mean number of embryos. Callusing decreased significantly with increasing NAA concentrations. Morphogenesis was stopped with 50 to 100 mg L^{-1} kanamycin and 7.5 to 15 mg L^{-1} hygromycin. Although early globular embryos were observed up to 15 mg L^{-1}, further embryo development was inhibited at 10 mg L^{-1}. Interestingly, cefotaxime (250 and 500 mg L^{-1}) promoted a marked effect on enhancing fresh weight of calli, accompanied by decrease in embryo regeneration, whereas timentin concentrations (150 and 300 mg L^{-1}) did not affect embryo differentiation as compared to the control treatment.

Introduction

Eggplant (*Solanum melongena* L.) is an important Solanaceous species, widely grown in tropical and subtropical regions of the world. It is considered to be an excellent system for *in vitro* studies since plant regeneration can be achieved via organogenic or embryogenic pathways (*Fári* et al. 1995a, 1995b; *Sharma & Rajam* 1995). Transferring genetic information into plant genomes by recombinant DNA techniques has become an important strategy in basic plant biology studies as well, in improving cultivated plants (*Billings* et al. 1997). It may be accomplished by genetic transformation, where genes are inserted in elite genotypes (*Iannacone* et al. 1995; *Jelenkovic* et al. 1998). Therefore, the success of plant transformation needs a system to transfer DNA, a reliable plant regeneration protocol, and an efficient system for selecting the transgenic individuals (*Eady & Lister* 1998). Studies on eggplant *in vitro* regeneration indicate interactions between phytohormones, their concentrations and the explants used

(*Gleddie* et al. 1983; *Fobert & Webb* 1988; *Filippone & Lurquin* 1989; *Sharma & Rajam* 1995; *Billings* et al.1997; *Magioli* et al. 1998). Various selective agents have been used, as different plant species react differentially to a particular selective agent (*Eady & Lister* 1998). Selection of transformed cells is mostly performed with kanamycin (*Rotino & Gleddie* 1990; *Fári* et al. 1995b; *Billings* et al.1997), although low efficiency is reported in some species (*Pollock* et al. 1983; *Mihalka* et al. 1998). Different effects of antibiotics and selective agents on *in vitro* morphogenesis are also observed (*Billings* et al. 1997; *Tarré* et al. 1997; *Eady & Lister* 1998). Besides selection, antibiotics such as augmentin, carbenicillin, cefotaxime and timentin are commonly used for suppressing *Agrobacterium* growth after co-cultivation in plant tissues (*Guri & Sink* 1988; *Billings* et al. 1997; *Nauerby* et al. 1997; *Ling* et al. 1998). The stimulatory effects of various antibiotics have been observed for a number of species (*Mathias & Boyd* 1986; *Holford & Newbury* 1992; *Yepes & Aldwinckle* 1994; *Sarma* et al. 1995;

Billings et al. 1997; *Nauerby* et al. 1997). Among these antibiotics, timentin has recently been made available with marked efficiency for elimination of *Agrobacterium*, displaying also stimulatory effects on organogenesis (*Nauerby* et al. 1997; *Cheng* et al. 1998; *Ling* et al. 1998; *Costa* et al. 1999). For eggplant organogenesis, augmentin (300 mg L^{-1}) was considered superior in promoting higher number of buds and shoots per explant as compared to cefotaxime (500 mgL^{-1}) (*Billings* et al. 1997). Likewise, cefotaxime (200-500 mg L^{-1}) was detrimental for eggplant somatic embryogenesis (*Tarré* et al. 1997). To date, effects of timentin as compared to other antibiotics on eggplant somatic embryogenesis have not been described yet.

Embú is the widest cultivated of the Brazilian eggplant cultivars, being a candidate for future genetic transformation works, aiming to incorporate desirable agronomic traits, among them, disease resistance genes. The aim of the present work is to evaluate possible effects of NAA concentrations and antibiotics timentin, cefotaxime, kanamycin and hygromycin, on *in vitro* embryogenesis of this cultivar.

Material and methods

Plant material and explant preparation

Eggplant (*Solanum melongena* L cv. Embú) seeds were purchased from local commercial establishments. 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, and then by four rinses in sterile distilled water. Thereafter, seeds were soaked for 24 h, at 26 ± 22 °C, in sterile distilled water on a rotatory shaker (100 rpm). Seeds were germinated in Phytacon (Sigma Chemical Co., USA) with 100 ml of basal medium with pH 5.8 and solidified with 0.28% (w/v) Phytagel (Sigma Chemical Co., USA). Cultures were maintained under 16/8 h light/dark regime, 24 mol m^{-2} s^{-1} light radiation, provided by two fluorescent tubes (Luz do Dia Especial, 20 W, Osram, Brazil). Growth room temperature was kept at 26 ± 22 °C. Cotyledons of 16 to 20-days seedlings were aseptically removed and used as explants. Each cotyledon was longitudinally cut into 2 segments and placed with its abaxial surface in contact with the regeneration medium, according to our previously published method (*Fári* et al., 1995b). All these media, otherwise stated, used MS basal salts (*Murashige & Skoog* 1962), 100 mg L^{-1} i-inositol, B5 vitamins (*Gamborg* et al. 1968), 2% (w/v) sucrose, and 0.8% (w/v) agar (Sigma Chemical Co., USA) along with NAA or antibiotics. The following NAA concentrations were evaluated for somatic embryogenesis induction: 0.0, 2.5, 5.0, 7.5 and 10 mg L^{-1}. Likewise, cefotaxime (União Química Farmacêutica Nacional S/A, Brazil) at 0, 250 and 500 mg L^{-1}, and timentin (SmithKline Beecham Farmacêutica, Brazil) at 0, 150 and 300 mg L^{-1}, were evaluated. Regarding the selective antibiotics, kanamycin (0, 50, 100, 150 and 200 mg L^{-1}) or hygromycin (0, 2.5, 5, 7.5, 10 and 15 mg L^{-1}) (Sigma

Chemical Co., EUA) were added to the medium, but also 300 mg L^{-1} timentin. The antibiotics were filter-sterilized with Millipore filters (0.22 μm; 2.5 cm diameter; Millex), and added to the medium following autoclaving (1.2 kg cm^2 at 121° C; 15 min) and cooling. After four weeks, data on callus fresh weight (FW) and number of embryos in torpedo stage (EM) per explant were taken. For NAA experiments, the total number of embryos (TE) was also evaluated. For kanamycin and hygromycin experiments, pigment contents were quantified. Pigments were extracted by homogenizing 1–2 g of fresh tissue with 80% acetone, the extract was filtered through filter paper Whatman 1. Carotenoid and chlorophylls "a" and "b" contents were estimated as described by *Lichtenthaler* (1987). Absorbances were determined at 451 and 503 nm for carotenoids, and 647 and 664.5 nm for chlorophyll. Normal embryos were transferred to MS medium with 150 nM GA$_3$. The flasks were sealed with two PVC film layers (Rolopack, Brasil). Afterwards, regenerated plants had their roots washed, and were transferred to recipients with distilled water and covered with plastic bags. Approximately a week later, they were transferred into organic substrate (Plantagro) and grown in standardized greenhouse conditions using 50% shading.

Statistical analysis

Statistical analysis was performed using a completely randomized design. Data were subjected to analysis of variance (ANOVA) and then appropriate regression analysis or Tukey's test (P = 0.05) were used. Treatments had five replicates, with 8 explants per Petri dish. Each experiment was replicated.

Results and discussion

The effect of NAA on somatic embryogenesis in cotyledon explants

NAA, in all concentrations, induced somatic embryogenesis in 100% of the cultured explants (*Figure 1*) and in the auxin-free treatment senescence was commonly observed. Likewise, these cultures produced neither callus nor embryo, therefore, they are not considered in the evaluation. In NAA-treated explants, all explants displayed high callusing and embryos were found either on the callus surface or within it. From the 10th to 14th day on, yellowish-green embryogenic calli were formed along the cut surface of the longitudinally-halved cotyledons on NAA-supplemented media. Callusing decreased linearly with NAA concentration, suggesting a negative effect on fresh weight of callus (*Figure 2A*). Although embryogenesis was promoted in all treatments, except for in control, NAA concentrations had no effect on the number of differentiated embryos (*Figure 2B*). Indeed, different responses among three independent experiments were observed (*Figure* 3). Probably, this may be related to different explant developmental and maturation stages, as seen for germination uniformity and seedling development (data not shown). About the third week, cotyledon
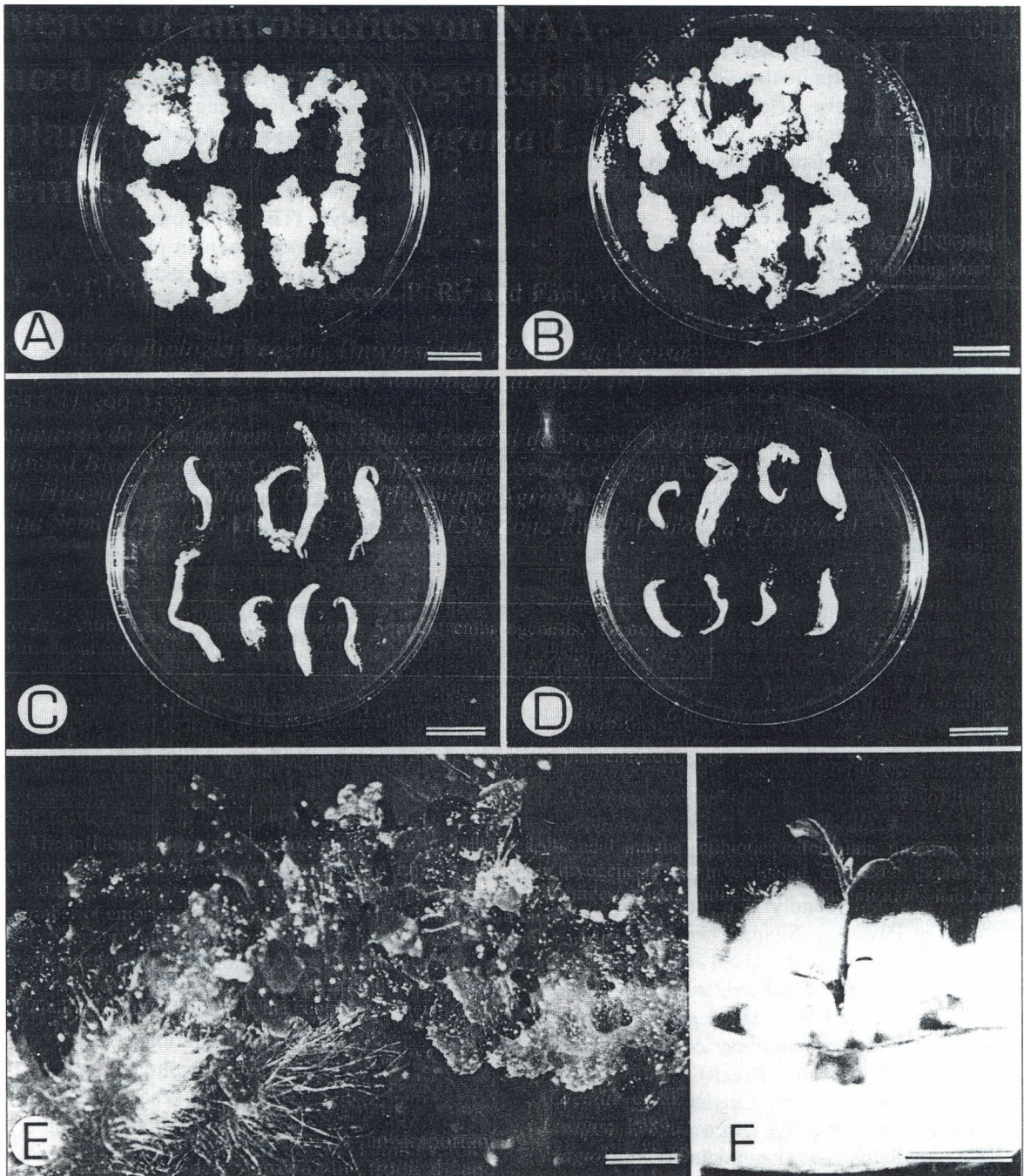
Figure 1 Eggplant somatic embryogenesis and antibiotic effects in NAA-supplemented media. **A** Control; **B** Cefotaxime (500 mg L-1); **C** Hygromycin (10 mg L-1); **D** Kanamycin (50 mg L-1) [Bar = 10 mm]; E Embryos regenerating from cotyledon explants [Bar = 5 mm]; **F** Embryo growing in maturation media [Bar = 10 mm].

senescence, epicotyl and hypocotyl swelling and radial expansion were frequently observed. Embryos were found to differentiate on the surface and inside the callus. Embryo development was asynchronous as seen by the different embryo stages, from globular to cotyledonar (*Figure 1E)*, within cultured explants. Malformed embryos were frequently observed, and most of them were hyperhydric, making difficult its conversion to plants and subsequent acclimatization. In eggplant, this was also reported by *Saito*
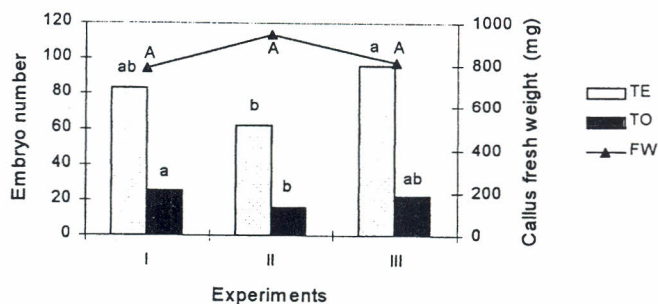
& Nishimura (1994). NAA has been assigned a major role in inducing somatic embryogenesis in eggplant (*Gleddie* et al. 1983; *Fobert & Webb*, 1988; *Fári* et al. 1995a). *Saito & Nishimura* (1994) pointed out better results using 50 µM 2,4-D for somatic embryogenesis induction of cultivar *'Nakate Shinkuro'*. *Gleddie* et al. (1983) & *Fobert & Webb* (1988) verified different optimal NAA concentrations for cultivar *'Imperial Black Beauty'* with values differing for leaf (10 mg L^{-1}) and cotyledon explants (5 mg L^{-1}),
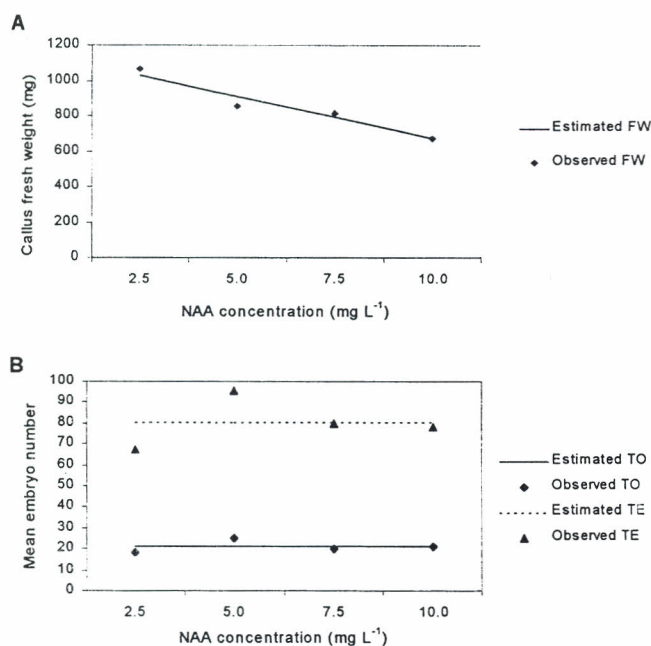
Figure 2 Observed and estimated values fresh weight (FW) of callus (A); (B) mean number of torpedo (TO) and total embryos (TE) per explant induced by different NAA concentrations on cotyledon explants of eggplant. Means of three independent experiments.

Figure 3 Mean number of total embryos (TE), torpedo embryos (TO) and fresh fresh weight (FW) of callus per explant from three independent experiments (I, II, III) as influenced by NAA concentration. Means followed by the same letter, capitals for FW and low case for TE and TO, are not statistically significant by Tukey's test (P = 0.05).

suggesting explant-dependent responses. Similarly, significant differences on somatic embryogenesis from leaf explants of 'Embú', as affected by NAA, were reported by *Marbach* (1998); while this was not observed for cotyledon explants in the present work. Nevertheless, 5 mg L^{-1} NAA was chosen based on higher mean values for total number of embryos (95.1) and number of embryo per explant (25.2) (*Figure 2B*). In the literature, contrasting NAA concentrations were used for eggplant somatic embryogenesis from various sources of explants (*Matsuoka & Hinata* 1979; *Fobert & Webb* 1988; *Sharma & Rajam* 1995; *Gleddie* et al. 1983; *Marbach* 1998). These differences may also be related with endogenous phytohormone variations of the explants (*Sharma & Rajam* 1995), the cultivar (*Gleddie* et al. 1983), and either environmental or physiological status of the donor plants. These results reinforce the importance of the donor plant for morphogenesis and suggest that this concentration must be optimized, for each cultivar as stated by *Sharma & Rajam* (1995), *Fobert & Webb* (1988), and *Marbach* (1998). The influence of genotype on eggplant regeneration was also observed by *Alicchio* et al. (1982) and *Matsuoka & Hinata* (1979). This is probably associated with different genes, alleles of the same gene or both, which may be related to regeneration. It seems to exist a number of alleles that confer different morphogenic abilities to cultivars, or either a group of genes regulating this characteristic.

Kanamycin and hygromycin effects

In the present work a drastic decrease of callogenesis was also observed (*Figures 1C, 1D*) and somatic embryogenesis (*Figures 4A, 4B*) which was due to kanamycin and hygromycin. Both antibiotics arrested embryogenesis, although the latter was more efficient at lower concentrations (*Figure 4B*). Kanamycin, at 50 mg L^{-1}, was enough for suppressing embryogenesis completely (*Figure 4A*). Interestingly, torpedo embryos were still observed (*Figure 4 B*) in media supplemented with hygromycinat 2.5 and 5.0 mg l^{-1}. This is not an unusual result, as *Park* et al. (1995) described a cytokinin-like effect at lower hygromycin concentrations. The higher concentrations tested of this antibiotic did not completely stop induction of embryogenesis, as globular embryos were observed in 15 mg L^{-1} hygromycin medium. Nonetheless, these embryos did not develop in hygromycin-supplemented media containing 10 or more mg L^{-1}. Similar results were achieved by *Bec* et al. (1998), where the development of friable globular structures in rice nodular embryogenic units were observed, even from 20 to 50 mg L^{-1} hygromycin-supplemented media. Several rice lines were compared, and histological analysis revealed that the regeneration pattern depended on the resistane of the differentiating embryos to the antibiotic, generating escapes. It was also verified that proembryos were more resistant to antibiotic selection. Eggplant leaf explants of the cultivar 'Hibush' were sensitive to kanamycin (*Billings* et al. 1997). No growth of any kind (swelling, callus or buds) was observed in the control, non-inoculated leaf discs grown on 10 to 100 µg mL^{-1} kanamycin. Higher number of GUS-positive individuals were regenerated in 50 µg mL^{-1} kanamycin, meanwhile at 70 and 80 µg mL^{-1} no GUS-positive regenerant was observed. The pH dependent activity is a disadvantage to this antibiotic class, although high kanamycin concentration (100 to 200 mg L^{-1}) is of widespread use for transgenic eggplant selection, except for *Guri & Sink* (1988), but without success to regenerate hygromycin resistant plants (*Filippone & Lurquin* 1989;
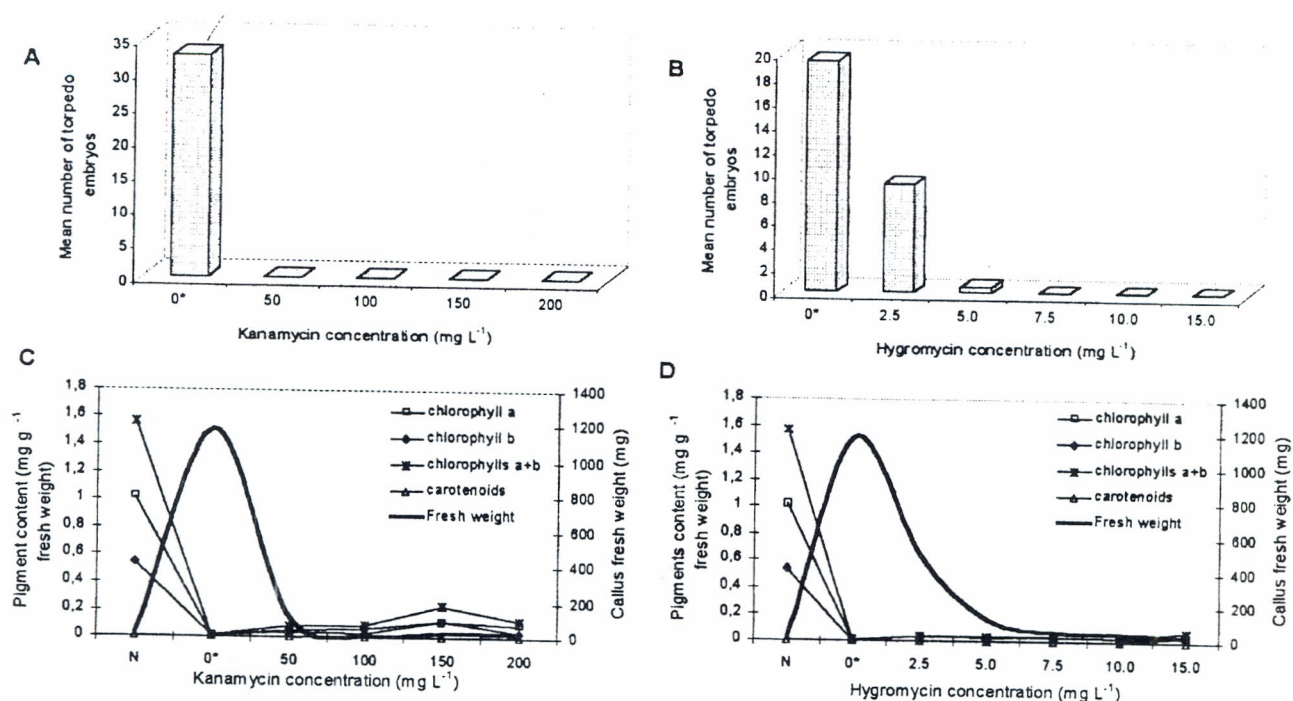
Figure 4 Influence of antibiotics on somatic embryogenesis and pigment contents of eggplant cotyledon explants. Mean number of torpedo embryos per explant: **A** Kanamycin, **B** Hygromycin; callus fresh weight and pigment contents: **C** Kanamycin, **D** Hygromycin. N corresponds to non-induced cotyledon (day zero); 0* corresponds to explants cultured on selective antibiotics-free medium, after 30 days in culture. All media contained timentin (300 mg L^{-1}) and NAA (5.0 mg L^{-1}). Data average of two independent experiments.

Rotino & Gleddie 1990; *Fári* et al. 1995b; *Billings* et al. 1997; *Szász* et al. 1998). Kanamycin at 4 mg L^{-1} and hygromycin at 0.8 mg L^{-1} were effective for transgenic selection, although hygromycin presented higher toxicity compared to kanamycin to *Vitis vinifera* explants (*Péros* et al. 1998), similarly to *Solanum melongena*. *Eady & Lister* (1998) observed that kanamycin was a less effective scorable marker in selecting embryogenic callus derived from immature embryos of onion. A gradual decrease in callus growth was also noted with increasing hygromycin concentration. Antibiotics of aminoglicosyde type showed different levels of toxicity levels as reported by *Pollock* et al. (1983) using *N. plumbaginifolia* cells as explants.

In respect to pigments, chlorophylls "a", "b", "a + b" and carotenoid contents were lower in explants grown in kanamycin or hygromycin containing media (*Figures 4 C, 4D*). Drastic decrease was observed also in the callusing response. Lower pigment contents were also verified in explants where only embryogenesis was induced without antibiotics (*Figures 4C, 4D*). We may attribute this to the natural degradation of the pigments and on the other hand to the callusing intensity, because the much higher fresh weight of callus diluted the existing pigments. Aminoglycoside antibiotics as, kanamycin, inhibit protein synthesis of mitochondria and chloroplasts resulting in chlorosis and in reduced growth of plant tissue (*Weide* et al. 1989; *Eady & Lister* 1998). Hygromycin, on the other hand, inhibit protein synthesis in eucaryotic systems (*Eady & Lister* 1998). The

scarsity of pigments may be associated to this inhibition, altering its production and/or its degradation. Selection of transformed cells is critical for recovering transgenic plants, the concentration of the selective agent should prevent regeneration without being toxic to the target explant (*Yepes & Aldwinckle* 1994). Antibiotics as geneticin (G-418), hygromycin, or herbicides, like methotrexate and phosphinotricin, proved to be better markers to score *C. annuum*, compared to kanamicyn (*Mihalka* et al. 1998). In the present work, the same was observed to eggplant since hygromicyn was suitable to arrest the morphogenic process at lower concentrations compared with kanamycin.

Effect of timentin and cefotaxime on somatic embryogenesis of eggplant

Callogenesis was stimulated in the presence of both timentin and cefotaxime (*Figures 5A, 5B*). Timentin did not affect embryo differentiation significantly (*Figure 5C*) whereas cefotaxime decreased somatic embryogenesis markedly (*Figure 5D*). An overall negative effect of cefotaxime was observed on embryogenesis similarly on organogenesis (data not shown). Up to nout, differential effects of timentin as compared to other antibiotics on the somatic embryogenesis of eggplant have not been described yet. As outlined by *Cheng* et al. (1998) timentin can be considered an alternative antibiotic for those species in which regeneration potential is negatively affected by carbenicillin and cefotaxime. Timentin at concentrations of
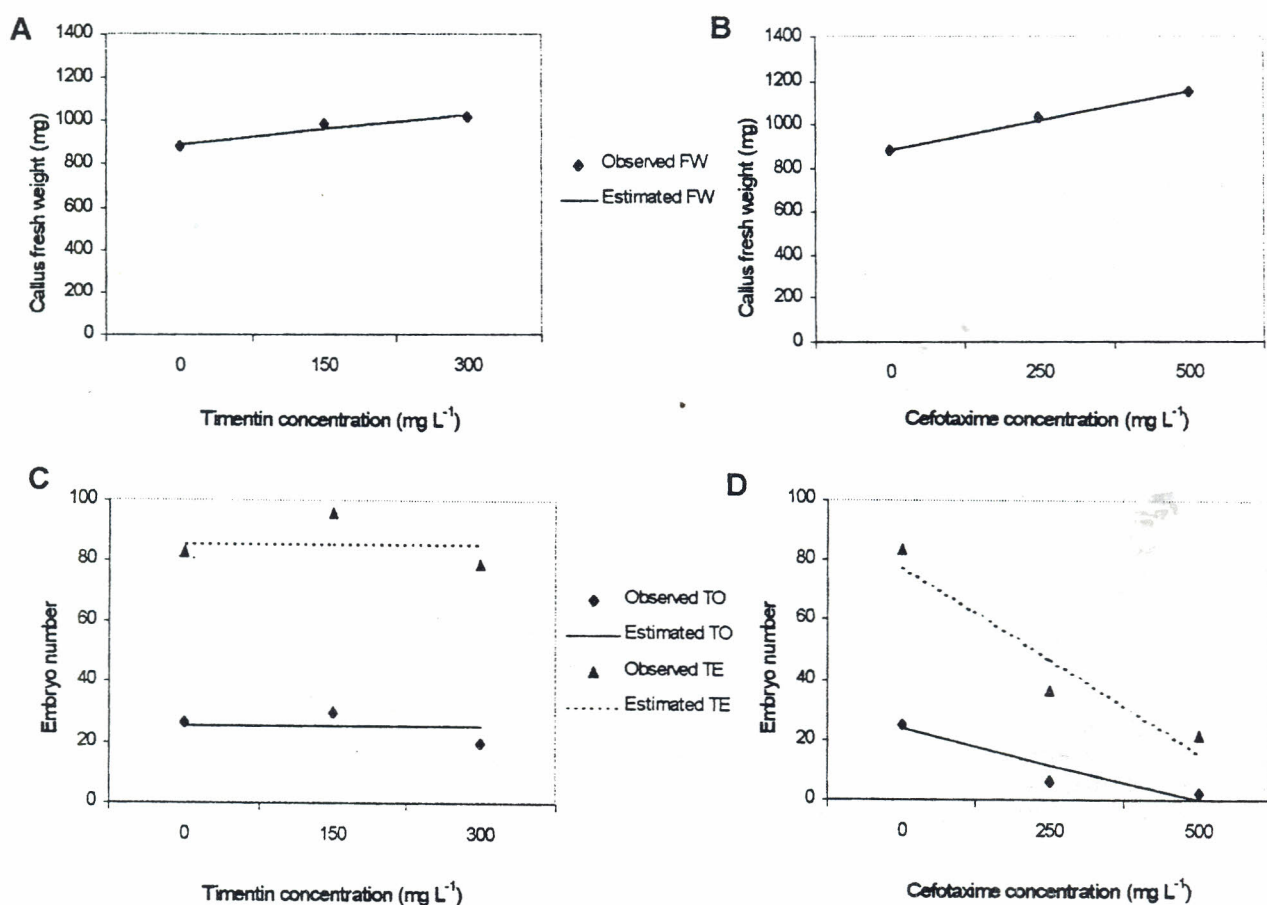
Figure 5 Influence of antibiotics on callus fresh weight (FW) and somatic embryogenesis in eggplant. **A** Timentin, $\underline{FW} = 0.890\underline{645} + 0.470626 \times 10^{-3} \times [C]$, $R^2 = 0.88$; **B** Cefotaxime, $FW = 0.882831 + 0.542042 \times 10^{-3} \times [C]$, $R^2 = 0.99$; **C** Timentin, $TE = \overline{TE}$ and $TO = \overline{TO}$; **D** Cefotaxime, $TE = 0.236759 \times 10^2 - 0.483258 \times 10^{-1} \times C$, $R^2 = 0.92$; and $TO = 0.778872 \times 10^2 - 0.123868 \times [C]$, $R^2 = 0.87$. [C] corresponds to the antibiotic concentration. TO corresponds to the number of torpedo embryos per explant, and TE to the mean number of total embryos per explant. Data average of three independent experiments

200–500 mg L^{-1}, employing a ratio of ticarcillin to clavulanic acid at 50:1 and 100:1, had little effect on shoot regeneration of tobacco (*Nicotiana tabacum*) and Siberian elm (*Ulmus pumila* L.). Timentin was also as effective as carbenicillin and cefotaxime in suppressing *A. tumefaciens* at concentrations commonly used in transformation. Likewise, *Tarré* et al. (1997) pointed out that cefotaxime caused a decrease on somatic embryogenesis of eggplant and that 400 mg L^{-1} ampicillin reduced the inhibitory effect on somatic embryogenesis more than the other antibiotica tested. Either the development of embryo or its induction was affected by cefotaxime (*Figure 6*). Our data confirm previous findings that cefotaxime led to negative effects on eggplant somatic embryogenesis (*Tarré* et al. 1997). Similarly, 500 mg L^{-1} cefotaxime reduced the production of embryo significantly in *Ipomoea batatas* (*Gama* et al. 1996). Inhibitory effects of cefotaxime were also observed in *Picea sitchensis* (*Sarma* et al. 1995), and *Antirrhinum majus*, *Arabidopsis*, *Solanum tuberosum* (*Nauerby* et al. 1997). Whereas its positive effects on *Triticum aestivum* (*Mathias* & *Boyd* 1986), *Pennisetum americanum* (*Pius* et al. 1993),

Malus x *domestica* (*Yepes* & *Aldwinckle* 1994) are also known. *Lin* et al. (1995) showed that carbenicillin and cefotaxime increased callus formation in tobacco. Cefotaxime is a cephalosporin (*Pius* et al. 1993), whereas timentin is an antibiotic composed of ticarcillin, a semi-synthetic penicillin, and clavulanic acid, a β-lactamase inhibitor (*Cheng* et al. 1998). They interrupt the peptideoglican synthesis by binding to the periplasm leading bacterial cells to death (*Nauerby* et al. 1997). The structure of these antibiotics similar to auxins and their hormonal activity suggest auxin-like effects besides their bactericidal effect (*Lin* et al. 1995). *Holford* & *Newbury* (1992) demonstrated that regeneration stimuli are related to the metabolism of penicillin; one of the products is the phenyl acetic acid, a weak natural auxin that should contribute to these responses. This positive effect was not observed in the eggplant '*Embú*', probably because NAA concentrations, ranging from 2.5 to 10 mg L^{-1}, had no influence on embryogenesis. Another hypothesis is that the compound with auxin function would have no effect on somatic embryogenesis of eggplant. These results suggest different

assimilation ratios of substances leading to a particular effect on embryogenesis depending on the species, genotype and antibiotic concentration studied.

Our results prove that timentin is a better option to be used in transformation protocols of eggplant compared to cefotaxime. It was based on the non-reduction of the mean embryo number and total number of embryos, where no negative effect could be observed, the same did not occur to cefotaxime. The data obtained from this research support the genetic transformation aimed to incorporate disease resistance genes as *SW-5*, which confers resistance to tospovirus (*Picoli* et al. 1999) and to optimize transformation protocols.

Acknowledgements

References

Alicchio, R., Del Grosso, E. & Boschieri, E. (1982): Tissue cultures and plant regeneration from different explants in six cultivars of *Solanum melongena*. Experientia, 38: 449–450, 1982.

Bec, S., Chen, L., Ferrière, N.M., Legrave, T., Fauquet, C. & Guiderdoni, E. (1998): Comparative histology of microprojectile-mediated gene transfer to embryogenic calli in japonica rice (*Oryza sativa* L.): influence of the structural organization of target tissues on genotype transformation ability. Plant Sci., 138:177–190.

Billings, S., Jelenkovic, G., Chin, C. K. & Eberhardt, J. (1997): The effect of growth regulators and antibiotics on eggplant transformation. J. Am. Soc. Hortic. Sci., 122:158–162.

Cheng, Z. M., Schunurr, J. A. & Kapaun, J. A. (1998): Timentin as an alternative antibiotic for suppression of *Agrobacterium tumefaciens* in genetic transformation. Plant Cell Rep., 17:646–649.

Costa, M. G. C., Nogueira, F. T. S., Figueira, M. L., Otoni, W. C., Brommonschenkel, S. H. & Cecon, P. R. (2000): Influence of the antibiotic timentin on plant regeneration of tomato (*Lycopersicon esculentum* Mill.) cultivars. Plant Cell Rep., 19:327–332

Eady, C.C. & Lister, C.E. (1998): A comparison of four selective agents for use with *Allium cepa* L. immature embryos and immature embryo-derived cultures. Plant Cell Rep., 18:117–121.

Fári M., Csányi M., Mitykó J., Peredi A., Szasz A. & Csillag A. (1995a): An alternative pathway of *in vitro* organogenesis in higher plants: plant regeneration via decapitated hypocotyls in three solanaceous vegetable genera. Hortic. Sci., 27:9–15.

Fári M., Nagy I., Csányi M., Mitykó J. & Andrásfalvy A. (1995b): *Agrobacterium* mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (*Solanum melongena* L. cv. 'Kecskeméti lila'). Plant Cell Rep., 15:82–86.

Filippone, E. & Lurquin, P. F. (1989): Stable transformation of eggplant (*Solanum melongena* L.) by cocultivation of tissues with *Agrobacterium tumefaciens* carrying a binary plasmid vector. Plant Cell Rep., 8:370–373.

Fobert, P. R. & Webb, D. T. (1988): Effect of polyamines, polyamine precursors, biosynthetic inhibitors on somatic embryogenesis from eggplant (*Solanum melongena*) cotyledons. Can. J. Bot., 66:1734–1742.

Gama, M. I. C. S., Leite Jr., R. P., Cordeiro, A. R. & Cantliffe, D. J. (1996): Transgenic sweet potato plants obtained by *Agrobacterium tumefaciens*-mediated transformation. Plant Cell Tiss. Org. Cult., 46:237–244.

Gamborg, O. L., Miller, R. A. & Ojima, K. (1968): Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50:151–158.

Gleddie, S., Keller, W. A. & Setterfield, G. (1983): Somatic embryogenesis and plant regeneration from leaf and cell suspension of *Solanum melongena* (eggplant). Can. J. Bot., 61:656–666.

Guri, A. & Sink, K. C. (1988): *Agrobacterium* transformation of eggplant. J. Plant Physiol., 133:52–55.

Holford, P. & Newbury, H. J. (1992): The effect of antibiotics and their breakdown products on the *in vitro* growth of *Antirrhinum majus*. Plant Cell Rep., 11: 93–96.

Iannacone, R., Fiore, M. C., Macchi, A., Grieco, P. D., Arpaia, S., Perrone, D., Mennella, G., Sunseri, F., Cellini, F. & Rotino, G. L. (1995): Genetic engineering of eggplant (*Solanum melongena* L.). Acta Hortic., 392: 227–233.

Jelenkovic, G., Billings, S., Chen, Q., Lashhomb, J., Hamilton, G. & Ghidiu, G. (1998): Transformation of eggplant with synthetic *cry IIIA* gene produces a high level of resistance to the Colorado potato beetle. J. Am. Soc. Hortic. Sci., 123:19–25.

Lichtenthaler H. K. (1987): Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Meth. Enzym., 148:3349–382.

Lin, J. J., Assad-Garcia, N. & Kuo, J. (1995): Plant hormone effects on the transformation efficiency of plant tissues by *Agrobacterium tumefaciens* cells. Plant Sci. 109:171–177.

Ling. H.–Q., Krieseleit, D. & Ganal, M. W. (1998): Effect of ticarcillin/potassium clavulanate on callus growth and shoot regeneration in *Agrobacterium*–mediated transformation of tomato (*Lycopersicon esculentum* Mill.). Plant Cell Rep., 17:843–847.

Magioli, C., Rocha, A. P. M., Oliveira, D. E. & Mansur, E. (1998): Efficient shoot organogenesis of eggplant (*Solanum melongena* L.) induced by thidiazuron. Plant Cell Rep., 17:661–663.

Marbach, P. A. S. (1998): Calogênese e embriogênese somática em berinjela (*Solanum melongena* L.). Master Thesis, Universidade Federal de Viçosa, Brazil

Mathias, R. J. & Boyd, L. A. (1986): Cefotaxime stimulates callus growth, embryogenesis and regeneration in hexaploid bread wheat (*Triticum aestivum* L. EM. TEHLL). Plant Sci., 46:217–223.

Matsuoka, H. & Hinata, K. (1979): NAA induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melongena* L. J. Exp. Bot., 30:363–370.

Mihalka V., Szász A., Fári M. & Nagy I. (1998): Gene transfer in pepper: comparative investigations on tissue culture factors and vector systems. Proceedings of the Xth Eucarpia meeting on genetics and breeding of *Capsicum* and eggplant, p.209–211.

Murashige, T. & Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant., 15: 473–497.

Nauerby, B., Billing, K. & Wyndaele, R. (1997): Influence of the antibiotic timentin on plant regeneration compared to carbenicillin and cefotaxime in concentration suitable for elimination of *Agrobacterium tumefaciens*. Plant Sci., 123:167–177.

Park, Y. D., Ronis, D. H., Boe, A. A. & Cheng, Z. M. (1995): Plant regeneration from leaf tissues of north dakota genotypes of potato (*Solanum tuberosum* L.). Am. Pot. J., 72:329–338.

Péros, J. P., Torregrosa, L. & Berger, G. (1998): Variability among *Vitis vinifera* cultivars in micropropagation, organogenesis and antibiotic sensitivity. J. Exp. Bot., 49:171–179.

Picoli, E. A. T., Lima, G. S. A., Lau, D., Brommonschenkel, S. H., Otoni, W. C. & Zerbini, F. M. (1999): Heterologous expression of the *SW-5* in eggplant (*Solanum melongena* L.) confers resistance to *Tospovirus* infection. Virus Rev. & Res., 4(1):155.

Pius, J., George, L., Eapen, S. & Rao, P.S. (1993): Enhanced plant regeneration in pearl millet (*Pennisetum americanum*) by ethylene inhibitors and cefotaxime. Plant Cell Tiss. Org. Cult., 32:91–96.

Pollock, K., Barfield, D.G. & Shields, R. (1983): The toxicity of antibiotics to plant cell cultures. Plant Cell Rep., 2:36–39.

Rotino, G.L. & Gleddie, S. (1990): Transformation of eggplant (*Solanum melongena* L.). Plant Cell Rep., 9:26-29.

Saito, T. & Nishimura, S. (1994): Improved culture conditions for somatic embryogenesis using an aseptic ventilative filter in eggplant (*Solanum melongena* L.). Plant Sci., 102:205–211.

Sarma, K. S., Evans, N. E. & Selby, C. (1995): Effect of carbenicillin and cefotaxime on somatic embryogenesis of sitka spruce (*Picea sitchensis* (Bong.) Carr.). J. Exp. Bot., 46:1779–1781.

Sharma, P. & Rajam M. V. (1995): Genotype explant and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). J. Exp. Bot. 46:135–141.

Szász A., Szilassy D., Salánki K., Fári M. & Balázs E. (1998): A simple and efficient method for the transformation of eggplant (*Solanum melongena* L.). Acta Bot. Hung., 46:201–207.

Tarré, E., Magioli, C., Rocha, A. P. M., Oliveira, D. E. & Mansur, E. (1997): Inhibitory effect of antibiotics on somatic embryogenesis of eggplant (*Solanum melongena* L.). Rev. Bras. Gen., 20[Suppl.]:188.

Weide, R., Koornneef, M. & Zabel, P. (1989): A simple, non destructive spraying assay for the detection of an active kanamycin resistance gene in transgenic tomato plants. Theor. Appl. Gen., 78:169–172.

Yepes, L. M. & Aldwinckle, H. S. (1994): Factors that affect leaf regeneration in apple, and effect of antibiotics in morphogenesis. Plant Cell Tiss. Org. Cult., 37:257–269.