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Effect of antibiotics and gelling agents in transformation of brinjal (*Solanum melongena* L.) cv. Manjarigota

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ABSTRACT

A study was conducted to find out the effect of antibiotics and gelling agents on *Agrobacterium*-mediated transformation using hypocotyl explants of brinjal cv. Manjarigota. Hypocotyl explants of brinjal were found to be sensitive even to the lowest level of kanamycin (25 mg/l) tested. Explants that showed increased callus initiation and regeneration response upon cocultivation with *Agrobacterium* and on kanamycin at 100 mg/l were selected as this indicated a highly effective selection pressure. Cefotaxime did not affect regeneration response and at 500 mg/l, it effectively inhibited *Agrobacterium* overgrowth completely on *Agrobacterium* cocultivated hypocotyl explants. There were marked differences in regeneration response in hypocotyl explants cultured on medium solidified with various gelling agents indicating the influence of gelling agent on the activity of kanamycin in culture medium, which indirectly affects selection and recovery of transformants. Antibiotics and gelling agents could therefore affect, directly or indirectly, transformation of brinjal cv. Manjarigota.

Key words: *Solanum melongena*, kanamycin, cefotaxime, gelling agents, transformation

INTRODUCTION

In transformation studies, once the target cells have been transformed, the transgenic cells or plants produced from them are identified on selection medium. A marker gene is necessary because only a low proportion of the cells exposed to the transformation processes subsequently become stably transformed (Klee *et al*, 1987). The use of selection medium confers an advantage to those cells that stably incorporate the transgene construct and are, therefore, resistant to the specific antibiotic in the selective medium. The use of a marker gene in a transformation process thus aims to give a selective advantage to transformed cells by allowing them to grow. The selectable marker gene confers the transformed cells an ability to metabolize compounds that are not usually metabolized by untransformed cells. One such widely used marker gene for transformation of plants is *nptII* (*neomycin phosphotransferase II*) gene, which confers resistance to the aminoglycoside antibiotic kanamycin by phosphorylation of the specific hydroxyl group (Nap *et al*, 1992). Antibiotics are added to the culture media to control *Agrobacterium* that may affect the plant regeneration process (since *Agrobacterium* itself is basically a plant pathogen (Hanur, 2004)) and to select transformants with an antibiotic resistance that gets cotransferred with the gene of interest. Sensitivity to kanamycin varies with crop and explant (Shaw *et al*, 1983).

Cefotaxime is a b-lactam antibiotic that inhibits bacterial cell wall synthesis. It inhibits the cross linking of peptidoglycan by binding and inactivating of transpeptidase leading to nicks in cell wall by which the cell membrane protudes into the hypotonic environment and, finally, ruptures as a consequence of osmotic shock. Although many antibiotics have been described for effective control of *Agrobacterium* cells, cefotaxime is known to exert minimal effect on most plant tissues (Mathias and Boyd, 1986) and has become most widely used antibiotic in *Agrobacterium*-mediated transformation (Yu *et al*, 2001; Magioli *et al*, 2000). Cefotaxime has been shown to have both negative and positive effect on callus formation and regeneration in crop plants.

Various gelling agents differ in their affinity to bind kanamycin and inhibit the latter's activity in the culture medium, which may indirectly affect transformation and recovery of transformants (Laine *et al*, 2000). Though the

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mechanism is not clear, efficiency of kanamycin that inhibiting regeneration differed with various gelling agents (Chauvin *et al*, 1999). In brinjal transformation, the effect of antibiotics, viz., cefotaxime and kanamycin, has been studied to a lesser extent (Billings *et al*, 1997) and there are no reports on the effect of gelling agents in brinjal transformation. In developing an efficient transformation protocol, finding out effect of various *in vitro* factors is an important step. Also, it is necessary to document the effect of such factors affecting transformation in various cultivars of a crop plant, so that importance of any particular factor at the species/varietal level can be studied. This is useful in determining factors for developing an efficient transformation protocol for a given cultivar. In this paper, an effort has been made to study the effect of antibiotics and gelling agents, which are critical factors in making a transformation protocol efficient, using hypocotyl explants in brinjal cv. Manjarigota.

MATERIAL AND METHODS

Plant material

The genuine breeder seed material of brinjal cv. Manjarigota was obtained from the Division of Vegetable crops, IIHR, Bangalore. Seeds were soaked in gibberillic acid (100 ppm) for three hours, surface sterilized for 1 minute in 70% ethanol, washed in sterile distilled water, treated for 8-10 min. in sodium hypochlorite (approximately 4% available chlorine) solution and washed five times in sterile distilled water. They were germinated on half-strength MS (Murashige and Skoog, 1962) basal medium containing 3% sucrose (w/v), 0.8% agar and pH adjusted to 5.8 using dilute NaOH/HCl prior to autoclaving. Hypocotyls from aseptically germinated seedlings were used as the explant donor source (Hanur *et al*, 2006).

Sterilization and culture incubation conditions

Sterilization of culture medium and instruments was done by autoclaving at 121^{o} C at 15-psi pressure for 15 min. Cultures were incubated in culture racks tilted with white, fluorescent tubes with a light intensity of 30-40µE m^{-2} s^{-1} under a 16 h photoperiod in a culture room maintained at 25^{o} ± 2° C.

Plant transformation

Plasmid pBinBt-1 (the pBinAR binary vector containing CaMV35S promoter, the coding region of synthetic *Cry1Ab* gene, *ocs* terminator and *nptII* selectable marker cassette) (Kumar *et al*, 1998) was used for standardizing the transformation protocol. The *nptII* gene conferring kanamycin resistance served as a selectable marker. Fifteen to twenty day old hypocotyl explants were precultured for two days on shoot regeneration medium for hypocotyl explants (SRMH) containing MS medium with 2 µM Benzyl Aminopurine (BAP) and 0.05 µM Naphthalene Acetic Acid (NAA) (Hanur *et al*, 2007). Explants were transferred to a sterile petri plate, infected with overnight-grown *Agrobacterium* culture for 20-25 min. and placed back onto the parent medium, cocultivated for two days, transferred onto culture media containing cefotaxime (500 mg/l) for two days and then transferred to SRMH containing cefotaxime (500 mg/l) and kanamycin (100 mg/l). Hypocotyl explants cultured without *Agrobacterium* cocultivation on SRMH served as the control. All the explants in all the treatments were subsequently subcultured on shoot elongation medium (SEM) and rooting initiation medium (RIM) for complete plant regeneration.

Kanamycin

To examine kanamycin sensitivity, hypocotyl explants (without cocultivation) were cultured on SRMH containing kanamycin at 0, 25, 50, 75, 100, 125, 150, 175 and 200 mg/l. Observations were recorded on callus initiation and regeneration response at four weeks from culture initiation. Observations on explant survival were recorded weekly upto four weeks from culture initiation. Similarly, hypocotyl explants were cultured after *Agrobacterium* cocultivation on SRMH containing kanamycin at different levels and observations were recorded and stringent selection pressure for selecting putative transformants was worked out.

Cefotaxime

After cocultivation, hypocotyl explants were cultured at different concentrations of cefotaxime (100, 250, 500, 750 and 1000 mg/l) and transformation procedure thereafter remained the same as above. Observations were recorded on callus induction and regeneration response at four weeks from culture initiation. Observations on explant survival and bacterial overgrowth were recorded weekly upto four weeks from culture initiation. Callus induction and regeneration (transformation) frequency was worked out.

Gelling agents

Three types of gelling agent [Agar, Gelrite (Phytagel) and Agargel (Agar + Gelrite)] were used in the culture medium as solidifying agents at all stages of the

transformation protocol. Observations were recorded on callus induction and regeneration response at four weeks from culture initiation. Callus induction and regeneration (transformation) frequency was worked out.

Data analysis

Sufficient numbers of replications were maintained in an experiment as required. Wherever necessary Analysis of Variance (ANOVA) was used to test significance of the results (details given below) observed.

RESULTS AND DISCUSSION

Effect of kanamycin on transformation and *in vitro* morphogenetic response of hypocotyl explants

In the experiment conducted to work out the minimum concentration of kanamycin required for complete killing of untransformed plant cells, hypocotyl explants showed varied sensitivity to various levels of kanamycin without *Agrobacterium* co-cultivation. After 4 weeks, 100 % hypocotyl explants remained viable (green) in the control i.e., zero kanamycin. Approximately, 92, 16 and 8 % callus induciton response was observed on hypocotyl explants cultured on SRMH containing 25, 50 and 75 mg/l kanamycin, respectively. However, these failed to regenerate shoots. Hypocotyl explants cultured on SRMH containing 100 mg/l kanamycin and above showed neither callusing nor regeneration response. However, Billings *et al* (1997) reported no growth of any kind with control, non-inoculated leaf discs (bulging/thickening) cultured on 10-100 mg/l kanamycin in brinjal. In the present study, initial callus induction response (bulging near the cut end) on hypocotyl explants on kanamycin containing culture medium, may be because of the nature of hypocotyl explants to respond as fast as compared to other explants like cotyledonary leaf or leaf (Curuk *et al*, 2002; Gaba *et al*, 1999). In the present study, explants gradually turned light yellow and ultimately died on SRMH containing kanamycin.

Time taken for chlorosis (yellowing/white) of explants reduced with increasing kanamycin concentration and differed markedly with kanamycin concentration. Shoots (untransformed) cultured on SEM containing 50 mg/l kanamycin did not elongate. Shoots cultured on RIM containing 25-mg/l kanamycin did not show root induction. Instead these turned yellow and died. Sensitivity to an antibiotic has been shown to differ with crop plant, cultivar and explant type (Sunilkumar and Rathore, 2001; Barcelo *et al*, 1998; Sriskandrajah and Goodwin, 1998; Sarmento *et al*, 1992) and the nature and size of explants may contribute to differed sensitivity to kanamycin (Chauvin *et al*, 1999). In the present study, hypocotyl explants cultured on kanamycin at 100 mg/l did not show any response.

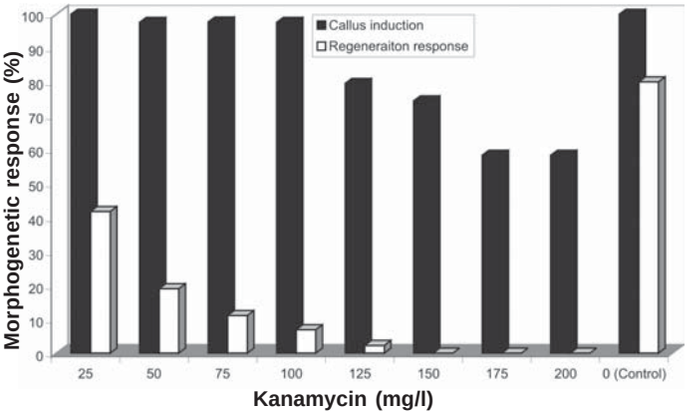

Fig 1. Effect of kanamycin on *Agrobacterium* mediated transformation and morphogenetic response in hypocotyl explants of brinjal cv. Manjarigota

Table 1. Effect of kanamycin on transformation and morphogenetic response of hypocotyl explants of brinjal cv. Manjarigota

Kanamycin (mg/l)	Callus initiation response (% ± SE)	Regeneration response (% ± SE)
25	100.0±0.00	41.86 ±2.51
50	97.6±2.22	19.04 ±1.11
75	97.7±2.22	11.11 ±2.22
100	97.6±2.56	6.90 ±0.34
125	79.5±3.57	2.27 ±2.22
150	74.4±4.03	0.00 ±0.00
175	58.3±5.87	0.00 ±0.00
200	58.3±4.44	0.00 ±0.00
Control	100.0±0.00	80.0 ±4.84

Fractions were converted into percentages; percentage data are with SE.

In the present study, after the hypocotyl explants were co-cultivated with *Agrobacterium*, their ability to show callus induction and regeneration in the presence of kanamycin increased showing that transformation of plant cells had taken place. Hypocotyl explants showed varied morphogenetic responses on various levels of kanamycin after *Agrobacterium* co-cultivation (Fig. 1; Table 1). Callus induction response occurred at all the levels of kanamycin tested (up to 200 mg/l). However, gradual reduction in callus initiation was observed with increase in kanamycin (>100 mg/l) concentration in the culture medium. Shoot regeneration response was observed upto 125 mg/l, which, sharply decreased at 150 mg/l or higher concentrations kanamycin. Billings *et al* (1997) found that 50 mg/l kanamycin was more effective for selecting transformed shoots and even transformed cells failed to regenerate at higher levels of kanamycin in cotyledonary leaves of brinjal.

Higher level of kanamycin has been found to inhibit chlorophyll synthesis even in transgenic tissues (Norouzi *et al*, 2005). However, in almost all previously reported brinjal transformation studies, high concentrations (100-200 mg/l) of kanamycin were used to select transformants (Rotino and Gleddie, 1990; Kumar *et al*, 1998). Reducing kanamycin concentration to sub-lethal levels during selection led to higher number of escapes. Application of higher kanamycin concentration in the selection medium reduced regeneration response, while, transformed plants remained mostly chimeric in nature in carnation (Zuker *et al*, 1999). In the present study, almost all the explants showed callus initiation (>97%) with regeneration response (6.9%) after *Agrobacterium* co-cultivation on 100mg/l kanamycin. This shows that there is scope for further improvement in regeneration response by optimizing other factors involved in the transformation procedure. Moreover, both callus initiation and regeneration response were completely inhibited in control explants at 100 mg/l of kanamycin. Kanamycin at 100mg/l was, therefore, identified as a stringent selection pressure for selection of transformants in hypocotyl explants of brinjal cv. Manjarigota.

Effect of cefotaxime on transformation and *in vitro* morphogenetic response of hypocotyl explants

Hypocotyl explants cultured on SRMH containing cefotaxime showed a little callus production all over the surface (Plate 1). Cefotaxime did not affect callus initiation response in hypocotyl explants and callusing was seen at 100% at all levels of cefotaxime. Cefotaxime did not significantly affect regeneration response. Complete exclusion of bacteria was possible by employing a culture medium with cefotaxime at 500 mg/l. Explants cultured on cefotaxime below 500 mg/l showed *Agrobacterium* overgrowth. It is known that once bacteria start surviving in the plant tissue, it is difficult to control their overgrowth. The only option was to exclud such explants from the culture to prevent spread of overgrowth to other explants. Similarly, it is reported that lower levels of cefotaxime do not completely eliminate *Agrobacterium* in brinjal cotyledons (Billings *et al*, 1997). Five hundred mg/l of cefotaxime was extremely effective in eliminating *Agrobacterium* from explants of brinjal up to 3 months (Billings *et al*, 1997; Kumar *et al*, 1998). Effective concentrations of antibiotic for elimination of *Agrobacterium* overgrowth was dependent on *Agrobacterium* strain, explant type and crop (Sriskandarajah and Goodwin, 1998; Hoque *et al*, 2005). Various concentrations of cefotaxime, 200-500mg/l, were effectively used to eliminate *Agrobacterium* overgrowth in transformation studies in rice (Hoque *et al*, 2005), strawberry (Barcelo *et al*, 1998) and apple (Sriskandaranjah and Goodwin, 1998). In the present study hypocotyl explants cultured on higher levels of cefotaxime (>500mg/l) turned brown at third week from culture initiation. This may be due to delayed sensitivity of explants to higher levels of cefotaxime. Similarly, it was seen that papaya callus turned brown in color on medium containing cefotaxime at 250 mg/l (Yu *et al*, 2001).

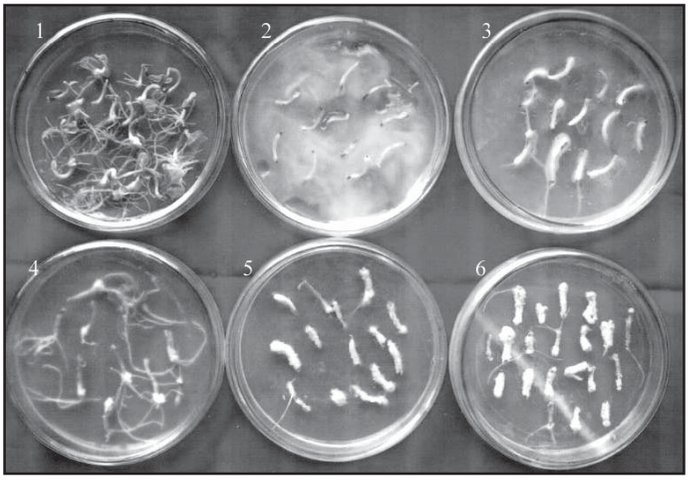

Plate 1. Regeneration response of hypocotyl explants after Agrobacterium cocultivation cultured on SRMH containing defferent levels of cefotaxime (mg/l):1, 0; 2, 100; 3, 250; 4, 500; 5,750 and 6, 1000 mg/l.

In the present study, slightly higher amount of callus production was visually observed on explants cultured on all levels of cefotaxime compared to the control. However, no significant differences in callus initiation response and regeneration response were observed. Picoli *et al* (2002) found that cefotaxime enhanced callus fresh weight, it also caused a decrease in the rate of embryo regeneration in brinjal. Magioli *et al* (2001) however, reported that presence of cefotaxime did not affect embryogenic callus formation and development from leaf and cotyledonary explants. Billings *et al* (1997) reported no effect on either callus production or regeneration. Stimulation of callus growth and regeneration due to cefotaxime have been reported in barley (Mathias and Mukasa, 1987), bread wheat (Mathias and Boyd, 1986**)** and many horticultural crops. However, in the present study, the maximum regeneration response observed in explants cultured on 500mg/l (21.81%) cefotaxime might be due to an effective control of *Agrobacterium* without harming the explants (Fig 2). Negative effects of cefotaxime on callus formation and plant regeneration have been described in carrot (Okkels and Pederson, 1988).

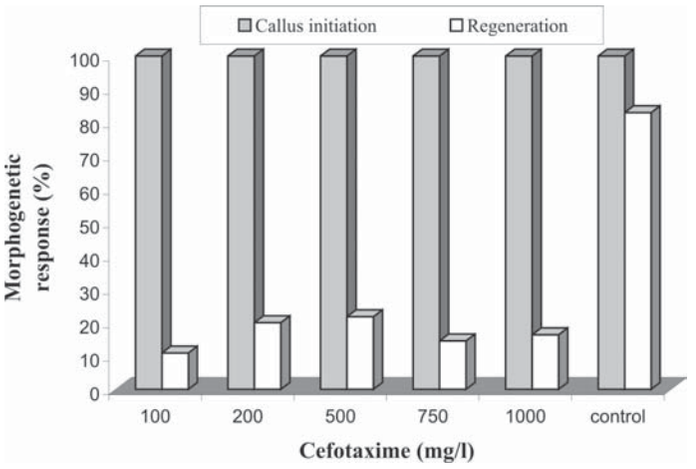

Fig 2. Effect of cefotaxime on transformation and *in vitro* morphogenetic response of hypocotyl explants in brinjal cv. Manjarigota

In tomato, cefotaxime did not by itself inhibit callus growth in the culture medium, but it clearly decreased shoot differentiation. Together with kanamycin, cefotaxime showed a strong negative effect on callus growth, shoot regeneration and transformation frequency in tomato (Ling *et al*, 1998). Okkels and Pederson (1988) reported stimulation of plant regeneration at low concentrations of cefotaxime (less than 100 mg/l) in carrot and inhibition at high concentration (300 mg/l). Enhanced callus formation and shoot regeneration in culture medium containing antibiotic may be possibly caused by release of auxin-like compounds (Halford and Newbury, 1992; Robert *et al*, 1989; Ling *et al*, 1998). Hence, it appears that the effect of cefotaxime on any tissue depends on the crop plant, genotype, explant type, concentration of cefotaxime and other transformation conditions. The present study showed that cefotaxime could promote a slight increase in callus production in explants without a marked effect on regeneration response of hypocotyl explants in brinjal cv. Manjarigota.

Effect of gelling agent in the culture medium on transformation and *in vitro* morphogenetic response of hypocotyl explants

In the present study**,** three types of gelling agents, viz., agar, agargel (agar + Phytagel at 1:1 ratio) and Phytagel were assessed for their effect on regeneration response of *Agrobacterium* cocultivated hypocotyl explants in the presence of kanamycin (Table 2). Gelling agents did not affect callus initiation response from hypocotyl explants and callus induction response was found to be 100% on all the gelling agents tried. Explants cultured on medium solidified with agargel showed better regeneration (35.71%)

Table 2. Effect of gelling agents on transformation and morphogenetic response from hypocotyl explants of brinjal cv. Manjarigota

Gelling agent	Callus initiation (%)	Regeneration response (% ± SE)
Agar	100	27.14±2.8
Agar+ Phytagel (Agargel)	100	35.71±2.0
Phytagel	100	31.42±2.6

Fractions were converted into percentages; percentage data were subjected to angular transformation; values in the parentheses are transformed values, CD=34.30, SEm=3.81. Differences among treatments were non-significant at 5%.

than Phytagel (31.42%) or agar (27.14%). Though the effect of gelling agent was not significant, it is clear that kanamycin has the maximum activity in a medium solidified with agar than with agargel and Phytagel. Similarly, agar encouraged maximum effect of kanamycin, whereas Phytagel and agargel showed reduced inhibitory effect of kanamycin in flax transformation studies (Laine *et al*, 2000). Kanamycin appears to bind to gelrite with higher affinity than to agar; hence, inhibition of regeneration by kanamycin on a medium solidified with gelrite was less, compared to that with agar (Chauvin *et al*, 1999; Wilmink and Dons, 1993). In the present study, agargel, an intermediate form of agar and Phytagel, showed the highest regeneration response and less kanamycin activity compared to that with agar and Phytagel.

Cassells and Collins (2000) reported that physical and chemical grading of gelling agents was not related to biological performance of the gelling agents. However, the small differences observed in regeneration response of cocultivated explants cultured on selection medium solidified with various gelling agents suggest that the gelling agent found to be optimal with respect to sensitivity of the explants to kanamycin should be continued to be used in transformation studies. It should not be changed at any stage of the transformation experiment because it may lead to altered response from explants between the experiments. Change in solidifying agent may either reduce regeneration response or increase regeneration of escapes due to change caused in the effect of kanamycin (selection). Generally, differences were observed in explants cultured on media solidified with various gelling agents in tobacco (Chauvin *et al*, 1999). So, there is also a possibility of the gelling agent itself affecting regeneration response of explants without kanamycin in the culture medium. However, in brinjal, reports indicate agar to be the best gelling agent for the realization of better *in vitro* regeneration response (Perrone *et al*, 1992). It can be therefore concluded that gelling agents have a role in deciding kanamycin activity,

which in turn, affect transformation and regeneration of hypocotyl explants in brinjal cv. Manjarigota.

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REFERENCES

Barcelo, M., Mansouri, E. I., Mercado, A. J., Quesada, A. M. and Pliego, F. 1998. Regeneration and transformation via *Agrobacterium tumefaciens* of the strawberry cultivar Chandler. *Pl. Cell Tiss. Org. Cult.,* **54**: 29-36.

Billings, S., Jelenkovic, G., Chin, C. K. and Eberhardt, J. 1997. The effect of growth regulation and antibiotics on eggplant transformation. *J. Amer. Soc. Hortl. Sci.,* **122**: 158-162.

Cassells, A. C. and Collins, I. M. 2000. Characterization and comparison of agars and other gelling agents for plant tissue culture use. *Acta Hort.,* **530**: 203-212.

Chauvin, E. J., Marhadour, S., Cohat, J. and Nard, L. M. 1999. Effects of gelling agents on *in vitro* regeneration and kanamycin efficiency as a selective agent in plant transformation procedures. *Pl. Cell Tiss. Org. Cult.,* **58**: 213-217.

Curuk, S., Elman, C., Schlarman, E., Sagee, O., Shomer, I., Cetiner, S., Gray, D. J. and Gaba, V. 2002. A novel pathway for rapid shoot regeneration from the proximal zone of the hypocotyl of melon (*Cucumis melo* L.). *In Vitro Cell. Devtl. Biol.-Pl.,* **38**: 260-267.

Gaba, V., Schlarman, E., Elman, C., Sagee, O., Watad, A. A. and Gray, D. J. 1999. *In vitro* studies on the anatomy and morphology of bud regeneration of melon cotyledons. *In Vitro Cell. Devtl. Biol.-Pl.,* **35**: 1-7.

Halford, P. and Newbury, H. J. 1992. The effects of antibiotics and their breakdown products on the in vitro growth of *Antirrhinum majus*. *Pl. Cell Rep.,* **11**: 93-96.

Hanur, V. S., 2004. Genetic transformation of human cells by a soil phytopathogen presents common molecular strategies. *Curr. Sci.,* **87**: 856-857.

Hanur, V. S., Prakash, D. P. and Asokan, R. 2007. Effects of explant, pre-conditioning and light on the regeneration of transgenic tomato. *Ind. J. Hort.,* **64**: 104-105.

Hanur, V. S., Prakash, D. P., Deepali, B. S., Asokan, R., Ramachandra, Y. L., Mahmood, R. and Anand, L. 2006. Synergistic use of hypocotyl explants and high BAP preconditioning for enhanced transformation frequency in brinjal. (*Solanum melongena* L.). *J. Hort. Sci.,* **1**: 116-119.

Hoque, M. E., Mansfield, J. W. and Bennett, M. H. 2005. *Agrobacterium*-mediated transformation of Indica rice genotypes: an assessment of factors affecting the transformation efficiency. *Pl. Cell Tiss. Org. Cult.,* **82**: 45-55.

Klee, H., Horsch, R. and Rogers, S. 1987. *Agrobacterium*-mediated transformation and its further applications to plant biology. *Ann. Rev. Pl. Physiol.,* **38**: 467-487.

Kumar, P. A., Mandaokar, A., Sreenivasu, K., Chakrabarti, S. K., Bisaria, S., Sharma, R. S., Kaur, S. and Sharma, R. P. 1998. Insect resistant transgenic brinjal plants. *Mol. Breed..,* **4**: 33-37.

Laine, E., Lamblin, F., La Coux, P. D., Roger, D., Sihachakr, D. and David, A. 2000. Gelling agent influences the detrimental effect of kanamycin on adventitious budding in flax. *Pl. Cell Tiss. Org. Cult.,* **63**: 77-80.

Ling, H., Kriseleit, D. and Ganal, M. W. 1998. Effect of ticarcellin/potassium clavulanate on callus growth and shoot regeneration in *Agrobacterium*-mediated transformation of tomato. *Pl. Cell Rep.,* **17**: 843-847.

Magioli, C., Rocha, A. P. M., Pinheiro, M. M., Martins, S. G. and Mansur, E. 2000. Establishment of an efficient *Agrobacterium*-mediated transformation system for eggplant and study of a potential biotechnologically useful promoter. *J. Pl. Biotech.,* **2**: 43-49.

Magioli, C., Rocha, A. P. M., Tarre, E., Santiago-Fernandes, L. D. Oliviera, D. E., Krul, W. R. and Mansur, E. 2001. Effect of morphological factors, antibiotics and *Agrobacterium* cocultivation in the efficiency of somatic embryogenesis of eggplant (*Solanum melongena* L.). *J. Pl. Biotech.,* **3**: 19-25.

Mathias, R. J. and Boyd, L. A. 1986. Cefotaxime stimulates callus growth, embryogenesis and regeneration of hexaploid bread wheat. *Pl. Sci.,* **46**: 217-223.

Mathias, R. J. and Mukasa, C. 1987. The effect of cefotaxime on the growth and regeneration of callus from four varieties of barley. *Pl. Cell Rep.,* **6**: 454-457.

Murashige, T. and Skoog, F. 1962. A revised method for rapid growth and bioassays with tissue cultures. *Physiol. Plant.,* **15**: 473-497.

Nap, J. P., Bijvoet, J. and Stiekema, W. J. 1992. Biosafety of kanamycin resistant transgenic plants. *Transgenic Res.,* **1**: 239-249.

Norouzi, P., Mahboobi, M. A., Zamani, K. and Samadi, B.

Y. 2005. Using a competent tissue for efficient transformation of sugarbeet (*Beta vulgaris* L.). *In Vitro Cell. Devtl. Biol.-Pl.*, **41**: 11-16.

Okkels, F. T. and Pedersen, M. G. 1988. The toxicity of plant tissue and *Agrobacterium tumefaciens* of some antibiotics. *Acta Hort.,* **225**: 199-207.

Perrone, D., Bico, I. V. and Rotino, G. L. 1992. Effect of gelling agents and activated charcoal on *Solanum melongena* plant regeneration. *Capsicum Newslett.*, **11**: 43-44.

Picoli, E. A. T., Cecon, P. R., Fari, M. G. and Otoni, W. C., 2002. Organogenesis in eggplant as affected by antibiotics and growth regulators. *Int'l J. Hortl. Sci.*, **8**: 76-82.

Robert, M. L., Flores, M. R. and Loyola-Vergas, V. M. 1989. Growth promoting activity of certain penicillins on cultivated cells of *Bouvardia ternifolia*. *Phytochem.*, **28**: 2659-2662.

Rotino, G. L. and Gleddie, S. 1990. Transformation of eggplant (*Solanum melongena* L.) using binary *Agrobacterium tumefaciens* vectors. *Pl. Cell Rep.*, **9**: 26-29.

Sarmento, G. G., Aipert, K., Tang, F. A. and Punja, Z. K. 1992. Factors influencing *Agrobacterium tumefaciens* mediated transformation and expression of kanamycin resistance in pickling cucumber. *Pl. Cell Tiss. Org. Cult.,* **31**: 185-193.

Shaw, C.H., Leemans, J., Van Montagu, M. and Schell, J. 1983. A general method for the transfer of cloned genes to plant cells. *Gene*, **23:** 315-330.

Sriskandarajah, S. and Goodwin, P. 1998. Conditioning promotes regeneration and transformation in apple leaf explants. *Pl. Cell Tiss. Org. Cult.,* **53**: 1-11.

Sunilkumar, G. and Rathore, K. S. 2001. Transgenic cotton: factors influencing *Agrobacterium*-mediated transformation and regeneration. *Mol. Breed.*, **8**: 37-52.

Wilmink, A. and Dons, J. J. 1993. Selective agents and marker genes for use in tranformation of monocotyledonous plants. *Pl. Mol. Biol. Rep.*, **11**: 165-185.

Yu, A. F., Yeh, D. S. and Yang, S. J. 2001. Effects of carbenicillin and cefotaxime on callus growth and somatic embryogenesis from adventitious roots of papaya. *Bot. Bull. Acad. Sci.,* **42**: 281-292.

Zuker, A., Ahroni, Tzfira, T., Meir, B. H. and Vainstein, A. 1999. Wounding by bombardment yields highly efficient *Agrobacterium*-mediated transformation of carnation (*Dianthus caryophyllus* L.). *Mol. Breed.*, **5**: 367-375.

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