J. Hortl. Sci.
Vol. 11(1):21-26, 2016

Effect of *Agrobacterium* infection time, co-cultivation and cell density on *in vitro* response in hypocotyl of eggplant (*Solanum melongena* L.)

D.P. Prakash, Y.L. Ramachandra[1] and Vageeshbabu S. Hanur[2]*
College of Horticulture
University of Horticultural Sciences, Munirabad - 583 233
Koppal, Karnataka, India
*E-mail: vageesh@iihr.res.in

ABSTRACT

The present study purports to assess the effect of *Agrobacterium* infection time, co-cultivation and cell density on *in vitro* response in hypocotyl explants of eggplant (brinjal) cv. Manjarigota. *Agrobacterium* (OD_{600} 0.3-0.5) infection for 10-15 minutes (24.44±2.34%) was found to be optimum, while, higher or lower infection-time resulted in reduced callus initiation, shoot regeneration and explant survival. Explants with no (only *Agrobacterium* infection) or short (1 day) co-cultivation, showed reduced callus-initiation response and turned yellow, with no regeneration. Callus-initiation response increased from Day 1 (96.66±03.33%), and reached a maximum on Day 2 and Day 3 (100±00.00%). It decreased on further increase in co-cultivation time. Explants co-cultivated for three days showed highest regeneration response (30.00±02.96%) which thereafter reduced with further increase in co-cultivation time. Explants infected with *Agrobacterium* culture at 0.05 OD_{600} showed hardly any regeneration, and turned yellow and necrotic on the selection medium. Highest regeneration response (28.33±02.33%) was obtained in explants infected with 0.1 OD_{600} culture, and this gradually reduced as cell-density increased (upto 1.0 OD_{600}), becoming zero in explants treated with cultures at 1.5 OD_{600} or above. *Agrobacterium* overgrowth was noticed on explants infected with cultures of 0.5 OD_{600} and above. Exposure of hypocotyl explants to higher cell-density, longer infection-time and prolonged co-cultivation regime resulted in severe necrosis of explants; time taken for development of *Agrobacterium* overgrowth was less with increase in the level of these factors. Regenerated shoots were healthy, green, elongated and showed root induction on culture medium containing Kanamycin.

Key words: Eggplant, Manjarigota, regeneration, *Agrobacterium*, PCR

INTRODUCTION

Eggplant (brinjal, *Solanum melongena* L.) 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 into Europe by Arabian traders and, then, brought to North America by early European settlers. In popular medicine, eggplant is suggested for treatment of several diseases including diabetes, arthritis, asthma and bronchitis. It is becoming increasingly evident that environmental factors such as drought, salinity, extremes of temperature, incident light, heavy metals and biotic stresses have profound effects on brinjal production worldwide. A technology for production of transgenic plants will have an important and powerful impact on some immediate problems such as abiotic stresses and phytopathogen attack, and, could reduce dependence on chemical pesticides or fungicides. Plant transformation is an essential tool both for experimental investigation of gene function and for improvement of crops, either by enhancing existing plus traits, or by introducing new genes. The technique of *Agrobacterium*-mediated transformation has proved to be a popular method favored for its practicality, effectiveness and efficiency. It is a widely used technique for gene transfer to various crops and often produces fertile, and morphologically normal, transgenic plants (Veluthambi *et al*, 2003). In the past decades, a large number of research reports on brinjal transformation were published, particularly on optimization of explant-type and source, plant growth regulators for regeneration, pre-culture period, *Agrobacterium* culture concentration, co-cultivation

[1]Department of Biotechnology, Kuvempu University, Shankaraghatta, Shimoga - 577 451, India
[2]Division of Biotechnology, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru - 560 089, India

period, *Agrobacterium* virulent gene inducers, and improvement in transformation efficiency (Magioli and Mansur, 2005; Kumar and Rajam, 2005). However, no effort has been made to study the effect of *Agrobacterium* infection-time on *in vitro* response in transformation studies in brinjal and other crops (Opabode *et al*, 2006). Previous studies on brinjal indicate a necessity for identifying factors affecting *in vitro* response to increase *in vitro* regeneration frequency in *Agrobacterium* co-cultivated explants and, in turn, transformation efficiency to reduce resources for production of transgenic plants in elite cultivars. 'Manjarigota' is a popular and preferred round-striped brinjal cultivar. We have earlier reported better growth regulator and explant combinations (Hanur *et al*, 2006; Prakash *et al*, 2007a; Prakash *et al*, 2008), gelling agents and concentration of antibiotics (Prakash *et al*, 2007b) in this cultivar. The present study purports to study the effect of *Agrobacterium* infection-time, co-cultivation and cell density on *in vitro* response in hypocotyl explants for maximizing plant regeneration.

MATERIAL AND METHODS

An efficient Shoot Regeneration Medium [SRM: full-strength basal MS medium (MS salts, organics and vitamins); Murashige and Skoog, 1962] supplemented with 3% sucrose and 0.8% agar, containing 2µM BAP (BAP – 6-benzyleaminopurine) + 0.05µM NAA (NAA - naphthalene acetic acid) was used (Prakash *et al*, 2008). pH of the medium was adjusted at pre-sterilization to 5.75±0.05 with NaOH (Sodium hydroxide) or HCl (Hydrochloric acid) (0.1N) after addition of growth regulators. Sterilization of the culture medium and instruments was done by autoclaving at 21°C and 15psi pressure for 20 minutes. Antibiotics were filter-sterilized before adding to the autoclaved medium (wherever necessary). The medium was cooled down to under 45°C, poured into *petri* plates under a laminar airflow chamber (Alpha Scientific Co., India). After solidification, the media were used in experiments. Cultures were maintained in culture racks placed in a culture room at 25± 2°C under 16h photoperiod with a light intensity (white, fluorescent tubes) of 30-40µE $m^{-2}s^{-1}$. Breeder seeds of eggplant cv. Manjarigota were obtained from Division of Vegetable Crops, ICAR-IIHR (Indian Institute of Horticultural Research), Bangalore. Hypocotyls from aseptically germinated seedlings (Prakash *et al*, 2008) were used as the source of explants after removing the apical meristem and basal stub.

Agrobacterium tumefaciens A208 strain harbouring pBinAR-1 binary vector containing CaMV35S (cauliflower mosaic virus 35S promoter) promoter-*nptII (neomycin phosphotransferase gene) - ocs* terminator cassette was employed (Source: NRC on Plant Biotechnology, New Delhi) in plant transformation studies. *NptII* gene conferring kanamycin resistance served as the selectable marker. A single colony of *Agrobacterium tumefaciens* A208 strain (Source: NRC, Biotechnology, New Delhi) was inoculated onto Yeast Extract Mannitol Broth (YEMB-mannitol 10g l^{-1}, yeast extract 0.4gl-$^{-1}$, NaCl 0.1gl-$^{-1}$, $MgSo_4.7H_2O$ 0.2gl-$^{-1}$ and K_2HPO_4 0.5g l-$^{-1}$) containing Kanamycin (Sigma, USA; 50 mgl^{-1}) and incubated in a shaker at 180 rpm and 28±1°C. Overnight grown culture of *Agrobacterium* was spun at 5000 rpm for 5 minutes, the supernatant discarded, the bacterial pellet dissolved in sterile, liquid half-strength MS medium, cell concentration measured at optical density of 600 nanometer (OD_{600}) using spectrophotometer (BioRad, USA). Thereupon, 0.3-0.5 OD_{600} culture was prepared using sterile, liquid half-strength MS medium for further use. 15-20 days old, medium-sized (1cm) hypocotyl explants were placed on SRM in *petri* plates for pre-culture for two days. Explants were collected into a sterile *petri* plate and immersed for 10-15 minutes in *Agrobacterium* culture (0.3-0.5 OD_{600}) for infection. The explants were then blotted onto sterile tissue paper towels to remove any excess *Agrobacterium* culture, placed back onto the medium used for pre-culture, and incubated under co-cultivation for two days. Explants were transferred onto a fresh culture medium containing Cefotaxime (Taxim, India; 500mg l^{-1}) for the next two days, and were finally transferred onto a culture medium containing Cefotaxime (250mg l^{-1}) and Kanamycin (100mgl^{-1}). Further subculture of explants was carried out at ten day intervals onto fresh SRM containing Cefotaxime (250mgl^{-1}) and Kanamycin (100mgl^{-1}).

Experiments to assess the effect of various factors on *in vitro* response were carried out to representing conditions in transformation studies, and, the procedure used was as mentioned above. To study the effect of infection-time, explants were immersed in *Agrobacterium* culture for 1, 5, 10, 15, 20, 30, 40, 50 or 60 minutes before co-cultivation; to study the effect of co-cultivation, explants were co-cultivated for 0, 1 2, 3, 4, 5, 6 or 7 days before transfer to SRM containing Cefatoxime; to study the effect of cell-density, explants were treated with bacterial culture containing *Agrobacterium* culture of 0.05, 0.1, 0.3, 0.5, 0.7, 1.0, 1.5 or 2.0 OD_{600}. In addition, the explants cultured

without *Agrobacterium* treatment on SRM and SRM containing Cefotaxime (250mg l^{-1}) and Kanamycin (100mgl^{-1}), served as the Controls.

Adequate number of explants and replications were used in all the experiments. Observations were recorded on callus-initiation (a bulge near the cut end) and shoot regeneration response at four weeks. Observations on survival of explants, sensitivity of the explants to *Agrobacterium* infection and *Agrobacterium* overgrowth in culture plates, were recorded weekly upto four weeks after culture initiation. Observations on shoot elongation were recorded at 3 weeks of subculture. Observations on rooting were recorded as and when root induction occurred. Frequency (per cent) of callus-initiation response (Number of explants showing callus-initiation response/ Number of explants cultured X 100) and frequency (per cent) of shoot regeneration response (Number of explants showing regeneration response/ Number of explants cultured X 100) was calculated. Percentage data was subjected to angular transformation and, then, subjected to Analysis of Variance (ANOVA) to test significance of the observed results. Comparison between mean values of treatment was made by Least Significant Difference (LSD) to identify the best treatment. Frequency (per cent) of shoot elongation and root induction was calculated.

Regenerated shoots were transferred onto shoot elongation medium (SEM: SRM containing 100mgl^{-1} Cefotaxime and 50mgl^{-1} Kanamycin) in test tubes. Shoots with well-developed primary meristem were transferred onto root induction medium (RIM: MS medium supplemented with 5µM IBA, 0.1µM BAP, 3% sucrose, 0.8% agar, 50mgl^{-1} Cefotaxime and 25mgl^{-1} Kanamycin in test tubes.

Molecular analysis of transformants was done using PCR for confirmation of presence of the marker transgene. Good quality DNA was isolated from 10 putative transformants using CTAB method, and, PCR was carried out with antibiotic-specific forward primer (*npt*-F) 5' GATGGATTGCACGCAGG 3' and reverse primer (*npt*-R) 3' GAAGGCGATAGAAGGCG 5' to confirm presence of the transgene. PCRs were performed in a total volume of 25µl, comprising 2.5µl of 10X reaction buffer containing 15mM $MgCl_2$ (Genetix), 0.25µl of 10mM dNTPs mixture (Genetix), 1µl of each primer (10µM, MWG), 0.33µl of *Taq* DNA polymerase (Genetix), 2.0µl (100-200ng) of DNA sample and 17.92µl of sterile water. As a positive Control, 2.0µl of pBinBt-01 DNA was used. Non-transformed plant DNA and sterilized water samples were used as negative Controls. PCR was carried out in MWG^R PCR system (MWG, Germany). DNA was denatured at 94^{o}C for 5 min, followed by 35 amplification cycles. Each cycle was programmed with three different thermal periods: 94^{o}C for 1 min to denature DNA, 56^{o}C for 40 sec to anneal the primers, and 72^{o}C for 1 min for the extension of DNA by *Taq* DNA polymerase. The final extension lasted 5 min at 72^{o}C. The amplified product was mixed with 6X loading buffer (30% sucrose, 0.05% xylene cynol and 0.05% bromophenol blue) and loaded along with 500bp (base-pair)/ 0.5kb (kilo base-pair) ladder (Genei, Bangalore) into 1.2 per cent agarose gel containing ethidium bromide (0.001%). Electrophoresis was conducted at 50 Volts for five hours, and the gel was photographed under UV light using Alpha Digi Doc system (Herolab, Germany). Gels were scored for presence or absence of the expected size of the amplified product.

RESULTS AND DISCUSSION

In this study, eggplant hypocotyl explants treated with *Agrobacterium* culture showed callus-initiation and shoot-regeneration response after 4-5 and 10-12 days of culture, respectively. The culture showed, on an average, 77% shoot-regeneration response. *Agrobacterium* co-cultivated hypocotyl explants showed callus-initiation and shoot-regeneration response after 10-12 days and 20-22 days of culture, respectively (Fig. 1-A). A delay and drastic reduction was seen in *in vitro* regeneration response, common in *Agrobacterium* co-cultivated explants. This could be due to the inhibitory effect of *Agrobacterium* and antibiotics present in the culture medium. Similar results have been reported in previous studies (Magioli *et al*, 2000; Billings *et al*, 1997).

In our study, *Agrobacterium* infection-time significantly affected callus-initiation and shoot-regeneration response in eggplant. Explants exposed to *Agrobacterium* cell cultures for under five minutes turned yellow and died on the selection medium. Explants infected for 5-20 minutes showed over 95% callus-initiation response and stayed green (survived), without any apparent *Agrobacterium* overgrowth. Highest regeneration-response was obtained in explants co-cultivated for 10-15 minutes (24.44±2.34%). This reduced in explants infected for less or more time (Table 1). Further, explants infected for 30 minutes or more showed reduced callus-initiation response, along with reduced or no regeneration response. This is the first study on the effect of *Agrobacterium* infection-time on *in vitro* response in explants of brinjal. Similar results

Table 1. *In vitro* response in hypocotyl explants of eggplant cv. Manjarigota infected with *Agrobacterium* for various time durations

Infection time (min.)	Callus initiation frequency (%)	Regeneration frequency (%)	Score for sensitivity of explant	Remarks
1	100.00^{a} ± 00.00	00.00 ± 00.00	D	Insufficient
5	100.00^{ba} ± 00.00	04.44^{d} ± 2.20	Y	Insufficient
10	97.7^{cba} ± 02.33	24.44^{a} ± 2.13	H	Optimum
15	95.55^{dcba} ± 04.67	24.44^{ba} ± 2.34	H	Optimum
20	95.55^{edcba} ± 02.33	04.44^{cd} ± 2.18	H	Optimum
30	73.33^{f} ± 04.10	02.22^{e} ± 2.20	B/AOG++ (3rd-4th week)*	Hypersensitive
40	68.88^{gfh} ± 03.07	02.22^{fe} ± 2.20	D/ AOG+++ (3rd week)*	Hypersensitive
50	62.22^{hgf} ± 02.09	00.00 ± 0.00	D/ AOG+++ (2nd week)*	Hypersensitive
60	40.00^{ih} ± 06.67	00.00 ± 0.00	D/ AOG+++ (2nd week)*	Hypersensitive
Control	100.00 ± 00.00	78.93 ± 3.84	H	-
CD	21.32**	2.19**	-	-

**Significant at $P \leq 0.01$; B-Brown; H-Healthy; Y-Yellowing; D-Dead; AOG++ prominent *Agrobacterium* overgrowth around the explant; AOG+++ *Agrobacterium* overgrowth covering the entire explant

Table 2. *In vitro* response in hypocotyl explants of eggplant cv. Manjarigota co-cultivated with *Agrobacterium* for various time durations

Co-cultivation (days)	Callus initiation frequency (%)	Regeneration frequency (%)	Score for sensitivity of explant	Remarks
0	16.66^{f} ± 01.93	00.00 ± 00.00	Y	Insufficient
1	96.66^{c} ± 03.33	00.00 ± 00.00	H	Optimal
2	100.00^{a} ± 00.00	25.00^{b} ± 02.87	H	Optimal
3	100.00^{ba} ± 00.00	30.00^{a} ± 02.96	H	Optimal
4	95.00^{d} ± 02.89	18.33^{c} ± 01.66	B/AOG+	Hypersensitive
5	65.00^{e} ± 07.27	10.00^{d} ± 02.76	D/AOG+++	Hypersensitive
6	00.00 ± 00.00	00.00 ± 00.00	D	Hypersensitive
7	00.00 ± 00.00	00.00 ± 00.00	D	Hypersensitive
Control	100.00 ± 00.00	76.68 ± 03.48	H	-
CD	1.249**	1.127**		-

**Significant at $P \leq 0.01$; B-Brown; H-Healthy; Y-Yellowing; D-Dead; AOG+*Agrobacterium* colonization starting around the explant; AOG+++ *Agrobacterium* overgrowth covering the entire explant

on effect of infection-time have been reported in cauliflower, however (Chakrabarty *et al*, 2002).

Insufficient time given for co-cultivation may end up in no-transformation of plant cells (Cardoza and Stewart, 2003), while, prolonged co-cultivation period affects regeneration competence of the explant (Fillatti *et al*, 1987). In our study, both callus-initiation and regeneration response were significantly affected by the co-cultivation schedule. Explants with no (only *Agrobacterium* infection), or short (1 day) co-cultivation, showed reduced callus-initiation response and turned yellow without regenerating. Callus-initiation response increased from day one (96.66±03.33%), and reached a maximum on day two and day three (100%); it decreased with further increase in co-cultivation period. Explants co-cultivated for three days showed highest regeneration response (30.00±02.96%), and, this reduced with further increase in co-cultivation time (Table 2). Short co-culture period may not be adequate for *Agrobacterium* infection to take hold, while, longer periods may result in excessive bacterial growth or affect regeneration/survival of the hypocotyl explant. Length of co-cultivation period required for achieving maximal gene transfer was genotype-dependent, ranging from two to five days in most plant species (Zhang *et al*, 1997). Magioli *et al* (2000) noticed that duration of co-cultivation was important in deciding the rate of Kanamycin-resistant calli and adventitious-shoot formation in eggplant; a maximum of two days of co-cultivation was used in all the transformation studies in eggplant hitherto. However, our study shows that co-cultivation period can be increased to three days, without adversely affecting survival/regeneration response in hypocotyl explants of eggplant cv. Manjarigota.

In the present study, *Agrobacterium* cell-density significantly affected callus-initiation and regeneration response in eggplant. Explants infected with *Agrobacterium* at 0.05 OD_{600} showed no regeneration and turned yellow on the selection medium. Highest regeneration response

Table 3. ***In vitro*** **response in hypocotyl explants of eggplant cv. Manjarigota treated with varying** ***Agrobacterium*** **cell-density**

Agrobacterium cell-density (OD_{600})	Callus initiation frequency (%)	Regeneration frequency (%)	Score for sensitivity of explant	Remarks
0.05	100.00^{a} ± 00.00	06.60^{e} ± 00.00	Y	Insufficient
0.1	100.00^{ba} ± 00.00	28.33^{a} ± 02.33	H	Optimum
0.3	100.00^{cba} ± 00.00	22.00^{b} ± 02.05	H	Optimum
0.5	82.22^{d} ± 02.20	13.33^{c} ± 04.04	B/AOG+	Hypersensitive
0.7	75.50^{ed} ± 05.86	08.80^{d} ± 02.43	D/AOG+++	Hypersensitive
0.1	68.88^{fe} ± 05.93	02.20^{f} ± 02.20	D/AOG+++	Hypersensitive
1.5	53.33^{g} ± 06.67	00.00 ± 00.00	D	Hypersensitive
2.0	42.22^{hg} ± 05.89	00.00 ± 00.00	D	Hypersensitive
Control (without co-cultivation)	100.00 ± 00.00	78.37 ± 04.13	H	-
CD	15.52**	1.88**		-

**Significant at $P \leq 0.01$; B-Brown; H-Healthy; Y-Yellowing; D-Dead; AOG+*Agrobacterium* colonization starting around the explant; AOG+++ *Agrobacterium* overgrowth covering the entire explant

(28.33±02.33%) was obtained in explants infected with bacterial colonies at 0.1 OD_{600}; it reduced gradually as cell-density increased (upto1.0 OD_{600}), and was nil in explants treated with 1.5 OD_{600} or above (Table 3). *Agrobacterium* overgrowth was noticed on explants infected with bacterial cultures of 0.5 OD_{600} or more. In the earlier studies, higher bacterial density (0.4-1.5 at OD_{600}) in eggplant hypocotyl explants increased transient GUS activity (Kumar and Rajam, 2005); however, it adversely affected survival of explants, callus growth, and subsequent regeneration (Kumar and Rajam, 2005; Billings *et al*, 1997; Magioli *et al*, 2000). Hence, in the present study, treatments containing very low levels of *Agrobacterium* cell-density (<0.4 at OD_{600}) were tested and were found to be beneficial for the health of the explants as well as *in vitro* regeneration in hypocotyl explants of eggplant cv. Manjarigota.

In our study, *Agrobacterium* colonization on eggplant hypocotyl explants was observed with infection-time, co-cultivation, *and Agrobacterium* cell-density. Exposure of hypocotyl to higher cell-density, longer exposure/infection time and prolonged co-cultivation resulted in severe necrosis of the explant, while, diluted culture reduced necrosis in the hypocotyl explants to a great extent. Time taken for *Agrobacterium* overgrowth reduced with increased level of infection-time, co-cultivation and cell-density. In these cases, explants turned brown and died, perhaps be due to hypersensitivity of the explants to *Agrobacterium*. Elimination of *Agrobacterium* overgrowth was difficult with prolonged co-cultivation of explants. Similar results were reported earlier in eggplant and cauliflower (Magioli *et al*, 2000; Kumar and Rajam, 2005). In the present study, regenerated shoots in various experiments were healthy,

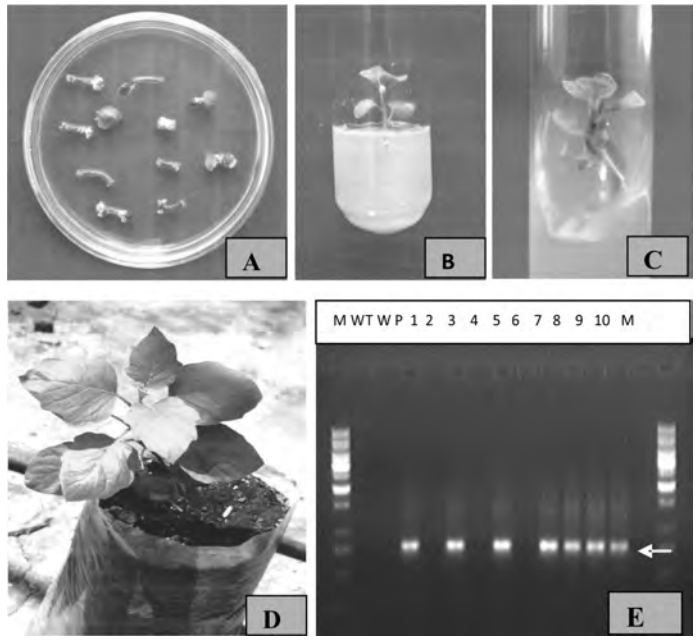

Fig. 1. A. Regeneration response in hypocotyl explants of brinjal cv. Manjarigota in SRM containing Cefotaxime (250 mgl^{-1}) and Kanamycin (100 ml^{-1}); B. Shoot elongation in SRM containing Cefotaxime (100 mgl^{-1}) and Kanamycin (50 mgl^{-1}); C. Rooting in MS medium containing Cefotaxime (50 mgl^{-1}) and Kanamycin (25 mg^{-1}); D. A hardened putative transformant plant of brinjal cv. Manjarigota; E. PCR amplification of ~0.9kb *nptII* gene (arrow) in transformed lines of eggplant (Lanes: M- 1kb marker (Genetix); WT-wild type; W-water control; P-plasmid DNA; 1-10 putative transformants).

green and showed improved growth on subculture onto SRM containing Kanamycin, and were elongated (79.23% of regenerated shoots; data not shown; Fig.1-B) and rooted (85.48% of elongated shoots; data not shown; Fig. 1-C) in the culture medium containing Kanamycin. This shows their

resistance to Kanamycin, while, Control shoots (regenerated without *Agrobacterium* treatment) showed stunted growth, and, gradually turned yellow in the culture medium due to chlorosis. Rooted plantlets were hardened. Transformed plants were morphologically normal and fertile (Fig.1-D). Molecular analysis of the transformants using PCR showed amplification of an expected size (750bp) PCR products for *nptII* gene in both treated (87.43%) and positive Control (Fig. 1-E), indicating the presence of the transgene. No amplification was found in negative Controls (untransformed plant and water Control).

In conclusion, the present study provides a baseline for working out optimum conditions for *Agrobacterium* infection-time, co-cultivation period and cell-density in transformation protocols using hypocotyl explants in eggplant cv. Manjarigota. In addition, it hints at reasons for poor explants-survival and low shoot-regeneration in explants co-cultivated with *Agrobacterium* in transformation studies previously reported. This would help design improved protocols for future transformation studies.

ACKNOWLEDGEMENT

The authors are grateful to Director, ICAR-IIHR, for encouragement.

REFERENCES

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

Cardoza, V. and Stewart, C.N. 2003. Increased *Agrobacterium*-mediated transformation and rooting efficiencies in canola from hypocotyl segment explants. *Pl. Cell Rep.,* **21**:599-604

Chakrabarty, R., Viswakarma, N., Bhat, S.R., Kirti, P.B., Singh, B.D. and Chopra, V.L. 2002. *Agrobacterium*-mediated transformation of cauliflower: optimization of protocol and development of Bt-transgenic cauliflower. *J. Biosci.,* **27**:495-502

Fillatti, J.J., Sellmer, J., McCown, B., Haissing, B. and Comai, L. 1987. *Agrobacterium* mediated transformation and regeneration of hybrid aspen and poplar clones. *Pl. Physiol.,* **93**:1110-1116

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

Kumar, V.S. and Rajam, M.V. 2005. Enhanced induction of *vir* genes results in the improvement of *Agrobacterium*-mediated transformation of eggplant. *J. Biochem. Biotech.,* **14:**89-94

Magioli, C. and Mansur, E. 2005. Eggplant (*Solanum melongena* L.): tissue culture, genetic transformation and use as an alternative model plant. *Acta Bot. Bras.*, **19**:139-148

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

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

Prakash, D.P., Deepali, B.S., Asokan, R., Ramachandra, Y.L., Lalitha Anand and Vageeshbabu S. Hanur. 2007a. Effect of explant type and growth regulators in the transformation of brinjal (*Solanum melongena* L.) cv. Manjarigota. *J. Hortl. Sci.,* **2**:94-98

Prakash, D.P., Deepali, B.S., Asokan, R., Ramachandra, Y.L., Lalitha Anand and Vageeshbabu S. Hanur. 2007b. Effect of antibiotics and gelling agents in the transformation of brinjal (*Solanum melongena* L.) cv. Manjarigota. *J. Hortl. Sci.* **2**:19-25

Prakash, D.P., Deepali, B.S., Asokan, R., Ramachandra, Y.L., Shetti, D.L., Lalitha Anand and Vageeshbabu S. Hanur. 2008. Effect of growth regulators on *in vitro* complete plant regeneration in brinjal (S*olanum melongena* L.). *Ind. J. Hort.,* **6**:112-116

Veluthambi, K., Gupta, K.A. and Sharma, A. 2003. The current status of plant transformation technologies. *Curr. Sci.,* **84**:368-379

Zhang, Z., Coyne, D.P. and Mitra, A. 1997. Factors affecting *Agrobacterium*-mediated transformation of common bean. *J. Amer. Soc. Hortl. Sci.,* **122**:300-305

(MS Received 07 May 2014, Revised 03 August 2015, Accepted 23 August 2015)