African Journal of Biotechnology Vol. 11(33), pp. 8218-8224, 24 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.252
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

In vitro regeneration from internodal explants of bitter melon (*Momordica charantia* L.) via indirect organogenesis

Muthu Thiruvengadam, Nagella Praveen and Ill-Min Chung*

Department of Applied Life Science, Konkuk University, Seoul 143-701, South Korea.

Accepted 22 March, 2012

Organogenic callus induction and high frequency shoot regeneration were achieved from internodal explants of bitter melon. About 97.5% of internodal explants derived from 30 day old *in vivo* grown plants produced green, compact nodular organogenic callus in Murashige and Skoog (MS) plus Gamborg et al. (1968) (B_5) medium containing 5.0 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.0 µM thidiazuron (TDZ) after two successive transfers at 11 days interval. Adventitious shoots were produced from organogenic callus when it was transferred to MS medium supplemented with 4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-glutamine with shoot induction frequency of 96.5% and regeneration of adventitious shoots from callus (48 shoots per explant). Shoot proliferation occurred when callus with emerging shoots was transferred in the same medium at an interval of 15 days. The regenerated shoots were elongated on the same medium. The elongated shoots were rooted in MS medium supplemented with 3.0 µM indole 3-butyric acid (IBA). Rooted plants were acclimatized in green-house and subsequently established in soil with a survival rate of 95%. This protocol yielded an average of 48 shoots per internodal explant after 80 days of culture.

Key words: Adventitious shoots, growth regulators, hardening, organogenic callus, *Momordica charantia*.

INTRODUCTION

Bitter melon (*Momordica charantia* L.) is an important cucurbit crop species and one of the major vegetable grown in the tropical regions of Asia, Amazon, east Africa and the Caribbean and is cultivated throughout the world. The fruits of bitter melon contain rich amount of vitamins, iron, minerals, phosphorous and presents good dietary fiber levels (Sultana and Bari Miah, 2003). Medicinal properties of the plant include anti-microbial, anti-cancerous, anti-mutagenic, anti-tumor, anti-infertility, anti-diabetic and anti-rheumatic properties (Singh et al., 1998). Recently, phytochemists have isolated a number of potential medical components from this plant, such as the ribosome inactivating protein (RIP), MAP30 (Momordica anti-Hiv protein), which suppresses HIV (human immunodeficiency virus) activity, *M. charantia* lectin (MCL), *M. charantia* inhibitor (MCI) and momordicoside A and B, both of which can inhibit tumor (Bourinbaiar and Lee-Huang, 1996; Beloin et al., 2005). Recent developments in biotechnology has opened up several ways for cucurbit breeding using genetic transformation in which heterologous genes can be introduced into existing cultivars (Sarowar et al., 2003). However, in many instances, the lack of an efficient regeneration system causes limitations for the utilization of gene transfer technologies for these crops.

An efficient plant regeneration system is therefore necessary for genetic transformation and propagation. During the past years, considerable efforts have been made for *in vitro* plant regeneration of this important plant

*Corresponding author. E-mail: imcim@konkuk.ac.kr. Tel: +82-2-450-3730. Fax: +82-2-446-7856.

Abbreviations: BAP, 6-Benzylaminopurine; **TDZ,** thidiazuron; **NAA,** α-naphthaleneacetic acid; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **IBA,** indole 3-butyric acid; **MSB_5,** Murashige and Skoog basal salts mixture + B_5 vitamins.

through organogenesis in nodal and shoot tips (Wang et al., 2001; Sultana and Bari Miah, 2003; Malik et al., 2007; Ma et al., 2012) and cotyledons (Islam et al.,1994). Paul et al. (2009) showed effect of exogenous polyamines (PAs) on enhancing somatic embryogenesis in *M. charantia*. *Agrobacterium*-mediated β-glucuronidase expression was detected in explants of immature cotyledonary nodes in *M. charantia* (Sikdar et al., 2005). In this study, we established for the first time, an efficient protocol for the regeneration of bitter melon using internodal explants.

In the present investigation, an attempt was made to evaluate the choice of auxin, cytokinin and amino acid concentrations and their combination on MS medium, in order to develop a standard reproducible protocol for rapid adventitious shoot regeneration and propagation via organogenesis from internodal explants of bitter melon. We believe that our findings could facilitate genetic transformation of this commercially important vegetable and may also be applicable to other related species.

MATERIALS AND METHODS

Collection of seeds and germination

Seeds of bitter melon (*M. charantia* cv. Coimbatore-1) were obtained from Arignar Anna Farm, Kudimianmalai, Pudukkottai, Tamilnadu, India. Seeds were potted in a mixture of peat : vermiculite : soil (1:2:1) and maintained in growth chamber (MLR-350H, Sanyo, Tokyo, Japan) at 27°C day/22°C night under 16 h light and 8 h dark photoperiod. The plants were fertilized and watered at weekly intervals. Internodal explants were excised from highly proliferating (30 day old) plants in growth chamber and rinsed thoroughly in running tap water for 2 h. The internodes were then surface sterilized by agitating in 5% laboline and 0.1% $HgCl_2$ for 10 min and rinsed five to seven times with sterile distilled water. The internodal explants were sliced into approximately 0.1 mm width and 0.7 mm length.

Callus induction

The callus induction media consisted of MS salts (Murashige and Skoog, 1962), B_5 vitamins (Gamborg et al., 1968) plus 3% sucrose and solidified with 0.8% agar; supplemented with different concentrations of 1.0 to 7.0 µM of naphthaleneacetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D) either separately or in combination with 1.0 - 4.0 µM thiadiazuron (TDZ) or benzyl amino purine (BAP) were tested for callus induction. The medium was adjusted to pH 5.8 prior to autoclaving at 121°C for 15 min. The cultures were maintained at 25 ± 2°C under 16 h light and 8 h dark photoperiod with a light intensity of 150 µmol m^{-2} s^{-1}. Two transfers were made at an interval of 11 days in the same induction medium.

Shoot bud induction and proliferation

Organogenic callus obtained from internodal explants was selected for shoot regeneration. Green, compact, nodular calluses obtained from internodal explants were transferred to MS medium plus 3% sucrose, solidified with 0.8% agar supplemented with different concentrations of auxins (NAA and 2,4-D; 1.0 to 3.0 µM), cytokinins (TDZ and BAP; 1.0 to 6.0 µM) and amino acids (L-asparagine and L-glutamine; 0 to 0.1 mM). The medium was adjusted to pH 5.8 prior to autoclaving at 121°C for 15 min. All compounds, plant growth regulators (PGRs) and amino acids were from Sigma-Aldrich. The calluses were subcultured at 15 day interval on the shoot induction medium. The cultures were maintained at 25 ± 2°C under 16/8 h photoperiod with the light intensity of 30 µmol m^{-2} s^{-1} for shoot bud induction. The adventitious shoots produced on calli were then separated from them and cultured to induce further shoot development. Calli with regenerating adventitious buds were subcultured twice at 15 day intervals in the same medium for shoot multiplication and elongation.

Rooting and acclimatization

Elongated shoots were transferred to rooting medium supplied with indole 3-butyric acid (IBA; 1.0 to 4.0 µM). The medium was adjusted to pH 5.8 prior to autoclaving at 121°C for 15 min. The cultures were maintained at 25 ± 2°C under 16/8 h photoperiod with light intensity of 30 µmol m^{-2} s^{-1}. After 3 weeks, the rooted plants were transplanted to paper cups containing sterile soil, sand and vermiculite (1:1:1, v/v/v) and were placed in the green house. The plants were watered daily with Hoagland's nutrient solution (Hoagland and Arnon, 1950). The potted plants were then covered with polyethylene to maintain a condition of high humidity (85% RH) and grown for 2 weeks at a photosynthetic photon flux density (PPFD) of 25 µmol m^{-2} s^{-1} before planting in the greenhouse. After the development of new leaves, the covers were removed, and hardened plants were transferred to earthen pots (diameter 18 cm) filled with soil mix (peat, perlite and vermiculite in equal proportions: 1:1:1, v/v/v) and grown to maturity. The survival percentage was calculated after 4 weeks in the greenhouse.

Histology

For histological observations, the organogenic callus derived from internodal explant with regenerating adventitious shoot buds was fixed in FAA (formaldehyde, acetic acid, alcohol, 0.5:0.5:9.0 (v/v/v) ratio) for 48 h, and it was dehydrated in a graded series of tertiary butyl alcohol (TBA) and finally embedded in paraffin wax. The blocks were sectioned at 10 mm thickness (Leica microtome, Germany, RM 2135). The ribbons were placed on the slides smeared with Meyer's albumin and flooded with 4% formalin. These slides were slightly warmed.

Statistical analysis

The data were collected 3 weeks after the initiation of callus induction, after 4 weeks of shoot regeneration culture and after 3 weeks in the rooting experiments. All experiments were conducted with a minimum of 10 replicates per treatment. The data were analyzed statistically using SPSS ver. 14 (SPSS, Chicago, IL). The significance of differences among means was carried out using Duncan's multiple range test at P = 0.05. The results were expressed as the mean ± standard error (SE) of three experiments.

RESULTS AND DISCUSSION

Callus induction

The callusing ability of internodal explants derived from 30 day old *in vivo* plants of bitter melon was evaluated on

Table 1. Effect of auxin and cytokinin on organogenic callus induction from internodal explants of *M. charantia*.

Plant growth regulators (µM)	Percentage of explants exhibiting callus induction	Nature of callus
5.0 2,4-D	56.3 ± 1.5^{i}	YF
7.0 2,4-D	65.5 ± 1.0^{g}	YBF
5.0 2,4-D + 2.0 BAP	77.0 ± 2.0^{e}	YGC
7.0 2,4-D + 2.0 BAP	82.1 ± 2.0^{d}	YBC
5.0 2,4-D + 2.0 TDZ	97.5 ± 1.0^{a}	GCN
7.0 2,4-D + 2.0 TDZ	91.0 ± 1.2^{bc}	GC
5.0 NAA	51.4 ± 1.0^{j}	YF
7.0 NAA	60.7 ± 1.2^{h}	YBF
5.0 NAA + 2.0 BAP	74.4 ± 1.0^{ef}	YGC
7.0 NAA + 2.0 BAP	80.0 ± 2.0^{de}	YBC
5.0 NAA + 2.0 TDZ	93.0 ± 1.6^{b}	GC
7.0 NAA + 2.0 TDZ	89.2 ± 1.5^{c}	GC

Each value represents the mean ± SE of 10 replicates per treatment. In the same column, significant differences according to LSD at the P = 0.05 level are indicated by different letters. YF, Yellowish friable; YBF, yellowish-brown friable; YBC, yellowish-brown compact; YGC, yellowish-green compact; GC, green compact; GCN, green compact nodular.

MS medium supplemented with individual treatment of different auxins (NAA and 2,4-D) or their combination, either with BAP or TDZ (Table 1). The quality of the callus was assessed after 3 weeks of culture. The combination of 5.0 µM 2,4-D and 2.0 µM TDZ produced greenish compact callus (Figure 1a and Table 1) with high callusing response (97.5%). Nabi et al. (2002), Devendra et al. (2009) and Selvaraj et al. (2006) found that the combination of BAP with NAA or 2,4-D produced organogenic callus in *Momordica dioica* and *Cucumis sativus*. The combination of 7.7 µM NAA with 2.2 µM TDZ produced greenish compact callus from leaf explants of *M. charantia*. Handley and Chambliss (1979) reported that NAA and Kn combination in MS medium produced nodular compact callus in cucumber. Selvaraj et al. (2006) obtained nodular, greenish compact and organogenic callus in the presence of 2,4-D and BAP for hypocotyl explants of cucumber. Punja et al. (1990), Seo et al. (2000) and Selvaraj et al. (2007) reported callus formation in cucumber cultivars in the combination of NAA and BAP for petiole, leaf and cotyledon explants, respectively.

Adventitious shoot formation

Nodular, green compact callus obtained from internodal explants in 5.0 µM 2,4-D and 2.0 µM TDZ medium were transferred to MS medium containing different concentrations of cytokinins (TDZ and BAP) combined with auxins (NAA and 2,4-D) for adventitious shoot induction (Table 2). MS medium with 4.0 µM TDZ and 1.5 µM 2,4-D produced 25.4 shoots from callus which reached 4.0 cm in length. Savitha et al. (2010) reported that MS medium with 2.5 mg/l 2,4-D and 0.5 mg/l TDZ produced high frequency shoot regeneration from leaf derived callus of *Citrullus colosynthis*. MS medium supplemented with 4.0 µM BAP and 1.5 µM 2,4-D produced 14.0 shoots from callus which attained 2.5 cm length after 4 weeks (Table 2).

In the present study, TDZ was found to be more effective in shoot regeneration as compared to BAP. The effectiveness of TDZ over other cytokinins has also been reported in other cucurbits such as *Cucurbita pepo* (Pal et al., 2007), *C. colosynthis* (Savitha et al., 2010) and *Melothria maderaspatana* (Baskaran et al., 2009). MS medium containing 4.0 µM TDZ and 1.5 µM 2,4-D and various concentrations of L-asparagine and L-glutamine significantly increased adventitious shoots from internode derived callus (Table 3). The highest number of shoots (48 shoots) was produced on MS medium containing 4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-glutamine from internode derived callus (Figure 1b and c). The shoots with 9.4 cm length were obtained after 4 weeks. NAA (1.34 µM), BAP (8.88 µM), zeatin (0.91 µM) together with L-glutamine (136.85 µM) produced large number of shoots in cucumber (Selvaraj et al., 2007). Addition of L-glutamine in the adventitious shoot regeneration medium greatly enhanced the production of shoots from callus. This is in agreement with the findings of Selvaraj et al. (2002) and Vasudevan et al. (2004) for *C. sativus* and *M. maderaspatana* (Baskaran et al., 2009).

Adding nontoxic glutamine (Gamborg et al., 1968) to the medium maintains a high growth rate of cells for a longer period. Locy and Wehner (1982) demonstrated that L-asparagine was the best nitrogen source for the

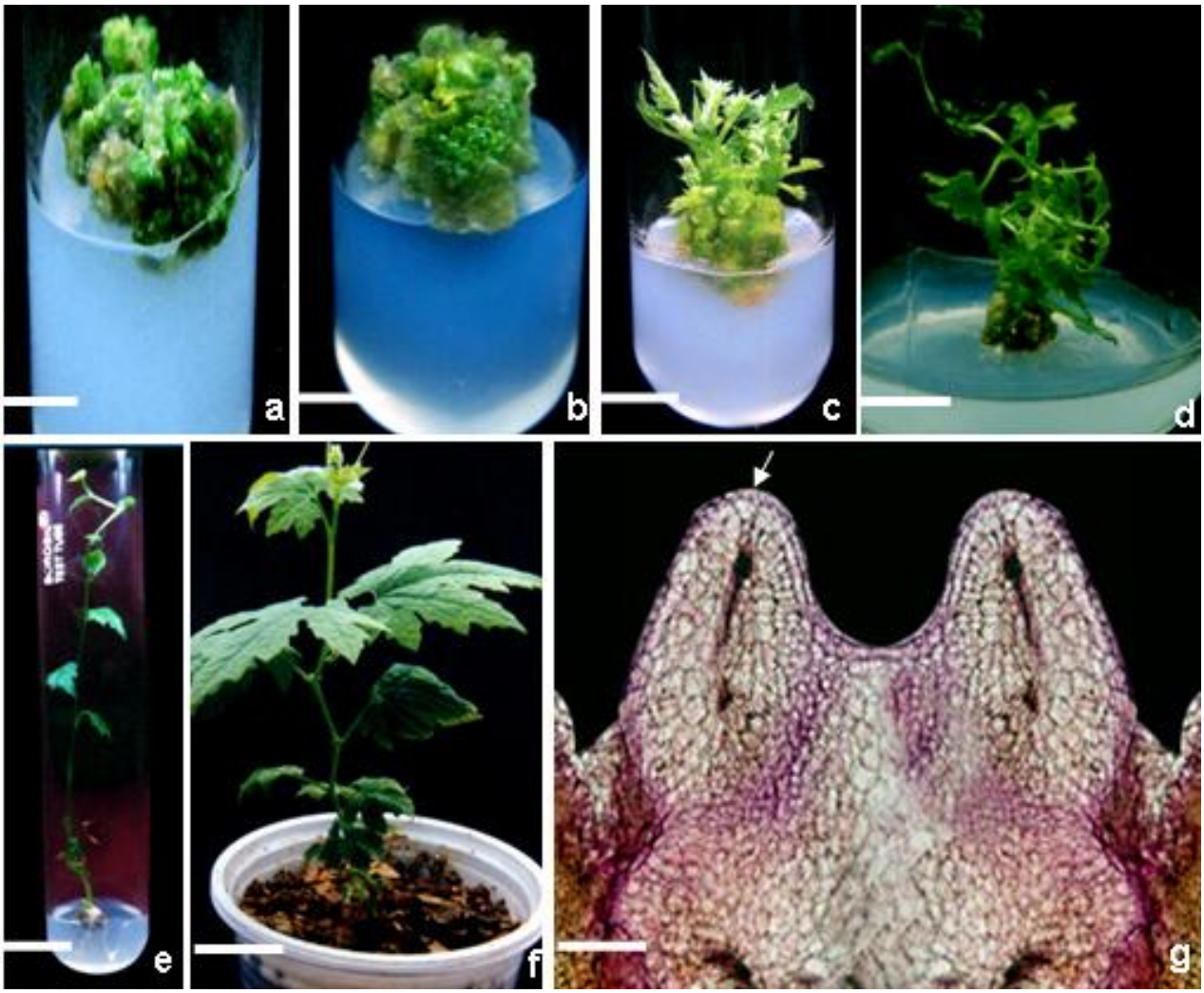

Figure 1. *In vitro* plant regeneration from internode derived callus of *M. charantia* L. (a) Greenish compact nodular organogenic callus (5.0 µM 2,4-D and 2.0 µM TDZ), bar: 10 mm; (b and c) Adventitious shoot regeneration and shoot proliferation (4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-glutamine), bar: 10 mm; (d) Elongation of shoots (4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-glutamine), bar: 10 mm; (e) *In vitro* rooting of shoots (3.0 µM IBA), bar: 10 mm, (f). Hardened plants, bar 3.0 cm; (g). Longitudinal section of internode derived callus showing the meristematic zone (bar = 50 mm). Arrows indicate emerging shoot primordia.

growth of cucumber shoots. The highest number of shoots (34.5 shoots) was produced on MS medium containing 4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-asparagine (Table 3). In our present investigation, L-glutamine is the best for induction of shoots as compared to L-asparagine. After two transfers in shoot induction medium at 15 days interval, adventitious shoots were produced from the protuberances of the callus. Malepszy and Nadolska-Orezyk (1983), Bergervoet et al. (1989) and Trulson and Shahin (1986) advocated repeated subcultures of callus to obtain high frequency shoot regeneration in cucumber. Earlier studies of bitter melon regeneration via organogenesis showed the production of 30 to 40 shoots per leaf explant (Thiruvengadam et al., 2010). Cucumber regeneration via organogenesis showed the production of 36 shoots per explant (Selvaraj et al., 2007). In the present study, we achieved a statistically significant better regeneration frequency (48 ± 1.0 shoots/internode) from internodal explants. Our study clearly indicates the requirement of high auxin : low cytokinin ratio for callus induction and low auxin : high cytokinin ratio for shoot induction from callus. The regenerated shoots when cultured in MS medium containing 4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-glutamine favoured for shoot proliferation and elongation after 1 week (Figure 1d). There are similar reports on other cucurbits such as *Cucumis melo* (Kathal et al., 1988), *Citullus vulgaris* (Srivastava et al., 1989) and *C. sativus* (Handley and Chambliss, 1979) where elongation of shoots occurred on MS medium fortified with either

Table 2. Effect of auxins and cytokinins on shoot regeneration from internode derived callus of *M. charantia*.

Plant growth regulators (µM)	Percentage of calluses that regenerated into shoots	Mean number of regenerated shoots per explant	Mean shoot length (cm)
BAP + NAA			
2.0 + 1.5	39.6 ± 1.5^{ij}	6.4 ± 1.0^{i}	1.2 ± 0.4^{h}
4.0 + 1.5	47.4 ± 1.0^{g}	10.5 ± 0.8^{g}	2.0 ± 0.5^{f}
6.0 + 1.5	40.3 ± 2.0^{i}	7.2 ± 0.5^{hi}	1.0 ± 0.2^{i}
BAP + 2,4-D			
2.0 + 1.5	42.4 ± 1.2^{h}	9.1 ± 0.6^{gh}	1.5 ± 0.5^{g}
4.0 + 1.5	55.8 ± 1.0^{e}	14.4 ± 1.2^{e}	2.5 ± 0.5^{d}
6.0 + 1.5	40.5 ± 1.5^{hi}	8.5 ± 1.0^{h}	1.0 ± 0.4^{i}
TDZ + NAA			
2.0 + 1.5	57.0 ± 2.0^{de}	17.0 ± 1.2^{d}	2.4 ± 0.6^{de}
4.0 + 1.5	66.5 ± 1.0^{bc}	21.4 ± 1.0^{b}	3.2 ± 0.5^{b}
6.0 + 1.5	53.2 ± 1.5^{f}	15.6 ± 0.5^{de}	2.0 ± 0.5^{f}
TDZ + 2,4-D			
2.0 + 1.5	69.2 ± 1.0^{b}	21.2 ± 1.0^{bc}	3.1 ± 0.8^{bc}
4.0 + 1.5	76.5 ± 2.0^{a}	25.4 ± 1.0^{a}	4.0 ± 0.5^{a}
6.0 + 1.5	60.6 ± 1.5^{d}	14.0 ± 0.5^{ef}	2.4 ± 0.6^{de}

Each value represents the mean ± SE of 10 replicates per treatment. In the same column, significant differences according to LSD at the P = 0.05 level are indicated by different letters.

Table 3. Effect of amino acids on shoot regeneration from internode derived callus of *M. charantia* in MS medium containing 4.0 µM TDZ and 1.5 µM 2,4-D.

Asparagine (mM)	Glutamine (mM)	Percentage of calluses that regenerated into shoots	Mean number of regenerated shoots per explant	Mean shoot length (cm)
0	-	76.5 ± 2.0^{f}	25.4 ± 1.0^{e}	4.0 ± 1.0^{e}
0.03	-	81.4 ± 1.0^{d}	29.2 ± 1.0^{d}	6.2 ± 0.8^{cd}
0.07	-	88.0 ± 1.5^{c}	34.5 ± 1.5^{bc}	7.0 ± 0.5^{c}
0.1	-	80.5 ± 1.0^{de}	30.4 ± 1.6^{cd}	6.0 ± 0.4^{d}
-	0.03	90.3 ± 1.2^{b}	37.5 ± 1.5^{b}	8.5 ± 0.5^{ab}
-	0.07	96.5 ± 1.0^{a}	48.0 ± 1.0^{a}	9.4 ± 0.6^{a}
-	0.1	89.0 ± 1.0^{bc}	33.2 ± 1.3^{c}	8.2 ± 0.8^{b}

Each value represents the mean ± SE of 10 replicates per treatment. In the same column, significant differences according to LSD at the P = 0.05 level are indicated by different letters.

BAP or KIN but not GA_3. In contrast, GA_3 showed a better response for shoot elongation as reported in *C. sativus* (Selvaraj et al., 2007) and *M. dioica* (Thiruvengadam et al., 2006).

Rooting and acclimatization

For rooting, the elongated shoots were transferred to MS basal medium containing different concentrations of IBA (1.0 to 4.0 µM) (Figure 2). A maximum of roots per shoot (9 roots) were obtained in MS medium with 3.0 µM IBA after 3 weeks culture period (Figure 1e). The effectiveness of IBA in rooting has been reported in *M. dioica* (Hoque et al., 1995; Nabi et al., 2002), *Benincasa hispida* (Thomas and Sreejesh, 2004) and *M. maderaspatana* (Baskaran et al., 2009). In contrast, NAA have been used for *in vitro* rooting in *Cucurbita pepo* (Kathiravan et al., 2006). BAP and IAA were used for rooting in *C. sativus* (Selvaraj et al., 2002). Thiruvengadam et al. (2006) and Han et al. (2004) reported that IAA is the best for root induction in *M. dioica* and *Lagenaria siceraria*. The rooted plants were gently removed from the vessels, washed initially to remove adhered agar and traces of the medium

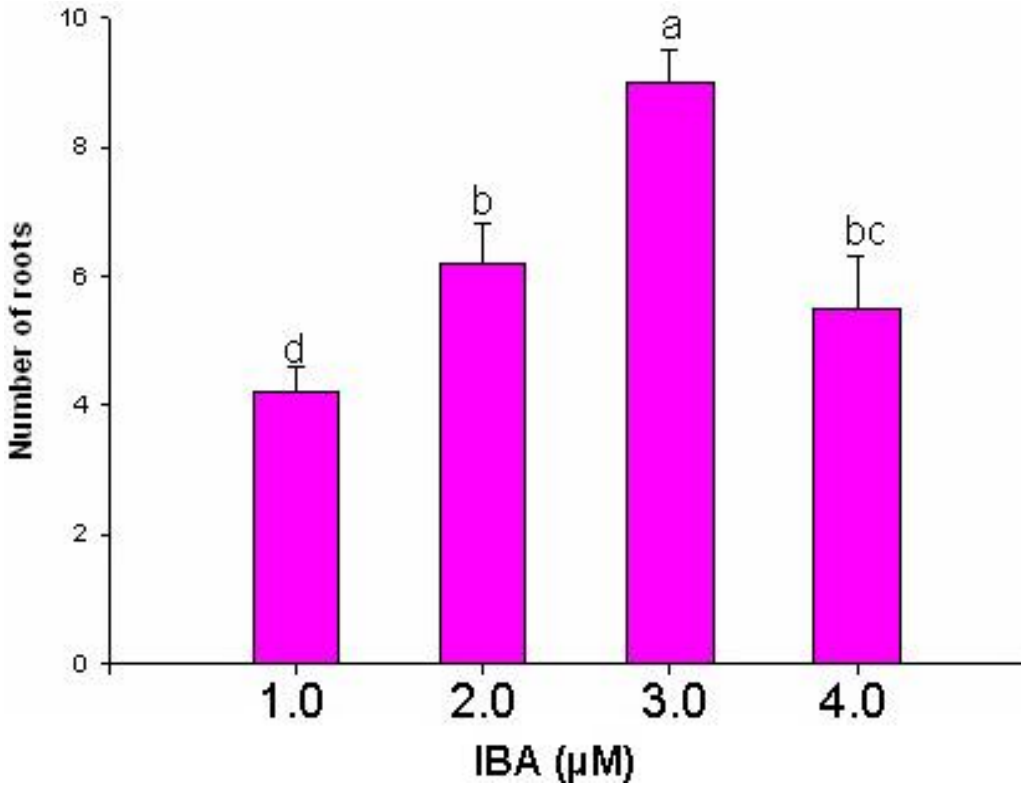

Figure 2. Effect of IBA on root induction from the *in vitro* shoot of *M. charantia*. Each value represents the mean ± SE of 10 replicates per treatment. The data were statistically analyzed using Duncan's multiple range test (DMRT). In the same column, significant differences according to the least significant difference (LSD) at the P = 0.05 level are indicated by different letters. The data were recorded after 3 weeks of culture.

to avoid contamination, and then washed for 10 min in distilled water. They were then transferred to plastic vessels containing a sterile soil, sand and vermiculite mixture (1:1:1, v/v/v) (Figure 1f), and after 2 weeks, they were transferred to pots. The hardening of potted plants for 15 days in a growth chamber was found to be essential. We had a 95% survival rate of plants derived from internodal explants when rooted plantlets were transferred from pots to field conditions. Regenerated plants transferred to the field became fully established and grew well and were similar to the parental plants in their morphology (Figure 1f).

Histology of organogenic callus

Histological examination of 3 weeks old organogenic callus revealed the initiation of bud primordium beneath the epidermal region of the meristematic dome. The bud primordium is flanked by two leaf primordia (Figure 1g). It reveals *de novo* origin of adventitious shoot buds. Similarly, histological studies were observed in leaf derived callus of *M. charantia*, hypocotyl and cotyledon explants of *C. sativus* (Selvaraj et al., 2006, 2007) and nodal explants of *M. dioica* (Thiruvengadam et al., 2006). In conclusion, high frequency regeneration of shoots was achieved using internodal explants of bitter melon via indirect organogenesis. MS medium containing 5.0 µM 2,4-D and 2.0 µM TDZ favoured organogenic callus induction, and 4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-glutamine combination induced adventitious shoots from organogenic callus. About 48 shoots were produced per internodal explants in culture duration of 80 days. We believe that this regeneration system could be used in the production of transgenic bitter melon plants by *Agrobacterium*-mediated genetic transformation as the protocol would yield higher number of shoots and the chance of recovering transformed plants at a higher frequency may be high.

ACKNOWLEDGEMENTS

The author Muthu Thiruvengadam is a recipient of the Konkuk University Brain Pool Postdoctoral Program, Konkuk University, Seoul, South Korea. The authors wish to thank the Arignar Anna Farm, Kudimianmalai, Pudukkottai, Tamilnadu, India, for supplying the seed materials.

REFERENCES

Baskaran P, Velayutham P, Jayabalan N (2009). *In vitro* regeneration of *Melothria maderaspatana* via indirect organogenesis. *In Vitro* Cell Dev. Biol. Plant. 45: 407-413.

Beloin N, Gbeassor M, Akpagana K, Hudson J, Soussa KD, Koumaglo K, Arnason JT (2005). Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. J. Ethnol. Pharmocol. 96: 49-55.

Bergervoet JHW, Van der Mark F, Custers JBM (1989). Organogenesis versus embryogenesis from long-term suspension cultures of cucumber (*Cucumis sativus* L.). Plant Cell Rep. 8: 116-119.

Bourinbaiar AS, Lee-Huang S (1996). The activity of plant derived antiretroviral proteins MAP30 and GAP31 against herpes simplex virus *in vitro*. Biochem. Biophy. Res. Commun. 219: 923-929.

Devendra NK, Subhash B, Seetharam YN (2009). Callus growth and plant regeneration in *Momordica dioica* (Roxb.) Willd. Cucurbitaceae. Am. Eurasian J. Sustain Agric. 3: 743-748.

Gamborg OL, Miller RA, Ojima K (1968). Nutrients requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50: 151-158.

Han JS, Oh DG, Mok IG, Park HG, Kim CK (2004). Efficient plant regeneration from cotyledon explants of bottle gourd (*Lagenaria siceraria* Standl.). Plant Cell Rep. 23: 291-296.

Handley LW, Chambliss OL (1979). *In vitro* propagation of *Cucumis sativus* L. Hort. Sci. 14: 22-23.

Hoagland DR, Arnon DI (1950). The water culture method for growing plants without soil. California Agric. Exp. Station Bull. p. 347.

Hoque A, Islam R, Joarder OI (1995). *In vitro* plantlets differentiation in kakrol (*Momordica dioica* Roxb.). Plant Tissue Cult. 5:119-124.

Islam R, Sarkar PK, Naderuzzaman ATM, Joarder OI (1994). *In vitro* regeneration of plants from cotyledons of *Momordica charantia* L. Plant Tissue Cult. 4: 105-109.

Kathal R, Bhatnagar SP, Bhojwani SS (1988). Regeneration of plants from leaf explants of *Cucumis melo* cv. *Pusa sharbati*. Plant Cell Rep. 7: 449-451.

Kathiravan K, Vengedesan G, Singer S, Steinitz B, Paris H, Gaba V (2006). Adventitious regeneration *in vitro* occurs across a wide spectrum of squash (*Cucurbita pepo*) genotypes. Plant Cell Tissue Organ Cult. 85: 285-295.

Locy RD, Wehner TC (1982). Cucumber shoot tip growth on 9 nitrogen sources in *in vitro* culture. Cucurbits Genet. Coop. Rep. 5: 10-11.

Ma C, Tang Y, Li X, Li J, Wang L, Li HX (2012). *In vitro* induction of multiple buds from cotyledonary nodes of Balsam pear (*Momordica charantia* L.). Afr. J. Biotechol. 11: 3106-3115.

Malepszy S, Nadolska-Orezyk A (1983). *In vitro* culture of *Cucumis sativus*. I. Regeneration of plantlets from callus formed by leaf explants. Z. Pflanzenphysiol. 111: 273-276.

Malik S, Zia M, Rehman R, Chaudhary F (2007). *In vitro* plant regeneration from direct and indirect organogenesis of *Momordica charantia*. Pak. J. Biol. Sci. 10: 4118-4122.

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

Nabi SA, Rashid MM, Al-Amin M, Rasul MG (2002). Organogenesis in teasle gourd (*Momordica dioica* Roxb.). Plant Tissue Cult. 12: 173-180.

Pal SP, Alam I, Anisuzzaman M, Sarker KK, Sharmin SA, Alam MF (2007). Indirect organogenesis in summer squash (*Cucurbita pepo* L.). Turk. J. Agric. Forest. 31: 63-70.

Paul A, Mitter K, Raychaudhuri SS (2009). Effect of polyamines on in vitro somatic embryogenesis in *Momordica charantia* L. Plant Cell Tissue Organ Cult. 97: 303-311.

Punja ZK, Abbas N, Sarmento GG, Tang FA (1990). Regeneration of *Cucumis sativus* vars, sativus and hardwickii, *C. melo* and *C. metuliferus* from explants through somatic embryogenesis and organogenesis. Influence of explant source, growth regulator regime and genotype. Plant Cell Tissue Organ Cult. 21: 93-102.

Sarowar S, Oh HY, Hyung NI, Min BW, Harn CH, Yang SK, Ok SH, Shin JS (2003). *In vitro* micropropagation of a *Cucurbita* interspecific hybrid cultivar-a root stock plant. Plant Cell Tissue Organ Cult. 75: 179-182.

Savitha R, Shasthree T, Sudhakar, Mallaiah B (2010). High frequency of plantlet regeneration and multiple shoot induction from leaf and stem explant of *Citrullus colosynthis* (L.) Schrad, an endangered medicinal cucurbit. Int. J. Pharma Biol. Sci. 6(2): 1-8.

Selvaraj N, Vasudevan A, Manickavasagam M, Ganapathi A (2006). *In vitro* organogenesis and plant formation in cucumber. Biol. Plant. 50: 123-126.

Selvaraj N, Vasudevan A, Manickavasagam M, Kasthurirengan S, Ganapathi A (2007). High frequency shoot regeneration from cotyledon explants of cucumber via organogenesis. Sci. Hortic. 112: 2-8.

Selvaraj N, Vengadesan G, Vasudevan A, Prem Anand R, Ramesh Anbazhagan V, Ganapathi A (2002). Micropropagation of *Cucumis sativus* L. from field grown plants. In: Maynard DN (Ed.), Proceedings of the Cucurbitaceae 2002, ISHS Press, Belgium, pp 149-156.

Seo SH, Bai DG, Park HY (2000). High frequency shoot regeneration from leaf explants of cucumber. J. Plant Biotechnol. 2: 51-54.

Sikdar B, Shafiullah M, Chowdhury AR, Sharmin N, Nahar S, Joarder OI (2005). *Agrobacterium*-mediated GUS expression in bitter gourd (*Momordica charantia* L.). Biotechology, 4: 149-152.

Singh A, Singh SP, Bamezai R (1998). *Momordica charantia* (Bitter gourd) peel, pulp, seed and whole fruit extract inhibits mouse skin papillomagenesis. Toxicol. Lett. 94: 37-46.

Srivastava DR, Rianov VM, Piruzian ES (1989). Tissue culture and plant regeneration of watermelon (*Citrullus vulgaris*) Schrad. cv. Melitopolski. Plant Cell Rep. 8: 300-302.

Sultana RS, Bari Miah MA (2003). *In vitro* propagation of karalla (*Momordica charantia* L). J. Biol. Sci. 3: 1134-1139.

Thiruvengadam M, Rekha KT, Jayabalan N (2006). An efficient *in vitro* propagation of *Momordica dioica* Roxb. ex. Willd. Philipp Agric. Scientist. 89: 165-171.

Thomas TD, Sreejesh KR (2004). Callus induction and plant regeneration from cotyledonary explants of ash gourd (*Benincasa hispida* L.). Sci. Hortic. 100: 359-367.

Trulson AJ, Shahin EA (1986). *In vitro* plant generation in the genus *Cucumis*. Plant Sci. 47: 35-43.

Vasudevan A, Selvaraj N, Ganapathi A, Kasthurirengan S, Ramesh Anbazhagan V, Manickavasagam M (2004). Glutamine: a suitable nitrogen source for enhanced shoot multiplication in cucumber (*Cucumis sativus* L.). Biol. Plant. 48: 125-128.

Wang S, Tang L, Chen F (2001). *In vitro* flowering of bitter melon. Plant Cell Rep. 20: 393-397.