Plant Tissue Cult. & Biotech. **21**(1): 75-82, 2011 (June)

PTC&B

Rapid Micropropagation via Axillary Bud Proliferation of *Coccinia grandis* (L.) Voigt. from Nodal Segments

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Key words: *Coccinia grandis,* Axillary bud, Micropropagation, Nodal segments

Abstract

An efficient and rapid micropropagation protocol for *Coccinia grandis* (L.) Voigt. was successfully developed on MS fortified with Kn, BA and TDZ in various concentrations and in combination with IBA and IAA. Higher percentage (80) of response was obtained from nodal segments with an average number of 8.3 ± 0.9 shoots on MS + 0.5 mg/l Kn + 1.0 mg/l BA + 0.3 mg/l IBA. The isolated microshoots rooted (100%) on MS fortified with 0.1 mg/l IBA. The plantlets were hardened successfully with 80% survival rate. This efficient protocol will help the rapid multiplication of *C. grandis* for commercial propagation of this valuable medicinal plant.

Introduction

Coccinia grandis (L.) Voigt. (Cucurbitaceae), a perennial, tendril climber commonly known as little gourd, grows widely throughout India and other tropical countries. The plant has been mentioned in Ayurveda and Unani systems of medicine for treatment of ringworm, psoriasis, smallpox and scabies (Perry 1980). The leaves, stem, root and whole plant is used in the treatment of jaundice, indigestion, asthma, bronchitis, skin eruption, and mainly in diabetes (Wasantwisat and Vriyapanich 2003). The fruits are rich source of carbohydrates, proteins, vitamin A and C. Medicinally this vegetable is gaining importance among diabetic patients (Dharmatti et al. 2008). As it was significantly effective in diabetes treatment, *Coccinia grandis* (syn. *C. indica*) was described as "Indian substitute for Insulin" (Chopra et al. 1958). The various phytoconstituents reported in *Coccinia grandis* are cephalandrol, tritriacontane, lupeal, β-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one, taraxerone and taraxerol (Ray and Kundu 1987; Rastogi and Mehrotra 1998).

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Coccinia grandis has been well documented for its uses as anthelmintic, antidyslepidemic, antimicrobial, antitussive, anti-inflammatory, analgesic and antipyretic, antiulcer and antioxidant activities (Dewanjee et al. 2007, Singh et al. 2007, Shaheen et al. 2009, Pattanayak and Sunita 2009, Niazi et al. 2009, Manoharan et al. 2010, Umamaheswari and Chatterjee 2008 and Chandira et al. 2010).

Normally in Cucurbits, the seed setting and seed germination is low, probably due to the presence of a thin nucellar membrane lending impermeability to water and gases and make them dormant for many days (Devendra et al. 2008). Problems associated with its cultivation include the shortage of seedling material from cuttings of mature stems and unrestricted exploitation by the pharmaceutical industries may lead to depletion of this plant resource. In this case, an attempt for the replenishment of this valuable genotype is deemed imperative.

As an alternative to vegetative propagation through seeds and stem cuttings, the main goal has therefore been developing an efficient and true to type micropropagation system using nodal segments. Limited number of shoot regeneration of *Coccinia grandis* from hypocotyls explants (Anugulati 1988), shoot tip and nodal segments (Sarker et al. 2008), leaf and nodal segment (Josekutty et al. 1993) have been reported earlier.

The objective of this present study is to establish rapid and reproducible *in vitro* regeneration system with less cost and high frequency survival success of regenerated plants through nodal explants of *C. grandis*.

Materials and Methods

Nodal segments from tender shoots of *C. grandis* were collected from Bharathidasan University campus, Tiruchirappalli, India. The defoliated explants were washed under continuous flashing of tap water for 30 min and then treated with few drops of Teepol (detergent solution) for 5 min. Later, the explants were disinfected with freshly prepared 70% alcohol for 30 sec; 3% sodium hypochlorite for 3 min and with 0.1% mercuric chloride for 3 min in Laminar Flow and the explants were washed thrice with sterile distilled water after every treatment.

Sterilized explants of 0.5 - 1.0 cm length were implanted in basal medium containing MS nutrients supplemented with Kn, BA, TDZ individually and in combination for shoot bud proliferation and multiplication. To enhance the number of shoots and quality, the proliferated shoots were transferred to the shoot multiplication medium containing different concentrations of IAA, IBA (0.1 - 2.0 mg/l and BA (1.0 mg/l) + Kn (0.5 mg/l). The medium contained 3% sucrose (w/v) and 0.8% (w/v) agar, the pH was adjusted to 5.7 ± 0.2 after adding

growth hormones and prior to gelling with agar. About 15 ml of the medium was dispensed in each culture tube and capped with plugs of non-absorbent cotton and the media were steam sterilized at 121ºC for 30 min. The cultures were incubated in sterilized culture room at 25 ± 2ºC, under 16/8 hrs light regime provided by cool white fluorescent lamp (60 μmol m^2/s) with 55 - 60% relative humidity.

Microshoots (2 - 8 cm length) were transferred to MS containing different concentrations of IAA or IBA (0.1 - 2.0 mg/l) individually for root initiation. The well-rooted plantlets were washed off adhering agar in sterile distilled water and were transferred to paper cups (2.5 cm diameter) containing autoclaved red soil, sand and coconut coir (1 : 1 : 1), under controlled growth chamber conditions (25 ± 2ºC, 16 hrs photoperiod, 75 - 80% relative humidity and 35 μmol m^2/s light intensity). The potted plants were fed with MS basal salt solutions at every 4 days intervals for two weeks and the plantlets were covered with porous polythene to maintain high humidity. After 15 days, the plants were transferred to green house.

The experiment was conducted as a randomized complete design. Observations based on percentage of culture response with regard to axillary bud induction, number of shoots per explant and shoot lengths were recorded after 8 weeks. Only data which showed some advantageous effect were included in the tables and presented in mean ± SD of 20 explants per treatment and repeated 3 times. The generated data were analyzed using DMRT at $p \geq 0.05\%$ level of significance.

Results and Discussion

Nodal explants from field grown mature plants of *C. grandis* were cultured on MS supplemented with Kn, BA and TDZ at different concentrations (0.1 - 2.0 mg/l). The axillary bud proliferation was observed that all individual concentrations of Kn and BA but showed varied response with respect to their shoot length and percentage of response. BA was the most effective cytokinin for bud proliferation and multiple shoot induction in many plants of the Cucurbitaceae *viz., Momordica charantia* (Islam et al. 1994), *Trichosanthes dioica* (Sanjeevakumar et al. 2003), *Trichosanthes cucumerina* (Devendra et al. 2008). But in the present study, Kn was more effective than BA. MS fortified with 0.5 mg/l Kn induced 100% shoot bud proliferation and shoots attained highest length of 8.2 ± 0.5 cm (Table 1, Fig. 1B).

The other cytokinin, thidiazuron used in this study produced profuse proliferated friable callus in the nodal segments, hence no further shoot regeneration was observed on MS supplemented with TDZ. Basal callusing of growing shoots was observed at higher concentrations (1.0 - 2.0 mg/l) of either BA or Kn in the culture medium.

Kathal et al. (1988) reported that, a mixture of more than one cytokinin was more effective in shoot multiplication. Hence, an attempt was made to know the synergistic effect of cytokinins on shoot multiplication of *C. grandis.* Among the combinations of BA and Kn tested, the synergistic effect of Kn (0.5 mg/l) and BA (1.0 mg/l) was found as much efficient for desirable morphogenic responses with the maximum number of shoots (6.2 ± 3.2) and shoot mean length (3.8 ± 0.3 cm) (Table 1, Fig. 1C), whereas Sarker et al. (2008) obtained maximum number of 2 - 3 shoots while testing synergism of BAP (1.5 mg/l) and Kn (1.0 mg/l). The combined effect of BA and Kn for promotion of shoot sprouting frequency and multiple shoot induction was also reported in *Gymnema sylvestre* (Komalavalli and Rao 2000), *Artemisia vulgaris* (Sujatha and Ranjithakumari 2007).

Table 1. Response of nodal segments of *C. grandis* on MS with different concentrations of Kn and BA on shoot bud proliferation and multiplication, after four weeks of culture. Values represent the mean (± SD) of three replicates with 20 explants.

Plant growth regulators (mg/l)	Days for shoot bud induction	No. of shoots/explant	Shoot length (cm)	Percentage of response	Morphogenic response
Kn					
0.1	22	1.0 ± 0.0^{i}	3.5 ± 0.4^{fg}	40	-
0.2	19	2.0 ± 0.0^{g}	3.3 ± 0.3^{gh}	40	-
0.3	13	1.0 ± 0.0^{i}	6.0 ± 1.5^{b}	60	-
0.5	12	3.0 ± 0.0^{d}	8.2 ± 0.5^{a}	100	-
1.0	12	1.6 ± 0.5^{h}	5.3 ± 0.8^{c}	80	C
2.0	14	1.0 ± 0.0^{i}	3.2 ± 0.7^{hi}	40	C
BA					
0.1	29	1.0 ± 0.0^{i}	-	20	C
0.2	29	1.0 ± 0.0^{i}	-	20	C
0.3	16	1.0 ± 0.0^{i}	1.5 ± 0.0^{mn}	20	C
0.5	12	2.0 ± 0.0^{g}	1.7 ± 0.3^{lm}	40	C
1.0	12	3.0 ± 0.0^{d}	4.0 ± 0.4^{d}	80	C
2.0	15	2.5 ± 0.5^{ef}	2.3 ± 0.4^{k}	60	C
Kn (0.5) + BA					
0.1	18	2.0 ± 0.8^{g}	1.9 ± 0.5^{kl}	40	C
0.2	16	1.0 ± 0.0^{i}	2.3 ± 0.7^{k}	30	C
0.3	16	2.7 ± 0.5^{de}	2.8 ± 0.2^{ij}	40	C
0.5	15	3.3 ± 0.5^{c}	2.8 ± 0.3^{ij}	60	C
1.0	14	6.2 ± 3.2^{a}	3.8 ± 0.3^{de}	80	C
2.0	14	4.1 ± 1.1^{b}	3.7 ± 0.2^{ef}	60	C

C = Presence of basal callus.

Mean values with the same letters within columns are not significantly different at $p \geq 0.05\%$ level, according to DMRT.

Sharma and Singh (1997) reported that cytokinins are required in optimal quantity for shoot proliferation in many genotypes; however, the results of the present study show that inclusion of a low concentration of an auxin along with cytokinin increases the rate of shoot multiplication. Addition of either IAA or IBA in the culture medium along with BA and Kn favored the shoot multiplication. The percentage of multiple shoots (80%) and two to threefold enhancements in shoot length (8.6 cm/shoot) was observed when IBA (0.3 mg/l) was combined with the 0.5 mg/l Kn and 1.0 mg/l BA. (Table 2, Fig. 1D).

Table 2. Response of nodal segments of *C. grandis* on MS with Kn 0.5 mg/l +BA 1.0 mg/l in combination with different concentrations of IAA and IBA on shoot multiplication and elongation after 8 weeks of culture. Values represent the mean (±SD) of three replicates with 20 explants.

Plant growth regulators (mg/l)	No. of shoots/explant	Shoot length (cm)	Percentage of response
IAA			
0.1	2.0 ± 0.0^{ki}	1.9 ± 0.1^{l}	40
0.2	2.5 ± 0.7^{jk}	2.8 ± 0.2^{jk}	40
0.3	3.3 ± 1.5^{hi}	3.3 ± 0.4^{i}	60
0.5	5.2 ± 0.5^{de}	4.3 ± 0.06^{fg}	80
1.0	4.6 ± 0.5^{f}	4.2 ± 0.2^{gh}	60
2.0	2.9 ± 0.5^{ij}	2.9 ± 0.4^{ij}	60
IBA			
0.1	3.5 ± 0.7^{gh}	4.4 ± 0.02^{ef}	40
0.2	5.3 ± 1.1^{cd}	5.7 ± 0.4^{d}	60
0.3	8.3 ± 0.9^{a}	8.6 ± 0.4^{a}	80
0.5	7.5 ± 1.0^{b}	8.1 ± 1.0^{ab}	80
1.0	5.6 ± 0.5^{c}	6.8 ± 1.0^{c}	60
2.0	3.6 ± 0.5^{g}	4.8 ± 0.2^{e}	60

Mean values with the same letters within a column are not significantly different at $p \geq 0.05\%$ level, according to DMRT.

In vitro rooting of regenerated shoots was observed on MS supplemented with various concentrations of IAA and IBA. In most of the Cucurbits, the root induction was achieved on either basal medium alone or with a very low level of an auxin (Mythili and Thomas 1999). In the present study, the highest frequency of root formation (100%), number of roots (7 ± 1.5) and root length (3.9 ± 0.2 cm) were achieved on MS supplemented with 0.1 mg/l IBA. This result is consistent with the findings in *Paederia foetida* (Amin et al. 2003), *Adhatoda vasica* (Abhayankar and Reddy 2007). Root development was however slow at higher concentration of IBA (0.5 - 2.0 mg/l) (Table 3, Fig. 1E). The rooted shoots were

Table 3. Effect of different concentrations of IAA and IBA on *in vitro* rooting of *C. grandis* on MS, after 2 weeks of culture. Values represent the mean (±SD) of three replicates with 20 explants.

Plant growth regulators (mg/l)	Percentage of response	No. of roots/micro shoot	Root length (cm)
IAA			
0.1	40	2.0 ± 0.0^{de}	3.3 ± 0.2^{cd}
0.2	60	2.0 ± 0.0^{de}	4.2 ± 0.0^{a}
0.3	60	1.0 ± 0.4^{fg}	2.6 ± 0.3^{e}
0.5	40	1.0 ± 0.7^{fg}	2.2 ± 0.2^{f}
1.0	-	-	-
2.0	-	-	-
IBA			
0.1	100	7.0 ± 1.5^{a}	3.9 ± 0.2^{ab}
0.2	80	4.7 ± 0.9^{b}	3.5 ± 0.3^{c}
0.3	60	3.3 ± 0.5^{c}	2.1 ± 0.6^{fg}
0.5	60	2.5 ± 0.7^{d}	2.1 ± 0.5^{fg}
1.0	40	1.5 ± 0.7^{ef}	1.4 ± 0.4^{h}
2.0	40	1.5 ± 0.7^{ef}	1.3 ± 0.3^{i}

Mean values with the same letters within columns are not significantly different at $p \geq 0.05\%$ level, according to DMRT.

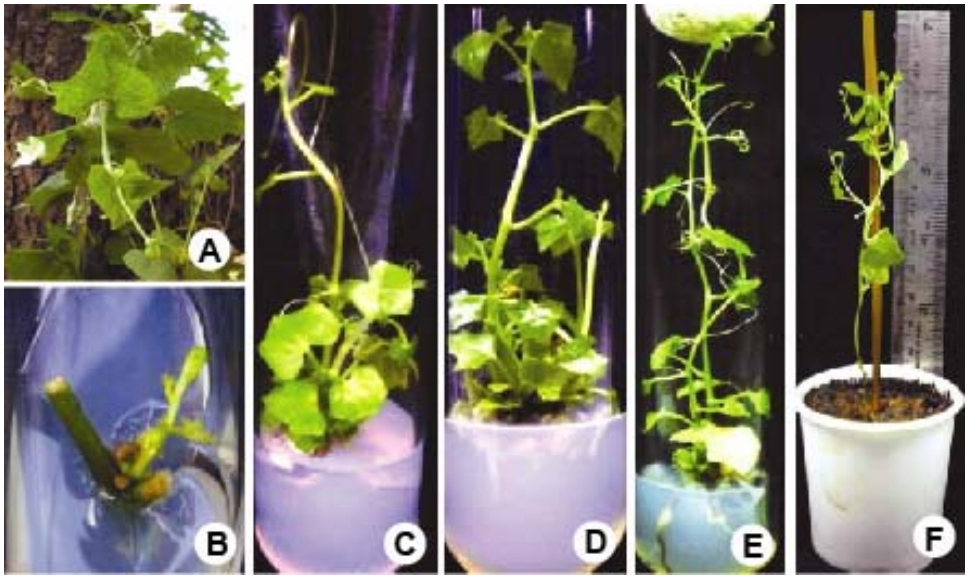

Fig. 1. Micropropagation of *C. grandis.* a. *C. grandis* – habit; b. Shoot bud proliferation on MS + Kn 0.5 mg/l; c. Shoot multiplication on MS + Kn (0.5) + BA (1.0) mg/l; d. Shoot multiplication and elongation on MS + Kn (0.5) + BA (1.0) + IBA (0.3) mg/l; e. Rooting of microshoots on MS + IBA (0.1) mg/l; f. A hardened plant.

transferred to paper cups containing autoclaved red soil, sand and coconut coir in the ratio of 1 : 1 : 1 (Fig. 1F). The acclimated plantlets of *C. granids* showed 80% survival and this result was more efficient than an earlier report on C. *grandis*, whereas Sarker et al. (2008) developed a protocol with 70% survival rate of regenerated plants.

In conclusion, the present study describes an efficient and reproducible protocol for *in vitro* regeneration of an anti diabetic drug plant. This report will help in mass propagation of the species with the maximum survival rate that can be used for commercial cultivation which is important for extraction for bioactive compounds.

Reference

Abhyankar G and **Reddy VD** (2007) Rapid micropropagation via axillary bud proliferation *Adhatoda vasica* Nees from nodal segments. Ind. J. Exp. Bio. **45**: 268-271.

Amin NM, Rahman MM and **Manik SM** (2003) *In vitro* Clonal propagation of *Paederia foetida* L. - A medicinal plant of Bangaladesh. Plant Tiss. Cult. **13**: 117-123.

Anugulati (1988) Tissue culture of *Coccinia grandis*. Curr. Sci. **57**: 1232-1235.

Chandira M, et al., (2010) Studies on anti-stress and free radical scavenging activity of whole plant of *Coccinia indica* Linn. Int. R. J. Pharm. Sci. **1**: 50-55.

Chopra RN, Chopra IC, Handa KL and **Kapur LD** (1958) Indigenous drugs of India, Seconnd ed. UN Dhur and Sons, Calcutta, pp. 314-316.

Devendra NK, Rajanna L, Sheetal C and **Seetharam YN** (2008) *In vitro* clonal propagation of *Trichosanthes cucumerina* L. var.cucumerina. Plant Tiss. Cult. & Biotechnol. **18**: 103-111.

Dewanjee S, Maiti A, Kundu M and **Mandal SC** (2007) Evaluation of anthelmintic activity of crude ectracts of *Diospyros peregrine*, *Coccinia grandis* and *Schima wallichi*. Dhaha Univ. J. Pharm Sci. **6**: 121-123.

Dharmatti PR, Patil RV, Patil SS, and **Athani SI** (2008) A new Coccinia (*Coccinia indica*) Variety DRC-1, a boon to vegetable growers. Karnataka J. Agric. Sci. **21**: 99-103.

Islam R, Sarkar PK and **Naderuzzaman M** (1998) *In vitro* regeneration of plants from cotyledons of *Momordica charantea* L. Plant Tiss. Cult. **4**: 105-109.

Josekutty PC, Swati Shah and **Prathapsenan G** (1993) Direct and indirect organogenesis in *Coccinia indica.* J. Hort. Sci. & Biotechnol. **68**: 31-35.

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

Komalavalli N and **Rao MV** (2000) *In vitro* micropropagation of *Gymnema sylvestre*- a multipurpose medicinal plant. Plant Cell Tiss. Org. Cult. **61**: 97-105.

Manoharan P, a Jhon S, Golla U and **Thangathirupathi A** (2010) Anti-ulcer effect of *Coccinia grandis* (Linn.) on pylorus ligated (Albino) Rats. I JPRD **2**: 1-9.

Mythili JB and **Thomas** (1999) Micropropagation of *T. Dioica* Roxb, Sci. Hort. **79**: 87-90.

Niazi J, Parabhdeep Singh, Yogita Bunsal and **Goel RK** (2009) Anti-inflammatory, analgesic and antipyretic activity of aqueous extract of fresh leaves of *Coccinia indica*. Inflammo. Pharmacol. **17**: 239-244.

Pattanayak SP and **Sunita P** (2009) *In vitro* antitussive activity of *Coccinia grandis* against irritant aerosol and sulfur dioxide - induced cough model in rodants. Bangladesh J. Pharmacol. **4**: 84-87.

Perry LM (1980) Medicinal plants of east and South East Asia, attributed properties and uses, MIT Press, London.

Rastogi RP and **Mehrotra BN** (1998) Compendiumof Indian medicinal plants, vol I. CDRI, Lucknow, India, p. 115.

Ray AB and **Kundu S** (1987) Chemical examination of *Coccinia indica* fruits. J. Ind. Chem. Soc. **54**: 776-777.

Sanjeevkumar, Majorsingh, Singh AK, Srivastava K and **Banerjee MK** (2003) *In vitro* propagation of pointed gourd (*Trichosanthes dioica* Roxb.) Cucurbit Genetics Cooperative Rep. **26**: 74-75.

Sarker P, FMS, Jahan R and **Rahmatullah M** (2008) *In vitro* regeneration of *Coccinia grandis* (L.) Voigt. An indigenous medicinal plant of Bangaladesh. Afr. J. Trad. Comp. Alt. Med. (Absrtact).

Shaheen SZ, Krishna Bolla, Kandukuri Vasu and **Singara Charya MA** (2009) Antimicrobial activity of the fruit extracts of *Coccinia indica*. Afri. J. Biotech. **8**(24): 7073-7076.

Sharma TR and **Singh BM** (1997) High frequency *in vitro* multiplication of disease-free *Zingiber officinale* Rosc. Plant Sci. **20**: 15-18.

Singh G, Gupta P, Rawat P, Puri A, Bhatia G and **Mourya R** (2007) Antidyslipidemic activity of polyprenol from *Coccinia grandis* in high-fat diet-fed hamster model. Phytomedicine **14**: 792-798.

Sujatha G and **Ranjithakumari BD** (2007) Effect of phytohormones on micropropagation of *Artemisia vulgaris* L. Acta Physiol. Plant **29**: 189-195.

Umamaheswari M and **Chatterjee TK** (2008) *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. Afr. J. Trad. CAM **5**(1): 61-73.