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Indirect organogenesis from nodal explants of *Coccinia grandis* (L.) Voigt

U Thiripurasundari and M V Rao*

Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, India

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The present study reports an efficient protocol for indirect shoot organogenesis and plantlets regeneration of *Coccinia grandis* (L.) Voigt from nodal explants. The maximum frequency of organogenic callus induction was observed in MS medium supplemented with 0.1 mg/L 1-naphthaleneacetic acid (NAA) and 1.0 mg/L N^6-benzyladenine (BA). Multiple shoot induction was achieved from the surface of callus on regeneration medium [MS nutrients supplemented with NAA, BA and various concentrations of kinetin (KIN) 0.1-2.0 mg/L]. The highest shoot multiplication (90%) with the increased numbers of shoots (8.7±1.8) and the shoot mean length (8.8±0.4 cm) was achieved on MS medium with 0.1 mg/L NAA, 1.0 mg/L BA and 0.5 mg/L KIN. The regenerated shoots were rooted *in vitro* on MS medium containing 0.1 mg/L IBA. Plantlets with well developed shoot and root systems were successfully acclimatized (83.5%) and exhibited normal morphology and growth characteristics.

Keywords: *Coccinia grandis*, indirect organogenesis, nodal explants

Coccinia grandis (L.) Voigt (syn. *C. indica* Wight et Arnold; Family: Cucurbitaceae) is a perennial creeper, described as 'Indian substitute for Insulin'[1]. It grows widely throughout India and other tropical countries. *C. grandis* has been extensively used in Ayurvedic and Unani practices in the Indian Subcontinent[2]. The plant is known to have antidiabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, antimicrobial, anthelmintic, cathartic and expectorant activities[3]. It contains several phytoconstituents, such as, cephalandrol, tritriacontane, lupeal, β-sitosterol, cephalandrine A, cephalandrine B, taraxerone and taraxerol[4,5], triterpenoids, alkaloids and tannins[6]. The plant also possesses hypoglycemic effects and acts as insulin mimetic[7].

In cucurbits, normally 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[8]. Problems associated with conventional propagation and unrestricted exploitation by pharmaceutical industries may lead to depletion of this plant resource. Few reports on *in vitro* propagation of *Coccinia* include direct shoot regeneration from hypocotyls explants[9], shoot tip and nodal segments[10] and direct and indirect regeneration from node and leaf explants[11]. However, these studies reported low regeneration frequency not exceeding 70%. The aim of present study was to elaborate an efficient protocol for high frequency regeneration of *C. grandis via* indirect organogenesis from nodal explants.

Nodal segments from tender shoots of field grown mature *C. grandis* were collected from the campus of Bharathidasan University, Tamil Nadu, India. The explants were washed under running tap water for 30 min, followed by few drops of Teepol (detergent solution) for 5 min. Later, the explants were surface sterilized with freshly prepare 70% alcohol for 30 sec, 3% NaOCl for 3 min and finally with 0.1% $HgCl_2$ for 3 min, and they were rinsed thrice with sterile distilled water after every treatment. Disinfected explants of 0.5-1.0 cm length were cultured on a sterile callus induction medium consisting of salts and vitamins of Murashige and Skoog[12] (MS) supplemented with different concentrations and combinations of IAA, IBA, NAA (0.1-2.0 mg/L) and BA (1.0 mg/L). After 4 wk, the well proliferated callus was transferred to regeneration medium supplemented with MS+BA (1.0 mg/L)+KIN (0.5 mg/L)+NAA (0.1-2.0 mg/L). The regenerated microshoots were then rooted in IBA (0.1 mg/L).

Commercial grade sucrose at 3% concentration was used as the carbon source. The medium was gelled with 0.8% agar and the *p*H was adjusted to 5.7±0.1 with 1 *N* NaOH or 0.1 *N* HCl before autoclaving at 121ºC for 15 min. Cultures were maintained at 25±2ºC under 16/8 h light regime provided by cool white fluorescent lamp (60 µmol m^{-2} s^{-1}) with 55-60% relative humidity. Experiments were carried out with 20 replicates and each experiment was repeated three times. Rooted plants were washed thoroughly in tap water and transplanted into paper cups containing autoclaved red soil, sand and coconut coir (1:1:1) as potting media. The plants were fed with MS basal salt

*Author for correspondence:
Tel: +91-431-2407061; Fax: +91-431-2407045
E-mail: mvrao_456@yahoo.co.in

solutions for 2 wk at 4 d intervals. The potted plants were grown under growth chamber conditions (25±2° C, 16/8 h photoperiod and 35 µmol m^{-2} s^{-1}) and the plants were covered with porous polythene sheets to maintain high (75-80%) humidity. After 2 wk of hardening, the plantlets were transferred to greenhouse conditions. Data were recorded after 8 wk of culture based on percentage of culture response with regard to callus formation, number of regenerated shoots per callus unit and shoot mean length. The observed data were analyzed using Duncan's multiple range test (DMRT) at P≥ 0.05% level of significance.

Callus was initiated from the surface of the nodal explants in MS medium containing either auxin or its combination with cytokinin. After 4 wk of culture, callus rating was accessed. Light green friable, pale green watery and pale green friable callus was produced by the individual effect of NAA, IAA and IBA, respectively, and all of them were non-organogenic. Adelberg *et al*[13] reported BAP as sole plant growth regulator successfully preferred for good texture callus development. However, in the present study, cytokinin BA (1.0 mg/L) combined with different concentrations of NAA (0.1-2.0 mg/L) produced much proliferated green nodular compact callus on nodal explants. The highest frequency of callus induction was observed in MS medium supplemented with NAA 0.1 mg/L and BA 1.0 mg/L with a callusing response of 90% (Table 1, Fig. 1A). In an earlier report[11], calli from nodal explants of *C. indica* was obtained in MS+BAP (2.0 mg/L)+KIN (0.5 mg/L)+NAA (1.0 mg/L) with the frequency of 77% in 25 d. Whereas, in our study, higher frequency (90%) of organogenic callus induction was achieved within 8-9 d of culture period compared to NAA/BA combinations.

The shoot proliferation response is dependent upon concentration of cytokinin supplemented in the medium. Cytokinin works as a signaling molecule that activates totipotent cells of callus for shoot organogenesis[13]. Four-wk-old nodular green compact callus obtained from nodal explants was transferred to regeneration medium containing MS+NAA (0.1 mg/L)+BA (1.0 mg/L) with various concentrations of KIN (0.1-2.0 mg/L). The highest frequency of shoot regeneration (90%), the maximum number of shoots (8.7±1.8; Fig. 1B) and shoot mean length (8.8±0.4 cm; Fig. 1C) was observed on MS+NAA (0.1 mg/L)+BA (1.0 mg/L)+KIN (0.5 mg/L) after 2 subcultures in the same medium composition (Table 2). *Memordica dioica*[14] and *M. charantia*[15] along with other Cucurbits also produced shoots from the callus culture on the medium containing the combination of auxins and cytokinins.

The regenerated shoots produced roots when transferred to MS medium supplemented with IBA. The maximum numbers of roots (8.2±0.8) with the root mean length of 4.7±0.3 cm was observed on MS+IBA (0.1 mg/L; Fig. 1D). In contrast, Josekutty *et al*[11], in an earlier study, reported root formation on hormones free ½ MS medium supplemented with only 3% sucrose. The rooted shoots were transferred to paper cups containing autoclaved red soil, sand and coconut coir (1:1:1; Fig. 1E). After 2 wk, the acclimatized plantlets of *C. grandis* were transferred

Table 1—Effect of NAA and BA on organogenic callus induction from nodal explants of *C. grandis* (L.) Voigt

NAA (mg/L)	BA (mg/L)	Callus induction (%)	Nature of callus
0.1	1.0	90	GNC*
0.2	1.0	80	GNC
0.3	1.0	80	GNC
0.5	1.0	70	GNC
0.7	1.0	60	GNC
1.0	1.0	50	GNC
2.0	1.0	50	GNC

*GNC-Green nodular compact
Values represent the mean of three replicates with 20 explants

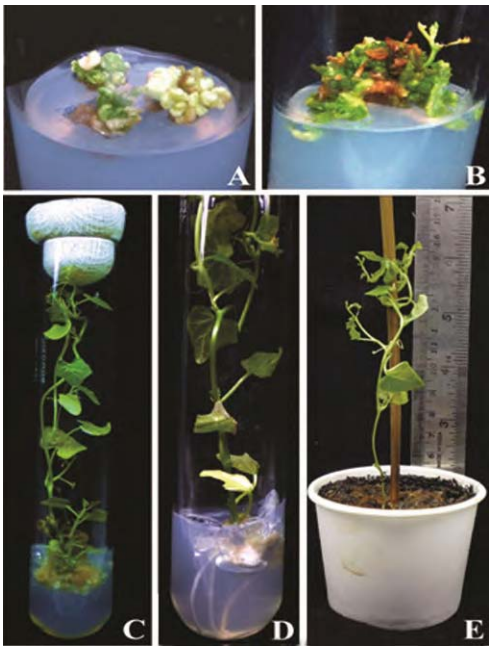

Fig. 1 (A-E)—Indirect organogenesis from nodal explants of *C. grandis* (L.) Voigt: A. Green nodular callus with shoot buds, B. Multiple shoot induction, C. Shoot elongation, D. *In vitro* rooting, & E. Hardened plantlet

Table 2—Effect of NAA (0/1 mg/L), BA (1.0 mg/L) and various concentrations and combinations of KIN on regeneration of shoots from organogenic callus of *C. grandis*(L.)Voigt

NAA	BA	KIN	% response	No. of shoots (explant^{-1})	Mean shoot length (cm)
0.1	1.0	0.1	50	4.8±0.7^{g*}	2.1±0.2^{ef}
0.1	1.0	0.2	60	5.6±0.5^{ef}	2.7±0.2^{e}
0.1	1.0	0.3	80	6.2±2.1^{cd}	4.2±1.0^{d}
0.1	1.0	0.5	90	8.7±1.8^{a}	8.8±0.4^{a}
0.1	1.0	1.0	90	8.3±2.2^{ab}	6.8±1.1^{ab}
0.1	1.0	2.0	80	6.8±2.3^{c}	6.3±1.0^{bc}

*Values represent the mean±SD of three replicates with 20 explants

Mean values with the same letters within columns are not differ significantly ($P \geq 0.05$)

to greenhouse, where they showed 83.5% survival rate. Thus, the present protocol showed more efficient results compared to the earlier report with only 10-15% survival success[11].

The protocol described here is simple and efficient with high regeneration frequency and can be employed for the large scale production and genetic manipulation of medicinal plant, *C. grandis*.

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