In vitro bud proliferation response in ivy gourd (*Coccinia grandis* (L.) Voigt.) cv. Sulabha nodal explants with cytokinin levels

A. Thasni, S. Sarada* and Swapna Alex

College of Agriculture, Kerala Agricultural University, Thiruvananthapuram 695 522, Kerala, India

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Abstract

MS medium supplemented with different concentrations of cytokinins, Kn (0.1-1.0 mg L^{-1}) and BA (0.1-1.0 mg L^{-1}), individually and in combination was used to study the bud proliferation and shoot induction responses in ivy gourd. The combination MS + 1.0 mg L^{-1} BA was the best with respect to the number of days for bud initiation (5.50), number of shoots per explant (1.75), shoot length (5.71) and percentage of response (100). The micro shoots obtained from the best medium (MS+BA1mg L^{-1}) have successfully produced shoots in MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1} and rooted in MS+BA1mg L^{-1}+IBA0.2mg L^{-1}.

Keywords: Bud proliferation, Cucurbit, Micropropagation, Nodal explants, Propagation, Tissue culture.

Introduction

Ivy gourd (*Coccinia grandis* (L.) Voigt), also known as little gourd/ scarlet gourd, is an underexploited vegetable belonging to the family Cucurbitaceae. It is a perennial, dioecious creeper, widely cultivated in India and other tropical countries. The name is derived from the Latin word *Coccineus*, meaning scarlet, which refers to the fruit colour. Vernacular names are kundru (Hindi), kovakka (Malayalam), kovakkai, kovai (Tamil) and thondakayi (Kannada). In India, ivygourd is grown extensively in West Bengal, Karnataka, Tamil Nadu, Maharashtra, Andhra Pradesh and Gujarat (Peter, 2007). In Kerala, total area under ivy gourd cultivation is 1643 ha, of which Kottayam district ranks first (326 ha) (Farm Guide, 2019).

Ivy gourd is propagated through vegetative methods by stem cuttings with three or four nodes and 30-40 cm length, selected from high yielding female vines, and planted at a spacing of 4m x 3m (KAU, 2016). Kerala Agricultural University (KAU) developed a high yielding ivygourd cv. Sulabha, which produces long fruits with a length of 9.25 cm and weighing about 18.48 g (Gopalakrishnan, 2007). The main problems associated with the cultivation of ivygourd is the shortage of planting material from cuttings of mature stems. Micropropagation has emerged as a promising technology for the rapid and large-scale propagation of plants. Establishment of a cost effective, rapid and reproducible *in vitro* regeneration system with high survival of regenerated plants, as an alternative to vegetative propagation through stem cuttings, is the need of the hour. Hence this study was undertaken to develop an efficient, large scale *in vitro* multiplication protocol for the regeneration of *C. grandis* from nodal explants.

Materials and Methods

The study was conducted at the Department of Vegetable Science, College of Agriculture, Kerala Agricultural University, Thiruvananthapuram, during 2018-'19. Nodal cuttings of ivy gourd cv. Sulabha, containing an axillary bud from tender shoots, were used as explants. Earlier studies by Haque et al. (2008) in pumpkin and ash gourd and

*Author for Correspondences: Phone: 9447074675, Email: sarada.s@kau.in

Ahmad and Anis (2005) in cucumber have reported the use of nodal segments as explants for *in vitro* propagation. Choudhary et al. (2017) observed that nodal explants obtained from the greenhouse maintained wild female spine gourd plants sprouted within 4–5 days of inoculation.

The explants were defoliated, washed under tap water and treated with detergent Tween 20 solution for 10-15 min. The explants were subsequently disinfected with 1% Carbendazim for 30 min. to prevent fungal contamination. After every treatment, the explants were washed several times with double distilled water. The explants were treated with 70% ethanol for 30 sec. followed by surface sterilization using mercuric chloride 0.1 % for 3 minutes. After every treatment, explants were washed 3-4 times with sterile distilled water. MS medium (Murashige and Skoog, 1962) was used as the basal media for the experiment. Sterilized nodal explants of 1-2 cm length were inoculated in basal MS medium supplemented with Kn and BA, individually and in combination, for bud proliferation and multiple shoot induction (Table 1). Each treatment was replicated thrice and the number of bottles per replication was four. Observations on days taken for shoot bud initiation, number of shoots/ explant, shoot length (cm) and percentage response were recorded. The shoots obtained from the best treatment were transferred to the shoot multiplication medium and then to the rooting medium. Different concentrations of IAA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) and IBA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) were tried for shoot multiplication and rooting. Rooted plants were transferred to protrays containing autoclaved red soil, sand and coir pith compost in 1:1:1 ratio for hardening under high humidity and subsequently after 15 days to small pots under greenhouse conditions for further establishment.

Table 1. Growth regulators and their combinations evaluated for *in vitro* bud proliferation and multiple shoot induction in *Coccinia grandis*

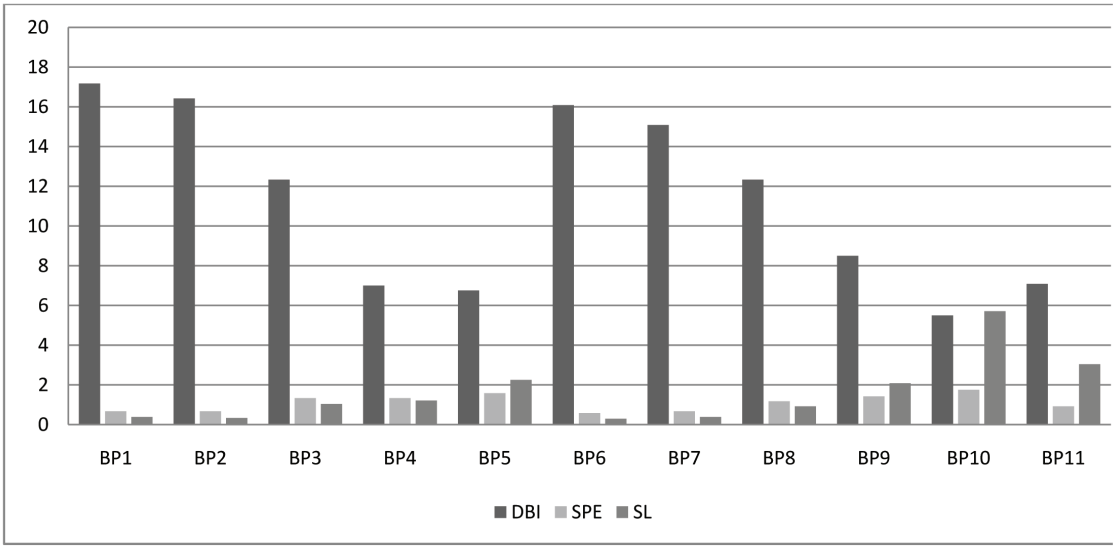
Treatments	Plant growth regulator con. (mg L^{-1})	
	BA	Kinetin
BP_1	-	0.1
BP_2	-	0.2
BP_3	-	0.3
BP_4	-	0.5
BP_5	-	1
BP_6	0.1	-
BP_7	0.2	-
BP_8	0.3	-
BP_9	0.4	-
BP_{10}	0.5	-
BP_{11}	Best of BA	Best of Kinetin

Results and Discussion

Significant difference was observed among the treatments with respect to the days to shoot bud initiation, number of shoots/ explant, shoot length

Table 2. Effect of Kinetin and BA individually and in combination on bud proliferation and multiple shoot induction in *Coccinia grandis*

Treatment No.	Plant growth regulators(mg L^{-1})		Days to bud initiation	Shoots per explant	Shoot length (cm)	Percentage of response (%)
	Kn	BA				
BP_1	0.1	-	17.17	0.67	0.38	33.33
BP_2	0.2	-	16.42	0.67	0.33	41.67
BP_3	0.3	-	12.33	1.33	1.04	66.67
BP_4	0.5	-	7.00	1.33	1.21	66.67
BP_5	1	-	6.75	1.58	2.25	83.33
BP_6	-	0.1	16.08	0.58	0.29	25
BP_7	-	0.2	15.08	0.67	0.38	33.33
BP_8	-	0.3	12.33	1.17	0.92	58.33
BP_9	-	0.5	8.50	1.42	2.08	83.33
BP_{10}	-	1	5.50	1.75	5.71	100
BP 11	1	1	7.08	0.92	3.04	91.67
SE(m)±			0.201	0.159	0.248	
CD (0.05)			0.593	0.469	0.731	



DBI – Days for shoot bud initiation,**SPE**– Shoots per explant,**SL** – Shoot length(cm)

Figure 1. Effect of Kinetin and BA individually and in combination on bud proliferation and multiple shoot induction in *Coccinia grandis*

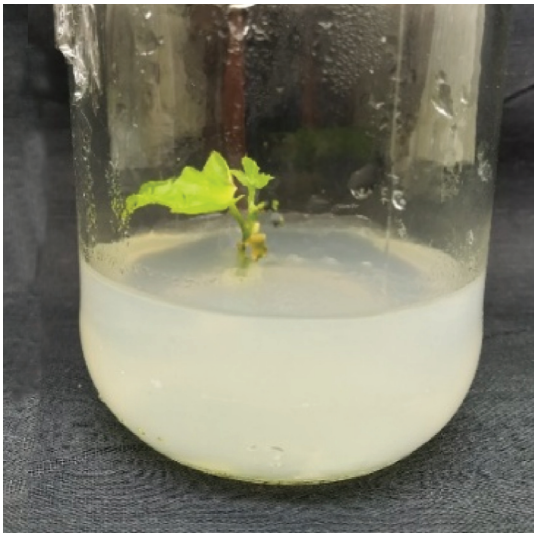


Figure 2.Bud proliferation and multiple shoot induction in *Coccinia grandis* on MS medium with 1.0 mg L^{-1} kinetin

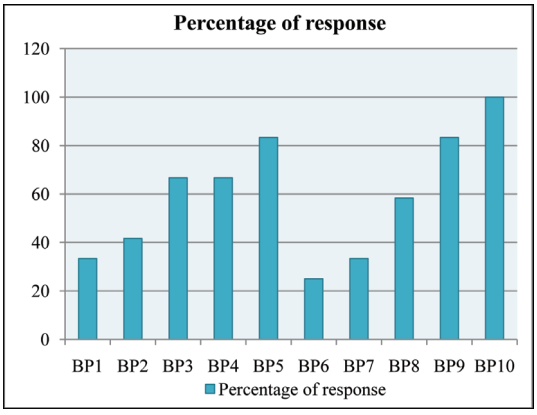


Figure 3. *In vitro* regeneration response (%) in *Coccinia grandis* to cytokinin levels and combinations

and percentage of response (Table 2) (Fig 1). The earliest shoot bud initiation (5.50 days) was observed in BP_{10} (MS + BA 1.0 mg L^{-1}). Bud initiation was late (17.17 days) in the treatment BP_1 (MS +Kn 0.1 mg L^{-1}). Shoot initiation after 5 days of inoculation was reported by Patel and Ishnava (2015) in *C. grandis* in MS+ Kn 0.2 mg L^{-1} medium. With respect to the number of shoots per explant, the maximum number of shoots (1.75) was obtained in the treatment BP_{10} (MS + BA 1.0 mg L^{-1}) which was on par with the treatments BP_5 (MS + Kn 1.0 mg L^{-1})(1.58) (Fig 2) and BP_9 (MS + BA 0.5 mg L^{-1}) (1.42). Lowest number of shoots (0.58) was observed in the treatment BP_6 (MS + BA 0.1 mg L^{-1}). It was on par with BP_7 (MS + BA 0.2 mg L^{-1}), BP_1 (MS + Kn 0.1 mg L^{-1}) and BP_2 (MS + Kn 0.2 mg L^{-1}) (0.67). The highest shoot length of 5.71 cm was recorded by the treatment, BP_{10} (MS + BA 1.0 mg L^{-1}), while the lowest (0.29 cm) in the treatment

Figure 4. Bud proliferation and multiple shoot induction in *Coccinia grandis* on MS medium with 1.0 mg L^{-1} kinetin and 1.0 mg L^{-1} BA

BP_6 (MS + BA 0.1 mg L^{-1}), which was on par with BP_2 (MS+ Kn 0.2 mg L^{-1}) (0.33 cm), BP_1 (MS + Kn 0.1 mg L^{-1}) (0.38 cm) and BP_7 (MS + BA 0.2 mg L^{-1}) (0.38 cm). Percentage of response was the highest in BP_{10} (MS + BA 1.0 mg L^{-1}) (100 %), followed by BP_9 (MS + BA 0.5 mg L^{-1}) and BP_5 (MS + Kn 1.0 mg L^{-1}) (83.33 %). The lowest response was recorded in BP_6 (MS + BA 0.1 mg L^{-1}) (25.00 %) (Fig 3). Kathal et al. (1988) observed that the combination of two cytokinins, BAP and 2 ip in the regeneration of plants from leaf explants of *Cucumis melo* was effective for multiple shoot induction. The synergistic effect of cytokinins, BA and Kn for multiple shoot induction was also reported by Komalavalli and Rao (2000) and Sujatha and Kumari (2007) in medicinal plants *Gymnema sylvestre* and *Artemisia vulgaris* respectively. Hence, the synergistic effect of Kn and BA was tested, in the present study. In the combination treatment BP_{11} (MS + Kn 1.0 mg L^{-1} + BA 1.0 mg L^{-1}), shoot buds initiated within 7.08 days with 0.92 shoots per explant, shoot length of 3.04 cm and with a percentage response of 91.67 % (Fig 4).

Among all the treatments tried for bud proliferation and multiple shoot induction, the treatment BP_{10} (MS + BA 1.0 mg L^{-1}) was found to be significantly superior to all other treatments with respect to days for bud initiation (5.50 days), number of shoots per explant (1.75), shoot length (5.71 cm) and percentage of response (100 %) (Fig 5). The individual concentration of BA exhibited best results than the individual concentration of kinetin and the combination of BA and Kn. Axillary bud proliferation from nodal explants of *C. grandis* was attempted by Sundari et al. (2011) and reported that MS medium fortified with 1.0 mg L^{-1} BA was found to induce only 80% shoot bud proliferation,with shoots attaining the highest length of 4.0 cm. BA was the most effective cytokinin for bud proliferation and multiple shoot induction in many plants of the family Cucurbitaceae *viz., Trichosanthes dioica* (Kumar et al., 2003), *Cucurbita maxima* (Mahzabin et al., 2008) and *Lagenaria siceraria* (Saha and Kazumi, 2007). Mahzabin et al. (2008) observed that use of BA alone was more effective than combination treatment with NAA and Kinetin for direct organogenesis from shoot tips in *Cucurbita*

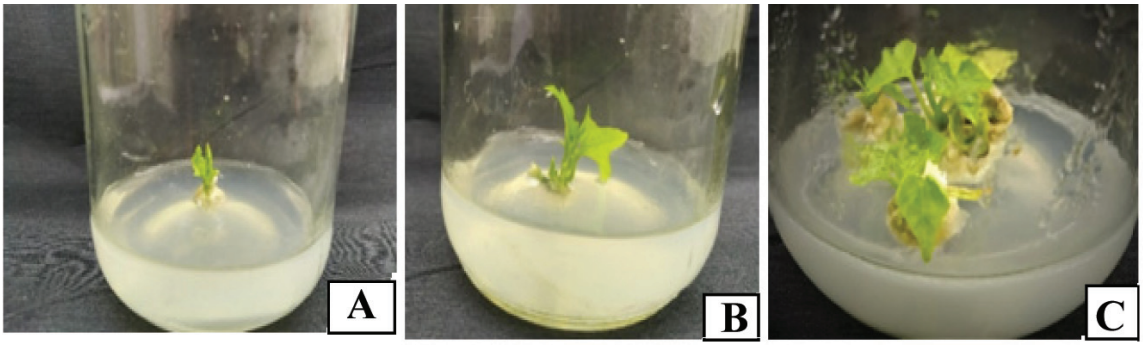


Figure 5.Bud proliferation and multiple shoot induction in *Coccinia grandis* on MS medium 1.0 mg L^{-1} BA; A) One week after inoculation B) Two weeks after inoculation C) Three weeks after inoculation

maxima.The differences observed in the response of different hormone combinations on plant regeneration can be due to either the presence of different hormone receptors with varying affinity to different auxins (Starling et al., 1986) or to specific enzyme systems that catabolize cytokinins with particular side groups (McGaw and Horgan, 1983). Cytokinins are adenine derivatives that are mainly concerned with cell division, modification of apical dominance and shoot differentiation in tissue culture. Lateral buds are released from dormancy and shoot formation is promoted by cytokinins. In the present study, multiplication rate, *i.e.* number of shoots per explant was low. For increasing the multiplication rate, addition of different additives could be suggested. Ahmad and Anis (2005) observed that MS medium containing 1.0 µM BA and 200 mg L^{-1}casein hydrolysate enhanced the number of multiple shoots in cucumber.The micro shoots obtained from the best treatment BP_{10}(MS+BA1mgL^{-1})successfully produced shoots in MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1} and rooted in MS + BA1mgL^{-1} + IBA0.2mg L^{-1}. New leaf emerged within 6- 7 days in the plantlets planted out inprotrays. The plants showed a survival rate of 72% after two weeks.

The present study describes an efficient and reproducible protocol for bud proliferation and multiple shoot induction of ivygourd (*Coccinia grandis)* cv. Sulabha. Among the treatments evaluated for bud proliferation and multiple shoot induction, the treatment BP_{10} (MS + BA 1.0 mg L^{-1}) was found to be significantly superior to all other treatments with respect to days for bud initiation (5.50 days), number of shoots per explant (1.75), shoot length (5.71 cm) and percentage of response (100 %). The newly optimised protocol can assure the mass multiplication of the superior lines and distribution to farmers to enable commercial cultivation.

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