

In vitro clonal propagation of *Momordica charantia* L.

Mala Agarwal^{1*} and Raka Kamal²

¹Department of Botany, Government College, Chimanpura (Shahpura), Jaipur 303 004, India

²Department of Botany, University of Rajasthan, Jaipur 302 004, India

Received 3 April 2003; accepted 19 August 2003

The present investigation outlines the *in vitro* propagation of *Momordica charantia* L. The explants from *in vitro* grown seedling were cultured on modified MS medium. Shoot differentiation was obtained on MS medium supplemented with BAP. Root, callus were formed on IBA and 2,4-D, respectively. Shoot as well as root differentiation was obtained on medium containing BAP+IBA/NAA. Multiple shoots with roots were formed on MS medium without hormones (MSO). Rooting on shoot grown occurred on medium containing IBA and 40% of the plants survived successfully, when transferred to the field.

Keywords: clonal propagation, *Momordica charantia*, *in vitro* regeneration, explant

IPC Code: Int. Cl.⁷ A 01 H 4/00, 5/00

Introduction

Momordica charantia L. (*Cucarbitaceae*), commonly known as bitter melon/gourd, is a slender, tendril climbing, annual vine. Bitter melon, a common vegetable, is used for the treatment of cancer, diabetes and many ailments¹. It is a potent hypoglycemic agent due to alkaloids, insulin like peptides, and a mixture of steroidal sapogenins known as charantin. Clinical studies with multiple controls have confirmed the benefit of bitter melon for diabetes². Momarcharin (α and β), found in bitter melon, are known to inhibit the AIDS virus³. *M. charantia* has not been much investigated for *in vitro* culture⁴.

In vitro culture is used to some degree in the improvement of almost every major agronomic, vegetable and fibre crop species⁵. The objective of this study was to develop a simple and efficient system for plant regeneration from seedling explants, which could be used for genetic transformation studies. The paper describes the first report of plant regeneration of *M. charantia* through direct shoot formation from the shoot apex, nodal and internodal explants.

Materials and Methods

Seeds of bitter gourd (*M. charantia* L. cv. Faizabadi) were collected in January-February from the plants grown in the Botany Department, Rajasthan University, Jaipur. They were pre-treated with 1% Bavistin solution and 400 ppm Chloramphenicol on a rotatory shaker and thoroughly washed with sterile distilled water. A final treatment with 0.1% HgCl₂ (w/v) was given for 5 min after which the seeds were thoroughly washed with sterile distilled water. The seeds were then inoculated on 15 ml aliquots of 0.8% agar gelled MS medium⁶ and incubated in dark for 20 days. After radicle initiation, the seeds were transferred to incubation at 25±2°C with a 16 hrs photo-period provided by cool fluorescent light (50 μ Em⁻² s⁻¹) (Philips, India). After 5 weeks, from 15 cm long seedlings (Fig. 1A), 5-6 mm shoot tip explants, 20-25 mm long nodal and internodal explants were dissected out and inoculated on MS medium with various concentrations and combinations of growth regulators [0.5-6 mg/l of 6-benzylaminopurine (BAP), Kinetin (Kn), Naphthaleneacetic acid (NAA), Indolebutyric acid (IBA), Indoleacetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D)]. The comparative morphogenetic responses of the explants taken from the *in vitro* grown seedling to plant hormones were observed regularly (Tables 1 & 2). Each treatment had 10 replicates of culture and experiments were repeated twice.

*Author for correspondence:

Tel: 91-141-2550098; Fax: 91-141-2721343

E-mail: htfc@satyam.net.in

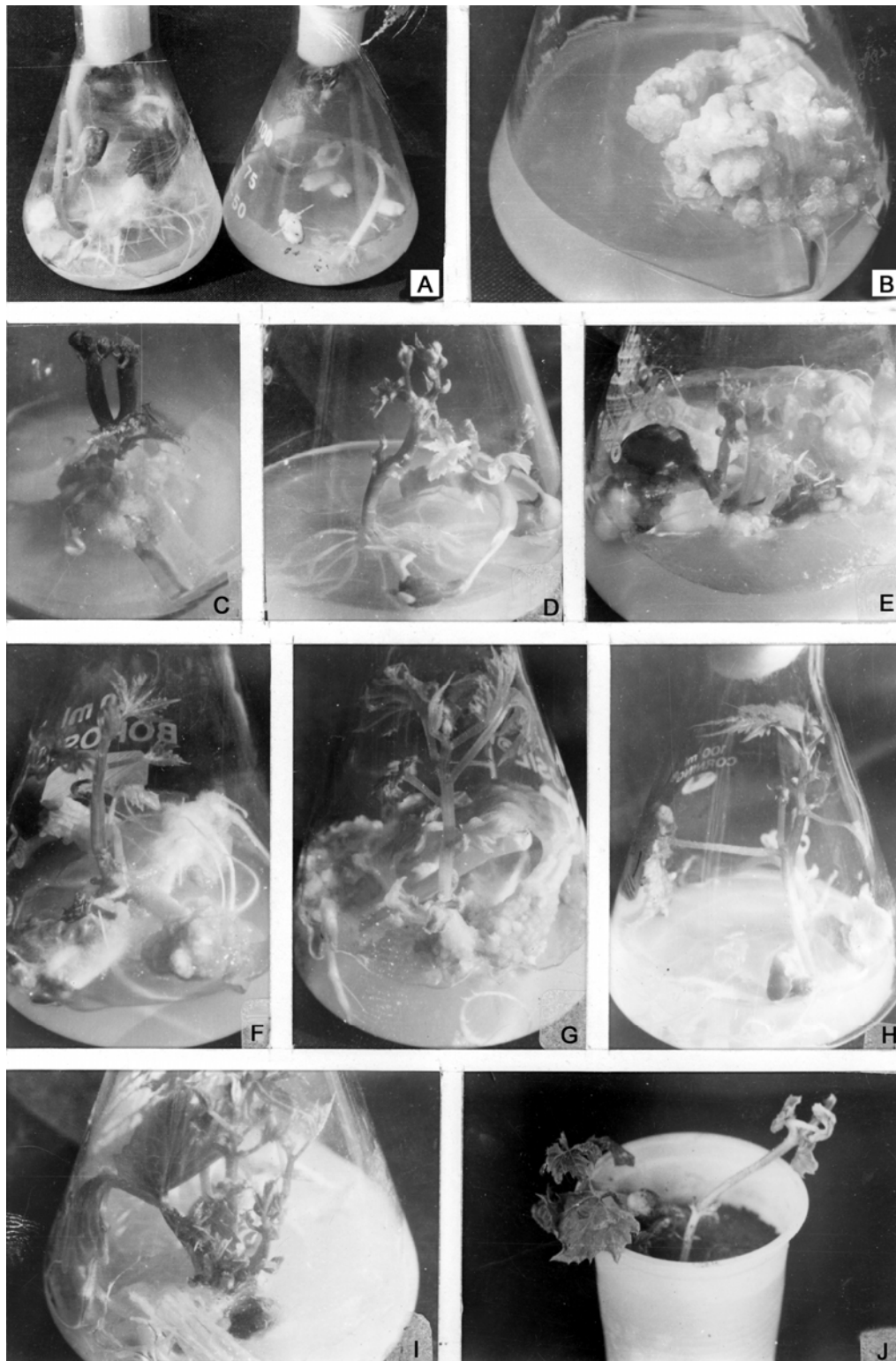


Fig 1 (A-J)—Clonal propagation of *M. charantia* L.: A, Germinated seedling; B, Callus formation; C, Shoot bud formation from callus; D, Regeneration of shoots and roots from shoot explant; E, Multiple shoot buds; F, Multiple shoots with callus; G, Multiple shoots with increased callus; H, Rooting on multiple shoots; I, Multiple shoots without callus & J, Regenerated and hardened plantlet.

Table 1—Shoot production of *M. charantia* grown on various hormonal concentrations supplemented in MS medium and MS medium without hormones (MSO)

Growth hormones mg/l	Explant							
	Shoot tip		Nodal		Internodal		Shoot tip with node	
	Growth response	Number of shoots	Growth response	Number of shoots	Growth response	Number of shoots	Growth response	Number of shoots
BAP								
0.5	Multiple shoots	4+0.54	Multiple shoots	4.7+0.58	Multiple shoots	4.9+0.61	Shoots with callus	4.5+0.57
2	Multiple shoots	4.1+0.53	Multiple shoots	4.3+0.53	Multiple shoots	4+0.54	Multiple shoots	4.2+0.51
4	Shoots with callus	1.5+0.25	Shoots with callus	1.6+0.24	Shoots with callus	1.7+0.23	Shoots with callus	1.5+0.25
6	Callus	-	-	-	Callus	-	Shoots with callus	1.8+0.25
Kn								
0.5	Multiple shoots	1.3+0.20	Multiple shoots	2.4+0.35	Multiple shoots	1.7+0.24	Shoots with callus	1.9+0.21
2	Multiple shoots	1.7+0.23	-	-	Multiple shoots	1.5+0.17	Shoots with callus	2.1+0.26
4	Multiple shoots	1.4+0.16	-	-	-	-	Shoots with callus	2.5+0.31
6	-	-	-	-	-	-	-	-
MSO	Multiple Shoots with roots	3.2+0.36	Multiple shoots with roots	4+0.24	Multiple shoots with roots	1.5+0.25	Multiple shoots with roots	4.5+0.57

Values are mean + SE (n=10,2)

Table 2—Effect of growth hormones on average rooting of *in vitro* developed shoots of *M. charantia*

Hormones mg/l	Explant		
	Shoot tip	Nodal	Internodal
IAA			
0.5	R(+)	R(++)	R(+)
1	-	R(+)	R(++)
2	R(+)	-	R(+)
3	R(++)	R(+)	-
4	R(+)	R(+)	-
5	R(+)	R(++)	-
IBA			
0.5	R(+)	R(+)	R(+)
1	-	R(++)	R(+)
2	R(++)	-	R(+)
3	R(++++)	R(+++)	R(++++)
4	R(++)	R(++)	R(++)
5	R(+)	-	R(+)
NAA			
0.5	R(+)	R(++)	R(+)
2	R(++)	-	R(+)
3	R(+++)	R(++)	R(+)
4	R(+)	-	R(+)
5	-	R(+)	-

R, rooting; +, poor; ++, moderate; +++, good; +++++, very good

Five weeks after root initiation, the plants were hardened in 5×5 cm pro trays filled with cocopeat+soil (1:1) and kept in a moist saturated greenhouse having 60-80% humidity. The plants were irrigated twice daily. After three weeks the established plants were transplanted to 15 cm pots containing, soil+biofertilizer for further growth.

Results and Discussion

Shoot and leaf explants were taken from aseptically grown seedling. Regeneration was observed from all types of explants but the best response was from shoot tip, nodal and internodal explants. This is in agreement with the earlier reports⁷⁻⁹ that the tissue organ used as a source of explant can also be a determinant for the success of a plant tissue culture. Regeneration of plants was carried out through the initiation of adventitious buds or somatic embryos¹⁰. Regeneration through organogenesis via shoot formation in *Cucumis sativus* was reported on MS media+IAA+ and MS media+NAA+BAP with the best shoot growth obtained through a balance of both auxins and cytokinins¹¹. Regeneration from callus of *C. melo* had also been reported¹².

Cytokinin (0.5-6 mg/l) produced shoots after 20 days in culture and the best response was observed on media containing 0.5 mg/l cytokinin and 2 mg/l BAP (Table 1). IBA/NAA were suitable for rooting with best response at 4 mg/l IBA and 2 mg/l BAP, the root formation was observed after 22 days in culture. Callus was formed on 2,4-D, with profuse callusing at 2 mg/l. A combination was most effective for callus formation with best response in 2 mg/l NAA+0.5 mg/l BAP+2 mg/l 2,4-D (Fig. 1B). Regeneration of shoots from callus was on BAP (Fig. 1C) and regeneration of shoots as well as roots from callus formed on explants taken from *in vitro* grown seedling and shoot tip, nodal and internodal explants taken from *in vitro* grown seedling with best response at 2 mg/l NAA+0.5 mg/l BAP and 4 mg/l IBA+2 mg/l BAP (Fig. 1D). The shoots were 55 mm in length with 2-3 nodes and 1-2 fully expanded leaves, after 22 days. There was *de novo* regeneration of shoots from callus and different explants.

This study supports that organogenesis is determined by auxins and cytokinins¹¹. Regeneration from *C. melo* and *C. sativus*, through somatic embryogenesis and organogenesis, respectively, is also reported¹³. In the present study, regeneration was observed through organogenesis only. This is in confirmation with the earlier reports¹⁴⁻¹⁶ that differences in the culture conditions required for organogenesis and somatic embryogenesis are not well defined because very few examples exist where both embryogenesis and organogenesis occur.

Auxin free media, containing Kn, stimulated the production of complete *C. sativus* plants with minimal callus¹⁷. On low concentration of cytokinin, initiation of multiple shoots from explants of *C. sativus* was observed with a dramatic increase in callus production and reduction in number of shoots produced after the addition of IAA¹⁸.

In the present experiment, three types of multiple shoot developments were observed on BAP, a low concentration of BAP (0.5 mg/l) resulted in high shoot production with minimal callus (Fig. 1E), shoot formation was moderate with associated callus at higher concentration (2 mg/l) (Fig. 1F) of BAP, on much higher concentration of BAP shoot production was moderate associated with increased callus growth (Fig. 1G; Table 1). From these observations, MS medium without hormones was chosen as basal medium, where multiple shoots and roots were formed (Table 2; Fig. 1H). The shoots attained a

length of about 60 mm with 2-3 nodes and 1-2 fully expanded leaves. While some callus was associated with these shoots it was only at the edge of explant and not on the site of *de novo* shoot production.

The shoots were separated and transferred on MS medium containing different concentrations of rooting hormones; best response was on 3 mg/l IBA, the roots were developed on shoots within ten days (Fig. 1I; Table 2). The complete plantlets, thus formed, were hardened in greenhouse and transferred to pots where 40% plants survived successfully (Fig. 1J). This is in agreement with previous reports^{17,18} that multiple shoots can be formed on MS medium without hormones. The single factor determining organ initiation was the relative quantities of auxins and cytokinins¹⁹. When tissues *in vitro* did not appear to require exogenous supply of auxins and cytokinin, it may be that sufficient endogenous levels of hormones existed in the culture system for organogenesis.

References

- 1 Duke J A, *Handbook of medicinal herbs* (CRC Press, Boca Raton FL) 1985, 315-316.
- 2 Raman A & Lau C, Anti-diabetic properties and phytochemistry of *M. charantia* L, *Phytomedicine* (1996) 349-362.
- 3 Zhang Q C, Preliminary report on the use of *M. Charantia* extract by HIV patients, *J Naturopath Med*, 3 (1992) 65-69.
- 4 Khanna P & Mohan S, Isolation and identification of diosgenin and sterols from fruits and *in vitro* cultures of *Momordica charantia* L, *Indian J Exp Biol*, 11 (1973) 58-60.
- 5 Cade R M, Wehener T C & Blazich F A, Organogenesis and embryogenesis from cucumber (*Cucumis sativus* L.) cotyledon-derived callus, *Hort Sci*, 22 (1987) 154.
- 6 Murashige T & Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol Plant*, 15 (1962) 473-497.
- 7 Murashige T, Suppression of shoot formation in cultured tobacco cells by gibberellic acid, *Science*, 134 (1961) 280.
- 8 Cheng T Y & Voqui T H, Regeneration of *Douglas fir* plantlets through tissue culture, *Science*, 198 (1977) 306.
- 9 Thorpe T A & Patel K R, Clonal propagation: Adventitious buds in *Cell culture and somatic cell genetics of plants*, vol 1, edited by I K Vasil (Academic Press, New York) 1984, 49-60.
- 10 Torrey J G, Morphogenesis in relation to chromosomal constitution in long-term plant tissue cultures, *Plant Physiol*, 20 (1966) 265.
- 11 Kim S G, Chang J R, Cha H C & Lee K W, Callus growth and plant regeneration from cotyledons in diverse cultures of cucumber (*Cucumis sativus* L.), *Plant Cell Tissue Organ Cult*, 12 (1988) 67-74.
- 12 Moreno V, Gracia S M & Granell J, Plant regeneration from callus of melon (*Cucumis melo* L.), *Plant Cell Tissue Organ Cult*, 5 (1985) 139-146.
- 13 Ziv M & Gadasi G, Enhanced embryogenesis and plant regeneration from cucumber callus by activated charcoal in solid/liquid double layer cultures, *Plant Sci*, 47 (1986) 115-128.

- 14 Matsuoka A M & Hinata K, NAA-induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melangena*, *Indian J Exp Biol*, 30 (1979) 363-370.
- 15 Reynolds T L, An effect of cytokinin concentration on morphogenesis in *Solanum xanthocarpum*, *Am J Bot*, 73 (1986) 914-918.
- 16 Rajasekaran K, Mullins M G & Niar Y L, Flower formation *in vitro* by hypocotyl explants of cucumber (*Cucumis sativus* L.), *Ann Bot*, 44 (1983) 665.
- 17 Handley L W & Chambliss O L, *In vitro* propagation of *Cucumis sativus* L., *Hort Sci*, 14 (1979) 22-23.
- 18 Rhonda L & William, *In vitro* technique for the production of *de novo* multiple shoots in cotyledon explants of *Cucumis sativus*, *Plant Cell Tissue Organ Cult*, 20 (1990) 177-183.
- 19 Skoog F & Miller C O, Chemical regulation of growth and organ formation in plant tissue cultures *in vitro*, *Symp Soc Exp Biol*, 11 (1957) 118.

BT-195....