Indian Journal of Plant Sciences ISSN: 2319-3824 (Online)
An Online International Journal Available at http://www.cibtech.org/jps.htm
2013 Vol. 2 (2) April-June, pp.17-21/Debnath et al.
Research Article

IN VITRO DIFFERENTIATION AND REGENERATION OF *MOMORDICA COCHINCHINENSIS* (LOUR.) SPRENG

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ABSTRACT
High frequency *in vitro* regeneration method of medicinally important, diploid, female *Momordica cochinchinensis* (Lour.) Spreng has been established. Callus was established on Murashige and Skoog's (MS) agar gelled medium supplemented with 2mg/l 2, 4-D and Coconut milk (15% v/v). Multiple shoot buds were developed from the callus when they were cultured on MS containing 4mg/l 6-Benzylaminopurine (BAP) and CM (15% v/v). Further shoot multiplication (70 ± 4.32) was achieved by sub culturing of regenerated shoot clump on the same fresh medium. The shoot multiplication could be repeated for more than five cycles with normal morphogenesis without returning to the original explants source. For rooting micro-propagated shoots were subsequently transferred to half strength MS containing 0.5mg/l Indole-3 butyric acid (IBA) with 100% success. Plants regenerated by organogenesis through callus are similar with the natural cytotypes. The present study highlights the rapid *in vitro* multiplication of *M. cochinchinensis*.

Key Words: *Momordica Cochinchinensis, Cucurbitaceae, In Vitro Regeneration, Callus, Cytotypes.*

INTRODUCTION
Giant spine gourd, *Momordica cochinchinensis* (Lour.) Spreng, commonly known as 'BattKakrol' (Tripura), belong to family Cucurbitaceae and is one of the non-conventional vegetable especially for tribal communities of the North East India. It is indigenous to Southeast Asia. It is grown in Thailand, Laos, Cambodia, Burma and other tropical areas and predominantly available in Bangladesh, China and India (Voung *et al.,* 2006). The plant is dioecious, woody climber, often climbing high on the trees with unbranched tendril. In Tripura it is occasionally grown in home orchard. Fruits, seeds and leaves are used for the treatment of lumbago, ulceration, fracture of bones, sores and obstractions of liver and spleen (Das *et al.,* 2012). Its root is used to cure hair falling, insect bite, cough, haemorhoid, to eliminate poisonous substances, and as a contraceptive in traditional medicine system (Nanthachit *et al.,* 2008). The seeds, known in traditional Chinese medicine "Mubfezi" are thought to have resolvent and cooling properties and are used for liver and spleen disorders, wounds, hemorrhoids, bruises, swelling and pus (De Shan *et al.,* 2001).
Phytochemical studies revealed that seed membrane and seed oil are the excellent source of bio-available Beta-carotenes and Lycopene which promotes healthy vision (Voung *et al.,* 2003), and inhibit the proliferation of cancer cells. Lycopene levels in fruit are higher than those in any of the plant sources reported by West and Poortvliet (1993), a good source of vitamin-E alpha-tocopherol (Voung *et al.,* 2006) and contain antimicrobial and anti-diabetic properties (D. SaiKoteswarSarma, *et al.,* 2011). In spite of the immense economic importance of the plant no information regarding the *in vitro* morphogenetic response of *M. cochinchinensis* was available. Therefore the present experiment was designed to develop an efficient protocol for *in vitro* regeneration of the species.

MATERIALS AND METHODS
Shoot tip, leaves and nodes taken from field grown mature female plants were used as explants. MS medium and different concentrations of growth regulators such as BAP, NAA, IAA and IBA were used.

Explants were washed thoroughly under running tap water for 10 to 15 min. Later plants were cut into pieces and washed with liquid detergent 5% Teepol (v/v) for 10 min and then sterilized with 0.1 % $HgCl_2$ solution for 5 min. followed by three to four rinses in autoclaved distilled water to remove traces of $HgCl_2$ under a laminar airflow. Small segments measuring 0.5 - 1.0 cm were cultured on MS medium supplemented with specific concentrations of growth regulators and coconut milkwith 3% sugar. The media were gelled with 0.7 % agar with a pH of 5.8. Subcultures were done every 14 days interval. Cultures were kept for callus induction and maintained for shoot initiation, proliferation and elongation. Each proliferated and adventitious shoot was cut from the basal end and sub-cultured again for further multiple shoot induction. Regenerated multiple shoots were cut and individual shoots were placed in halfstrength MS containing different concentrations of IBA, NAA and IAA for rootinduction. All cultures were kept at a temperature of 25 ± 1^0C under 16 hour's photoperiod at 2000 - 3000 lux from fluorescent tubular lamps. For somatic chromosome studies, root tips were pretreated in saturated solution of Para-dichlorobenzene at 10 – 12°C for four hour followed by fixation in 1:3 acetic acid ethyl alcohol mixtures. The root tips were then kept in 45% acetic acid for 8-10 min. thoroughly washed with distilled water and hydrolyzed with 1N HCl at cold for 5 min. After washing with distilled water root tips were stained with 2% aceto-orcein for 2 hours and squashed in 45% acetic acid. Five metaphase plates from each plant were taken into account for karyotype analysis.

RESULT AND DISCUSSION

Different degrees of callus induction and growth were achieved in MS media supplemented with different concentration of growth regulators along with coconut milk. MS media containing 2mg/l 2,4-D along with either 0.5mg/l BAP or CM (15% v/v) supported better callus formation and growth. MS medium supplemented with 2mg/l 2,4-D along with 0.5mg/l BAP was found suitable for better callus growth and differentiation. Among the different explants, nodal segments were found to produce suitable callus.

Table 1: Induction and multiplication of adventitious shoots from 14 days old callus

MS media with growth regulators (mg/l)	Response After 21 days	Number of shoot buds initiated after 6 weeks
BAP(2.0)+ CM (15% v/v)	Initiation of adventitious	11.2±0.80
BAP (4.0)	Initiation of adventitious	15.4±0.44
BAP (4.0) + CM (15% v/v)	Initiation of adventitious	19.2±1.22

Table 2: Response of regenerated shoots in different rooting media

Media	Treatment concentration (mg/l)	No. of shoots with root (%) After 14 days	After 21 days	Nature of response	No. of roots per shoot after 21 days (mean of ten shoots)
Half MS+IBA	0.1	40	100	R	10.1
	0.2	60	100	R	12.4
	0.5	60	100	R	18.2
	1.0	50	100	R + C	10.4
Half MS+NAA	0.1	60	100	R	8.6
	0.2	70	100	R	12.2
	0.5	65	70	R +C	10.5
	1.0	50	50	R +C	5.9
Half MS+IAA	0.1	10	50	R	3.0
	0.2	20	50	R	5.0
	0.5	40	50	R +C	11.4
	1.0	54	60	R +C	9.5

Table 3: Comparative karyotype of source plant with regenerated plant

Plants	2n Chromosomes	Ploidy	Total Chromosome length	Karyotype formula	TF %	Chromosome arm symmetry index	Stebbins categorization (1971)
Source plant	28	Diploid	41.80 ±0.50	A2 B22 C4	41.02	70.16	1A
Regenerated	28	Diploid	42.01 ±0.32	A2 B22 C4	41.52	70.72	1A

In vitro morphogenesis of *M. Cochinchinensis*

Figure 1: Shoot bud differentiation from the callus

Figure 2: Shoot budsMultiplication in MS medium containing BAP 4mg/l and coconut milk (15% v/v)

Figure 3: Rooting of shoots

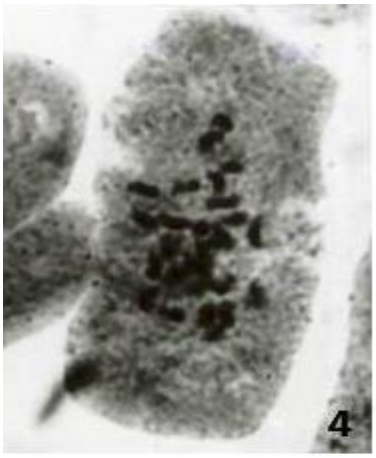

Figure 4: Somatic chromosome number (2n=28) of regenerated shoot

This result corroborate with the findings of other researcherthat were observed in other species of *Momordica* (Shekhawat *et al.,* 2011, Agarwal and Kamal *et al.,* 2004 and Sultana *et al.,* 2005). Thus, 14 days old callus derived from the nodal segments were subsequently used for shoot buds initiation, growth and differentiation (Figure 1). MS media containing high cytokinin (BAP 4mg/l) along with CM (15% v/v) induced maximum number of adventitious buds (Figure 2) and shoots during 42 days of culture (Table-1). Shoot buds thus produced after 42 days were sectioned in to small groups (4-8 shoot buds in each groups) and sub-cultured on the same inductive media for further multiplication and growth. The maximum number of shoot bud (70±4.32) was found on the 16 weeks of culture. The shoot buds multiplications were recorded up to five multiplication cycles with normal morphogenesis. Multiple shoots were excised and cultured in half strength MS supplemented with different concentration of IBA, IAA and NAA (Table-2). Most of the shoot produced root within 14 to 21 days (Fig.3). Optimum induction and differentiation of root was achieved in half strength MS media supplemented with 0.5mg/l Indole-3 butyric acid (IBA). It has also been observed that all the auxins used for rooting at higher concentration (1mg/l) induced rooting as well as callus at the base of the shoot. Root produced through the *in vitro* regeneration were cytologically analyzed and somatic chromosome number was found to be 2n=28 (Fig.4). The observation clearly indicates cytological stability at chromosomal level. Thus present *in vitro* regenerants could be used in true clonal propagation of the species.

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