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Research Article

RAPID *IN VITRO* DIFFERENTIATION AND REGENERATION OF *MOMORDICA DIOICA* ROXB.

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ABSTRACT
High frequency *in vitro* regeneration and multiplication of shoots of female *Momordica dioica* Roxb. has been established from callus derived from nodal explants culture. Callus was established on Murashige and Skoog's (MS) agar gelled medium supplemented with 2mg/l 2, 4-D and 0.5mg/l BAP/ Coconut milk (15% v/v). Multiple shoot buds were developed from the callus when they were cultured on MS medium containing 4mg/l 6-Benzylaminopurine (BAP) and CM (15% v/v). Further shoot multiplication (86 ± 3.44) was achieved by sub culturing of regenerated shoot clump on the same fresh medium. The shoots 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 medium 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. dioica.* Roxb.

Key Words: *Momordicadioeca, Cucurbitaceae, In Vitro Regeneration, Cytotype*

INTRODUCTION
Momordic adioica Roxb. (Kakrol) belonging to Cucurbitaceae is an important vegetable in North East India. It is also widely cultivated in different districts of Bangladesh (Karim and Ullah, 2011). In Tripura it is one of the important vegetables of home garden in every villages and its cultivation is popular for its high economic return as compared to other vegetables of summer seasons. Its fruit is the important source of vitamin-C, carotenoides and proteins (Bhuiya *et al.,* 1977). Traditionally the fruits, leaves and tuberous roots are used by the diabetic patient. The plant was also reported to possess anti-allergic, antimalarial, anti-feedant, nematocidal and antibacterial properties (Nabi *et al.,* 2002), anti-oxidants, hepatoprotective (Jain *et al.,* 2008) and used as a remedy of jaundice and bleeding pile (Deokule, 2008).
Germination of seeds of *M. dioica* is very difficult and the propagation of this dioecious plant depends on underground tuberous roots which have low multiplication rate (Mondal *et al.,* 2006). The maintenance of quality of tubers in the field as well as in the store is difficult. An attempt of *in vitro* propagation of this plant was demonstrated by many workers (Hoque *et al.,* 1995; Nabi *et al.,* 2002; Karim and Ullah, 2011, Devendra *et al.,* 2009; Rai *et al.,* 2012; Mustafa *et al.,* 2013). Till now, this popular vegetable is considered as underutilized vegetable (Bharati *et al.,* 2007; Ali *et al.,* 1991) and does not receive any research priority. It is essential to develop improve technology for higher production of this crop. Therefore the present work may help the researcher to develop essential technique for large scale *in vitro* propagation.

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% Ezee (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 milk with 3% sugar. The media were gelled with 0.7 % Bacto agar (BDH) 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 half strength MS media containing different concentrations of IBA, NAA and IAA for root induction. 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.

RESULTS 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 0.5 mg/l BAP or CM (15% v/v) supported better callus formation and growth. Among the different explants, nodal segments were found to produce suitable callus. This result corroborate with the findings of other researcher that were observed in other species of *Momordica* (Debnath *et al.*, 2013; Shekhawat *et al.*, 2011; Agarwal and Kamal, 2004; Sultana *et al.*, 2005). Thus, 14 days old callus derived from the nodal segments were subsequently used for shoot buds initiation, growth and differentiation. MS media containing high cytokinin (BAP 4mg/l) along with CM (15% v/v) induced maximum number of adventitious buds (Figure A) and shoots during 86 days of culture (Table 1). However, earlier reports suggest that the lower concentration of cytokinin support optimum shoot regeneration from leaf and cotyledon explants of the species (Mustafa *et al.*, 2013; Devendra *et al.*, 2009; Nabi *et al.*, 2002). Requirement of high cytokinin (4mg/l) and CM (15% v/v) in nodal callus culture clearly indicate the differential morphogenetic potential of the explants. The reported result is significant in terms of high shoot multiplication and successful regeneration of *M. dioica* using nodal explant. Shoot buds thus produced after 42 days were sectioned in to small groups (8-10 shoot buds in each groups) and sub-cultured on the same inductive media for further multiplication and growth. The maximum number of shoot bud (86±3.44) was found on the 126 days of culture (Table 2). The shoot buds multiplications were recorded up to five multiplication cycles with normal morphogenesis.

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	15.4±0.81
BAP (4.0)	Initiation of adventitious	18.6±0.85
BAP (4.0) + CM (15% v/v)	Initiation of adventitious	22.2±1.98

Multiple shoots were excised and cultured in half strength MS supplemented with different concentration of IBA, IAA and NAA (Table 3). Most of the shoot produced root within 14 to 21 days (Figure B). 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 (Sinha *et al.*, 1996) was found to be 2n=56 (Table 4, Figure C&D). The observation clearly indicates cytological stability at chromosomal level. Thus present *in vitro* regenerants could be used in large scale propagation of the species.

Table 2: Proliferation rate of adventitious shoots in different period of subcultures on MS medium supplemented with BAP (4.0) + CM (15% v/v)

Age of culture	63 Days	84 Days	105 Days	126 Days	147 Days
No. of shoot buds/subculture	24.2±0.81	41.4±1.32	79±2.44	86±3.44	75.1±6.75

Each subculture of a group of 8-10 shoot buds was done after 21 days of interval

Table 3: Response of regenerated shoots in different rooting media

Media	Treatment concentration (mg/l)	No. of shoots with root (%)		Nature of response	No. of roots per shoot after 21 days (mean of ten shoots)
		After 14 days	After 21 days		
Half MS+IBA	0.1	40	100	R	15
	0.2	60	100	R	21
	0.5	60	100	R	35
	1.0	50	100	R + C	30
Half MS+NAA	0.1	70	100	R	11.6
	0.2	80	100	R	18.2
	0.5	50	70	R +C	10.5
	1.0	40	50	R +C	8.6
Half MS+IAA	0.1	10	60	R	5.0
	0.2	20	60	R	6.0
	0.5	60	70	R +C	14.5
	1.0	40	50	R +C	11.5

R=Rooting, R+C= Rooting +Callusing

Table 4: Comparative karyotype of source plant with regenerated plant

Plants	2n Chrom-osomes Numbe r	Total Chromosome length (µm±SE)	Karyotype formula	TF %	Chromosome arm symmetry index	Stebbins categorization (1971)
Source plant	56	78.28 ±0.60	A4 B46 C6	43.17	70.16	1A
Regener ated	56	79.64 ±0.32	A4 B46 C6	44.22	70.42	1A

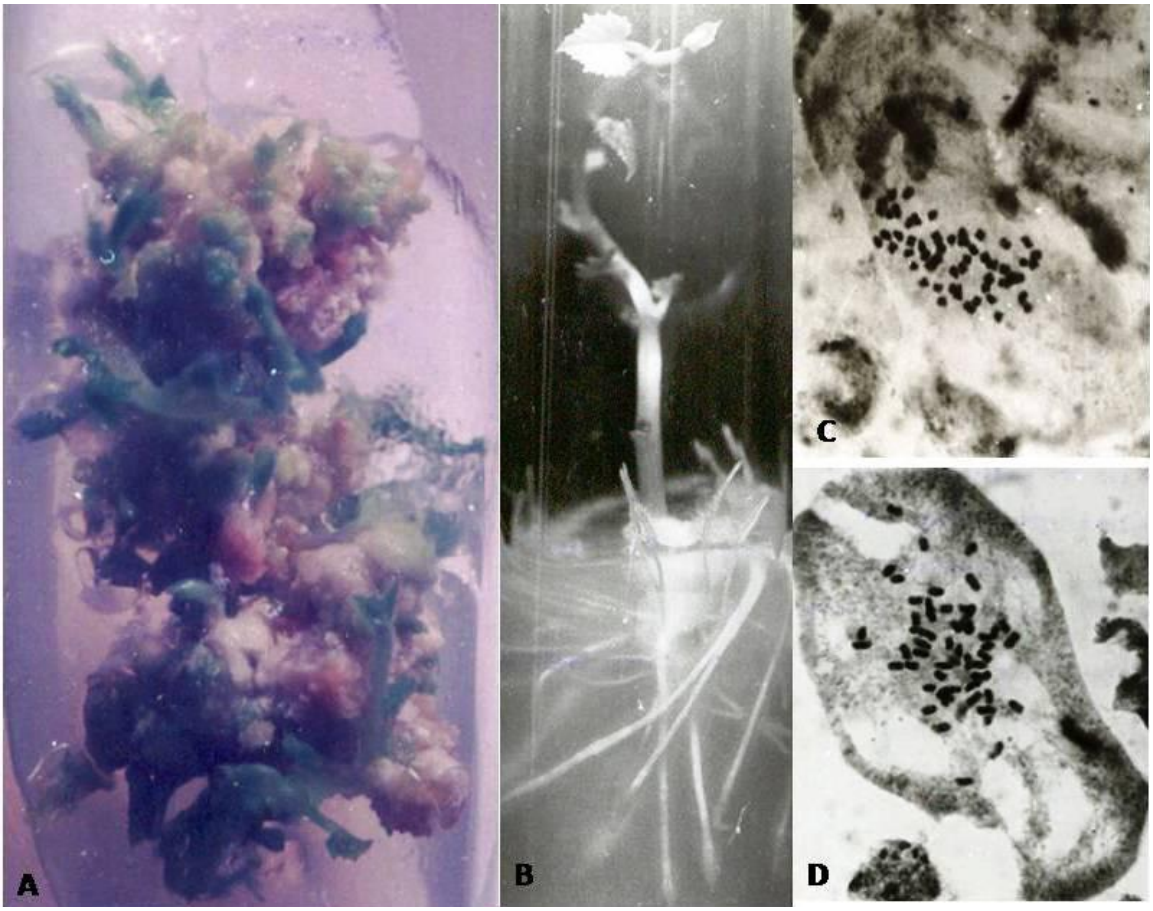

Figure: A- Shoot buds differentiation from callus, B- Rooting of shoot, C- Somatic chromosome number of source plant, D- Somatic chromosome number of regenerated plant

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