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In vitro regeneration from *Momordica dioica* (Roxb.) willd. cucurbitaceae

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ABSTRACT

The present investigation outlines the *in vitro* regeneration of *M.dioica*. The explants from in vivo were cultured on modified MS medium. Highest percentage of shoot differentiation was obtained on MS supplemented with BAP and Kn. from node explants then BAP alone. Rooting on shoot grown occurred on medium containing IBA and 60 percentages of hardened plants survived successfully, when transferred to field.

KEYWORDS

Momordica dioica;
Regeneration;
In vitro.

INTRODUCTION

Momordica dioica is a significant medicinal plant originated in Indo-Malayan region[28]. Fruits are used as a vegetable in India, Bangladesh and other neighboring countries, as it is a rich source of vitamin C, carotenoids and proteins[4]. Preference for this vegetable is more evenwith the bitterness of this fruit.

Traditionally it is being used for treating eye diseases, poisoning and fever[25]. Fruits, leaves and tuberous roots are used as a folk remedy for diabetes. The plant was reported to posses anti-diabetic[33], Analgesic, postcoital anti-fertility[29], nematocidal, anti-allergic, anti-malarial, anti-feedant, and anti-bacterial[21], antioxidants and hepatoprotective[16], jaundice and bleeding pile properties[6].

The commercial improvement of cultivation of this vegetable crop has not been attempted, because of its dioecious nature and vegetative mode of propagation[2]. Normally in cucurbits, the seed setting and seed germination is low, probably due to presence of a thin nucellar membrane lending impermeability to water and gases and make them dormant for many days[48]. In nature, male and female plants are shown together at a ratio 1:15. Due to above mentioned reason, the natural population of the plant is gradually decreasing and hence conservation of this plant is necessary. In this direction, plant biotechnology plays an alternative tool for large scale multiplication, propagation and conservation.

The perusal of literature on tissue culture aspect indicated that, few reports are available for conservation and propagationthe *Momordica dioica* female plant is selected for *in vitro* regeneration.

MATERIALS AND METHODS

Collection, identification of plants and explant sources

The *Momordica dioica* plant tubers were collected from forest range of Chikli (U) Aurad (B) Taluk, Bidar District and cultivated in Botanical Garden Gulbarga

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University, Gulbarga. Identified and authenticated with the help of Flora of Presidency of Madras by[10], Fascicle of Flora of India[5], Flora of Karnataka[23] and Flora of Gulbarga District[26] and a voucher specimen are deposited in the Herbarium of Botany Department, Gulbarga University, Gulbarga (Vouch. No. HGUG-803).

Elongated shoots 4-5 nodes were collected from plants grown at the Botany Department, Gulbarga University, Gulbarga. (India) with their cut ends placed in distilled water, leaves were removed from the stem and the stem is washed under running tap water for at least 10-15 min, followed by soaking in 5% (v/v) detergent solution (Teepol Qualigen, India) for 5 min. After thorough washing in sterilized distilled water, the explants were surface sterilized with freshly prepared 0.1% (w/v) aqueous mercuric chloride solution for 3 min. Followed by repeated washing with sterile distilled water, the stems were cut transversely in to 0.5-1 cm segments the explants were inoculated onto culture media.

Media evaluation

The best explants were selected and cultured on two different media i.e., MS[19] and B_5[11] supplemented with BA 1.0 mg/l and 3% sucrose. The morphogenic responses of the explants on different media were recorded.

Culture media and culture conditions

Shoot tip and nodal explants were placed on semi-solid MS medium supplemented with Sucrose 3% (w/v), Polyvinylpyrrolidone (PVP) 0.1% (w/v) and mesoinositol in all the experiments. Different concentrations of plant growth regulators like 6-benzyl aminopurine (BA: 0.5-2.5mg^{-l}) and Kinetin (0.5-2.5 mg^{-l}) were tested for shoot multiplication. The pH of the media was adjusted to 5.7 using 0.1N NaOH or 0.1N HCl prior to adding 0.8% agar (Hi-media, Mumbai). Medium was dispensed in 20 ml aliquots in to culture tube (25150 mm), which were covered with an aluminum foil. Media were steam sterilized at 121^0C and 1.05 kg cm^{-2} $s^{-1}$for 20 min. The cultures were incubated under a 16 h photoperiod in cool white florescent light (55µ mol m^{-2} s^{-1}) (Phillips, India) and maintained at a constant temperature of 25 ± 2^0C. The cultures were maintained by sub culturing at 4-week intervals to fresh medium with the same composition.

Induction of rooting and acclimatization

The elongated shoots (4-5 cm) were excised from the 4-week old culture grown on MS medium supplemented with 0.5mg^{-l} BA + 0.1mg^{-l}Kn. The excised shoots were transferred to half strength MS basal semi-solid medium supplemented with different concentrations of IAA (0.5, 1.0 and 1.5mg^{-l}), IBA (0.5, 1.0 and 1.5mg^{-l}) and NAA (0.5, 1.0 and 1.5mg^{-l}) with 3% (w/v) sucrose tested individually for root initiation. One excised shoot was cultured in each tube (25×150 mm) containing 15 ml of culture medium. Temperature and photoperiod were same as for shoot multiplication. Rooted micropropagules were thoroughly washed to remove the adhering gel and planted in earthen pots containing a mixture of soil, sand and farmyard mixture in the ratio of 1:1:1 and grown in the green house for acclimatization. Watering was made at 2 day intervals. Percentage of survival was recorded 1 month after transfer.

Statistical analysis

All the experiments were repeated three times with 10 replicates per treatments, Data on the bud proliferation, percentage of regeneration, number of shoots per explant and shoot length were statistically analyzed using the procedure of SPSS package version X, correlation significance were assessed at 0.05 level.

RESULTS AND DISCUSSION

Media evaluation

The highest rate of micropropagation not only depends on the suitable explants, but also of the correct basal medium. For organ or tissue culture[18] the nutritional requirement varies according to the cells, tissue organs and even protoplast with respect to particular plant species.

The leaf, node, internode, tendril and shoot tip segments were inoculated in two different media. i.e., MS[19] and B_5[11] supplemented with 2,4-D (1.0 mg/l) and 3 % sucrose were tested for callus induction.

In the present investigation the callus induction is high (70%) and fresh weight of 2100.00 ± 55.07, dry weight of 120.00 ± 5.77 on MS medium as compared

to B_5 medium (TABLE 1). Hence, MS media was selected for further callus studies as well as organogenesis.

Direct organogenesis

Effect of various cytokinins BAP and Kn (0.5-2.5 mg/l) alone and in combinations for direct shoot regeneration, using leaf, node, internode and shoot tip and tendril explants in *M.dioica* on MS basal medium were tested

The node and shoot tip explant remained quiescent for about 8-10 days later swelling appeared at the sides of auxiliary buds in node and shoot tip explants. Multiple shoots initiated on 15th day from both the explants. Among different concentrations tested, BAP 1.0 mg/l was proved to be the best concentration for shoot induction in both node (90%) and shoot tip (80%). The highest number of shoot elicitation was observed in nodal segment on MS medium supplemented with BAP 1.0 mg/l with an average 10.00 ± 0.00 number of shoots with 1.48 ± 0.05 cm an average shoot length were recorded (TABLE 2; Figure 1a).

While, in shoot tip explant an average number 8.66 ± 0.88 of shoots with an average length 1.40 ± 0.57 cm were recorded. Similar, effect of BAP on regeneration of multiple shoots were also observed in different plants like *Trichosanthes dioica* by[24] and in *M.charantia*[15] and in *T.cucumerina* var. *cucumerina*[9]. As the concentration of BAP increases, there was gradual decrease in number of shoots.

Nodal explant is proved to be best explant for multiple shoot induction as compared to shoot tip explant. The present result support the results obtained in *Cucumis sativus*[3]. Leaf and internode explant display poor response to BAP alone at higher or lower concentrations, it can produce callogenic responses. Kn alone failed to induce multiple shoots from leaf, node, internode and shoot tip explants tested.

Combined effect of BAP and Kn on direct shoots regeneration from node and shoot tip explant

The node and shoot tip explant (0.5-1.0 cm length) treated on MS medium supplemented with BAP (1.0 mg/l) and various concentrations of Kn (0.5-2.5 mg/l) was found to be best and effective, for formation of multiple shoots.

The poor performance of Kn was observed in lone concentration. But with combination of BAP (1.0 mg/l) multiple shoot bud were initiated on both explants. In node explant multiple shoots were initiated on 6th day of inoculation on MS medium containing BAP (1.0 mg/l) and Kn (1.0 mg/l), an average number of shoots 18.00 ± 0.57 with an average shoot length of 2.00 ± 0.05 cm (Figure 1b) were obtained. Similarly, in shoot tip explant, multiple shoots were initiated on 8th day which produced an average number of 12.0 ± 0.05 shoots and 1.93 ± 0.05 cm length on medium with BAP (1.0 mg/l) and Kn (1.0 mg/l). The nodal explant produced maximum number of shoots compared to shoot tip explant. As the concentration of Kn was increased with BAP 1.0 mg/l) formation of shoots reduced (TABLE 3). Similar result have been reported using shoot tip explant in bitter melon[35], node and shoot tip explant in *M.charantia*[30], shoot tip, node and internode explants from *M.charantia*[1], immature embryo of *M.dioica*[13] and nodal segment of *M.charantia*[31].

Among the node and shoot tip explant, node explant showed maximum number of shoots than shoot tip explant. From the above data it is clear that node

TABLE 1: Influence of different media on induction of callus from leaf segment of *M.dioica*

Plant growth regulator	Medium	Percent of response	Fresh weight of callus (mg/culture)	Dry weight of Callus (mg/culture)
2,4-D 1.0	MS	70	2100.00±55.07	120.00±5.77
mg/l	B_5	30	960.00±0.08	067±0.05

TABLE 2 : Effect of BAP on direct shoot regeneration from node and shoot tip explant in *M.dioica* on MS medium

Sl. no	Hormone (mg/l)	Node			Shoot tip		
		Percent response (%)	Average no of shoots	Average shoot length	Percent response (%)	Average no of shoots	Average shoot length
1	BAP 0.5	60	8.66 ± 0.33	1.46 ± 0.08	50	5.66 ± 0.33	0.96 ± 0.03
2	BAP 1.0	90	12.00 ± 0.57	1.48 ± 0.05	80	8.66 ± 0.88	1.40 ± 0.57
3	BAP 1.5	75	10.00 ± 0.00	1.30 ± 0.05	60	7.33 ± 0.33	1.30 ± 0.57
4	BAP 2.0	60	7.66 ± 0.33	1.46 ± 0.05	40	4.66 ± 0.33	1.10 ± 0.57
5	BAP 2.5	40	5.33 ± 0.33	0.96 ± 0.03	20	3.66 ± 0.33	0.96 ± 0.03

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TABLE 3: Combined effect of cytokinin on direct induction of shoot regeneration from node and shoot tip explant in *M.dioica* on MS medium

Sl. no.	Hormone (mg/l)	Node			Shoot tip		
		Percent response (%)	Average no of shoots	Average shoot length	Percent responce(%)	Average no of shoots	Average shoot length
1	BAP 1.0 + Kn 0.5	70	11.66 ± 0.33	1.66 ± 0.03	60	9.66 ± 0.33	1.40 ± 0.05
2	BAP 1.0 + Kn 1.0	100	18.00 ± 0.57	2.00 ± 0.05	90	12.00 ± 0.57	1.93 ± 0.05
3	BAP 1.0 + Kn 1.5	80	12.00 ± 0.57	1.66 ± 0.05	80	10.33 ± 0.33	1.76 ± 0.03
4	BAP 1.0 + Kn 2.0	70	9.66 ± 0.33	1.10 ± 0.12	50	7.66 ± 0.33	1.40 ± 0.05
5	BAP 1.0 + Kn 2.5	50	5.66 ± 0.33	0.96 ± 0.03	40	5.33 ± 0.33	1.20 ± 0.05

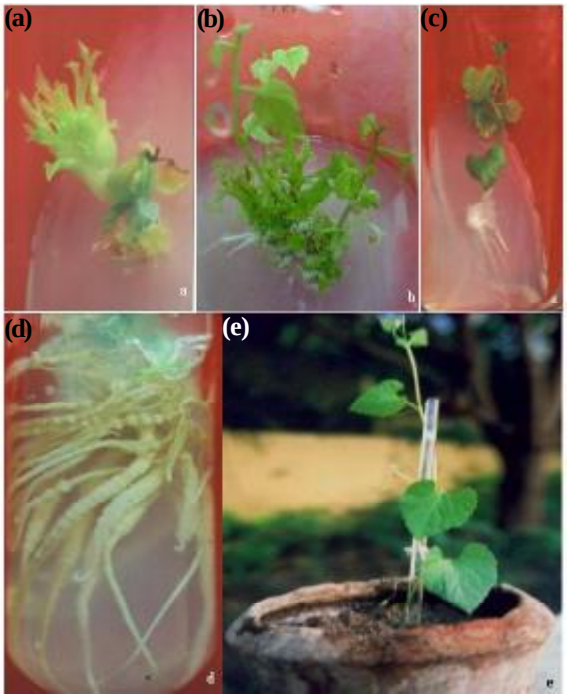

Figure 1 : (a) Initiation of multiple shoot on MS basal medium with BAP (1.0 mg/l); (b) Shoots multiplication and elongation from nodal explant on MS basal medium supplemented with BAP (1.0 mg/l) with GA3 (1.0 mg/l) after 35 days; (c) Initiation of *in vitro* roots on 1/2 strength MS medium with IBA (1.0 mg/l); (d) Thick and long tuberous roots on 1/2 strength MS basal medium with IBA (1.0 mg/l); (e) Completely hardened plantlet in the earthen pot

responded better than shoot tip explant and leaf, tendril have only callogenic response.(TABLE 3).

Proliferation and elongation of multiple shoots

The micro shoots of different length were excised and further cultured on MS medium supplemented with BAP (1.0 mg/l) and varied concentration of GA_3 (0.5-2.0 mg/l) for proliferation and elongation of multiple shoots and the results are summarized in the TABLE 4. Maximum shoots proliferation and elongation observed on MS medium supplemented with combination of BAP

TABLE 4: Effect of GA3 on shoot elongation of multiple shoots derived from nodal explant in *M.dioica* on MS medium

Hormone (mg/l)	Average no. of shoots	Average length of shoots (cm)
BAP1.0 + GA_3 0.5	12.66 ± 0.33	5.20 ± 0.66
BAP1.0 + GA_3 1.0	18.00 ± 0.57	8.70 ± 0.51
BAP1.0 + GA_3 1.5	13.66 ± 0.33	7.60 ± 0.70
BAP1.0 + GA_3 2.0	10.66 ± 0.33	6.70 ± 0.58

(1.0 mg/l) and GA_3 (1.0 mg/l) the average number 18.00 ± 0.57 of shoots were obtained with an average 8.7 ± 0.51 cm length on 35 days culture (Figure 1, b).

It is evident from results there was a marked increased of shoot length in GA_3 treated cultures. GA_3 have been extensively used in apical meristem culture. The relative concentration of GA_3 plays a vital role in organogenesis[34]. There is superiority of BAP and GA_3 in combination has been found to increase shoot length and micropropagation of several plant such as *Cucumis melo cv* pusa sharabati[22].

Effect of auxins on *in vitro* rooting of excised shoots of *M.dioica* on half strength MS medium

Elongated leafy shoots, derived from different explant, cultured on full and half strength MS medium supplemented with IBA, IAA and NAA 0.5-2.0 mg/l concentrations with 3% (w/v) sucrose. The *in vitro* rooting was successful on half strength MS medium supplemented with various concentrations of IBA, NAA and IAA (0.5-2.0 mg/l) as shown in TABLE 5.

The excised shoot showed rooting in all treatments, when micro shoots were inoculated on half strength MS medium supplemented with IBA (1.0 mg/l) roots emerged within 10-15 days which developed in to good root system after 35 days of culture. A 100 percent of rooting with maximum number of (24.0 ± 0.28) roots per culture with an average (6.5 ± 0.28 cm) root length were observed (Figure 1, d). The root produced in all the cultures of IBA shows the presence of tuberous

TABLE 5: Effect of auxins on in vitro rooting of excised shoots derived from node and shoot tip explants of *M.dioica* on ½ strength MS medium with 3% (w/v) sucrose

Growth regulator	Hormone concentration (mg/l)	Percentage of shoots rooted	Average no. of roots/ shoot	Days taken for rooting	Average root length (cm)
NAA	0.5	50	11.00± 0.57	18	4.66± 0.33
	1.0	60	13.00± 0.57	16	3.33± 0.33
	1.5	40	8.33 ± 0.33	14	2.21 ± 0.03
	2.0	30	5.33 ± 0.33	15	2.56 ± 0.03
IBA	0.5	80	21.66 ± 0.88	18	4.83 ± 0.16
	1.0	100	24.00 ± 0.57	15	6.50 ± 0.28
	1.5	70	17.66 ± 0.57	19	5.50 ± 0.28
	2.0	40	12.33 ± 0.88	20	4.50 ± 0.28
IAA	0.5	40	8.00 ± 0.38	16	3.33 ± 0.33
	1.0	50	9.00 ± 0.05	18	4.33 ± 0.05
	1.5	40	10.00 ± 0.50	19	2.66 ± 0.50
	2.0	30	6.00 ± 0.57	20	2.56 ± 0.50

roots which is not observed in other cultures containing rooting hormone. According to Hu et al., (1983), the auxin treatment must be limited to a period of time. Auxin at this stage of process may have undesirable side effects i.e., callus production and inhibition of root elongation.

IAA proved as the poor hormone for induction of roots in *M.dioica* compared to IBA and NAA. The nature of roots formed was thick and long in case of IBA treated cultures. Whereas, thin and stout in both IAA and NAA. The similar results were reported *C.sativus*[3]. *M.dioica*[12,20].

IBA is the effective phytohormone for induction of root and the same was reported in other members of Cucurbitaceae. Agarwal, in *M.charantia*[1], in *M.dioica*[13].

Hardening and pot transfer

Plantlets grown *in vitro* have been continuously exposed to a microenvironment to provide minimal and optimum stress. Plantlets were developed within the culture vessels under low level of light, aseptic condition on medium containing ample sugar and nutrients to allow for heterotrophic growth and in an atmosphere with high level of humidity. *In vitro* cultivated plant lacks the necessary anatomical futures to withstand variations in the natural environment[32]. Plantlets cannot survive in the environmental conditions when directly placed in a green house or field. Decreasing the water potential of the medium and reducing the humidity in cultural vessel can be achieved *in vitro* to harden micro propagated plantlets.

After 35 days of *in vitro* regenerated plantlets with developed shoot and roots were harvested, washed with sterile distilled water and planted in a mini pots filled with mixture of soil, sand and farmyard manure.

The potted plants were covered with polythene cover to ensure high humidity and irrigates every three days. With half strength MS nutrients free from sucrose for 2 weeks.

Presence of low concentration of sucrose or omitting it altogether can give faster and more successful acclimatization[7,27,17]. The plantlets are maintained in culture room conditions for two week and gradual exposure of plants to out side conditions. The polythene covering helps to conserve and develop a proper balance of relative humidity and there by increased the rate of survival.

After two weeks, hardened plantlets were transferred to earthen pots and maintained under shade for one more week (Figure 1, e) then plants are exposed to sunlight for few hour, week and then plants are transferred to soil and watered with tap water. The rooted plants ware successfully established in soil with 60% survival rate.

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