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Multiple shoot induction from the nodal cultures of teasle gourd (*Momordica dioica* Roxb.)

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Abstract

Momordica dioica is a dioecious cucurbit. The fruits are used as costly vegetable in southern part of India. The biotypes were collected form Warangal and Khammam districts of Andhra Pradesh. When the nodal region were cultured on MS medium supplemented with 2.0 mg/l 6-Benzylaminopurine BAP +2.0 mg/l L-Glutamic acid, the explants produced little amount of callus and shoot buds. The shoot buds on successive subcultures for twice on the same medium produced multiple shoots. Shoot proliferation was further continued even after six months.

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Introduction

Momordica dioica is an annual dioecious herb belongs to the family cucurbitaceae, mainly grows during rainy season as a wild crop. The fruit is used as vegetable and having high medicinal value to cure number of diseases, the root is used as sedative in fevers. Several cucurbitaceae members have been investigated for *in vitro* multiplication (Halder *et al.*, 1982). Information on such works in *Momordica dioica* is scanty (Halder *et al.*, 1982). As a Teasle gourd has number of problems, including poor natural pollination of female flowers and low yield, fruits become inedible at maturity owing to the presence of large number of hard seeds. Germination is very difficult or impossible because of its hard seed coat (Rasheed M. M. M., 1976). More over it is impossible to predict sex of the seed producing plants before flowering. Propagation by tuberous roots is limited due to low multiplication rate (Mondal *et al.,* 2006*)* and occupies the valuable cultivation land until next planting season (Ram *et al.*, 2001). Attempts have also been made for *in vitro* propagation of *Momordica dioica*, wherein shoots were regenerated from callus cultures obtained from various explants (Hoque *et al.*, 2007, Karim and Ahmed, 2010) but not from nodal segments. Callus culture may also lead to risk of somaclonal variations, which can seriously limit the broader utility of micropropagation methods. On the other hand, multiple shoot induction method ensures clonal uniformity among the regenerates. The study of genetic fidelity of micro propagated spine gourd plants is not available. In this paper we have established simple, short way method for the production of genetically identical plants of spine gourd. Unfortunately there have been very few reports on tissue culture of Teasle gourd (Islam R. *et al.*, 1994). The present study is therefore carried out to develop a protocol of in *vitro* multiple shoot production that would be used for the improvement of this crop for large scale production of plant material.

Material and methods

Explants like internodes, nodes were collected from *Momordica dioica* during rainy season for tissue culture studies. These explants were thoroughly washed under running tap water for 10 minutesand surface sterilized with 0.1%$Hgcl_2$ for 7-8 minutes, rinsed 3- 4times with sterilized distilled water. The sterilized nodes and internodes were cut in to small pieces aseptically by using steriled forcep and scalpel.These explants were inoculated on MS medium supplemented with 6 – Benzylaminopurine 0.5, 1.0, 2.0 mg/l and L – Glutamic acid 0.5, 1.5, 2.0, 2.5, 3.0 mg/l (table). Among all the explants nodal cuttings have shown better results for callus and multiple shoot induction. All the cultures were maintained at 25 ± 2˚C, 2000 lux light intensity with a photo period of 16 hours. Cultures were grown under florescent light on successive subculture and small adventitious buds were proliferated from the explant. Small multiple shoots were transferred to Ms basal medium for further development. The developed shoots were rooted on Ms medium supplemented with 3.0 mg/l Indole-3 butyric acid (IBA) and then to green house.

Results

The explant was enlarged with little amount of callus on MSO (Fig. A).The explant pieces inoculated on MS medium (Murashige T. and Skoog F., 1962) supplemented with different concentration and combinations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and L- Glutamic acid (0.5, 1.0, 1.5, and 2.0 mg/l) (table1). After two weeks of culture, the explants induced little amount of callus and few shoots from the basal end of the explant. (Fig: 1. B, C). Each adventitious shoot was cut from the basal end and sub cultured again on the M S medium fortified with 2.0 mg/l BAP and 0.5 mg/l L-Glutamic acid. The subcultures were maintained at an interval of four weeks. The frequency of multiple shoots was enhanced on the same medium. (table).The results indicated that the nodal explants were more capable of producing multiple shoots compared to internodes. Large numbers of shoots in short time were produced on MS medium. fortified with

2.0mg/l BAP and 2.0mg/l L- Glutamic acid. (table1), (Fig1E). The developed shoots were rooted on MS medium supplemented with 3.0 mg/l IBA and then for hardening. Callus induction was less on this medium, where as internodal explants showed higher percentage of callus induction.

Table 1. Effect of different combination of BAP and L- Glutamic acid with MS medium on In vitro multiple shoot induction.

MS medium with Growth regulators (mg/ l)	% of cultures responding	Number of shoots/explant (Mean±SE)	Lengh of shoot(cm) (Mean±SE)
BAP + L-Glutamic acid			
0.0 + 0.0	40 (explant enlarged)	Nil	Nil
0.5 + 0.5	8	1.8 ± 0.11	4.2 ± 0.06
0.5 + 1.5	10	2.1 ± 0.14	4.0 ± 0.05
0.5 + 2.0	20	3.9 ± 0.12	3.8 ± 0.05
0.5 + 2.5	40	4.4 ± 0.16	3.5 ± 0.06
0.5 + 3.0	50	5.5 ± 0.23	3.2 ± 0.06
1.0 + 0.5	30	2.3 ± 0.17	4.6 ± 0.04
1.0 + 1.5	40	4.1 ± 0.21	4.2 ± 0.05
1.0 + 2.0	60	4.6 ± 0.22	3.7 ± 0.06
1.0 + 2.5	70	5.1 ± 0.17	3.2 ± 0.08
1.0 + 3.0	70	6.1 ± 0.14	2.8 ± 0.08
2.0 + 0.5	60	7.8 ± 0.17	3.0 ± 0.07
2.0 + 1.5	70	8.4 ± 0.21	2.4 ± 0.06
2.0 + 2.0	80	12.1 ± 0.25	1.8 ± 0.08
2.0 + 2.5	60	8.6 ± 0.24	2.2 ± 0.08
2.0 + 3.0	40	6.7 ± 0.25	2.6 ± 0.09

Data was collected at the end of fourth week of subculture. Values are given as the Mean ± Standard error (SE) of 20 replicates per treatment.

Discussion

High frequency of shoot formation was achieved from *Momordica charantia* with 7 to 9 shoots explants in MS medium supplemented with 2.0mg/l BAP (Sultana R. S. and Ban M. A., 2003). The frequency of adventitious shoots was enhanced from stem segments of *Trichosanthes anguina* on MS medium fortified with BAP+NAA (Mustafa Md. and Mallaiah B., 1991). Multiple shoot regeneration of *Cucumis melo* as explants on the MS medium supplemented with 2.5mg/l L-Glutamic acid and 1.0mg/l BAP was obtained (Moreno V. M. *et al.*, 1985). The synergistic effect of L-Glutamic acid in combination with BAP in the formation of multiple shoots has been reported for other members of cucurbitaceae such as *Citrullus lanateus* and *Momordica charanitia*. Swelling of shoot bases were also accompanied by formation of adventitious buds (Sultana R. S. Ban M. A., 2003) (Sultana R. S. *et al.* 2004).

Fig. 1. Induction of callus and multiple shoots from nodal explants of *Momordica dioica* Roxb. (A)Enlargement and scanty callus induction from nodal explant on MSO. (B), (C) Induction of callus and few shoots. (D) Induction of multiple shoots on MS+ 2.0 mg/l BAP+1.0 mg/l L-Glutamic acid. (E), (F) High frequency of multiple shoot induction on

MS + 2.0mg/l BAP + 2.0 mg/l L- Glutamic acid. (G)Hardening of plant.

The present results are similar to Hoque *et al*., 1995. They found that a combination result of 1.5 mg/l L-Glutamic acid was more suitable for adventitious multiple shoot induction (Hoque *et al.,* 1982), where as in our investigation 2.0 mg/l BAP +2.0 mg/l L-Glutamic acid was to be the proved best for the production of multiple shoots. (fig..E, F). (Whener P. C. and Locky R., 1981) , achieved adventitious shoot formation from the callus of cotyledon culture of *Cucumis sativus*. Lee C. W. and Thomas J. C., 1985 succeeded by obtaining multiple shoots proliferation from shoot tips and stem nodes of *Cucurbita foetidissima* in MS medium supplemented with 1.0 mg/l BAP L-Glutamic acid. The level of sucrose in the medium significantly influenced the formation of shoot buds (Moreno V. M. et al., 1985). On the whole 3.5 gm/l of sucrose is proved to be better for the above results.

In our investigations, a novel method is developed by which multiple shoot can be induced on MS-medium supplemented with cytokinin (BAP) and amino acid (L-Glutamic acid). It is a simple one step protocol for the raped propagation of medicinally important wild herbs.

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