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OPTIMIZATION OF CULTURE MEDIUM FOR HIGHER MULTIPLICATION AND EFFICIENT MICRO PROPAGATION OF SPINE GOURD (*Momordica dioica* ROXB.)

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ABSTRACT

Spine gourd is diocious and perennial creeper which can conventionally propagate through seeds, vine cuttings and dividing tubers. The main difficulty in the conventional propagation is associated with the quality and storage of tubers; on the other hand, the micropropagation technique of spine gourd gives true to type plants in large numbers throughout the year. In present study, routine micropropogations method including MS medium for the entire experiment was used. Nodal explants were sterilized by 0.1% mercuric chloride for 10 minutes this was followed by three sterile distilled water wash. Sterile explants were inoculated in full strength MS medium supplemented with different combination and concentration of hormones i.e. 6-Benzyl amino purine (BA), Indole acetic acid (IAA) and Naphthalic acetic acid (NAA) for shoot multiplication. Results of present study revealed that supplementation of NAA (1mg/l) along with BA (1mg/l) induced vigorous and healthy shoots with highest (4.75 ± 0.25) mean no of shoots with average shoot length (cm) of around 5.10 ± 0.04. The regenerated shoots of 4 cm in length were used for rooting purpose. Half strength of MS medium with different concentrations of hormones i.e. Indole butyric acid (IBA), Indole acetic acid (IAA) and Naphthalic acetic acid (NAA) were also used for rooting. The highest rooting percent (86.67 %), number of roots per culture (9.6 ± 0.50) and root length (cm) (4.59 ± 0.09) with lowest percent callusing at cut ends (33.33 %) was reported in the treatment M2 (½ MS + IBA (2.0 mg/l). The well developed shoots with roots were deliberately transferred to the polythene small size glass containing equal mixture of soil and vermicompost. The established plants were finely transplanted in the field conditions.

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1 Introduction

Spine gourd (*Momordica dioica* Roxb.) is diocious perennial cripper (vine) and native of tropical Asia (Bharathi et al., 2007). It is an indigenous and distributed throughout India, China, Nepal, Bangladesh, Myanmar, Pakistan and Srilanka (Trivedi & Roy, 1972). It belongs to family *cucurbitaceae*. Tuberous roots (Patel et al., 2015) and herbaceous climbers (Ghive et al., 2006) are most important parts in the plant, which can be used for the propagation. Vegetative propagation of Spine gourd has various limitations such as unavailability of root tubers to the farmers as per their demand (Mondal et al., 2006) and has very low multiplication rate however there is a number of difficulty with seeds and supply of stem cutting late in the season as well (Rai et al., 2012). Tuberous root occupies cultivable land until next planting year (Ram et al., 2001). Further, seed germination rate of spine gourd also very low and it is close to 10%, moreover hardness of the seed coat creates obstacles in large scale cultivation (Raju et al., 2015**).** Further**,** it has prolonged seed dormancy period about 120-150 days and before flowering prediction the sex of seed grown plant is almost impossible The fruit is the main edible part of the plant and naturally only female plants bear the fruits, therefore there is an extreme need to have predetermined sex types of the plants. The stem cutting too takes too long time to make it available for large land area in fruiting season and only 36% of the plant germinate and stay alive (Ram et al., 2001). To overcome all these drawbacks, plant tissue culture is extremely desirable for commercial production of spine gourd with predetermined sex (Patel & Ishnava, 2015). With the help of various tools of Micropopagation, one can generate large quantities of true to type plants in short period at any given season. There are several reports in the literature indicated that shoots could be regenerated from callus (Hoque et al., 2007; Karim & Ahmed, 2010), however there is always chances of somaclonal variants. Therefore, nodal explants are the methods of choice to ensure clonal uniformity among the regenerants (Bopana & Saxena, 2008). Study has been carried out for the formulation of effective tissue culture media for the better and rapid growth of spine gourd tissue. Here in our study, optimization of the culture media for high multiplication of spine gourd has been carried out. For shoot multiplication media different combinations and concentrations of hormones i.e. 6- benzyl amino purine (BA), Indole acetic acid (IAA) and naphthalene acetic acid (NAA) are used. Similarly, for root development different concentration of auxins like Indole acetic acid (IAA), Indole butyric acid and naphthalene acetic acid are used to optimize the media for micropropogations of spine gourd.

2 Materials and method

2.1 Source of explants and decontamination treatment

Nodal explants with 6-7 nodes were collected from the Vegetable Research Station, NAU, Navsari, Gujarat, India. This experiment was carried out at tissue culture lab of Department of Plant Molecular Biology and Biotechnology ACHF, Navsari Agriculture University, Navsari. The nodal segment was treated with systemic antibiotics (Bavistin, Tween-20 and streptocyclin) periodically for one week prior to cutting vines. Spine gourd vines were washed with running tap water and cut with sterile blade. These internodal cuttings of spine gourd were treated with 0.1% mercuric chloride in laminar hood for 5 minute and this was followed by three washes of sterile distilled water to remove traces of mercuric chloride on internodal explants of spine gourd. One tendril with single node of about 3-4 cm were excised and placed on the sterile media bottle.

2.2 Optimization of Culture Medium

Spine gourd explants were inoculated in Mc Cartney bottle containing full strength of MS medium (Murashige & Skoog 1962) with 3% sucrose, 0.8% agar, pH 5.8 and different combinations and concentrations of plant growth hormones i.e. BA, IAA and NAA for shoot multiplication (Table 1). The culture was incubated at 25-27^{0}C for 16h light and 8h dark conditions. Culture was subculture after every three weeks on fresh medium containing same concentration of hormones (Table 2).

Table 1 Effect of different plant hormones on Percent response of spine gourd during continuous sub culturing.

T. No.	Plant hormones concentration (mg/l)	Percent response		
		Sub culture - 1	Sub culture - 2	Sub culture - 3
M1	BA (1.0) + NAA(0.1)	72.50	80.00	77.50
M2	BA (2.0) + NAA(0.1)	80.00	82.50	85.00
M3	BA (2.0) + NAA(0.2)	82.50	85.00	87.50
M4	**BA (1.0) + NAA(1.0)**	**87.50**	**90.00**	**92.50**
M5	BA (2.0) + IAA (0.2)	77.50	75.00	82.50

Table 2 Effect of different plant hormones on Number of shoots of spine gourd during continuous sub culturing.

T. No.	Plant hormones concentration (mg/l)	Number of shoots (Mean ± S.E)		
		Sub culture - 1	Sub culture - 2	Sub culture - 3
M1	BA (1.0) + NAA(0.1)	3.2 ± 0.2	3.5 ± 0.28	3.75 ± 0.25
M2	BA (2.0) + NAA(0.1)	3.8 ± 0.37	4.0 ± 0.40	4.25 ± 0.47
M3	BA (2.0) + NAA(0.2)	4.0 ± 0.44	4.5 ± 0.28	4.5 ± 0.5
M4	BA (1.0) + NAA(1.0)	4.4 ± 0.24	4.75 ± 0.25	4.71 ± 0.23
M5	BA (2.0) + IAA (0.2)	3.4 ± 0.24	3.25 ± 0.25	3.5 ± 0.28

Table 3 Effect of different plant hormones on Shoot length (cm) of spine gourd during continuous sub culturing.

T. No.	Plant hormones concentration (mg/l)	Shoot length (cm) (Mean ± S.E)		
		Sub culture - 1	Sub culture - 2	Sub culture - 3
M1	BA (1.0) + NAA(0.1)	3.44 ± 0.11	3.59 ± 0.05	3.68 ± 0.08
M2	BA (2.0) + NAA(0.1)	4.33 ± 0.13	4.57 ± 0.02	4.72 ± 0.08
M3	BA (2.0) + NAA(0.2)	4.53 ± 0.07	4.70 ± 0.09	4.90 ± 0.04
M4	BA (1.0) + NAA(1.0)	4.99 ± 0.06	4.91 ± 0.04	5.10 ± 0.04
M5	BA (2.0) + IAA (0.2)	3.54 ± 0.18	3.96 ± 0.03	4.12 ± 0.08

Table 4 Effect of different plant hormones on Number of nodes of spine gourd during continuous sub culturing.

T. No.	Plant hormones concentration (mg/l)	Number of nodes (Mean ± S.E)		
		Sub culture - 1	Sub culture - 2	Sub culture - 3
M1	BA (1.0) + NAA(0.1)	2.4 ± 0.24	2.68 ± 0.23	2.72 ± 0.24
M2	BA (2.0) + NAA(0.1)	2.8 ± 0.20	3.00 ± 0.40	3.07 ± 0.41
M3	BA (2.0) + NAA(0.2)	3.01 ± 0.31	3.65 ± 0.23	3.70 ± 0.23
M4	BA (1.0) + NAA(1.0)	3.61 ± 0.23	3.73 ± 0.24	4.02 ± 0.05
M5	BA (2.0) + IAA (0.2)	2.80 ± 0.20	2.83 ± 0.31	3.03 ± 0.02

2.3 Shoot initiation, proliferation and multiplication

Surface sterilized tendril was cultured distinctly on full-strength MS basal medium with different combination of hormones. After developing 3-4 nodes, the single node explants was again excised from the long primary vine and subculture onto shoot multiplication media. This was repeated for four times after every 21 days of incubation (Table 2, 3 & 4).

2.4 Root initiation

To study the rooting response, individual shoot of approximately 3-4 cm was excised and cultured on the rooting media having different concentration of IAA, IBA and NAA as mentioned in Table 5. For root initiation study, half strength MS media for entire rooting experiments and incubated them for about 21 days in culture room with standard culture condition.

2.5 Acclimatization

Well developed rooted plants were removed from the media washed with running tap water and dipped in the 0.1% $CaNO_3$ solution. They were transferred to plastic cups containing 1:1 mixture of sterilized cocopit and soil and kept in the polycarbonate house with optimum temperature and humidity. After getting primary hardening and sufficient height almost week

Table 5 Effect of different plant hormones on *in vitro* rooting response of spine gourd.

T. No.	Plant hormones concentration (mg/l)	% response to rooting	% Callusing at cut ends	No. of roots per culture (Mean ± S.E)	Length of longest root (cm) (Mean ± S.E)
M1	IBA (1.0)	76.67	26.67	8.4 ± 0.24	4.1 ± 0.10
M2	**IBA (2.0)**	**86.67**	**33.33**	**9.6 ± 0.50**	**4.59 ± 0.09**
M3	IBA (3.0)	70.00	46.67	6.2 ± 0.37	3.65 ± 0.09
M4	IAA (1.0)	60.00	36.67	8.0 ± 0.31	3.33 ± 0.03
M5	IAA (2.0)	50.00	53.33	7.0 ± 0.44	4.77 ± 0.05
M6	IAA (3.0)	46.67	63.33	6.0 ± 0.54	3.70 ± 0.02
M7	NAA(1.0)	43.33	40.00	6.6 ± 0.24	1.52± 0.04
M8	NAA(2.0)	43.33	56.67	5.2 ± 0.2	2.17 ± 0.11
M9	NAA(3.0)	36.67	66.67	4.2 ± 0.37	1.50 ± 0.01

Values are given as the Mean ± Standard error (SE) of 10 replicates per treatment.

later, the plants were kept in the green house for two weeks. These hardened plants are ready for transfer in the soil after 15-21 days of hardening in the green house.

3 Result and Discussion

3.1 Shoot initiation, proliferation and multiplication

Among the various tested combinations, altogether 90% response was reported in the hormone combination M4 viz., BA (1.0 mg/l) + NAA (1.0 mg/l) and this was followed by the hormonal combination M3 and M2 in Nodal segments of Spine gourd (Table 1, Figure 1). Finding of present study are in the agreement of previous researches which suggested that the use of BA and NAA in combination gave good regeneration in spine gourd (Hoque et al., 1995; Hoque et al., 2000; Nabi et al., 2002; Mohammad & Shorif 2010; Rai et al., 2012; Patel et al., 2015). The frequency of callus formation increased with respect to increasing the concentration of BA and it was well reported that high rate of callus formation hindered the growth and lead to the formation of somaclonal variants therefore minimum concentration of BA was used in present study. In contrast to the finding of present study, Rai et al. (2012) obtained 100 % shoot regeneration from the female genotype on MS medium supplemented with 0.9 µM BA and 200 mg l^{-1} casein hydrolysate (CH). In the present study NAA (1mg/l) along with BA (1mg/l) induced vigorous and healthy shoots with highest 4.75 ± 0.25 mean number of shoots (Table 2) with average shoot length (cm) of 5.10 ± 0.04 in M4 (Table 3) and this was followed by M3 and M2 treatments which was found significant previously with culture establishment. On contrary, Rai et al. (2012) obtained 6.2 shoots per explant with average shoot length of 3.4 cm from the female genotype, which was significantly higher than the results of present study. Moreover, Patel et al. (2015) reported that BA with 0.5 mg/l gave 2.7±0.30 cm shoot length and 2±0.83 shoot number. Nabi et al. (2002) used same explant and observed better shoot initiation ad proliferation response with 1 mg/l BA concentration. The results of the Mohammad & Shorif (2010) indicated that, the nodal explants were more capable of producing multiple shoots compared to other explants. In contrary with Patel et al. (2015), who observed 1.0 $mg1^{-1}$ BA + 0.1 $mg1^{-1}$NAA as best combination to get maximum number of shoots and longest shoot, in present study, maximum growth was observed with NAA 1mg/l along with BA 1mg/l. Jadhav et al. (2015) standardized of procedure for shoots and root initiation in Spine

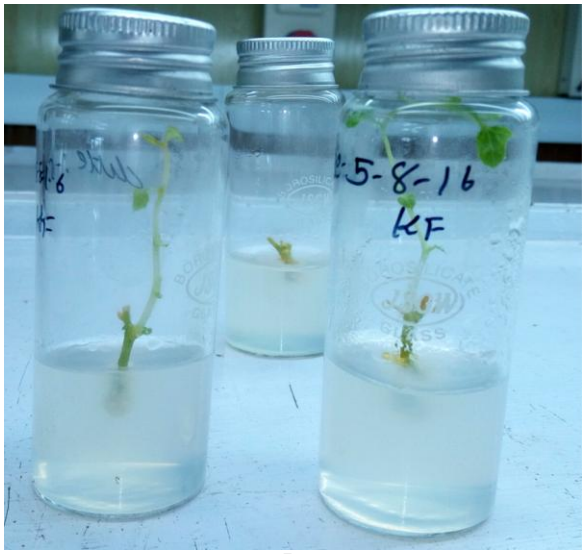
Figure 1 Shoot initiation from nodal segment during culture initiation of (Kankoda) (*Momordica dioica*).

Gourd (*M. dioica Roxb*.). They concluded that, MS + 1.0 mg/l BA + 0.2 mg/l NAA medium gave early shoot, highest shoot length and higher number of shoots. According to them, the shoot length of 3 to 4 cm is ideal for root induction. Although Karim & Ahmed (2010) reported 5.1 shoots per nodal explant of *M. dioica* but they used somatic embryogenesis rather than the nodal segment. Ghive et al. (2006) showed that MS medium with BA 1.5 mg. l^{-1} + NAA 1.0 mg.l^{-1} gave good response for establishment and initiation of explant. Addition of AdSO4 (70mg.l^{-1}) + BA (1.0 mg. Litre-1) + NAA (1.0 mg. Litre-1) in shoot regeneration medium greatly increased the production of multiple shoots. Sub culturing of shoot was carried out on the same media containing BA (1mg/l) and NAA (1 mg/l) after every 21 days of inoculation. The shoot numbers were increased with increasing the numbers of subcultures. There were 4.02 ± 0.05 nodes of spine gourd during continuous sub culturing after 3rd sub culture cycle in spine gourd (Table 4 Figure 2 A, B, C). Ajithkumar & Seeni (1998) reported that subculturing of *Aegle marmelos* nodal and leaf explants gave continuous production of healthy callus free shoots through 5th cycle without any decline sign of culture.

3.2 Root induction and acclimatization

There is universal law regarding efficiency of hormone and nitrogen content in the medium. The nitrogen supplements are generally added less in concentration in the medium when the aim is to target rooting response (Driver & Suttle, 1987). The reduced concentration of macro and micro nutrients are used to get better rooting response (Andrade et al., 1999) with less callusing at cut end. Rooting Effect of different plant hormones on *in vitro* rooting response of spine gourd (Kankoda) (*Momordica dioica*) was studied by incorporating IBA/IAA/NAA in the MS basal medium. The highest percent rooting (86.67 %), number of roots per culture (9.6 ± 0.50), root length (4.59 ± 0.09) and lowest percent of callusing at cut ends (33.33 %) was observed in the treatment M2 (½ MS + IBA (2.0 mg/l) (Table 5, Figure 3). Frequency of callus formation increased with an increase in the concentration of growth hormones IBA or NAA. There was better rooting observed on half strength MS medium supplemented with IBA (2mg/l) further, it was associated with less callus formation and with highest number of roots. Ghive et al. (2006) obtained well developed roots with MS + AdSO4 (80 mg/l) + IBA (1mg/l). Jadhav et al. (2015) showed that MS medium containing 0.5 mg/l NAA was the most appropriate medium for the root initiation, moreover the highest root length was reported with MS + 1.0 mg/l IBA. Rai et al. (2012) pointed out that addition of NAA in the medium induced callus formation, in accordance to this; our results also indicated the similar pattern with NAA, IBA, and IAA for the rooting response. They used rooting treatments consisted of full- and half strength MS medium supplemented with 0.0 to 24.6µM IBA. In previous reports also, IBA proved to be the best

A



B

C

Figure 2 Proliferation of axillary shoots from (Kankoda) (*Momordica dioica*) nodal segment on MS medium supplemented with hormone.

Figure 3 Root regenerated from shoot cultured on half-strength MS medium containing IBA 2mg/l

Figure 4 In vitro regenerated plantlet of (Kankoda) (*Momordica dioica*) transferred into plastic cup.

for root regeneration of *M. dioica* (Hoque et al., 2007; Karim & Ahmed, 2010) rather present investigation contradicted with this claim and said that NAA with half strength MS medium had significant impact on frequency of root induction, % of callus formation, number of roots, and root length.

3.3 Hardening

Rooted plants with shoot length of 6 cm were transferred from culture bottles into plastic cups containing mixture of cocopit and sand. These hardened plants have been transfer in the soil after 21 days of hardening in the polycarbonate house with humidity controller. Ghive et al.(2006) used soil rite: cocopit (3:1) while Jadhav et al. (2015) used polythene bags containing sand, soil and FYM in 1:2:1 ratio, rather we had used only simple and cheap media for primary hardening purpose (Figure 4). This is close to highest reported 85 % survival of female x female clones of *M. dioica* (Hoque et al., 2007).

Conclusion

In the present investigation, NAA (1mg/l) and BA (1mg/l) adding together to induced vigorous and healthy shoots with highest shoot length. The half strength MS medium with IBA (2mg/l) gave very good response for rooting. The rooted plant was transferred to the soil for better acclimatization. This is a viable and commercial protocol for mass multiplication of spine gourd.

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Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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