ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 4 (3): 221-224, 2005
ISSN 1682-296X

In vitro Propagation of Pointed Gourd (*Trichosanthes dioica* Roxb.) from Shoot Tips

S.M. Abdul-Awal, Md. Jahangir Alam, Md. Raihan Ali, Md. Nabiul Hasan,
Sazzad Reza Basunia and S.M. Mahbubur Rahman
Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh

Abstract: *In vitro* propagation of *Trichosanthes dioica* Roxb. locally known as patal has been studied. Shoot tips were cultured aseptically on MS media supplemented with different concentrations of cytokinins (BA and 2-ip) and their combinations with NAA, GA_3 and CW. The effectiveness of BA was proved to be superior to that of 2-ip alone with respect to shoot proliferation. Combinations of 2-ip with CW and GA_3 showed better response than 2-ip alone. The frequency of multiple shoot formation was 100% for shoot tips in MS medium containing BA 1.0 mg L^{-1} and BA 1.0 mg L^{-1} + NAA 0.2 mg L^{-1}. A best result with BA was found when explants were cultured on MS medium supplemented with 1.0 mg L^{-1} BA where the average length was 15.90±0.90 mm. In combinations the highest length was 18.25±0.42 mm at 1.0 mg L^{-1} 2-ip +0.5 mg L^{-1} GA_3 and the highest number of shoots was 3.25±0.22 at 1.0 mg L^{-1} 2-ip+ 10% CW. Efficient rooting was achieved on MS+IBA 0.5 mg L^{-1}. *In vitro* raised plantlets were then transferred to potted soil.

Key words: Propagation, *Trichosanthes dioica*, shoot tips

INTRODUCTION

Pointed gourd (*Trichosanthes dioica* Roxb.) is one of the most nutritive cucurbit vegetables belong to the family Cucurbitaceae. It is a tropical perennial vegetable crop with origin in the Indian subcontinent. It is morphologically distinct from the other cucurbitaceous species due to its well-established dioecism and vegetative means of propagation. It holds a coveted position in the market during summer and rainy seasons. It is highly popular to the people due to its availability for eight months in a year (February-September)[1]. It grows as a vine. Flowers are tubular white with 16-19 days initiation to anthesis time for pistillate flowers and 10-14 days for staminate flowers. Stigma remains viable for approximately 14 h and 40-70% of flowers set fruit[2]. The fruit is edible and rich in vitamin and minerals (9.0 mg Mg, 2.6 mg Na, 83.0 mg K, 1.1 mg Cu and 17.0 mg S/100 g)[3]. It is purported that pointed gourd possesses the medicinal property of lowering total cholesterol and blood sugar. These claims are supported by preliminary clinical trials with rats[4] and rabbits[5,6]. The fruits are easily digestible and diuretic in nature. It also has antiulcerous effects[7].

T. dioica is multiplied through seeds, vine cuttings, stem cuttings and root cuttings[1]. Vines require training on some form of aerial support system to achieve maximum fruit production[8,9]. Singh[3] reported 14% higher yield on vines trained on bower system compared to those growing on the ground. Both pre-rooted and fresh vine cuttings are used for propagation. Propagation through seeds is not desirable due to poor germination and inability to determine the sex of plants before flowering and imbalanced male-female ratio[1]. Seed based populations have a tendency to give more male than female plants and in some cases the ratio goes up to 85:15[7], limiting their use as their utility ends with pollination. Due to dioecy and resulting cross-pollinated, the maintenance of true to type plant is another major problem. To propagate from root suckers, tuberous roots of pointed gourd are dug in the early spring, subdivided and replanted. Stem and root cutting requires bulk amount of vines/roots, which restricts their multiplication at commercial level[1]. The present investigation was carried out to establish the *in vitro* propagation techniques with the objectives of evaluations of *in vitro* growth responses of shoot tips of field grown mature crop and selection of suitable auxins and its concentration for inducing roots *in vitro*.

MATERIALS AND METHODS

The experiment was conducted at Plant Biotechnology Laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna,

Corresponding Author: Md. Jahangir Alam, 207, Chhatra Hall, Khulna University, Khulna-9208, Bangladesh

Bangladesh. Shoot tips were collected from field grow mature plants of pointed gourd for this study. Healthy, disease free and actively growing shoots having tips (1.5-2 cm) were collected from mature plant. The plant materials were washed thoroughly under running tap water in the laboratory to reduce the dust. Then surface sterilization induces treatment of the shoot tips with 1% Savlon for 10 min with constant shaking. Then the materials were washed 3-4 times with distilled water and were immersed in 0.1% $HgCl_2$ solution with shaking for 4 min under laminar airflow cabinet. To remove the trace of the sterilant the materials were then washed with sterile distilled water at least 4-5 changes of sterilized water. The shoot tips (1 cm) were prepared from the surface sterilized material and were used as explants. Media used for shoot proliferation were MS[10] medium supplemented with BA and 2-ip alone (0.5, 1.0, 2.0 and 3.0 mg L^{-1}) and 1.0 mg L^{-1} BA and 2-ip combined with NAA (0.05, 0.1, 0.2 and 0.5 mg L^{-1}). BA and 2-ip (0.5, 1.0, 2.0 mg L^{-1}) were also combined with 0.5 mg L^{-1} GA_3 and 10% CW. For root initiation, MS medium supplemented with IBA and NAA (0.1, 0.5, 1.0 and 2.0 mg L^{-1}). The pH was adjusted to 5.7-5.8 using an digital pH meter with the help of 0.1 N NaOH or 0.1 N HCl, which ever needed and 0.7% agar was added to solidify and dissolved by heating in microwave oven for 2-3 min. Each treatment consisted of 10 test tubes. The media were sterilized by autoclaving at 121°C for 20 min at 15-psi pressure. GA_3 was filter sterilized.

The elongated shoots (usable shoots) were excised from the proliferated cultures and each of the microcutting was transferred individually to the rooting media. Some of the and recultured to freshly prepared medium for multiplication of axillary shoots. Inoculated test tubes were maintained at 25±2°C under light illumination (3000 lux) provided from white cool fluorescent tubes. For root initiation, tubes were maintained at dark for 7 days and then transferred to light. Relative humidity of the culture room was maintained about 65% and photoperiod was maintained as 16 h light and 8 h dark. Data on different parameters from different treatments were recorded after 6 weeks of culture. In the table, the mean data of different replications of each treatment are accomplished by Standard error[11]. Complete plantlets were taken out from the test tubes and rinsed with tap water to remove the medium. Plantlets were then transferred to pot containing soil and sand. Some plantlets died due to fungal, bacterial contamination and dehydration. However, 65% plants survived and hardened properly and then transferred to soil (Fig. 4).

RESULTS AND DISCUSSION

Effects of cytokinins (BA, 2-ip) alone on axillary shoot proliferation: Generally shoot tips responded to single shoot induction when MS medium was supplemented with different concentrations of cytokinins (BA, 2-ip) alone. Shoot tips required 6-9 days to initiate axillary shoots. Shoot tips were cultured on MS medium supplemented with BA and 2-ip individually at concentrations of 0.5, 1.0, 2.0 and 3.0 mg L^{-1} for selecting optimum cytokinin concentration for the maximum shoot proliferation. The results obtained from this experiment clarify that BA is better for axillary shoot formation than 2-ip. The highest percentage (100%) of shoot formation and the highest growth rate (2.65±0.15 mm/week) was found at 1.0 mg L^{-1} BA. The highest length of shoot was 15.90±0.90 mm at 1.0 mg L^{-1} BA. On the other hand, best results with 2-ip were found when explants cultured on MS medium fortified with 3.0 mg L^{-1} 2-ip whereas the longest shoot was 7.33±0.31 mm. Similarly other concentrations (0.5, 2.0 and 3.0 mg L^{-1}) of BA showed better response on axillary shoot formation than same concentrations of 2-ip (Table 1).

Effects of cytokinins (BA and 2-ip) in combination with NAA, GA_3 and CW on axillary shoot proliferation: In this experiment explants from field grown mature plants were cultured on MS medium supplemented with one concentration of BA and 2-ip with different concentrations (0.05, 0.1, 0.2 and 0.5 mg L^{-1}) of NAA and different concentrations (0.5, 1.0 and 2.0 mg L^{-1}) of BA, 2-ip with 10% CW, 0.5 mg L^{-1} GA_3. Generally shoot tips required 5-8 days to initiate shoots.

The highest percentage (100%) of shoot formation was found at concentration of 1.0 mg L^{-1} BA+ 0.2 mg L^{-1} NAA (Table 2). The highest growth rate (3.04±0.07 mm/week) and the longest length (18.25±0.42 mm) of shoot was found at concentration of 1.0 mg L^{-1} 2-ip +0.5 mg L^{-1} GA_3 (Fig. 1) but numbers of shoot per explant (3.25±0.22) were found at concentration of 1.0 mg L^{-1} 2-ip + 10% CW. Combinations of 2-ip with GA_3 and CW were found to be the best than single application. Some combinations were responded to induce callus. The highest callus induction frequency (+++) was

Table 1: Mean±SE of effects of different concentrations of cytokinins (BA and 2-ip) alone in MS medium on shoot formation

Hormone concentration (mg L^{-1})	Days to shoot initiation	Average length of shoot (mm)	Average No. of shoots/ explant	Percent-age of shoot formation	Average growth rate (mm/week)
BA					
0.5	7.11±0.37	10.22±1.35	1.00±0.00	90	1.70±0.22
1.0	6.30±0.25	15.90±0.90	1.00±0.00	100	2.65±0.15
2.0	5.57±0.19	11.29±0.85	1.00±0.00	70	1.88±0.14
3.0	8.89±0.43	8.88±0.66	1.00±0.00	90	1.48±0.11
2-ip					
0.5	8.25±0.49	7.13±0.33	1.00±0.00	80	1.19±0.05
1.0	8.44±0.36	6.89±0.51	1.00±0.00	90	1.15±0.09
2.0	6.67±0.16	6.78±0.49	1.00±0.00	90	1.13±0.08
3.0	7.00±0.27	7.33±0.31	1.00±0.00	90	1.22±0.05

Table 2: Mean±SE of effects of different concentrations and combinations of cytokinin along with auxin, GA_3 and CW on shoot formation from shoot tips in MS medium

Hormone concentration (mg L^{-1})	Days to shoot initiation	Average length of shoot (mm)	Average No. of shoots/ explant	Percentage of shoot formation	Average growth rate (mm/week)	Callus formation frequency*
BA+NAA						
1.0+0.05	6.00±0.31	6.89±0.55	1.00±0.00	90	1.15±0.09	+
1.0+0.1	5.00±0.18	5.75±0.55	1.00±0.00	80	0.96±0.09	++
1.0+0.2	5.90±0.30	5.70±0.35	1.00±0.00	100	0.93±0.05	+++
1.0+0.5	5.00±0.22	6.11±0.29	1.00±0.00	90	1.02±0.05	+++
2-ip+NAA						
1.0+0.05	-	-	-	-	-	-
1.0+0.1	-	-	-	-	-	-
1.0+0.2	-	-	-	-	-	-
1.0+0.5	-	-	-	-	-	-
BA+GA_3						
0.5+0.5	6.57±0.34	12.57±1.48	2.86±0.13	70	2.10±0.25	-
1.0+0.5	7.00±0.00	11.00±0.00	3.00±0.00	10	1.83±0.00	-
2.0+0.5	7.50±0.25	10.00±0.00	3.00±0.00	40	1.67±0.00	+++
2-ip+GA_3						
0.5+0.5	5.50±0.18	15.50±1.64	2.50±0.18	80	2.59±0.27	-
1.0+0.5	5.38±0.17	18.25±0.42	2.50±0.18	80	3.04±0.07	-
2.0+0.5	5.78±0.26	11.67±0.52	2.33±0.16	90	1.95±0.09	-
Hormone conc. (mg L^{-1}) + cw						
BA+CW						
0.5+10%	6.2 ±0.28	7.60 ±1.61	3.20±0.15	70	1.27±0.27	++
1.0+10%	-	-	-	-	-	-
2.0+10%	-	-	-	-	-	-
2-ip+CW						
0.5+10%	6.33±0.27	12.33±1.91	2.33±0.72	30	2.06±0.32	-
1.0+10%	5.75±0.41	10.75±1.08	3.25±0.22	40	1.79±0.18	-
2.0+10%	7.67±0.27	9.00 ±2.87	2.00±0.47	30	1.50±0.48	-

*+ =10-30%, ++ = 40-50%, +++ = 60-100%

Table 3: Mean±SE of effects of different concentrations of auxins (IBA and NAA) in MS medium on root formation

Hormone concentration (mg L^{-1})	Days to roots initiation	Average length of root (cm)	Range of roots (lowest to highest)	Average No. of roots per explant	Average growth rate (cm/week)	Percentage of root formation
IBA						
0.1	14.25±0.41	1.35±0.08	02-20	7.50±3.63	0.23±0.01	66.66
0.5	14.3±0.32	4.53±0.64	14-80	52.70±5.18	0.75±0.11	100.00
1.0	10.8±1.53	6.25±0.34	118-150	130.00±5.09	1.04±0.06	83.33
2.0	8.55±0.23	3.83±0.34	150-180	172.78±3.34	0.64±0.06	90.00
NAA						
0.1	15.0±0.35	1.88±0.13	88-110	103.25±4.52	0.31±0.02	66.66
0.5	11.13±0.7	4.91±0.19	65-115	85.63±5.76	0.82±0.03	80.00
1.0	15.0±0.47	0.90±0.06	18-50	34.33±7.55	0.15±0.01	50.00
2.0	13.17±0.4	2.68±0.16	30-60	52.33±4.31	0.45±0.03	60.00

observed at 1.0 mg L^{-1} BA+0.2 mg L^{-1} NAA, 1.0 mg L^{-1} BA+0.5 mg L^{-1} NAA and 2.0 mg L^{-1} BA+ 0.5 mg L^{-1} GA_3.

In these combination treatments some explants swelled, became pale and only elongated. Some combinations i.e. 1.0 mg L^{-1} BA+ 10% CW, 2.0 mg L^{-1} BA +10% CW and 1.0 mg L^{-1} 2-ip + (0.05, 0.1, 0.2 and 0.5) mg L^{-1} NAA did not response.

In these treatments, the highest percentage (100%) of shoot formation was found at concentration of 1.0 mg L^{-1} BA+ 0.2 mg L^{-1} NAA. Debnath *et al.*[12] found 92% multiple shoot regeneration from shoot tips when cultured on a medium containing MS + 2 mg L^{-1} BAP + 0.1 mg L^{-1} NAA and they found the longest shoots was 8.6 cm for

Fig. 1: Photograph showing the growth and development of shoots from shoot tip cultured on MS medium with 1.0 mg L^{-1} 2-ip + 0.5 GA_3

Fig. 2: Photograph showing the development of roots from the base of shoot cultured on MS-medium with 0.5 mg L^{-1} IBA

Fig. 3: Photograph showing the development of roots from the base of shoot cultured on MS medium with 0.5 mg L^{-1} NAA

Fig. 4: Photograph after transfer into pot

shoot tips cultured on MS medium supplemented with 1.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA + 10 mg L^{-1} adenine sulphate (AS).

Rooting of *in vitro* manipulated shoots: Induction and development of roots at the base of *in vitro* grown shoots is an indispensable step to establish tissue culture derived plantlets on the soil. The auxins used in the rooting media were IBA and NAA at different concentrations (0.1, 0.5, 1.0 and 2.0 mg L^{-1}). The percentage of root formation, number of roots per shoot and the length of roots were recorded after 6 weeks of culture. While lowest percentage (50%) was found at 1.0 mg L^{-1} NAA (Table 3).

Effect of IBA: Generally 9-15 days were taken to initiate roots. The root formation varied from 66-100% in different concentrations of IBA. The highest percentage (100%) of roots formation was observed at 0.5 mg L^{-1} IBA (Fig. 2). The highest growth rate was 1.04±0.06 cm/week at 1.0 mg L^{-1} IBA and the lowest growth rate was 0.23±0.01 cm/week at 0.1 mg L^{-1} IBA. The highest number of roots/shoot (172.78±3.34) formed at 2.0 mg L^{-1} IBA whereas the lowest number of roots/shoot (7.50±3.63) found at 0.1 mg L^{-1} IBA. The highest root length (6.25±0.34 cm) was found at 1.0 mg L^{-1} IBA; while the lowest root length (1.35±0.08 cm) was found at 0.1 mg L^{-1} IBA.

Effect of NAA: Generally 12-15 days were taken to initiate roots. The root formation varied from 50-80% in different concentrations of NAA. The highest growth rate was 0.82±0.03 cm/week at 0.5 mg L^{-1} NAA and the lowest growth rate was 0.15±0.01 cm/week at 1.0 mg L^{-1} NAA. The highest number of roots/shoot (103.25±4.52) formed at 0.1 mg L^{-1} NAA whereas the lowest number of roots/shoot (34.33±7.55) found at 1.0 mg L^{-1} NAA. The highest root length (4.91±0.19 cm) was found at 0.5 mg L^{-1} NAA (Fig. 3), while the lowest root length (0.90±0.06 cm) was found at 1.0 mg L^{-1} NAA.

There are too much SE because the ranges of root numbers from lowest to highest varied greatly. Percentage of root formation was greater in IBA than NAA. Kumar *et al.*[1] reported that a very low concentration of IBA (0.49 μM) produced significantly higher number of roots per shoot and higher concentrations of IBA favored formation of malformed and thick roots. Debnath *et al.*[12] found efficient rooting was achieved on half strength of MS + 0.5 mg L^{-1} NAA.

REFERENCES

1. Kumar, S., M. Singh, A.K. Singh, K. Srivastava and M.K. Banerjee, 2003. *In vitro* propagation of pointed gourd (*Trichosanthes dioica* Roxb.) Cucurbit. Genetics Cooperative Report, 26: 74-75.
2. Singh, A.K., R.D. Singh and J.P. Singh, 1989. Studies on floral biology of pointed gourd (*Trichosanthes dioica* Roxb.). Vegetable Sci., 16: 185-190.
3. Singh, K., 1989. Pointed gourd (*Trichosanthes dioica* Roxb.). Indian Hortic., 33: 35-38.
4. Chandrasekar, B., B. Mukherjee and S.K. Mukherjee, 1988. Blood sugar lowering effect of *Trichosanthes dioica* Roxb. in experimental rat models. Intl. J. Crude Drug Res., 26: 102-106.
5. Sharma, G. and M.C. Pant, 1988. Effects of feeding *Trichosanthes dioica* (parval) on blood glucose, serum triglyceride, phospholipid, cholesterol and high density lipoprotein-cholesterol levels in the normal albino rabbit. Curr. Sci., 57: 1085-1087.
6. Sharma, G., M.C. Pant and G. Sharma, 1988. Preliminary observations on serum biochemical parameters of albino rabbits fed on *Trichosanthes dioica* (Roxb.). Indian J. Medical Res., 87: 398-400.
7. Som, M.G., T.K. Maity and P. Hazra, 1993. Pointed Gourd in Genetic Improvement of Vegetable Crops (Eds.) Kalloo, G. and B.O. Berg, Pergamon Press, Oxford, UK.
8. Prasad, V.S.R.K. and D.P. Singh, 1987. Effect of training on pointed gourd (*Trichosanthes diocia* Roxb.) for growth and yield. Progressive Hortic., 19: 47-49.
9. Yadav, J.P., K. Singh, R.C. Jaiswal and K. Singh, 1989. Influence of various spacings and methods of training on growth and yield of pointed gourd (*Trichosanthus dioica* Roxb.). Vegetable Sci., 16: 113-18.
10. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plants, 15: 473-497.
11. Mian, M.A. and M.A. Mian, 1984. An Introduction to Statistics. 4th Edn., Ideal Library Dhaka.
12. Debnath, R.K., S.K. Roy, G. Ahmed and M. Hossain, 2000. Micropropagation of patal (*Trichosanthes dioica* Roxb.) from nodal segment and shoot tip. Plant Tissue Cult., 10: 125-130.