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IN VITRO PROPAGATION OF POINTED GOURD *(Trichosanthes dioica* Roxb.) THROUGH ENCAPSULATED SHOOT TIPS

M. A. MALEK [1]

Abstract

Plants were regenerated from encapsulated shoot tips of pointed gourd. Shoot tips isolated from multiple shoot cultures of AM-8 and AM-15 cultivars of pointed gourd were encapsulated in sodium alginate beads. For germination and shoot proliferation, encapsulated shoot tips (artificial seed) were cultured in MS basal medium containing different concentrations and combinations of BAP and NAA. Use of MS medium resulted in 90% conversion of encapsulated shoot tips into plantlets. The results exhibited that BAP and combinations of BAP and NAA play an important role in germination of artificial seed being encapsulated by sodium alginate beads. The plantlets were successfully established in earthen pot. Under the present study, limited experimental efforts have been made to establish the protocol for encapsulating the shoot tips for the production of artificial seed and their subsequent regeneration. It is the first report in Bangladesh in developing artificial seed production technique using vegetative tissue of pointed gourd.

Key Words: *In vitor* propagation, pointed gourd, shoot tips.

Introduction

Pointed gourd is a popular, comparatively costly and delicious vegetable of high demand in Bangladesh. Fruits are rich in vitamins and minerals and contain 9.0 mg Mg, 2.6 mg Na, 83.0 mg K, 1.1 mg Cu and 17.0 mg S per l00g edible part (Singh, 1989). It has also high industrial value as different types of jam, jelly and pickles can be made from this vegetable. Pointed gourd has a good medicinal value. It is easily digestible, diuretic and laxative, invigorates the heart and brain and is useful in disorder of the circulatoiy system (Yawalker, 1969). It was reported that pointed gourd possesses the medicinal property of lowering the total cholesterol and blood sugar (Chandrasekar *et al.,* 1988; Sharma and Pant, 1988; Sharma *et al.,* 1988). Traditionally pointed gourd is mainly propagated by stem and root cuttings. Propagation through seeds is not desirable due to poor germination and imbalanced male-female ratio. Seed based populations have a tendency to give more male than female plants and in some cases the ratio goes upto 85: 15 (Som *et al.,* 1993). Maintaining stem and root cuttings quality in field conditions as well as to conserve it in storage is difficult. Additionally, due to dioecy and cross-pollination. the maintenance of true to the type plant from true seeds is another major problem . Stem and root cutting are labour intensive and also requires bulk amount of vines per root, which restricts their multiplication at commercial level.

[1] Plant Genetic Resources Centre, BARI, Joydebpur, Gazipur 1701, Bangladesh.

The production of artificial seeds by encapsulating either somatic embryos or vegetative parts like shoot tips or axillary buds have received considerable attention in recent years (Redenbaugh *et al.,* 1986; Bapat *et al.,* 1987; Bapat and Rao, 1988, 1990: Furamanowa *et al.,* 1991). Many workers have encapsulated and germinated the axillary buds (Bapat *et al.,* 1987). These artificial seeds can be used for specific purposes, notably multiplication of non-seed producing plants, ornamental hybrids or the propagation of polyploid plant with elite traits. Cryopreserved artificial seeds may also be used for germplasm preservation, particularly in recalcitrant species (such as mango, jackfruit, cocoa and coconut), as these seeds will not undergo desiccation. The present study describes the production of artificial seeds from encapsulation of shoot tip through solid cultures. The result indicated that this method provides a mechanism for handling tissue cultured plants in a manner similar to that of seed grown ones.

The concept of somatic seed formation has been extended to cover the possibilities of production of artificial seeds through encapsulating all types of regenerative tissues for developing true parent plants. The main objective of this study was to develop a procedure for the production of artificial seeds. Under the present investigation, attempts were taken to develop the avenue for the production of artificial seeds by encapsulating highly regenerative shoot tips of pointed gourd genotypes.

Materials and Method

The experiments were conducted in the Tissue Culture Laboratory, Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur 1701. Shoot tips of 3-4 mm long were aseptically excised from *in vitro* cultured plants of pointed gourd genotypes regenerated by the method described by Maruyama (1996). Sodium alginate beads were produced by encapsulation according to the method of Kinoshita and Saito (1990). The methods involved in this investigation were described under the following separate heads:

Culture media preparation

Culture media were prepared following MS (Murashige and Skoog, 1962) medium preparation technique and 8 gram agar was added to each 1000 ml solution and then autoclaved for 20 minutes under 121° C.

Preparation of Alginate and $CaCI_2$ solution

Media preparation

200 ml MS medium was prepared. 8.0 g (4%) sucrose was first added to 150 ml of MS solution and then different concentrations and combinations of BAP (0.5,

1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/I) was also added to it. After mixing the solution, it was made up to 200 ml.

Alginate solution

20 ml of above mentioned solution (MS + Sucrose + hormones) was taken and 0.8 g of sodium alginate was added in a 50 ml beaker. With a small piece of glass rod efforts were made to mix the alginate in solution. During autoclaving, alginate was completely dissolved and the mixture was stored at 4 °C for further use.

$CaCI_2$ solution

50 ml of above mentioned solution (MS + Sucrose + hormones) was taken in a small beaker. An amount of 0.7 g $CaC1_2$ was added to it and dissolved. 70 ml of mixture (50+20) was used during the preparations of alginate and $CaCI_2$ solution and another 130 ml was kept reserved. After autoclaving, it was used during washing the encapsulated explants.

Autoclaving

Culture media, alginate solution, $CaC1_2$ solution, several petridishes and the remaining 130 ml (MS+Sucrose+hormones) solution were autoclaved, for 20 minutes under 121^0C.

Encapsulation

Sodium alginate beads were produced by encapsulation according to the method of Kinoshita and Saito (1990). Only *in vitro* grown plants were used for this experiment. Shoot tips explants were taken in an autoclaved petridishes and cut carefully. The excised shoot tips were placed to the beaker containing sodium alginate solution. The explants were dipped in alginate solution and kept inside the solution for about 30 minutes. The dipping explants were taken by a forceps and placed to the beaker of $CaCI_2$ solution. After 30 minutes, each explant becomes a hard ball encoded by alginate. They were then washed well with remaining solution of MS + Sucrose + hormones (BAP, NAA and combinations of BAP and NAA).

After washing, the encapsulated shoot tips were cultured in culture tubes containing MS medium supplemented with different concentrations of BAP (0.5, 1.0, 1 .5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l) and combinations of BAP and NAA and also on various substrates such as sterile soil, cotton and filter paper moistened with $^1/_2$ MS solution. The germination responses of the encapsulated shoot tips were scored after three weeks of culture. For each treatment, 20 shoot tips were tested and all the experiments were conducted

under controlled conditions of light (1000 lux), temperature (25±2°C) and relative humidity (50-60%).

Results and Discussion

Shoot tips were taken from *in vitro* grown plantlets for artificial seed production. Two genotypes of pointed gourd (AM-8 and AM-15) were used to exhibit different levels of morphogenic growth response in tissue culture media. The encapsulated shoot tips were cultured in MS (Murashige and Skoog, 1962) agar gelled media supplemented with different concentrations and combinations of BAP (0.5, 1.0, 1.5 and 2.0 mg/I) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l) to investigate the induction of shoot regeneration. Data on days to shoot proliferation and percentage of shoot forming explants were recorded after culture of encapsulated shoot tips. The results are presented under the following separate heads:

Encapsulation of shoot tips in sodium alginate

In the present study, various concentrations of sodium alginate were tested to find out the optimum level of encapsulation for production of artificial seeds. Among the various concentrations of sodium alginate tested, best results were obtained with 4% alginate (Table 1). Higher and lower levels of sodium alginate reduced the conversion frequency. Redenbaugh *et al.* (1987) noted that variables related to encapsulation method, including alginate type and concentration, medium and methods used to produce the synthetic seeds were responsible for significant variations in conversions percentages for alfalfa, carrot and celery. The present finding is similar with the report for artificial seed production in coriander (Stephen and Jayabalan, 2000).

Table 1. Effect of different concentrations of sodium alginate on germination of artificial seed of pointed gourd.

Sodium alginate (%)	No. of seeds cultured	No. of seeds germinated	% of seeds germination
3	40	16	40
4	40	38*	95*
5	40	12	30
Mean	40.00	22.00	55.00
SE (±)	0.000	8.083	20.207

*, ** Significant at 5% and 1% level, respectively.

Artificial seed regeneration derived from shoot tips

In the present investigation, encapsulated shoot tips of *in vitro* grown plantlets were cultured in MS media supplemented with different concentrations and

combinations of BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l). Shoot proliferations began from 7 to 9 days. Data were recorded after 4 weeks of culture. Results obtained on morphogenic response of the cultured

Table 2. Effect of different concentrations and combinations of BAP and NAA on artificial seed proliferation from shoot tip explants of AM-8 and AM-1*5* genotype.

Treatments (mg/l)	AM-8		AM-15	
	Days to shoot proliferation	% of shoot forming explants	Days to shoot proliferation	% of shoot forming explants
BAP 0.5	7	75	7	80*
BAP 1.0	7	85**	7	90**
BAP 1.5	7	70	7	72
BAP 2.0	7	*55*	7	58
Mean	7.00	71.25	7.00	75.00
S E (±)	0.000	6.250	0.000	6.757
NAA 0.5	8	65*	8	70*
NAA 1.0	8	70**	8	75**
NAA1.5	8	45	8	47
NAA 2.0	8	30	8	30
Mean	8.00	52.50	8.00	55.50
S E (±)	0.000	9.242	0.000	10.460
BAP + NAA				
1.0+0.5	8	*55*	8	60
1.0+ 1.0	8	75**	8	80**
1.0+ 1.5	8	50	8	55
1.0+2.0	8	30	8	35
Mean	8.00	52.50	8.00	57.50
S E (±)	0.000	9.242	0.000	9.242
NAA + BAP				
1.0+0.5	9	50	9	60
1.0+ 1.0	9	75**	9	80**
1.0+1.5	9	50	9	*55*
1.0+2.0	9	40	9	40
Mean	9.00	*53.75*	9.00	58.75
S E (±)	0.000	7.465	0.000	8.260

*, ** Significant at 5% and 1% level, respectively.

explants are shown in Table 2. Morphogenic responses of the encapsulated explants were found to vary with hormonal formulations present in the culture media. Shoot regeneration was observed for all the treatments and genotypes (AM-8 and AM-15) of pointed gourd under study. In case of AM-8, shoot proliferation ranged from 30 to 85%. Highest percentage (85%) of shoot proliferation was observed in MS medium containing 1.0 mg/I BAP followed by MS + 1.0 mg/l BAP + 1.0 mg/l NAA (75%). The lowest percentage (30%) of shoot formation was observed in MS + 2.0 mg/I NAA and MS + 1.0 mg/l BAP + 2.0 mg/l NAA.

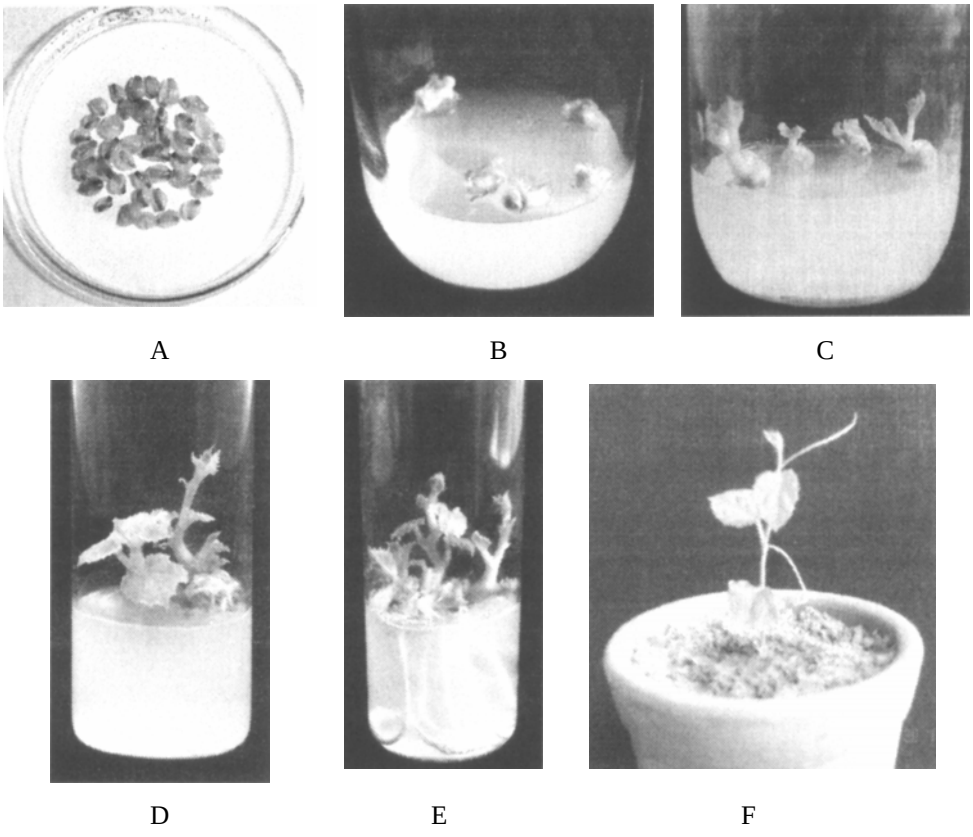

Fig. 1. Regeneration of encapsulated shoot tips (artificial seeds) of pointed gourd.

A. Encapsulated shoot tips in sodium alginate bead
B. Germination of encapsulated shoot tips
C. Shoot induction of encapsulated shoot tips
D. Shoots from encapsulated shoot tips
E. Rooted multiple shoots of encapsulated shoot tips
F. Established plant in earthen pot

In case of AM-15, shoot proliferation ranged from 30 to 90%. Among the different concentrations and combinations of BAP and NAA, the highest 90% shoot formation was observed in MS medium containing 1.0 mg/l BAP followed by 80% shoot proliferation that was obtained in MS + 0.5 mg/l BAP and MS + 1.0 mg/l BAP + 1.0 mg/l NAA. The lowest percentage (30%) of shoot formation was observed in MS + 2.0 mg/l NAA.

Plantlet development on various substrates

Encapsulated shoot tips were also cultured on different substrates, such as cotton, sterile soil and filter paper stripes moistened with $^{1}/_{2}$ strength MS salts and also in MS media. Plantlet formation was also noticed on these substrates, but in general, the frequency was low compared to plantlet development on MS media (90%). Among these substrates, the percentage of plantlet formation was comparatively good on moist cotton (35%) followed by filter paper (30%). No regeneration was noted on sterile soil as all the encapsulated shoot tips dried completely within a week (Fig. 2). Encapsulated shoot tips directly sown in petridishes containing autoclaved soil showed no emergence of shoots within a week. Possibly the nutrients in the matrix may not be sufficient for the encapsulated shoot tips to develop a balanced shoot and root system.

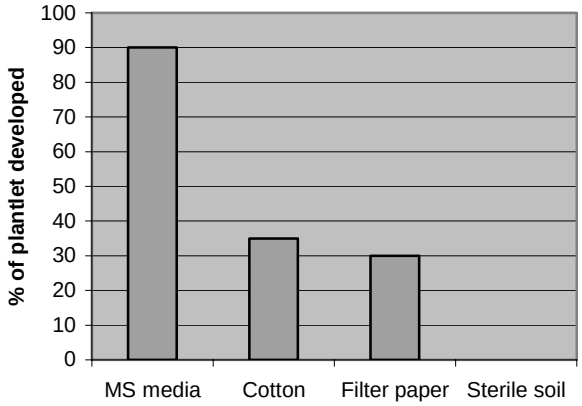

Fig. 2. Development of plantlet form encapsulated shoot tips of pointed gourd in different substrates.

Plantlet formation and their establishment in the soil

The regenerated healthy rooted plantlets were replaced from culture room and kept in room temperature (20-25°C). Plantlets were carefully removed from the culture vessels. After thoroughly washing the roots in tap water to remove the

traces of nutrients, the rooted plantlets were placed in plastic pots containing unsterilized garden soil and cow manure (1:1). The transplanted plantlets were covered with a transparent polyethylene bag (size 12 × 20 cm) to maintain high humidity. The plantlets were hardened in a green house for three weeks before transferring in the earthen pot.

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