Bangladesh J. Genet Pl. Breed., 21(1): 15-20, 2008

ARTIFICIAL SEED PRODUCTION IN POINTED GOURD (*TRICHOSANTHES DIOICA* ROXB.)

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ABSTRACT

Encapsulation of nodal segments was successfully developed for pointed gourd towards the formation of artificial seed with sodium alginate. Encapsulated nodal segments (artificial seed) were cultured in MS basal medium containing different concentrations and combinations of BAP and NAA to induce germination and shoot proliferation. Highest (95%) shoot formation was obtained in MS + 1.0 mg/l BAP followed by MS + 0.5 mg/l BAP. The encapsulated nodal segments also regenerated *in vitro* on different substrates. Frequency of plantlet formation was low on these substrates compared to plantlet development on MS media. Among these substrates, the percentage of plantlet formation was better on moist cotton (42%) followed by filter paper (35%). The hardened plants were transferred successfully to soil in the earthen pots. The protocol for encapsulating the nodal segments for the production of artificial seeds and their subsequent regeneration is a new area of research to develop *in vitro* conservation strategies for pointed gourd.

Key words: Pointed gourd, plant propagation, artificial seed

INTRODUCTION

Plant propagation using artificial or synthetic seeds developed from somatic tissue but not zygotic embryos opens up new vistas in plant biotechnology. Artificial seeds make a promising technique for propagation and conservation of vegetatively propagated crops with elite traits and those having problems in seed production. Traditionally pointed gourd is cultivated through stem and root cuttings. Propagation through seeds is not desirable due to poor germination, imbalanced male-female ratio and heterozygous form of heritable traits. Seed propagated plants normally do not produce flowers within one year, if they do, it requires more than one year for fruit setting. *In vivo* traditional vegetative propagation techniques are time consuming and expensive and the propagules carry the inborn diseases and pests from the mother plants. Development of artificial seed production technology is currently considered as an effective and efficient alternative method of germplasm conservation and propagation in commercially important agronomic and horticultural crops those can not be propagated through conventional seed

The artificial seeds would be powerful tool for mass propagation of such elite plant species with high commercial value (Saiprasad, 2001). Recent advances in this area have revealed that besides somatic embryos, encapsulation of cells and somatic tissues obtained from tissue culture techniques has become popular as a simple way of handling cell and tissue,

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protecting them against strong external gradients and as an efficient delivery system (Datta *et al*., 1999). The most suitable encapsulating agent used is sodium alginate due to its solubility at room temperature and its ability to form completely permeable gel with calcium chloride (Sharma *et al.,* 1992). The present study describes the production of artificial seeds from encapsulation of nodal segments of pointed gourd through solid cultures. This method provides a mechanism for handling tissue cultured plants in a manner similar to that of plants grown from seeds.

MATERIALS AND METHODS

The experiments were conducted in the Tissue Culture Laboratory, Biotechnology Division, BARI, Joydebpur, Gazipur 1701. Nodal segments (3-4 mm) were aseptically excised from *in vitro* grown plantlets of two pointed gourd cultivars (AM-8 and AM-15) regenerated by the method described by Maruyama (1996). Data on days to shoot proliferation and percentage of shoot forming explants were recorded after culture of encapsulated nodal segments.

Culture media preparation

Culture media were prepared following MS (Murashige and Skoog, 1962) media and 8.0 gram agar was added to each 1000 ml solution.

Preparation of Alginate and $CaCl_2$ solution

Media preparation

To prepare 200 ml MS medium 4% sucrose was first added to 150 ml of 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/l) were added to it. After mixing the solution was filled up to 200 ml.

Alginate solution

20 ml of the above mentioned solution (MS + Sucrose + hormones) was taken in a 50 ml beaker and 0.8 gm of sodium alginate was added and mixed with a glass rod. The alginate was completely dissolved during autoclaving

$CaCl_2$ solution

50 ml of above mentioned solution (MS + Sucrose + hormones) was taken in a small beaker. An amount of 0.7 gm $CaCl_2$ was added to it and dissolved. Out of 200 ml, 70 ml (50+20) was used during the preparations of alginate and $CaCl_2$ solution and remaining 130 ml was used for washing the encapsulated explants. Culture media, alginate solution, $CaCl_2$ solution and the washing solution were autoclaved for 20 minutes under 121^0 C.

Encapsulation

Sodium alginate beads were produced by encapsulation according to the method of Kinoshita and Saito (1990). Only *in vitro* grown plantlets were used for this experiment. *In vitro* plantlets were taken in autoclaved petridishes and nodal segments were cut carefully. The excised nodal segments with active buds were placed in a beaker containing sodium alginate solution. The explants were kept in the solution for about 30 minutes. Then they were placed in the beaker of $CaCl_2$ solution with a pair of forceps. During picking the forceps also took some alginate solution together with the explants. After 30 minutes each

explant become a hard ball encapsulated by alginate. They were then washed well with remaining solution of MS + Sucrose + growth regulators. After washing, the encapsulated nodal segments were cultured in culture tubes containing MS medium 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) and also on various substrates such as sterile soil, cotton and filter paper moistened with half strength MS solution. The germination responses (viz. development of plants) of the encapsulated nodal buds were scored after three weeks of culture. For each treatment 20 nodal buds were cultured and all the experiments were conducted under controlled conditions of light (1000 lux), temperature (25 ± 2^0C) and relative humidity (50-60%).

RESULTS AND DISCUSSION

Regeneration of artificial seed derived from nodal segment

Morphogenic responses of the encapsulated nodal segments were found to vary with hormonal formulations present in the culture media (Table 1). Shoot proliferations began from 5-8 days. Shoot regeneration from encapsulated nodal buds were observed in all MS media combinations. In case of AM-8, shoot formation ranged from 35-90%.

Table 1. Effect of different concentrations and combinations of BAP and NAA on artificial seed proliferation from nodal segment explants of AM-8 and AM-15 cultivar

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	5	80	5	90**
BAP 1.0	5	90**	5	95**
BAP 1.5	5	70	5	75
BAP 2.0	5	65	5	70
Mean	5.00	77.50	5.00	82.50
S E (±)	0.000	5.204	0.000	5.951
NAA 0.5	7	65*	7	70*
NAA 1.0	7	70*	7	75*
NAA 1.5	7	55	7	60
NAA 2.0	7	35	7	35
Mean	7.00	56.25	7.00	60.00
S E (±)	0.000	7.739	0.000	8.897
BAP + NAA				
1.0 + 0.5	7	60	7	60
1.0 + 1.0	7	80**	7	85**
1.0 + 1.5	7	40	7	50
1.0 + 2.0	7	35	7	30
Mean	7.00	52.50	7.00	55.00
S E (±)	0.000	9.242	0.000	10.408
NAA + BAP				
1.0 + 0.5	8	55	8	65
1.0 + 1.0	8	80**	8	85**
1.0 + 1.5	8	50	8	55
1.0 + 2.0	8	40	8	45
Mean	8.00	55.00	8.00	61.25
S E (±)	0.000	7.359	0.000	7.465

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

Table 2. Effect of substrates on plantlet development from encapsulated nodal segment of pointed gourd

Substrates	No. of seeds cultured	No. of plantlet developed	% of plantlet developed
MS media	40	38**	95**
Sterile soil	40	0	0
Cotton	40	17	42
Filter paper	40	14	35
Mean	40.00	17.25	43.00
S E (±)	0.00	7.846	19.617

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

The highest percentage of shoot formation was observed in MS medium containing 1.0 mg/l BAP followed by MS medium containing 0.5 mg/l BAP and 1.0 mg/l BAP + 1.0 mg/l NAA (Table 1). The lowest percentage of shoot formation was observed in MS + 2.0 mg/l NAA and MS + 1.0 mg/l BAP + 2.0 mg/l NAA. While in cultivar AM-15, shoot formation ranged from 30-95%. Among the different concentrations and combinations of BAP and NAA, the highest of shoot formation was observed in MS medium having 1.0 mg/l BAP followed by MS + 0.5 mg/l BAP. The lowest percentage of shoot formation was observed in MS + 1.0 mg/l BAP + 2.0 mg/l NAA (Table 1).

In the present study, MS + 1.0 mg/l BAP gave the best result in both the cultivars of pointed gourd. The experimental results indicated that the cultivar AM-15 showed better performance than AM-8 for artificial seed regeneration. The results also indicated that different concentrations and combinations of both BAP and NAA play an important role in germination of artificial seeds of pointed gourd.

Plantlet development on various substrates

Encapsulated nodal segments were also cultured on different substrates such as cotton, soil and filter paper stripes moistened with half strength MS solution. Plantlet formation was noticed on these substrates, but in general, the frequency was low compared to plantlet development on MS media. Among these substrates, the percentage of plantlet formation was comparatively better on moist cotton (42%) followed by filter paper (35%). This result is in agreement with the findings of Ganapathi *et al*. (1992) in banana. No regeneration was observed on soil as all the encapsulated nodal segments dried completely within a week (Table 2). Encapsulated nodal segments directly sown in petridishes containing autoclaved soil showed no emergence of shoots within a week. Possibly the nutrients in the matrix was not sufficient for the encapsulated nodal segments to develop into a complete shoot and root system.

Establishment of plantlet in the soil

The regenerated healthy rooted plantlets were transferred from culture room and kept in room temperature (20-25^{0}C) for 7 days. Plantlets were carefully removed from the culture vessels and after thorough washing of roots in tap water; the plantlets were placed in plastic pots containing garden soil and cow manure (1:1). The transplanted plants were covered with a transparent polyethylene bag (size 12 x 20 cm) to maintain high humidity. The plants were hardened in a green house for three weeks before transferring to the earthen pots. Artificial seed regeneration from nodal segments of pointed gourd is shown in Fig 1.

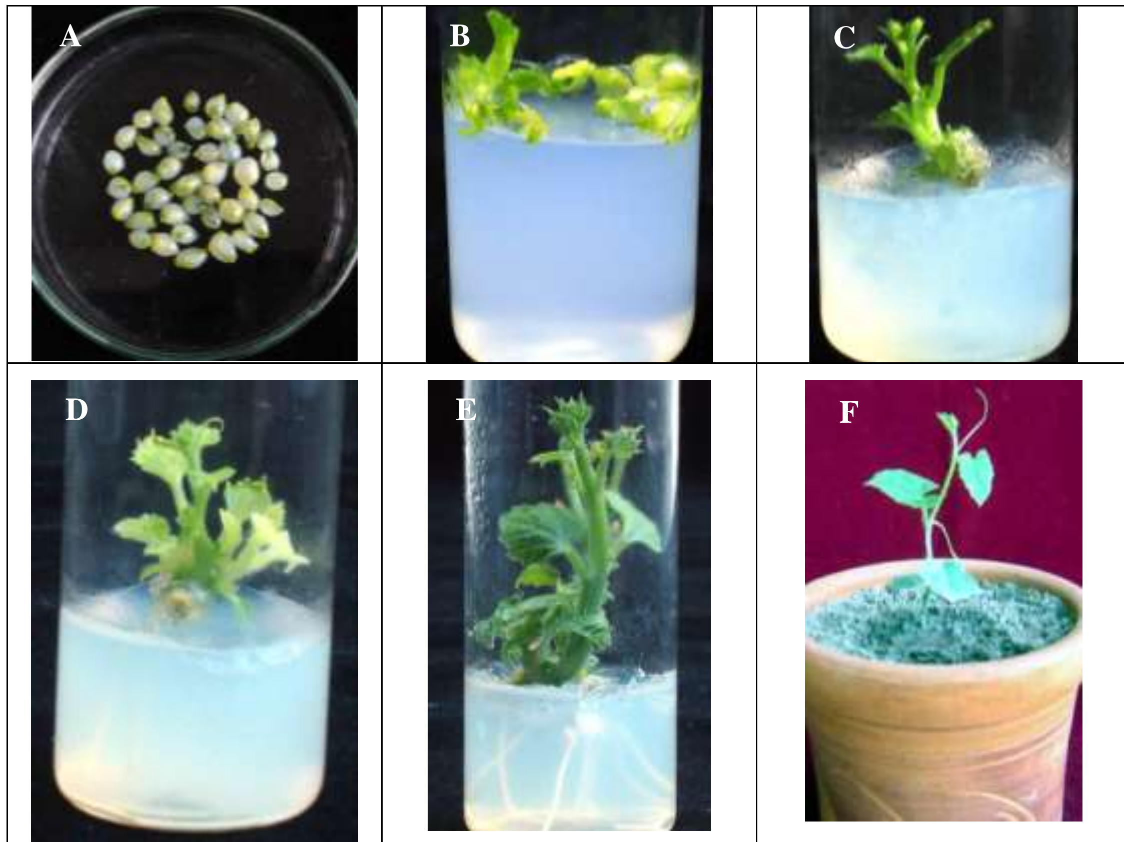

Figure 1. Artificial seed regeneration from nodal segments of pointed gourd. Encapsulated nodal segments in sodium alginate bead (A); Germination of artificial seeds (B); Shoot induction of artificial seeds (C). Multiple shoots from artificial seeds (D); Rooted multiple shoot of artificial seeds (E) and Established plant in earthen pot (F).

In the present investigation, nodal segment was successfully encapsulated by sodium alginate to perform as artificial seed beads. Artificial seeds developed from nodal segments were also subjected to hormonal treatments in order to enhance germination. It is the first report in Bangladesh to develop artificial seed production using vegetative tissue of a crop plants.

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