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Effect of Carbon Source in Alginate Bead on Synthetic Seed Germination in Eggplant (*Solanum melongena* L.)

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Abstract: This study describes the investigation on the synthetic seed technology in two varieties (Loda and China) of eggplant with a particular focus on the carbon source in seed bead. To assess exact type and concentration of carbon source, three types of carbon sources (viz., sucrose, mannitol and sorbitol) and their different concentrations were used in alginate seed bead to achieve the optimum germination of synthetic seed on MS_0. Among the different carbon sources, sucrose showed better performance for both varieties when nodal segment with bud was used as explants. But when somatic embryos were used as explants sucrose + sorbitol (1:1) was found to be more efficient for both varieties in germination of synthetic seeds. On the other hand, among the different concentrations 1% sucrose gave good result for the germination of synthetic seed made of nodal segment. In case of somatic embryo 3% sucrose showed best performance for both varieties.

Key words: Synthetic seed, sodium alginate, encapsulation, eggplant, carbon source

INTRODUCTION

Numerous techniques are now available for rapid and large-scale commercial multiplication of elite and desirable plant species *in vitro*. Somatic embryogenesis can provide a more efficient method for mass propagation. In order to make the system economically viable many workers have encapsulated the somatic embryo (Radenbaugh *et al*., 1986). In some instances, instead of embryos, axillary buds also have been encapsulated and germinated (Machii, 1992; Kinoshita and Satto, 1990). Encapsulation of embryos to produce synthetic seeds could serve as a substitute for the seeds produced *in vivo*. Eggplant is only propagated by seeds without having opportunity of asexual propagation. Attempts at crossing eggplant with its wild relatives resulted in limited success due to sexual incompatibilities. However, the ability of eggplant to response well in plant tissue culture, notably plant regeneration, has allowed the application of Biotechnology, particularly the exploitation of somaclonal variation, haploidization, somatic hybridization and gene transfer.

Synthetic seed technology offers many useful advantages towards commercial application of these biotechnological approaches. The resultant plant population from the synthetic seed will be uniform and the direct delivery of somatic embryos will save many subcultures to obtain plantlets from regenerated embryos. The encapsulated embryos could also be packed with pesticides, fertilizers, nitrogen fixing bacteria (Redenbaugh *et al*., 1986; Ohishi *et al*., 1995). Concentration and source of carbon in the bead medium has been found to affect photosynthetic potentiality of plant growing from synthetic seed. But Hyndman *et al*. (1982) and Landford and Wainwright (1988) reported that high concentration of sucrose cause to reduce the photosynthetic activity and germination rate may be decreased.

In this present study an attempt was made to assess the influence of carbon source in seed bead medium on synthetic seed germination and *in vitro* shoot development.

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MATERIALS AND METHODS

The experiment was conducted in the Institute of Biological sciences, Rajshahi University, Bangladesh in 2006.

Induction of Explant

In this experiment, two varieties of *Solanum melongena* viz. Loda and China were used and the seeds of these two varieties were cultured on MS0 (Murashig and Skoog without hormone) medium for seedlings and seedlings were cultured on nutrient medium. Nodal segment with axillary bud formed within 30 days of culture which was used as explants following the methods of Maruyama (1996). On the other hand, the cotyledon pair of seedlings was cultured as explant on callus inducing medium (2.0 mg L^{-1} NAA + 0.05 mg L^{-1} BAP) for callus induction. Best calli derived from cotyledonary explants of the two varieties were subcultured separately on MS medium having 2 mg L^{-1} NAA with 0.05 mg L^{-1} BAP. Somatic embryos, formed and matured within 7-28 days, were used as explant for making synthetic seed (Saiprasad, 2001). Carbon sources used were: Sucrose, Sorbitol and Mannitol.

Preparation of Solutions for Encapsulation

Encapsulation of synthetic seed was done according to the method reported by Kinoshita and Saito (1990). Two hundred milliliters MS media was prepared with 6 g sugar (sucrose, sorbitol and mannitol) supplemented with 0.5 mg L^{-1} BAP + 0.1 mg L^{-1} GA_3 for nodal segment but in case somatic embryo the medium was supplemented with 1.0 mg L^{-1} BAP + 0.1 mg L^{-1} GA_3. Twenty milliliters of the above mentioned solution was taken and 0.8 g of sodium alginate was added and taken in a small beaker (50 mL beaker). With a small piece of glass rod efforts were made to mix the alginate in solution. Alginate was partially dissolved and it was then kept aside. During autoclaving alginate was completely dissolved. Fifty milliliters of the above mentioned solution (200 mL MS) was taken in a small beaker and 0.7 g (70 mg) $CaCl_2$ was added to it and dissolved. Out of 200 and 70 mL (50+20) was used during the preparation of alginate and $CaCl_2$ solutions and rest 130 ml was remained as reserved. After autoclaving it was used during washing of the encapsulated beads. All prepared solutions were autoclaved at 15-lb/sq inch's pressure and at 120-121°C temperatures for 21 min for sterilization.

Encapsulation of Explants

The nodal segments with active buds and somatic embryos were placed to the beaker containing alginate solution. The explants were dipped in alginate solution and taken by a forcep and placed to the beaker of $CaCl_2$. During picking up the explants, the forceps, also took some addition alginate solution together with explants. The rolling explants with the liquid of alginate were dropped in to the $CaCl_2$ solution. The explants were kept inside the alginate solution for about 30 min. After 30 min each explant became a hardball encoated by alginate.

Culture of Synthetic Seed

The encapsulated explants or synthetic seeds were washed with 130 mL MS liquid medium (above mentioned). After washing, synthetic seeds were cultured on MS0 medium under *in vitro* condition in growth chamber where photoperiod was maintained generally 16 h light 8 h dark.

RESULTS AND DISCUSSION

In order to understand the regeneration potentiality of different carbon source in seed bead medium two types of explants were studied for eggplant germination. In this experiment, somatic

embryo and nodal segment with axillary buds were used as explants for artificial seed production and data were taken for germination percentage, days to germination and shoot length. The results are given in Table 1 and 2.

Table 1: Effect of different types of carbon sources on synthetic seed formation and germination

Carbon source (30 g L^{-1})	Variety	Name of the explants	Days to germination	Percentage of germination	Shoot length (cm) ($\bar{X}$ ±SE)
Sucrose	Loda	Node	4-7	85	1.6±0.2
		Somatic embryo	5-7	67	1.9±0.1
	China	Node	5-7	82	1.5±0.3
		Somatic embryo	16-21	50	1.5±0.3
Manitol	Loda	Node	4-6	61	1.7±0.1
		Somatic embryo	5-8	65	1.8±0.1
	China	Node	4-5	59	1.7±0.2
		Somatic embryo	15-22	49	1.5±0.1
Sorbitol	Loda	Node	4-5	68	3.7±0.6
		Somatic embryo	4-8	66	1.9±0.2
	China	Node	4-5	65	3.7±0.6
		Somatic embryo	15-21	49	1.4±0.8
Surcose+Manitol (1:1)	Loda	Node	4-6	81	1.8±0.3
		Somatic embryo	5-8	66	1.8±0.3
	China	Node	4-7	79	1.6±0.3
		Somatic embryo	15-21	49	1.5±0.3
Sucrose+Sorbitol(1:1)	Loda	Node	4-6	78	2.9±0.1
		Somatic embryo	5-7	70	1.8±0.6
	China	Node	4-7	78	2.6±0.3
		Somatic embryo	15-20	54	1.5±0.1
Manitol+Sorbitol (1:1)	Loda	Node	4-5	73	2.7±0.8
		Somatic embryo	5-8	65	1.8±0.2
	China	Node	4-6	71	2.8±0.1
		Somatic embryo	15-21	48	1.4±0.2

Table 2: Effect of different levels of sucrose (carbon source) on synthetic seed formation and germination

Amount of source	Variety	Name of the explants	Days to germination	Percentage of germination	Shoot length (cm) ($\bar{X}$ ±SE)
Controlled	Loda	Node	---	---	---
0%		Somatic embryo	---	---	---
	China	Node	---	---	---
		Somatic embryo	---	---	---
0.5%	Loda	Node	7-10	76	1.8±0.3
		Somatic embryo	12-15	30	1.5±0.5
	China	Node	8-12	69	2.1±0.1
		Somatic embryo	24-25	25	1.0±0.1
1%	Loda	Node	4-6	95	2.3±0.6
		Somatic embryo	7-10	56	1.6±0.3
	China	Node	5-6	90	2.0±0.1
		Somatic embryo	19-24	40	1.4±0.1
2%	Loda	Node	5-7	91	2.0±0.1
		Somatic embryo	6-8	60	1.8±0.7
	China	Node	6-7	86	1.7±0.3
		Somatic embryo	17-23	46	1.5±0.2
3%	Loda	Node	6-8	86	1.9±0.2
		Somatic embryo	5-7	67	1.9±0.1
	China	Node	6-9	80	1.5±0.1
		Somatic embryo	16-21	50	1.5±0.3
4%	Loda	Node	6-10	75	1.5±0.1
		Somatic embryo	7-9	64	1.5±0.3
	China	Node	7-10	65	1.2±0.1
		Somatic embryo	17-23	47	1.2±0.1
5%	Loda	Node	8-10	60	1.3±0.1
		Somatic embryo	8-10	56	1.3±0.2
	China	Node	8-12	51	0.9±0.2
		Somatic embryo	20-25	42	1.0±0.3

In the present investigation, three types of carbon sources viz., sucrose, sorbitol, mannitol and their combination in the ratio 1:1 were used in alginate bead (Table 1). Best result was found when sucrose was used alone using nodal segment and in this case 85% seeds of Loda variety were germinated within 4-7 days of culture and 82% seeds of China were germinated within 5-7 days of culture. But highest shoot length (3.7±0.6) was observed when sorbitol was used instead of sucrose. Among all the carbon sources used in the experiment, sucrose and its combination gave better result in artificial seed germination.

In case of somatic embryo, best result for synthetic seed germination was obtained when sucrose + sorbitol (1:1) was used as carbon source (Fig. 1C and D) and in this case 70% seeds of Loda variety were germinated within 5-7 days of culture and 54% seeds of China variety were germinated within 15-20 days of culture. Second highest result was obtained when sucrose was used alone and in this case, 67% seeds of Loda variety and 50% seeds of China variety were germinated.

As sucrose and its combination gave better results then it was necessary to understand the right concentration of sucrose, best effective in artificial seed germination. For this purpose different concentrations of sucrose were used in seed bead medium using the cultivars of eggplant and their performance was presented in Table 2. It was evident from this experiment that among different sucrose levels, treated in MS medium, 1% (10 mg L^{-1}) sucrose showed the best result for germination of synthetic seed made of nodal segment (Fig. 1F). In this sucrose level, 95% seeds of Loda variety and 90% of China variety were germinated on artificial medium. Second highest result was observed, when 2% (20 g L^{-1}) sucrose was used but germination rate was gradually decreased when the amount of sucrose was increased. It was observed under the increased dose of sucrose, germination period was delayed and the growth of shoot was decreased.

On the other hand, when somatic embryos were used as explant for synthetic seed production, 3% (30 g L^{-1}) sucrose level showed the better performance for seed germination (Fig. 1C). In this concentration, 67% seeds of Loda variety were germinated within 5-7 days of culture and 50% seeds of China variety were germinated within 16-21 days of culture. The optimal concentration of sucrose was 1% for synthetic seed containing nodal segment as explant and in case of somatic embryo it was 3%. In this experiment it was also found that in absence of carbon source, no germination was initiated. Lakshmana and Singh (1991) conducted similar types of experiments with eggplant using different levels of alginate concentrations in encapsulating somatic embryo and 3% sodium alginate and 75 mM calcium chloride were found to be optimum in plantlet regeneration. In their case the frequency of plantlet regeneration varied from 27.0-49.7% *in vitro* condition. But in our investigation, in case of somatic embryo, 70% germination was obtained using 4% sodium alginate with 1.4% calcium chloride. They used phytohormones in culture medium but we used phytohormone in seed bead medium and cultured on hormone free MS; here is the basic difference between these two investigations.

Artificial seeds consisting of somatic embryos enclosed in protective coating have been proposed as a low-cost, high volume propagation system (Radenbaugh *et al*., 1986) and somatic embryo possess shoot and root primordia and are usually able to develop directly into complete plant without any pretreatment (Ara *et al*., 2000). In addition to using somatic embryos, axillary buds, adventitious buds and shoot tips have also been used in preparation of synthetic seeds (Redenbaugh, 1993; Bapat and Rao, 1988, 1990; Ganapathia *et al*., 1992, 1994). Among the different encapsulating agents, sodium alginate was used for encapsulation of somatic embryo due to it's solubility at room temperature and ability to form completely permeable gell with calcium chloride (Bapat *et al*., 1987). It was found in carrot or celery that a suitable synthetic endosperm was required to provide nutrients including carbon sources necessary for optimum germination and conversion (Redenbaugh *et al*., 1988). Kinoshita and Satto (1990) also suggest that sucrose is essential for the germination of encapsulated buds.

In the present investigation, three types of carbon source viz., sucrose; sorbitol, mannitol and their combination in the ratio 1:1 were used in alginate bead (Table 1). Nieves *et al*. (1998) also used

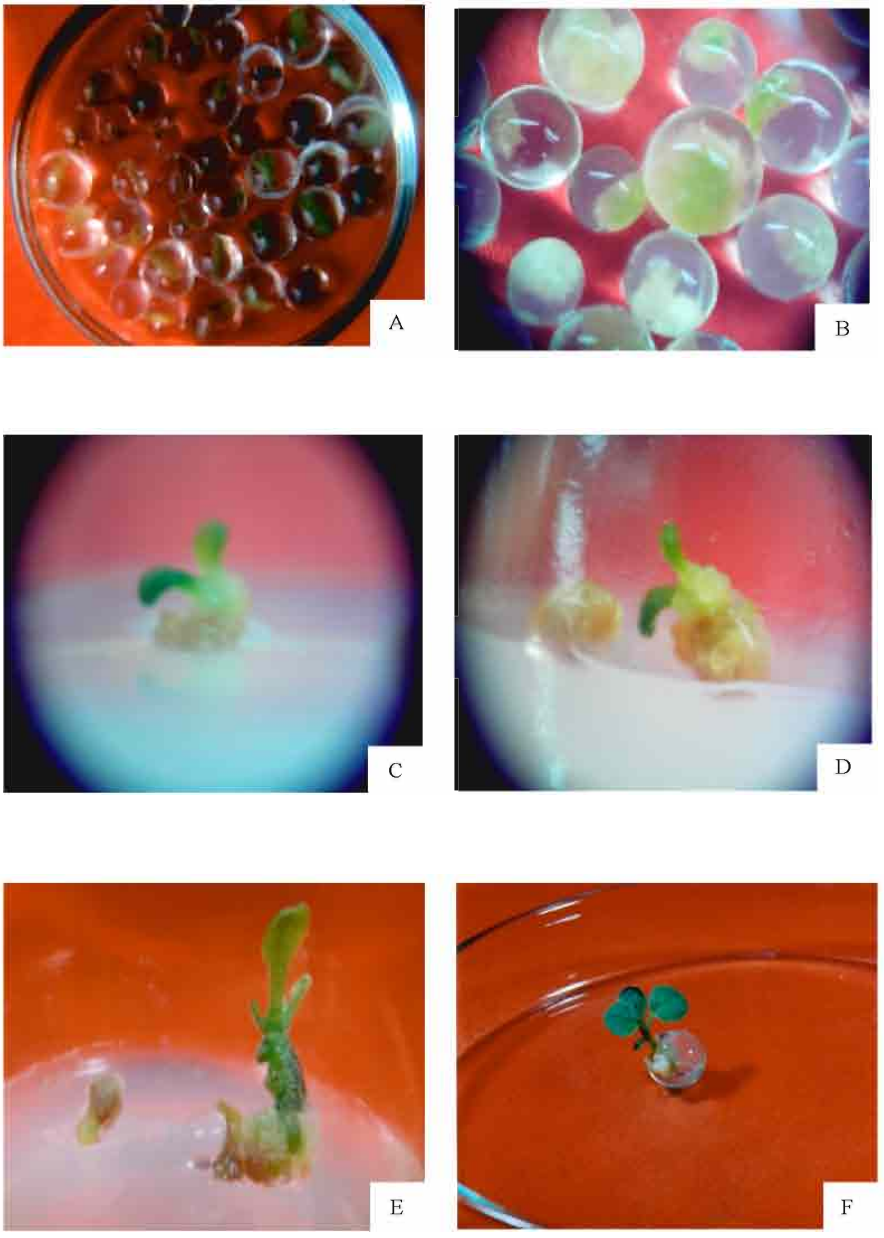

Fig. 1: (A) Encapsulated nodal segments, (B) Encapsulated somatic embryos, (C) Germination from seed bead containing low concentration of sucrose. (Somatic embryo) (D) Germination from seed bead containing high concentration of sucrose. (Somatic embryo), (E) Germination of seed from seed bead containing high concentration of sucrose. (Nodal segment) and (F) Germination from seed bead containing low concentration of sucrose. (Nodal segment)

two types of carbon source viz., corn starch and sucrose in alginate seed bead of *Cleopatra tangerine*. Artificial endosperm of *Cleopatra tangerine* assays showed that mannitol with abscisic acid delayed germination and conversion of artificial seed where zygotic embryos were used as explant for encapsulation. In contrast of *Cleopatra tangerine*, the present investigation also suggest that mannitol is less desirable among the three carbon sources because it gave lowest germination rate of synthetic seed. *In vitro* propagation of mulberry by encapsulating auxillary buds also reported by Machii (1992) obtained 10% seed germination using 20 mg L^{-1} (2%) sucrose in both seed bead and culture medium. But when he used only distilled water in seed bead germination rate was zero supporting the result of this investigation. On the other hand Kinoshita and Satto (1990) used abundant amount of sucrose in gell matrices of encapsulated axillary buds of Japanese White Birch to examine whether or not the beads germinate and concluded that the sucrose concentration in alginate gell should be as much as 20%. But the germination rate slows down when gell matrices contain 5% sucrose. The present investigation indicated that similar to White Birch, eggplant also required low concentration of sucrose in seed bead medium for better germination. This result suggests that if one wants to produce artificial seed using nodal segment with axillary buds as explant in eggplant, should use sucrose as carbon source and in case of somatic embryo combination of sucrose and sorbitol would be recommended.

REFERENCES

Ara, H., U. Jaiswal and V.S. Jaiswal, 2000. Synthetic seed: Prospect and limitations. Curr. Sci., 78: 1438-1444.

Bapat, V.A., M. Mhatre and P.S. Rao, 1987. Propagation of *Morus indica* L. (Mulberry) by encapsulated shoot buds. Plant Cell Rep., 6: 393-395.

Bapat, V.A. and P.S. Rao, 1988. Sandal wood plantlets from synthetic seeds. Plant Cell Rep., 7: 434-436.

Bapat, V.A. and P.S. Rao, 1990. *In vitro* growth of encapsulated auxillary buds of mulberry (*Morus indica* L.). Plant Cell Tiss. Org. Cult., 20: 69-70.

Ganapathia, T.R., P. Suprasanna, V.A. Bapat and P.S. Rao, 1992. Propagation of banana through encapsulated shoot tips. Plant Cell Rep., 11: 571-575.

Ganapathia, T.R., V.A. Bapat and P.S. Rao, 1994. *In vitro* development of encapsulated shoot tips of cardamom. Biotech, Techniques, 8: 239-244.

Hyndman, S., P.M. Hasengawa and R.A. Brassen, 1982. Stimulation of root initiation from cultured rose shoots through the use of reduced concentration of mineral salts. Hortic. Sci., 17: 82-83.

Kinoshita, I. and A. Saito, 1990. Propagation of Japanese white birch by encapsulated axillary buds. J. Jpn. For. Soc., 72: 166-170.

Lakshmana, P.V.R. and B. Singh, 1991. Plantlet regeneration from encapsulated somatic embryos of hybrid *Solanum melongena* L. Plant Cell Rep., 10: 7-11.

Landford, P.J. and H. Wainwright, 1988. Influence of sucrose concentration on the photosynthetic ability of *in vitro* grown rose shoots. Acta Hortic., 227: 305-310.

Machii, H., 1992. *In vitro* growth of encapsulated adventitious buds in Mulberry, *Morus alba* L. Japan J. Breed., 42: 553-559.

Maruyama, E., 1996. Micropropagation of bolaina blanca (*Guazuma crinita Mart.*), a fast growing tree in Amazon region. J. For. Res., 1: 211-217.

Nieves, N., C. Jose, L. Maria de Los and A. Blanco *et al*., 1998. Artificial endosperm of Cleopatra tangerine zygotic embryos: A model for somatic embryo encapsulation. Plant Cell Tiss. Org. Cult., 54: 77-83.

Ohishi, N., Y. Sakamoro and T. Hirosawa, 1995. Synthetic seed as an application of mass production of somatic embryos. Plant Cell Tiss. Org. Cult., 39: 137-145.

Redenbaugh, K., B.D. Paasch, J.W. Nichol, M.E. Kossler, P.R. Viss and K.A. Walker, 1986. Somatic seeds: Encapsulation of asexual embryos. Biotechnology, 4: 797-801.

Redenbaugh, K., J. Fuji and D. Slade, 1988. Encapsulated Plant Embryos. In: Mizrahi, A. (Ed.), Biotechnology in Agriculture. AR Liss. New York, pp: 225-248.

Redenbaugh, K., 1993. Syn. Seed: Application of Synthetic Seeds to Crop Improvement. CRC Press. Boca Raton, USA.

Saiprasad, G.V.S., 2001. Artificial seeds and their application. Resonance, pp: 19-47.