http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

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

Pakistan Journal of Biological Sciences 12 (15): 1085-1089, 2009
ISSN 1028-8880

Possibility of Sweet Corn Synthetic Seed Production

[1]P. Thobunluepop, [2]E. Pawelzik and [3]S. Vearasilp
[1]Department of Agricultural Technology, Faculty of Technology, Maha Sarakham University,
Talard Sub-District, Muang District, Maha Sarakham 44000, Thailand
[2]Institute of Agricultural Chemistry, Section of Quality of Plant Products,
Georg-August University of Goettingen, Carl-Sprengel-Weg, D-37075, Goettingen, Germany
[3]Faculty of Agriculture, Department of Agronomy, Chiang Mai University, 50000, Thailand

Abstract: Somatic embryogenesis in sweet corn has been reported by a number of workers. However, the knowledge maintaining storage life, vigor and viability of these somatic embryos are limited. A model system of synchronous somatic embryos production combined with encapsulation to synthetic seed was studied in sweet corn (*Zea mays* var. *saccharata*). In this study immature zygotic embryo cultured on N6 medium, contained 2, 4-D 2 mg L^{-1} and sucrose 60 g L^{-1} form the embryogenic callus. Higher 2, 4-D levels did not show increasing in inducing embryogenic callus. If the concentration of 2, 4-D decreased globular-stage, somatic zygote form the roots. Somatic embryo develop without surrounding nutritive tissues and protective seed coat has been devoted to causing somatic embryos to functionally mimic embryo, then was encapsulated by 3% (w/v) sodium alginate with 4-6 mm in diameter. It was found that when synthetic seed were treated with 60 g L^{-1} sucrose and stored at 15±2 degree Celsius for 2 weeks, the survival rate of synthetic seed were 44%, after 8 days of germination test, it was found that there were 91% of which were normal seedling and 9% were abnormal seedling. This result indicated that there is a possibility in sweet corn synthetic seed production. Anyhow, more research for better technique are further required.

Key words: Somatic embryo, synthetic seed, sweet corn, artificial seed, tissue culture

INTRODUCTION

Plant regeneration via somatic embryos start with one or only a few cells, this type of regeneration is important for plant multiplication, mass production and plant biotechnology such as clonal propagation and especially genetic transformation (Gordon-Kamm *et al*., 1990). Synthetic seed technology is an exciting and rapidly-growing research area in plant cell and tissue culture (Fujii *et al*., 1987; Datta *et al*., 1999). Even though this technique is still at a non-commercial phase because of several constraints that limit its practical use (Kozai *et al*., 1991; Carlson and Hartle, 1995), synthetic seeds offer the potential advantages of being genetically identical materials, easy to handle and transport and which display an increased efficiency for *in vitro* propagation in terms of space, time, labor and overall costs. The usefulness of encapsulation of *in vitro*-cultured explants has also been demonstrated in other fields of application, such as cryopreservation of plant genetic resources (Ishikawa *et al*., 1997; Bouafia *et al*., 1996; Mix-Wagner, 1999). The concept of synthetic seeds having first been publicly discussed by Murashige and Skoog (1962) and initially, the first synthetic seeds were simply somatic embryos produced from tissue cultured vegetative cells that had the particular advantage of enabling rapid clonal multiplication. Presently, the development of synthetic seed technology as expanded to the artificial encapsulation of somatic embryos, shoot, cell aggregates, or any other tissue that can be used for sowing and that retains this potential after storage (Capuano *et al*., 1998).

The encapsulation materials and methods for the production of synthetic seeds have seldom varied since the concept was introduced by Murashige and Skoog (1962). A series of studies dealing with the encapsulation of somatic embryos in conventional calcium alginate solid beads have been published in the past two decades (Redenbaugh *et al*., 1987; Sheeba and Padmaja, 1999). In these cases, the embryos protruded or were located close to the surface, so that complete protection was ensured by used of calcium alginate beads. These beads offer complete protection and allow for continued development of the explant within the beads (Patena *et al*., 2002).

Corresponding Author: Pitipong Thobunluepop, Department of Plant Production Technology, Faculty of Technology, Mahasarakham University, Talard Sub-District, Muang District, Maha Sarakham 44000, Thailand

Little attention has been directed towards encapsulation in calcium alginate hallow beads and storage of the encapsulated sweet corn propagules. Furthermore, storage of synthetic seeds using an alginate-encapsulation protocol has been attempted in only a few species, with minimal success (Redenbaugh *et al*., 1986, 1987; Fujii *et al*., 1987). In this study, results of the optimized production, storage and regeneration of sweet corn somatic embryos encapsulated in calcium alginate under various storage conditions and the re-growth under *in vitro* condition are presented.

MATERIALS AND METHODS

The experiment was conducted at Department of Agricultural Technology, Faculty of Technology, Maha Sarakham University and Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Thailand in 2007-2009. Sweet corn variety of FAH 01 somatic embryos were cultured from immature zygotic embryos 11 days after pollinated. Callus initiation with sterilized zygotic embryos was cultured on agar-solidified N6 medium, containing with different levels of sucrose (30 and 60 g L^{-1}) and 2, 4-D (2, 3 and 4 mg L^{-1}). In all experiments culture temperature was 25±2°C in the dark. Callus maintenance was incubated under temperature 25±2°C in the dark. Sweet corn somatic embryos were developed by transfer friable type II callus aggregates on regenerate medium N6 contained with 1 mg L^{-1} NAA and incubated under temperature 25±2°C in the dark for 2 weeks. Then, embryogenic callus were transferred for 2 weeks on plant growth regulator- free MS medium and cultured under temperature 25±2°C in the light condition. The *in vitro* plantlets were mutiplied in liquid medium culture; one cycle lasted 3-4 weeks. The single cell of somatic embryo raised on MS liquid medium at 25±2°C in the 14 h photoperiod condition. The single cell sweet corn somatic embryos of *in vitro*-propagated were dissected and trimmed as described by Schäfer-Menuhr *et al*. (1996) and pre-cultured on liquid MS medium. Under sterile conditions, the precultured somatic embryos (3-4 mm in length) were then mixed with 10 mL of 100 mmole calcium chloride solution. Using a hypodermic syringe the suspension was dropped in 500 mL 3% sodium alginate solution. After 20 min of polymerization, the alginate hollow beads were collected on a sterile sieve and washed with 500 mL of sterile water. After that, the experiment was conducted in factorial in complete randomized design with 4 replications, while synthetic seeds were stored under various conditions; 15±2°C and 25±2°C, various dessicated levels; 20, 40 and 60% by silica gel method and then storage for 2 weeks. After synthetic seed germinated, the percentage of sweet corn synthetic seed germination, normal seedling and abnormal seedling was observed. The analysis of variance was performed for data analysis and differentiated with Last Significant Different (LSD) test at $p<0.05$ using the software SX release 8.0 (Analytical software, Tallahassee, USA).

RESULTS AND DISCUSSIONS

The sweet corn synthetic seeds capsules were consisted of the somatic embryos in a liquid core of nutrients surrounded by a calcium alginate. The nutrients were added in the capsule core during incubated capsules in the MS liquid medium. Of the 400 measured capsules, a mean were between 0.54-0.68 cm diameters. The encapsulated somatic embryos that were not stored were 100% regenerated on MS solid medium. It was indicated that trimming and encapsulating had no effect on viability of somatic embryos and they could maintained their meristematic characteristics. Emergence occurred when the elongating shoot tips with new leaves burst through the calcium-alginate capsule walls. The first shoot tip emergence was observed at 7 days and the last emergence at 10 days after transferred to the regenerated MS solid medium. 10 days after regenerated, the seedling developed in 1-2 cm high plantlets. The synthetic seeds after storage under 15±2°C and 25±2°C condition were germinated 43 and 55% and emergence in 9 and 8 days, respectively (Fig. 1). For the seedling characteristics, the results was indicated that the seedling of sweet corn synthetic were normal seedling for 90 and 95%, abnormal seeding 10 and 5% (Fig. 2). Aggrey *et al*. (2003) reported regeneration of 80% of potato synthetic seeds on MS solid medium, while attained 60% regeneration of banana encapsuled. The somatic embryos were inoculated with various concentration of sucrose (0, 30 and 60 g L^{-1}), that affected on synthetic seeds germinated to 43, 57 and 46% and plantlets were regenerated in 7-9 days (Fig. 3).

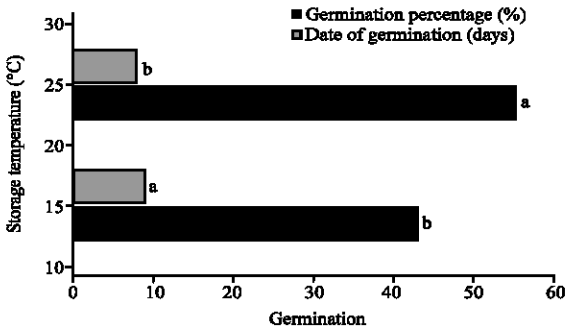

Fig. 1: The effect of storage temperature (15 and 25°C) on sweet corn synthetic seed germination percentage and date of germination

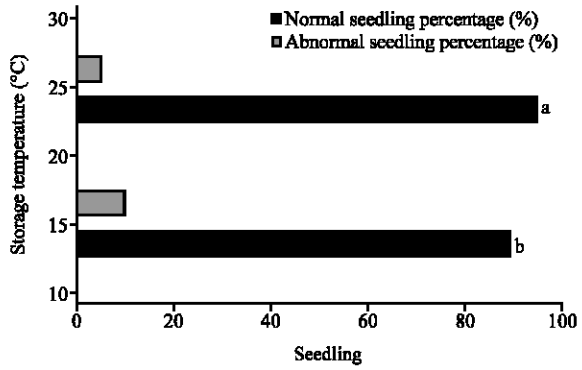

Fig. 2: The seedling characteristics of sweet corn synthetic seed after germinated under various storage temperatures (15 and 25°C)

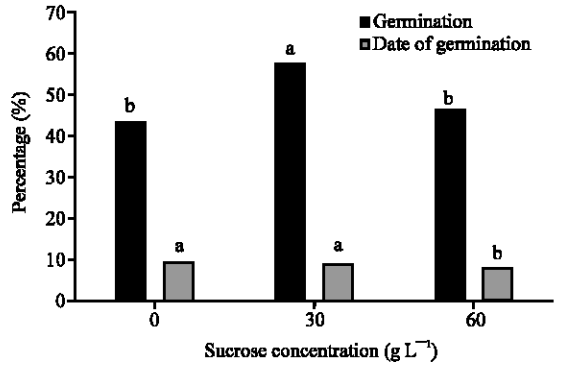

Fig. 3: The effect of various sucrose concentration (0, 30 and 60 g L^{-1}) on sweet corn synthetic seed germination percentage and date of germination

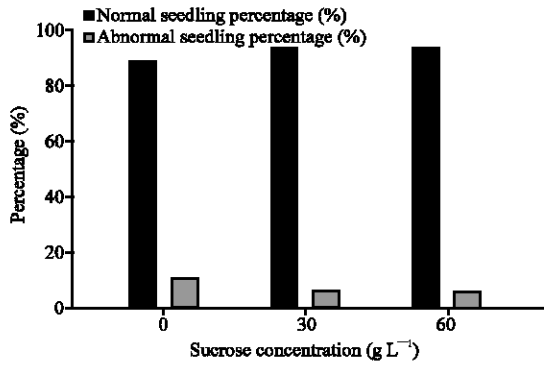

Fig. 4: The seedling characteristics of sweet corn synthetic seed after germinated under various sucrose concentrations (0, 30 and 60°C)

Moreover, the result was showed 89-94% of normal seedlings and 5-10% of abnormal seedlings (Fig. 4).

Synthetic seeds were decreased their germination to 60, 44 and 13% and normal seedling characteristics were also reduced 92, 89 and 81%, the abnormal seedling 7, 11 and 19% and wide range of germination date when desicated at level 20, 40 and 60%, respectively (Table 1, Fig. 5A-D).

The lack of external nutrients support necessary for synthetic seed germinated growth and developed and did not regenerate when transferred onto medium (Armstrong and Green, 1985). Chand and Singh (2004) showed that the uncapsulated axillary buds of six mulberry species failed to develop shoots after 7 days after storage at 4°C. It was also observed that the hollow beads capsules progressively become drier and shrunk in size during storage, probably due to decreasing humidity within storage condition. It is also further thought that the decline in the regeneration frequency observed among the stored encapsulated propagules may be due to the inhibited respiration of plant tissue by the alginate and accumulated of metabolic wastes in the alginate capsules during the long storage (Redenbaugh *et al*., 1987). The result indicated that at present the maximum storability of the synthetic seeds is 2 weeks. A 20% desicated were 46% regenerated is achieved and shown significantly on normal seedling characteristic, but it was shown that, synthetic seeds viability reduced when stored time and desicated level was increased (Table 2).

Other studies have shown lower regeneration levels under various storage conditions, Ghosh and Sen (1994) acheived a maximum regeneration of only 34% after 90 days storage of asparagus encapsulated somatic embryos. Datta *et al*. (1999) found that artificial seeds of an endangered orchid stored at 4°C for 120 days shown no reduction in viability, whereas non-encapsulated protocorn-like bodies showed no viability after 30 days at 4°C. Unlike zygotic embryos, synthetic seeds do not accumulate storage reserves, do not develop tolerance to drying, can be stored for a limited time and simply regenerate into plantlets, developing by emerging through the capsule, a process that can be slowed down by lowering the temperature (Duncan *et al*., 1985). The high viability of sweet corn synthetic seeds stored at 15±2°C indicated that they can be useful alternatives to be handled and transported cheaply like seeds due to their small size. Synthetic seeds can also allow breeders to store unique gene combinations that cannot be maintained by conventional seed production due to genetic recombination (Emons and Kieft, 1991). The usefulness of synthetic seeds was storage of vegetative propagules, especially for *ex situ* conservation germplasm as an alternative for field or greenhouse nursery is brought into focus in this paper, which was congruence with Fransz and Schel (1991), their high frequently regeneration, viability and re-growth imply that they can be very useful to sweet corn seed producers Fransz and

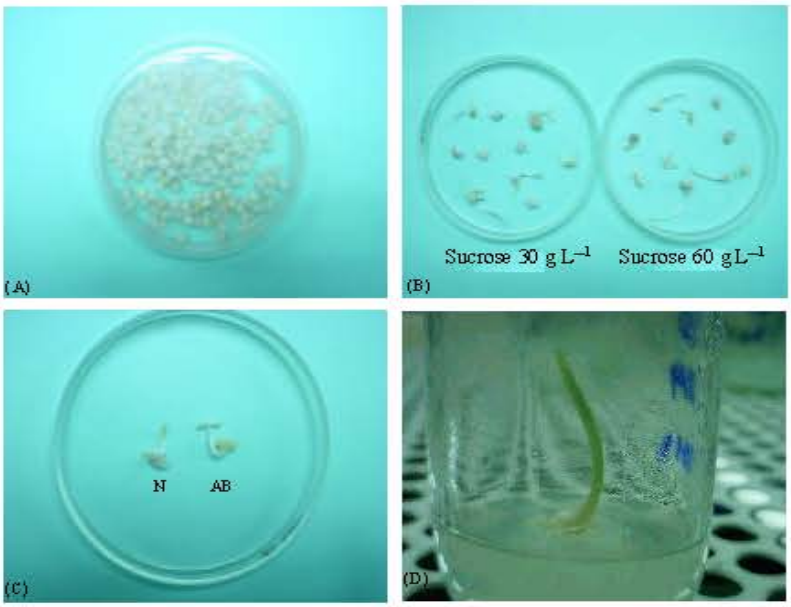

Fig. 5: Sweet corn synthetic seeds; (A) Sweet corn somatic embryos were encapsulated with calcium-alginate polymerized beads, (B) Germinated of sweet corn synthetic seeds, (C) Seedling characteristic of sweet corn synthetic seed; N: Normal seedling, AB: Abnormal seedling and (D) Plantlet was regenerated from sweet corn synthetic seeds

Table 1: The effect of desiccation levels on synthetic seeds viability

Desicated level	Germination	Normal seedling	Abnormal seedling	Days of germination
	(%)			
20	60.50^{a}	92.76	7.23	7.70^{b}
40	44.20^{b}	89.13	10.97	8.50^{a}
60	13.00^{c}	80.93	19.06	8.70^{a}
$LSD_{F\ 0.05}$	10.625	ns	ns	0.8022

The different letters indicate the statistically significant difference by LSD at 5% level. ns: Not significant

Table 2: The effect of storage time and desiccated level interaction on sweet corn synthetic seed storability and viability

Storage time (Week)	Desicated level	Germination	Normal seedling	Abnormal seedling
		(%)		
1	20	53.41^{a}	91.53^{a}	8.50
	40	45.27^{a}	89.71^{a}	10.40
	60	29.70^{c}	85.61^{b}	14.41
2	20	46.30^{a}	88.83	11.20
	40	38.20^{b}	87.02^{ab}	13.02
	60	22.60^{d}	82.92^{c}	17.03

The different letters indicate the statistically significant difference by LSD at 5% level

Schel (1994). This study was successful in developing and optimizing a procedure of the production and storage of sweet corn synthetic seeds by encapsulating somatic embryos in calcium alginate hollow beads and retrieving plantlets, as an alternative sweet corn propagation practice. However, a detailed study towards the development of a cost-effective delivery protocol for synthetic seeds in the field is under way. In order to confirm their possible economic use in agricultural system and whether they could be handled as traditional plant seeds or seedling transport as the natural true seeds.

ACKNOWLEDGMENTS

This research is an activity of the programme Subject-related partnership between the University of Göttingen (Germany) and Faculty of Agriculture, Department of Agronomy, Chiang Mai University (Thailand) in the area of Academic Co-operation in Teaching and Research and acknowledged to Department of Agricultural Technology, Faculty of Technology, Maha Sarakham University, Thailand.

REFERENCES

Aggrey, N., S. Siegfried, G. Mix-Wagner and G. Jörg-Micheal, 2003. Production, storability and regeneration of shoot tips of potato (*Solanum tuberosum* L.) encapsulated in calcium alginate hollow beads. *In Vitro* Cell. Dev. Biol. Plant, 39: 540-544.

Armstrong, C. and C. Green, 1985. Establishment and maintenance of friable, embryogenic maize callus and involvement of L-proline. Planta, 164: 207-214.

Bouafia, S., N. Jelti, G. Lairy, A. Blanc, E. Bonnel and J. Dereuddre, 1996. Cryopreservation of potato shoot tips by encapsulation-dehydration. Potato Res., 39: 69-78.

Capuano, G., E. Piccioni and A. Standardi, 1998. Effect of different treatments on the conversion of M.26 apple rootstock synthetic seeds obtained from encapsulated apical and axillary micropropagated buds. J. Hortic. Sci. Biotechnol., 73: 299-305.

Carlson, W.C. and J.E. Hartle, 1995. Manufactured Seeds of Woody Plants. In: Somatic Embryogenesis of Woody Plants, S.M. Jain, P.K. Gupta and R.J. Newton (Eds.). Vol. I. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp: 253-263.

Chand, S. and A.K. Singh, 2004. Plant regeneration from encapsulated nodal segments of *Dalbergia sissoo* Roxb, a timber-yielding leguminous tree species. J. Plant Physiol., 161: 237-243.

Datta, K.B., B. Kanjilal and D. Sarker, 1999. Artificial seed technology development of protocol in *Geodorum densiflorum* (Lam) Schltr. An Endangeted Orchid. Curr. Sci., 76: 1142-1145.

Duncan, D.R., M.E. Williams, B.E. Zehr and J.M. Widholm, 1985. The production of callus capable for plant regeneration from immature embryos of numerous *Zea mays* genotypes. Planta, 165: 322-332.

Emons, A.M.C. and H. Kieft, 1991. Histological comparison of single somatic embryos of maize from maturation of embryogenic calli of *Zea mays* L. Can. J. Bot., 71: 1349-1356.

Fransz, P.F. and J.H.N. Schel, 1991. Cytodifferentiation during the development of friable embryogenic callus of maize (*Zea mays* L.). Can. J. Bot., 69: 26-33.

Fransz, P.F. and J.H.N. Schel, 1994. Ultrastructural studies on callus development and somtic embryogenesis in *Zea mays* L. In: Biotechnology in Agriculture and Forestry, Bajaj, Y.P.S. (Ed.). Vol. 25. Maize Springer, Berlin Heidelberg, New York, pp: 50-65.

Fujii, J.A., D.T. Slade, K. Redenbaugh and Wlaker, 1987. Artificial seeds for plant propagation trends. Biotechnology, 5: 334-339.

Ghosh, B. and S. Sen, 1994. Plant regeneration from alginate encapsulated somatic embryos of *Asparagus cooperi* baker. Plant Cell Rep., 13: 381-385.

Gordon-Kamm, W.J., T.M. Spencer, M.L. Mangano, T.R. Adams and R.J. Daines *et al*., 1990. Transformation of maize cells and regeneration of fertile transgenic plants. Plant Cell, 2: 603-618.

Ishikawa, K., K. Harata, M. Mii, A. Sakai, K. Yoshimatsu and K. Shimomura, 1997. Cryopreservation of zygotic embryos of a Japanese terrestrial orchid Bletilla striata by vitrification. Plant Cell Rep., 16: 754-757.

Kozai, T., 1991. Micropropagation under Photoautotrophic Conditions. In: Micropropagation: Technology and Applications, Deberg, P.C. and R.H. Zimmerman (Eds.). Kluwer Academic Publishers, Dordrecht, pp: 447-469.

Mix-Wagner, G., 1999. The conservation of potato cultivars. Potato Res., 42: 427-436.

Murashige, T. and F. Skoog, 1962. A revised medium of rapid growth and bioassays with tobaccotissue cultures. Physio. Plant., 15: 473-497.

Patena, L.F., L.R. Carlos-Refuerzo and R.C. Barba, 2002. Somatic embryogenesis and plantlet regeneration in mango (*Mangifera indica* L.). *In vitro* Cell. Dev. Biol. Plant, 38: 173-177.

Redenbaugh, K., P. Viss, D. Slade and J.A. Fujii, 1986. Scale-up: Artificial seeds. Proceedings of the 6th International Congress, Plant Tissue Cell Culture, Aug. 3-8, University of Minnesota, USA., pp: 473-493.

Redenbaugh, K., D. Slade, P. Viss and J.A. Fujii, 1987. Encapsulation of somatic embryos in synthetic seed coats. Hortscience, 22: 803-809.

Schäfer-Menuhr, A., E. Mueller and C. Mix-Wagner, 1996. Cryopreservation an alternative for the long-term storage of old potato varieties. Potato Res., 39: 507-513.

Sheeba, R. and G. Padmaja, 1999. Mechanism of cyanogen reduction in cassava roots during cooking. J. Sci. Food Agric., 75: 427-432.