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Full Length Research Paper

The somatic embryogenesis and plant regeneration from immature embryo of sweet corn inbred line

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Synthetic seed consisting of somatic embryos enclosed in protective coating are a suitable tool for clonal mass propagation of elite plant varieties. The *in vitro* study was aimed to evaluate the optimizing medium for sweet corn somatic embryogenesis, synthetic seed production which leads to increasing germination and seed viability percentage. The *in vitro* study was aimed to evaluate the optimizing medium for sweet corn somatic embryogenesis, synthetic seed production which leads to increasing germination and seed viability percentage. Sweet corn (*Zea mays* var. *saccharata*) variety FAH01 embryogenic callus were derived from culturing immature zygotic embryos at 11 days after pollination on N6 medium that contained 2, 4-D 2 mgl^{-1} and sucrose 60 gl^{-1}. Somatic embryos was developed after transferred embryogenic callus to N6 medium contained with 2 mgl^{-1} 2, 4-D and 30 gl^{-1} sucrose. Sweet corn synthetic seed was produced by somatic embryos encapsulated into a protective calcium-alginate matrix with provides mechanical support, protection and is coated with a wax film to prevent desiccation. Synthetic seed were produced, it was found that when synthetic seed were treated with 60 gl^{-1} sucrose and stored at 15 ± 2 degree Celsius for 2 weeks, the percentage of germination of synthetic seeds were 42%, percentage of normal seedling was 91% and abnormal seedling was 8%, and they germinated for 8 - 9 days and could produce normal plantlet. When the synthetic seed were dehydrated by silica gel until remained 60% of their moisture content and then stored for 2 weeks, they could germinated at level 23%, which 83% of normal seedling and 17% of abnormal seedling. The survival ratio in sweet corn synthetic seed in this investigation indicated that there is still some more research required to increase number of the survival seeds and the optimum storage technique to prolong their viability is also needed

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

INTRODUCTION

Sweet corn is a herbaceaus monocot with an annual cycle. Its embryo lies embedded in the endosperm at one side. Abbe and Stein (1954) have described eight stages of zygotic embryo development and this order of events has been confirmed and more detail by Van Lammeren (1986). Sweet corn has contributed extensively in the area of genetics, biochemistry, physiology and specially molecular biology. However, the development of somatic embryogenesis system capable of plantlet regeneration is still in its beginning. Somatic embryogenesis has been demonstrated in immature embryos culture. Sweet corn somatic embryogenesis has been reported by a number of workers e.g. Armstrong and Green (1985), Vasil (1986), Fransz and Schel (1991a), Emans and Kieft (1991). Plant regeneration via somatic embryogenesis starts with one or only a few cells, this type of regeneration is important for plant production and plant biotechnology such as somaclonal propagation, multiplication and especially genetic transformation. This collection of techniques can be directed toward production of identical plants or to induce variability (Gordon-Kamm et al., 1990). The conventional techniques of crop improvement in agricultural system involve a search for stain of plant. Redenbaugh (1991) suggested that it is possible to produce asexual embryos in vitro. Synthetic seeds technology is one of the important applications of somatic embryogenesis. In these first synthetic seed system, somatic embryo is encapsulation in protective

Table 1. Effect of medium culture compositions on sweet corn callus initiation.

Medium	Sucrose (gl^{-1}.)	2, 4–D (mgl^{-1}.)	Callus generated (Percentage)		
			Time (Weeks)		
			2	6	10
N6	30	2	54c	75c	89c
		3	57ab	80bc	92b
		4	54c	78c	90c
	60	2	73a	89a	96a
		3	69b	84bc	92b
		4	69b	86b	94b

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

encapsulation in protective alginate matrix which provides mechanical support, protection and was coated with a wax film to prevent dessication Radenbaugh (1986). However, the stage life and vigor of synthetic seed is limited. The main objective of the study was to find the optimizing medium for sweet corn somatic embryogenesis, synthetic seed production which lead to increasing germination rate and seed viability.

MATERIALS AND METHODS

The experiment was conducted at Department of Agricultural Technology, Department of Technology, Maha Sarakham University, and Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Thailand in 2007 - 2009. Sweet corn var. FAH01, fresh immature zygotic embryos; 11 days after pollination were collected and were sterilized with 10% clorox solution for 5 min, followed to through rinsed with sterile water and were incubated on various culture mediums. The experiment was conducted in factorial in complete randomized design with 4 replications. Sweet corn callus was induced from sterilized zygotic embryos which were cultured on agar-solidified N6 medium, containing with different levels of sucrose (30 and 60 gl^{-1}) and 2, 4-D (2, 3 and 4 mgl^{-1}). In all experiments culture temperature was 25 ± 2℃ and was incubated in the dark. Fast-growing friable type II embryogenic callus was selected and maintained by sub-cultured once every 2 weeks intervals depending on growth rate. Callus maintenance was incubated under temperature 25 ± 2℃ in the dark. Somatic embryo was developed by transferring callus aggregates on regenerate-medium N6 contained 1 mgl^{-1} NAA and incubated under temperature 25 ± 2℃ in the dark for 2 weeks. Then, embryogenic callus were transferred to plant growth regulator-free MS medium and cultured under 25 ± 2℃ in the light condition. Sweet corn synthetic seeds were produced by inserting single somatic embryo into visco liquid beads of 3% (w/v) sodium alginate solution, single embryo was encapsulated by putting into 100 mM calcium nitrate solution for 20 min to develop calcium alginate gel complex and then, rinsed in sterilized water. Sweet corn synthetic seed germination, normal seedling, abnormal seedling were recorded. 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 DISCUSSION

The effect of sucrose and 2, 4 - D supplemented in N6 medium was significantly affected on sweet corn callus initiation. 11 days after pollinated immature zygotic embryos were placed on N6 medium supplemented with 2 mgl^{-1} of 2, 4 - D and 60 gl^{-1} of sucrose and resulted in the highest of callus induction rate. The callus initiation percentages were 73, 89 and 96 after cultured 2, 6 and 10 weeks respectively. On the other hand, the application of 30 g L^{-1} of sucrose and 2 mgl^{-1} of 2, 4 - D resulted in the lowest of callus induction (Table 1). This result congruence with Bates (1993) who reported the increasing of sucrose concentration increased the induction and development of callus.

The embryogenic callus was obvious that entirely developed from scutellum node, non-embryogenic callus was developed from shoot apex and the radicle of immature zygotic embryo was not developed to callus at all (Figure 1A). Two type of embryogenic calli were distinguished in 2 types; type I callus was compacted, type II callus so called friable callus was differentiated lesser than regenerable callus (Figure 1B). Type I callus might consist of aggregates of undifferentiated calli, interspersed with vascular cells, and groups of small isodiametric meristemic cells on the outside of the aggregated as similar results reported by Emons and Kieft (1991) (Figure 1C), which are tightly packed, thin-walled, richly cytoplasmic, many small vascular (Fransz and Schel 1991a, 1994). These groups of cell are comparable to the proembryogenic masses. After sub-culturing, these embryogeic calluses remained similar. In the fact, they were the dividing cells giving rise to new embryogenic cells. (Figure 1D). Single globules are unable to regenerate into plantlets; they form only roots (Figure 1E), which was correlated with Carvolho et al. (1997) results.

The embryogenic calli was selected and incubated on N6 medium contained with 2 mgl^{-1} of 2, 4-D and 60 gl^{-1} of

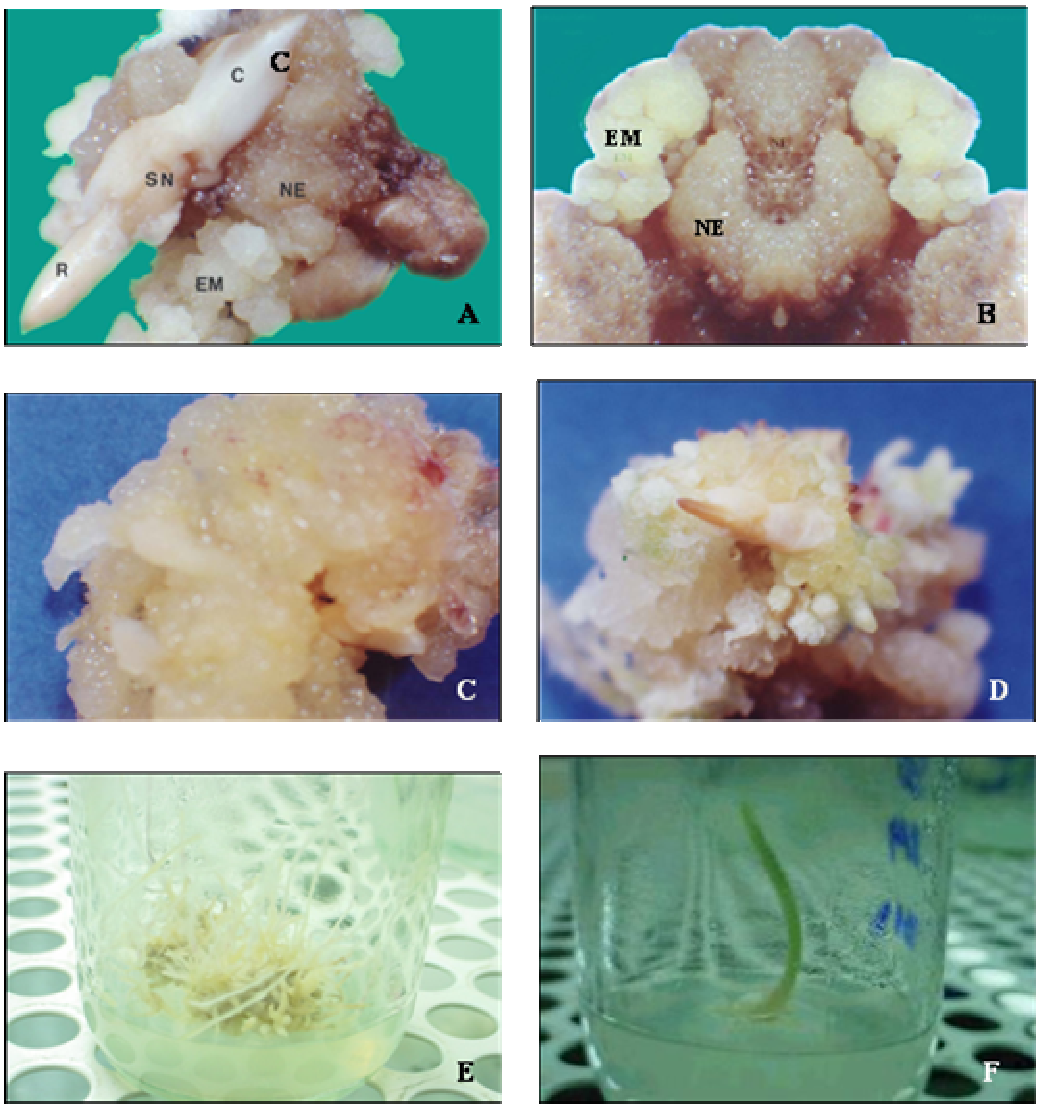

Figure 1. Sweet corn somatic embryogenesis via immature zygotic embryo, A: embryogenic and non-embryogenic callus development position, C: colepotile, R: radicle, SN: scutellum node, EM: embryogenic callus, NE: non-embryogenic callus, B: compact embryogenic callus and non-embryogenic callus, C: softy yellow-white friable non-embryogenic callus, D: friable embyogenic callus, E: rhizogenic callus, and F: plantlet regeneration via somatic embryogenesis

of sucrose, callus growth was monitored, callus size and fresh weight increased significantly through this culturing. It was shown that, callus fresh increased 0.0990 - 0.1103 g and callus size 2.16 - 4.88 mm. after 4 and 10 weeks of culturing (Table 2). Fahye et al. (1986) reported that sucrose was provided as carbon source for callus cell using for the increasing of cell metabolism, which was finally resulted the induction of callus size and fresh weight.

After the embryogenic callus were cultured on N6 medium contained with 30 gl^{-1} of sucrose and 2 and 3 mgl^{-1} of 2,4-D, then, the embryogenic callus were transferred to plant growth regulator-free MS medium containing 60 gl^{-1} of sucrose. The somatic embryos were allowed to regenerated and mature, the shoot meristem from these single somatic embryos was highest developed for 65 and 62% (Table 3). The somatic embryo continued to develop to plantlet and ready to be transplanted within 4 weeks (Figure 1F). While, the culture medium containing the higher levels of 2, 4 - D resulted the decreasing of percentage of sweet corn somatic embryos development. Based on this observation, it refers to the developmental sequence as sweet corn somatic embryogenesis. Green and Philips (1975) and Green et al. (1974) reported the optimum concentration of plant growth hormone especially on auxin and cytokinin was play a important role of sweet corn somatic embryogenesis, however, the over requirement levels

Table 2. Effect of Medium culture composition on sweet corn callus size.

Medium	Sucrose (gl^{-1})	2, 4–D (mgl^{-1})	Callus size (mm)		
			Time (weeks)		
			2	6	10
N6	30	2	2.16	2.94b	3.99c
		3	2.19	3.04ab	4.08b
		4	2.11	2.91b	3.93c
	60	2	2.16	3.10a	4.88a
		3	2.19	3.20a	4.40a
		4	2.11	3.12a	4.23ab

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

Table 3. Effect of medium culture compositions on percentage of sweet corn somatic embryos development.

Medium	Sucrose (gl^{-1})	2,4 - D (mgl^{-1})	Somatic embryos (percentage)
N6	30	2	65a
		3	62a
		4	54b
	60	2	54b
		3	52b
		4	43c

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

Table 4. The effect of sucrose concentration and storage temperature conditions on sweet corn synthetic seed viability.

Temperature (°C)	Sucrose (gl^{-1})	Germination (%)	Normal seedling (%)	Abnormal seedling (%)
15 ± 2	0	43^{c}	90^{c}	10^{a}
	30	50^{b}	92^{b}	8^{b}
	60	45^{c}	92^{b}	8^{b}
25 ± 2	0	49^{bc}	92^{b}	8^{b}
	30	56^{a}	95^{a}	6^{c}
	60	51^{b}	95^{a}	5^{c}

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

requirement levels of their compounds could also reducing the somatic embryo induction. Its might be the toxicity of their compounds (Table 4).

Single somatic embryos were encapsulated with protective seed coat calcium-alginate matrix, so-called synthetic seed. Seed qualities were observed. It was found that, somatic embryos were treated with 30 and 60 gl^{-1} sucrose significantly affected on germination percentage and date of germination, while synthetic seeds germinated for 57 and 46% respectively and their germinated after 8 and 9 days after planted (Figure 2). For seedling characteristics, the sweet corn synthetic seed could produce 89 - 94% of normal seedlings and 6 - 11% of abnormal seedlings (Figure 3). After stored at 25 ± 2°C for 2 weeks, the high level of sucrose concentration significantly produced the highest level of germination and normal seedling percentage. The percentage of germination was 56%, normal and abnormal seedling was 91 and 8%, respectively, they germinated after 6 days to produce normal plantlet. On the other hand, the germination and normal seedling percentage was decreased significantly when reducing the concentration

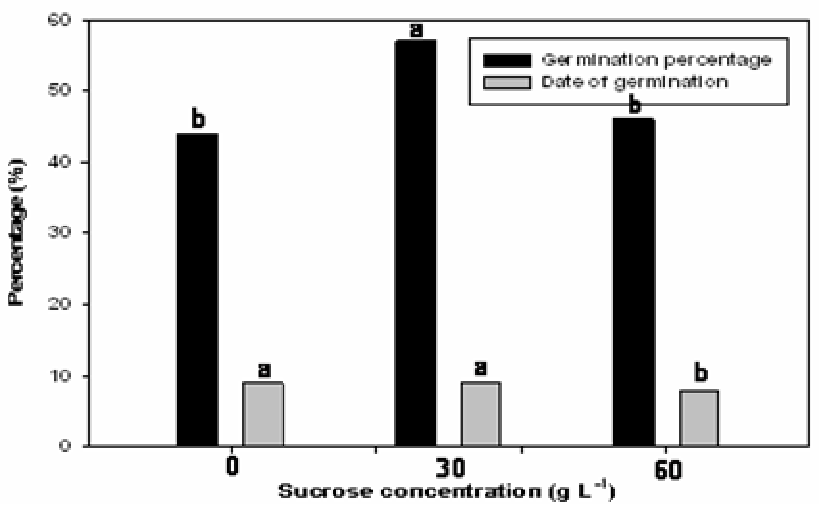

Figure 2. The effect of sucrose concentration on sweet corn synthetic seed germination.

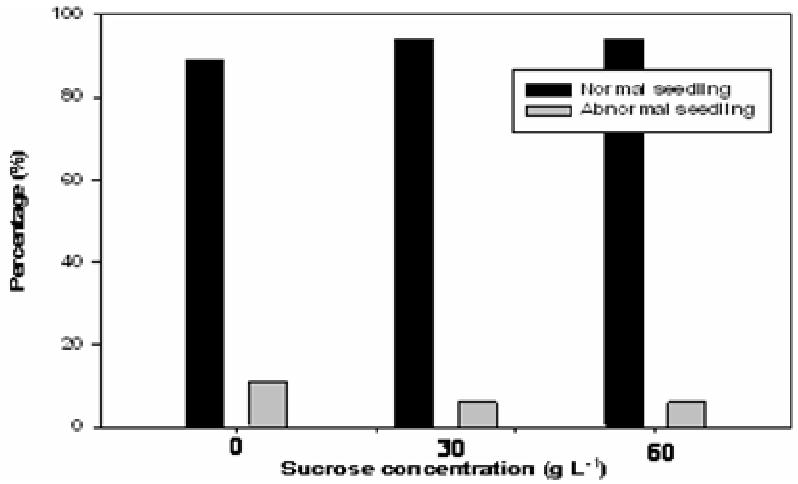

Figure 3. The effect of sucrose concentration on sweet corn seedling characteristics after germinated from sweet corn synthetic seeds

of sucrose (Table 5). Lecouteux et al. (1994) reported that pretreatment of sucrose was an important factor for the preservation of somatic embryos before the dehydration. Sucrose was provided as energy source to supported cell metabolism. Moreover, it could be incorporated into the cell wall to improve cell wall structure to tolerance to the dehydration. After synthetic seeds were dehydrated by silica gel method until remained 60% less of their moisture content and then stored 2 weeks, the synthetic seeds were shown low germinated was 23%, which was resulted the normal and abnormal seedling for 83 and 17% respectively. However, the prolongation of sweet corn synthetic seed viability could do under the high level of moisture content, which was resulted the highest percentage of germination and normal seedling (Table 5). Machii (1993) reported the best storage condition of somatic embryo especially under synthetic seeds form was cold temperature and high moisture content.

Sweet corn somatic embryogenesis by using N6 medium contained with sucrose and 2, 4-D could be produce embryogenic calli from immature zygotic embryos. This friable type II callus can be maintained in culture and regenerated (Takahata et al., 1993). The callus consists of aggregated, globular stage somatic embryos attached to a mass of vaculoe cells; regeneration can take place in small cell clusters that first from globules and then develop into callus aggregate. Single globules are unable to regenerate into plantlets; they form only roots (Pareddy and Petolino, 1990). The somatic embryos developed depending on abundant sucrose is necessary. Without this phase, calli were regenerate via organogenesis (Duncan et al., 1985). The survival rate of sweet corn synthetic seeds in this

Table 5. The effect of dehydrated levels and storage time on sweet corn synthetic seed viability.

Storage time (Weeks)	Moisture lose (%)	Germination (%)	Normal seedling (%)	Abnormal seedling (%)
	20	53[a]	92[a]	8[c]
1	40	45[b]	90[a]	10[b]
	60	30[c]	86[b]	14[b]
	20	46[b]	89[b]	11[b]
2	40	38[c]	87[b]	13[b]
	60	23[d]	83[c]	17[a]

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

investigation indicated that there are still some more research required to increase the number of the survival seeds and the optimum storage techniques to prolong their viability is also need.

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