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PRESERVATION EFFECT OF PEG (POLYETHYLENE GLYCOL) ON SYNTHETIC SEED OF SUGARCANE (*Saccharum officinarum*) VAR. NXI 1,3

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Abstract

Encapsulation is a method for embryonic tissue-coating system using Na-alginate for synthetic seed preservation. In order to produce synthetic seed of sugarcane, *coleoptillar* embryonic callus and apical bud were encapsulated by encapsulation media (MS0 + 4% Na-alginate + 100 mM $CaCl_2$). In this study, addition of PEG solution (0%, 1%, 2%, 3% (w/v)) in encapsulation media showed desiccation effect. This effect not only resulted in triggered the growth of synthetic seeds but also preserved the synthetic seed. Retarding the growth of synthetic seed is the effect of PEG on preserving the seed. PEG treatment also implied on longer storage periode of the synthetic seed. The result showed that 2% PEG was the most effective concentration of encapsulation media. Desiccation effect of 2% PEG prolonged storage period of synthetic seed until 3 weeks with 89,32 *planlets* generated and chlorophyll concentration of 26,03 μg/mL.

Keywords : *Encapsulation, Coleoptillar Embryonic Callus, Apical Bud, Synthetic Seed, PEG*.

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Introduction

Sugarcane (*Saccharum officinarum*) is ones of the most important sugar crops and agriculture commodities in Indonesia. The common propagation method applied by Indonesian farmers is cutting method using lateral bud sugarcane plant. This traditional sugarcane propagation method has limitation such as longer growth period and higher cost needed. Somatic embryogenesis is a method for rapid mass propagation and it can be used as embryonic material for synthetic seed production (Purnamaningsih, 2002). Somatic embryogenesis as cloned propagation method has been studied as a potential way to develop high yield clones. This advanced technique offers mass propagation with high yield, genetic improvement, lower *somaclonal* value and embryonic material for synthetic seed production (Purnamaningsih, 2002).

Somatic embryogenesis using solid media culture technique has been successfully developed in many plant species. Inducing somatic embryos from apical bud of sugarcane on MS medium has been reported by Soleha (2015). In this study, hormone modification of MS media culture showed that 4 ppm of 2,4-D was the optimal hormone for inducing somatic embryonic tissues. Somatic embryos have been applied as material in synthetic seed development, so it also offers great potential application for somatic embryonic of sugarcane synthetic seed production.

Synthetic seed produced by encapsulation method was a tissues coating system method which used Na-alginate and $CaCl_2$ as basic materials. This coating system is mixed with

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culture media (MS0), hormone, and sucrose (as carbon source) to improve growth and development of synthetic seed embryo (Reddy *et al.*,2012).

Synthetic seeds had many advantages over those produced for propagation by somatic embryogenesis, including their ease of handling and potential for long-term storage, a higher scale-up potential and their low cost of production. Artificially encapsulated somatic embryos can be shown under *in vitro* conditions, producing uniform clones (Inpuay, 2012).

Synthetic seed in *in vitro* cultures should be able to multiple efficiently and one of them had middle-term storage ability. The approach of this study is named dehydrated preservation. This approach was known an ideal way to make synthetic seed storaged for mid-term period (week). Seeds that show tolerant to desiccation through dehydrated preservation will retard its ability to growth. The time needed to recover the growth from retardation period called storage period. PEG has been kown as desiccators triggering for many tissues (Bapat, 2000). According to Sundararaj *et al.*, (2010), 0,5 mM and 0,75 mM of PEG can preserve ginger synthetic seed until 4 weeks. As mentioned by Widoretno (2003) this preservation process due to reversible inhibition of cell proliferation.

The effective concentration of PEG to induce storage period on sugarcane synthetic seed is still unknown until recently. In this study we have shown the most effective PEG concentration that give growth retardation effect and induce the longer storage period of sugarcane var.NXI 1,3 synthetic seed.

Materials and Method

Sugarcane Var.NXI 1,3 Somatic Embryogenesis Propagation

- **Embryogenic Callus Induction**

Spindle leaves of sugarcane (6 months-old) were sterilized with 96% alcohol, and 14 leaf rolls cultured on optimized embryonic callus induction media (MS0 + 4 ppm 2,4-D + 300 ppm CH (Casein Hydrolysate) + 30 g/L sucrose) (Soleha, 2015). It were kept for 30 days at 23 ± 1^0C in the dark condition.

- **Callus Embryogenic Proliferation**

Sugarcane embryonic calluses were transferred into proliferation media (MS0 + 2 ppm 2,4-D + 300 ppm CH + 30 g/L sucrose + 11 g/L agar) (Dewanti,2015). They were kept for 2 weeks at 23 ± 1^0C in 16 hours light - 8 hours dark condition. Callus development as marked by changing of callus from globular to coleoptillar stage was observed every a week using stereo microscope. Then, coleoptillar stage of the callus was used as a material for synthetic seed. Characteristics of coleoptillar callus observed were dorsal with protrusion formed, the opposite of second protrusion formed a notch between the bulge. The bulge will grow and elongate as a suspensor and then evolved into a torpedo shape or scutelum structure. In the subsequent development, the polar regions of the embryo formed to be plumula primordial (Guiderdoni & Demarly, 1988).

Sugarcane Synthetic Seed Production and Synthetic Seed Growth Retardation

Apical bud and coleoptillar callus stage were used as an explant. Explants were encapsulated with encapsulation media (MS0 + Na-alginate 4% + BA 0,3 mg/L + 30 g/L sucrose + IBA 0,5 mg/L), added with PEG (0%, 1%, 2% and 3% (w/v)) and dropped in the complex solution ($CaCl_2.2H_2O$ 100 mM) for 30 minutes. Then, the capsules were washed 3 times in sterilized water to remove the excess of $CaCl_2$ residues. 10 capsules were kept in *in vitro* condition, incubated at 25 ± 1^0C, with photoperiodity of 800-100 lux for 8 weeks.

Germinated capsules were observed every day and then transferred into regeneration media (MS0 + 30 g/L sucrose + 11 g/L Agar + 100 ppm Glutamine).

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Results

Somatic Embryonic Callus

Somatic embryonic callus was induced on somatic embryonic induction culture media for 4 weeks. The development of somatic embryonic callus is shown in figure 1. Sugarcane tissues began swelling in 7 days after planting and reaced maximum condition in 14 days. Callus had enlarged in 21 days. Callus entered pre-embryo mass phase in 28 days.

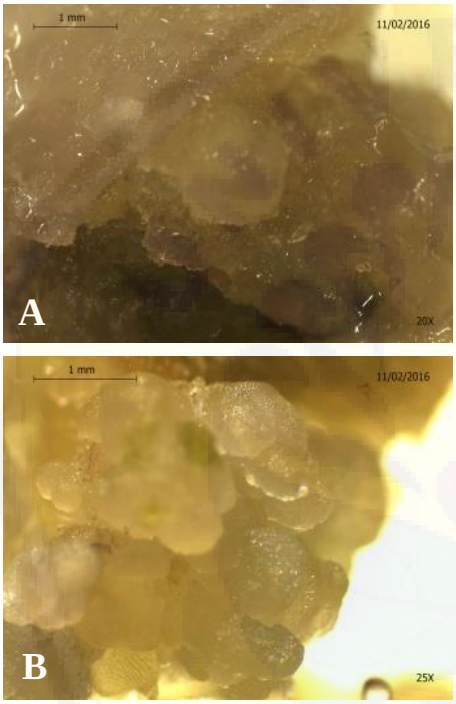

Figure 1. Induction of sugarcane Var.NXI 1,3 somatic embryonic callus: A. 21 and B. 28 days after planting.

Characteristics of embryonic callus were white to yellowish, friable and dried callus. Calluses were proliferated on proliferation media to evaluate stage of calluses development. The stages of callus development are showed in Figure 2.

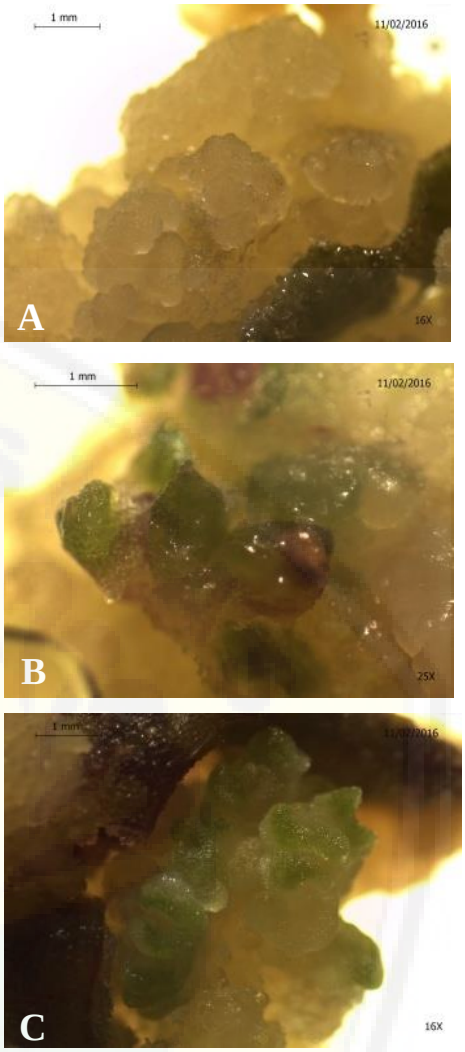

Figure 2. Callus development phases: A. *Globular* (5 weeks), B. *Scutellar* (6 weeks), C. *Coleoptillar* (7 weeks).

Synthetic Seed Production

Coleoptillar calluses or apical buds were encapsulated to produce sugarcane var.NXI 1,3 synthetic seed. Encapsulated media MS0 containing 4% Na-alginate and 100 mM $CaCl_2$ were the optimum media that induced 100% sprouting synthetic seed. Sugarcane synthetic seed growth is shown in Figure 3.

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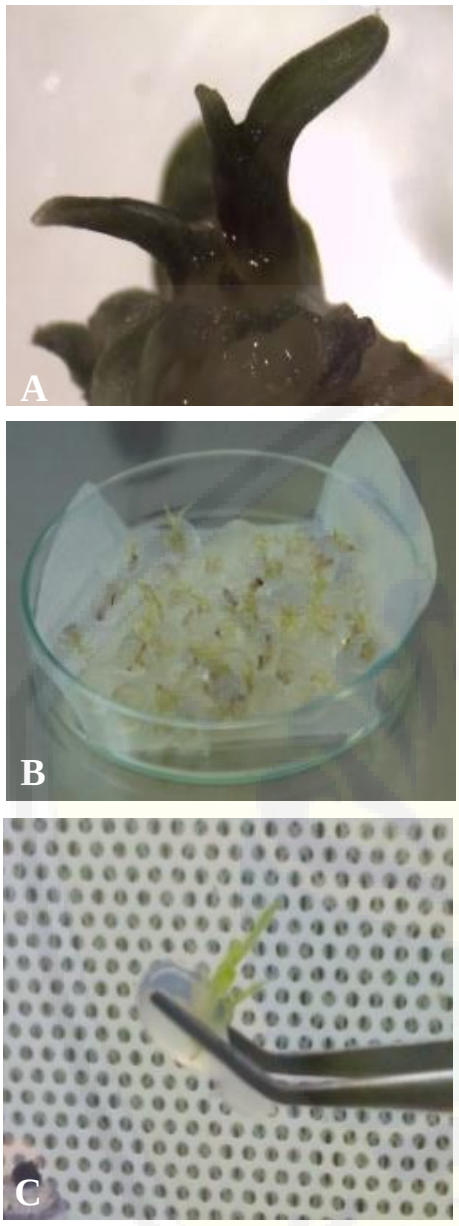

Figure 3. Synthetic seed production.A. *Coleoptillar* callus, B. Synthetic seed,C. germinated synthetic seed.

Retardation Effect of PEG

PEG 6000 at concentration of 0%, 1%, 2% and 3% (w/v) had been chosen to prevent embryo growth and development of synthetic seed. PEG 2% was the most optimum concentration to prolonge the storage period of the synthetic seed until 3 weeks (Table 4). Regeneration ability of synthetic seed treated by 2% of PEG produced the highest plantlets number, 89,32 plantlets (Table 5), but it had the lowest chlorophyll concentration, 26,03 μg/mL (Table 6).

Table 4. Sugarcane synthetic seed regeneration rate (%) on different PEG concentration (w/v)

Treatment	Weeks			
	1	2	3	4
PEG 0%	100±0.05	0	0	0
PEG 1%	15± 0.04	88±0.04	0	0
PEG 2%	0	28± 0.04	83±0.04	0
PEG 3%	0	13± 0.05	41± 0.06	84±0.05

Note: SD = Standard Deviation

Table 5. Total number of planlets of regenerated sugarcane synthetic seed

Treatment	Planlet Number
PEG 0%	69.32± 2.8
PEG 1%	78.00± 1.4
PEG 2%	89.32±0.7
PEG 3%	74.40± 2.8

Table 6. Chlorophyll concentration of sugarcane plantlets after PEG treatment

Treatment	Chlorophyl Concentration (μg/mL)
PEG 0%	31.202±1.8
PEG 1%	32.764± 1.9
PEG 2%	26.035± 1.5
PEG 3%	34.815± 2

Discussion

Sugarcane somatic embryos were the main materials to produce synthetic seed in this study. Somatic embryonic production in this study used modified MS media by adding of 4 ppm 2,4-D which has function to induce explant to somatic embrionic stage (Soleha, 2014). Chitra*et al.*, (2005) reported that embryonic callus was formed by inducer effect of synthetic *auksin*.

Morphological observation each phase of callus proliferation was focused to observe the characteristics of embryonic callus that differentiated further into the stages of somatic embryogenesis. Observation result showed characteristics of embryonic callus as pointed in Fig.1. The early stages of embryo formation began with the formation of pro-embryo mass (PEM) or the pre-globular embryonic callus which had the structure of a glossy, transparent,

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and dried (Figure 1A and 1B). Proembryo mass (PEM) would further developed into nodular embryonic callus-shaped, glossy, crumbs, dry, and transparent. Dewanti, *et al.*, (2015) had observed that the combination of 2,4-D (3 mg l-1) and BAP (4.5 mg l-1) induced callus embryonic up to 89% and 100%.

Somatic embryos as shown in our result have been produced from embryonic callus in proliferation media with characteristics: glossy, creamy, and transparent. These embryos further developed into embryonic stage such as globular stage (Fig.2A), scutellar stage (Fig.2B) and coleoptillar stage (Fig.2C) and finally regenerated into new plants (Roy *et al*., 2011). The development of sugarcane somatic embryonic was divided into several phases which are globular phase, scutellar phase and colleoptilar phase (Guiderdoni & Demarly, 1988).

An embryo of synthetic seed derived from Coleoptillar stage as capacity as an encapsulation material for synthetic seed. It was meristematic tissue that has fastest cleavage ability. Naturally, meristematic tissue has potential to support the development of plant tissue to be complete plants. Coleoptiles will develop into a complete structure with the shoot and root meristem or bipolar tissues (Purnamaningsih, 2002).

Coleoptillar stages and apical buds had great cleavage ability. Based on these abilities, they were chosen as materials on synthetic seed production. Coleoptiles and apical bud dropped into 4% Na-alginate + MS 100 mmol + BA 0.3 mg/L + 30 gr/L sucrose + IBA 0.5 mg/L. This encapsulation media composition is optimum media to make a sugarcane embryonic capsule and has resulted 100% synthetic seed germinated on seven days (Iffah, 2015). In that study, the combination of Na-alginate and $CaCl_2$ are not enough to preserve synthetic seed growth. Therefore it need the addition treatment or compound to create middle-term storage for synthetic seed.

In this study, PEG was a compound used to preserve development of the synthetic seed. Result shown that PEG 2% was the optimum compound that can prolong preservation of synthetic seed until 3 weeks, 83% of seeds can regenerate becoming plants with 89,32 plantlets generated and chlorophyll concentration 26.035 μg/mL.

PEG influenced the osmotic pressure and give desiccation effect, so that the transportation of nutrition in synthetic seed has run slowly. PEG has ability to decrease the water potential which make negative influence for embryo growth or this condition can make the growth velocity of sugarcane synthetic seed running very slowly. PEG in media will decrease the proliferation, development and regeneration of embryo (Tewary *et al*., 2000). Retardation mechanism shown by PEG on synthetic seeds is similar with dryness condition where polyamine endogen composition changed. Polyamine had function to retard embryonic carrot growth (Bastola & Minocha, 1995) and *Piceaglauca* (Kong *et al*., 1998).

The second mechanism of PEG to retard embryo growth is by decreasing the water potential. This system is a selective condition to expose the adaptive embryo on dryness condition. Adaptive cell or tissues can grow normally, without changing genetic arrangement of adaptive cultured cell or tissue in this selection media. Dried condition can induce tolerant adaptation of synthetic seed embryo by activating dried tolerant genes, LEA (Late Embryogenesis Abundant) and dehidrin protein. Both of them can active when dried condition, and they have function to protect cell water loss (Hundertmark & Hincha, 2008).

Acknowledgements

This work was supported by Higher Education Superior Research Project by BOPTN 2014 and Research and Development Trade Division PT.PN XI Persero.

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