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PRODUCTION POTENTIAL PHARMACEUTICAL VARIETY OF SUGARCANE WITH MICROSPORE CULTURE

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Abstract

Sugarcane is popular crops that have a high economic value to product sugar and bioethanol. In addition to these products, it turns out that sugar cane plants can also be used as herbal remedies in the healing of the urinary system and some liver diseases. Based on the increasing need for sugar cane and the benefits contained in sugarcane, it is necessary to develop new varieties that have potential pharmaceutical variety. Microspore culture techniques can produce pure homozygous lines in one generation so that selection of superior varieties is easier. This study used a completely randomized design with microspore culture donor plants of sugarcane varieties isolated at different storage temperatures. The results of Kruskal-Wallis data analysis showed that the variation of microspore culture storage temperature had a significant effect of 0.000 on the number of microspore sugarcane embryogenesis. The low-temperature storage 4^0C produces 10 Callus-like, while the room temperature storage 25^0C can produce as many as 300 Embryos-like.

***Keyword**: embryogenesis, microspore culture, sugarcane*

INTRODUCTION

Sugarcane species (*Saccharum officinarum* Linn.) is the poace family that has potential crops value. This plant is widely found in tropical and sub tropical regions (Anbanandan and Eswaran, 2018). This plant is one of important crops around the world. Statistic show that 80 % of the world's sugar commodities come from sugarcane (Fairtrade Foundation, 2013). Sugarcane plants are widely cultivated in Indonesia and its reached 445.520 ha in 2016 with a productivity approximately 360713 tons/year (Bantacut, *et al*., 2009). Indonesia is a tropical country that is suitable in developing sugarcane. An increase in Indonesian sugar production is expected to improve the country's economy.

In general, sugarcane plants are used for sugar production dan bioenergy/bioethanol (Zhao and Li (2015). Sugarcane also has other potential in the health pharmaceutical aspect. Sugarcane is widely used as sugarcane juice. Sugarcane juice is actually known as a raw material for the production of refined sugar. And the other is in the processing sugarcane juice, secondary products are also produced in the form of brown sugar, molasses, and jaggery (Singh *et al*., 2015). Sugar cane juice is very popular in India. This drink is used in traditional medicine systems for the healthy treatment such as urinary deseases (hemorrhage, dysuria, anuria etc.) (Chinnadurai, 2017). Based on the research Kadam *et al*., (2013), Sugar juice can cure

jaundice and liver disorders. Several sugarcane varieties tested have antioxidant aspects and also protect DNA damage so that it can counteract free radicals, reduce iron complexes and inhibit lipid peroxidation.

The use of sugar cane as a medicinal beverage has not been developed. currently, the development of sugarcane varieties only leads to the amount of sucrose content to be processed into sugar. In addition, conventional sugarcane breeding takes a long time. Plant breeding programs for the selection of genotypic varieties that have potential as medicines have been carried out by Alam *et al*., (2017). The conventional selection of genotypes takes a long time. The selection was done in the form of leaf blade length, leaf width, fresh leaf weight, dry leaf weight, number of tillers, millable sugar cane, shoot size, sugarcane diameter, number of segments, segment length, plant height, long stem, brix% and sugarcane weight individuals. The results of this study indicate that sugarcane genotypes have high variability and need further research to obtain higher sugarcane yields due to juice and brix content.

Chance the selection of sugarcane varieties with microspore culture gives great hope for the development of sugarcane varieties which have the potential pharmaceutical. Based on the data above, it is necessary to develop sugarcane varieties that can be used as pharmaceutical product.

Biotechnology Sugarcane breeding has been developed in Indonesia. Biotechnology offers plant breeding techniques quickly (Suslow *et al*., 2002). One of the modern plant breeding techniques that have been widely applied to plantation crops is microspore culture. Microspore culture techniques are able to produce haploid and even double-haploids. With pure line varieties, selection of varieties will be easier because that are completely homogenous and homozygous in one generation (Al-Khayri *et al*., 2015). So that in this study microspore culture techniques were carried out production potential pharmaceutical variety of sugarcane with microspore culture.

MATERIAL AND METHOD

Plant material preparation

This research used Bululawang variety as donor plants of sugarcane microspore culture. The anther was collect after sugarcane flowering at 8-10 months. *panicle selection based on shapes that* still covered with flags leafs. Panicle harvested wrapped in newspaper and then stored in growth camber for 24 hours.

Microspore isolation

The first technique is pre pre treatment stress for anther. panicle is opened inside the laminar airflow to remove anther. Anther selection based on anther color, specifically yellow anther and then stored in a period of time 7 days before isolation of microspore culture. The isolation microspore culture began with 200 pounding anther which were slowly mixed with a mortar and stamfer in the mannitol 0.3M medium. if the microspores have appeared out then filtered using a 100 µm filter. suspended filtration was centrifuged using 10 ml mannitol medium as much as 10 ml by 4^0C cold temperature centrifugation at 750 rpm for 5 minutes. After that the pellet produced was transferred in a 4 ml petri dish containing MS medium at a density of 3×10^4 microspores per petridish. each petridish is coated with parafilm to prevent contamination. Isolation results are stored at different storage temperature variations 25^0C and 4^0C in dark conditions. data observed after 4 weeks. And data was analized by Kruskal-Wallis Test in SPPS aplications.

RESULT AND DISCUSSION

The application of microspore culture techniques to sugarcane plants has a very big challenge. this is because there is no literature on the success of microspore culture sugarcane techniques into perfect plants. The data reported in this study is the initial data on optimization of micropore culture techniques. Embryonic development will then be reported in the next article.

Based on observations made for 30 day culture, it was found that different storage temperatures could produce variations in embryo shape. at low temperatures 4^0C produced the form of the callus likes and at room temperature storage 25^0C produced *embryos-like* (Table 1).

Table1. Average Number of embryonic microspores based on storage temperature

	S1	S2	Sign
Number of embryos	10	598	0.000
Type of embryo	*Callus-like*	*Embryos-like*	0.000

** S(storage temperature): S1= 4^0C, S2 = 25^0C)*

The number of embryos produced is also very different. room temperature storage 25^0C can produce as many as 300 *Embryos-like*, while low temperature storage produces *Callus-like* (Figure 1).

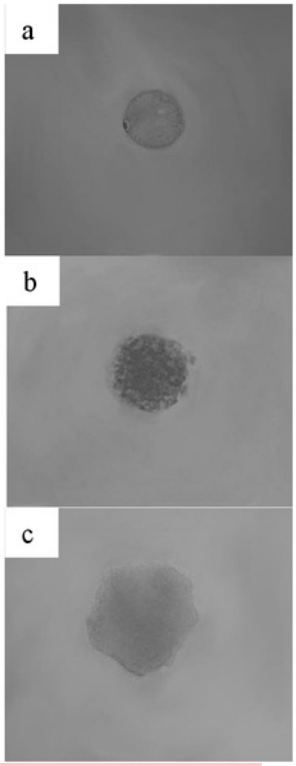

Figure 1. Microspore embryogenesis a. microspore b. *embrio-like* c. *callus-like*

There are several factors that can influence microspore culture such as genotype, stress treatments, culture media, microspore density and developmental stage in microspores (Chaar *et al*., 2014). Embryogenesis process in microspore culture is a unique system in which microspore cells are programmed specifically with stress treatment towards the embryogenesis pathway. stress treatment can be in the form of variations in incubation temperature to trigger embryogenesis (Prem *et al*., 2012). The statement is in line with research Suaib *et al*., (2013) which states that the stress of temperature treatment can trigger embryogenesis. Microspore culture techniques in this study used stress pre-treatment on anther before isolation of microspore culture. Selected anther was stored in mannitol 0.3M solution for 7 days at 4^0C. In the study of Ayed *et al*., (2010) at the other family poace (wheat) isolation, mannitol is effective in triggering microspore embryogenesis. Isolation of microspore culture of sugarcane in this study uses two different storage temperature variations to determine the temperature that is suitable to produce high embryogenesis.

Low temperature for storage of isolation results of microspore culture has not been developed much. Low temperature between 1-5^{0}C is developed as a stress treatment at anther culture in wheat (as in some other species) (Zheng, 2003),. in this study the low temperature of 4^0C which was applied as the storage temperature of culture results had an effect on embryogenesis. but the number of embryos produced just 10 embryos average and callus-like shaped. Storage of isolation of microspore culture at 25^0C room temperature is more effective in increasing the number of embryos-like. Storage at room temperature 25^0C in isolation of sugarcane microspore culture produces 589 average embryos. This

technique is widely applied to other plants such as brassica (Takahata and Keller, 1991). and has been shown to successfully increase embryogenesis.

Microspores have the ability to develop into haploid plants using in vitro culture by changing pathways to sporophytes by preventing the development of pollen (gametophytic pathways). This sporophytic process is carried out with stress treatment in microspores so that embryogenesis. The resulting embryo is truly homozygous making it easier to study plant breeding and variety selection (Touraev *et al*.,1997). So that in this study the treatment of microspore isolation was effective in producing sugarcane embryos.

CONCLUSION

Application of microspore culture techniques on sugar cane effectively produces embryos. the next stage is the regeneration of plants to become green plants so that the selection of potential sugarcane varieties in pharmacy can be done in a fast time.

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