Basic Study Microspore Development of Indonesian Sugarcane (Saccharum officinarum L.)

by Septarini Dian Anitasari

Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(2): 913-917. **OPEN ACCESS**

Basic Study Microspore Development of Indonesian Sugarcane (*Saccharum officinarum L.*)

Septarini Dian Anitasari[1], Dwi Nur Rikhma Sari[1], Ida Ayu Astarini[2] and Made Ria Defiani[2]

[1]Biology Education, FP.MIPA IKIP PGRI Jember, **Indonesia**
[2]Biology Departement, FMIPA Udayana University, **Indonesia**

***Correspondence:** septarini@ikipjember.ac.id **Accepted:** 14 Mar 2018 **Published online:** 08 June 2018

There is no standart protocol for microspore culture sugarcane. This reseach showed the first method to determine microspore development for preparation microspore culture sugarcane. This study used BL variety. The Stage of microspore was checked under microscope. All the experiment was designed according to CRD (completely random design) and the data was analyzed using descriptions technique. the result is each anther color has a different percentage of stages of microspores. Besides that from three sampling location that is jember, lumajang and bondowoso, percentage of number of microspore stages obtained also vary. The highest percentage of uninucleate stage was found in the yellow anter of 94.27% from Jember sampling area.

Keywords: saccharum, embryogenesis, uninucleate stage

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a tropical and subtropical plant belonging to the grass family (Poaceae). This plant is classified the cereal genera such us sorghum and Zea (corn) (OGTR, 2013). Sugar cane is widely used as one of the most efficient plants in the world as biomass and sucrose. The high economic value of sugarcane is for important foods and bioenergy such as sugar production, molasses and ethanol (Moore and Botha, 2013).

Sugarcane is one of the most important crops in Indonesia that has economic value with the increasing demand of sugar production (USDA Foreign Agriculture Service, 2016). Plant breeding programs are essential for developing high yielding sugarcane varieties with high productivity, high sucrose content, drought tolerance, ethanol production and high biomass (Morais et al., 2015).

Conventional sugarcane breeding program requires time, land, cost, plant material sources and a lot of labor (Suaib et al., 2013). This conventional breeding program for selection of new varieties takes 10-15 years (Tarique et al., 2010). Microspores culture technique is the latest technology of tissue culture to produce good varieties quickly compared with other techniques (Segui-simaro dan Nuez, 2008).

Quick technique in producing height quality sugarcane varieties that is with microspore culture. Microspore culture has an excellent system for breeding programs. The technique performed is microspore induction so that embryogenesis and can produce double haploid plants. Production of double haploid plants from microspores is an important technique used in plant breeding and basic research (Ferrie and Caswell, 2011). The main advantage of Microspore culture methods can produce homozygous line within a year as compared to conventional methods that needed the long

inbreeding method (Islam et al., 2013).

Breeding program through microspore culture techniques has been successfully applied to various horticultural crops such as brassica group (Kalashnikova et al., 2011), wheat (Ayed *et al.*, 2010), chili (Yin et al., 2010). In addition to some families of grasses have also been successfully applied such as rice (Islam et al., 2013), Wheat (Scagliusi, 2014), maize, barley.

The efficiency of this method is influenced by several factors such as physical and growth conditions of the donor plants, cultural conditions, growth regulators, plant media, pretreatments, pollen isolation procedure, etc (Islam et al., 2013). No reports about successfully propagation with microspore culture that product haploid plant in sugarcane. The first research report that the effect of temperature and of sucrose and 2,4-D in the media isolation microspore culture resulted in procalli and calli form (Hinchee and Fitch, 1984). Aplied microspore culture Microspore embryogenesis under different temperature and time duration pretreatments of spike segments resulted embrio like structure (Suaib et al., 2008).

The efficiency of this method is influenced by several factors such as physical and growth conditions of the donor plants , cultural conditions, growth regulators, plant media, pretreatments, pollen isolation procedure, etc (Islam et al., 2013). One of the important factor for isolation microspore is developmental stage of microspore in donor plant. The microspore development stage is a complex factor that strongly affects the success of microspore culture. The stage of microspores at the time of inoculation is one of the most critical factors for induction of androgenesis (Mishra and Goswami, 2014). The high success of Androgenesis is related to age of microspores and pollen development. The most responsive pollen stage may range between early uninucleate to late binucleate stage (Pratap et al., 2009).

Many parameters have been recognized to determine microspore stage for isolation culture. A preliminary study on the sugarcane haploid breeding through in vitro microspore culture has been initiating by Suaib (2013), result of the research showed that the unsheated panicles were contained less than 50% of uninucleate (early-and late-uninucleate) microspore development and the sheated panicles tend to be in high proportion of early-and late-uninucleate microspore development, and multinucleate or pollen grains, and the more away of spikelets or anthers positioned in the panicle or sub-panicle, the more number or percentage of uninucleate microspores development were tend to be gradually decreased.

There is no universal protocol that will result in microspore embryogenesis in all species, as differences occur among species and among genotypes within a species in terms of embryogenic response (Ferrie and Caswell, 2011). This reseach showed the first method to determine pollen development for preparation microspore culture sugarcane.

MATERIALS AND METHODS

Variety BL sugarcane were used as plant material. The anthers were collect in different color (white, yellow and brown) from three area in east java that is Bondowoso, Jember and Lumajang. The panicle were harvested in the morning and wrapped on newspapers then stored in an ice box. The anthers were place in 100 ml of aquadest, squeezed using glass rod and then filtered through 100 µm filter paper. Five anther from each length were collected into the laboratory. The Stage of microspore was checked under microscope. All the experiment was designed according to CRD (completely random design) and the data was analyzed using descriptions technique.

RESULTS AND DISCUSSION

There are only a limited number of reports successes of microspore culture to product doubled haploids in Sugarcane. Many researchers used bud size as a parameter in determining microspores stage in family poaceae (Mishra, 2016; Moraes, 2008; Suaib, 2007). Color parameters have not been widely used as parameters. Stage of microspore development is very important in microspore culture techniques. In this study used anther color differences and sampling area as a parameter to determine the percentage of stages and the number of microspores produced. Based on the anther color it is found that each anther color has a different percentage of stages of microspores. Besides that from three sampling location that is jember, lumajang and bondowoso, percentage of number of microspora stages obtained also vary. Based on the research that has been done. There was the percentage development stage of microspores:

Table 1. Percentage microspore development stage

Microspore development stage	Number microspore (%)								
	White			Yellow			Brown		
	J	L	B	J	L	B	J	L	B
Early uninucleate	81.44	52.66	62.94	5.73	23.16	33.33	0	0	0
Uninucleate	18.56	42.02	37.06	94.27	76.84	58.73	0	11.62	0
Multinucleate	0	5.32	0	0	0	4.23	18.85	39.89	44.57
Pollen grain	0	0	0	0	0	3.7	81.15	48.48	55.43

(Sampling area: J: Jember; L: Lumajang; B: Bondowoso)

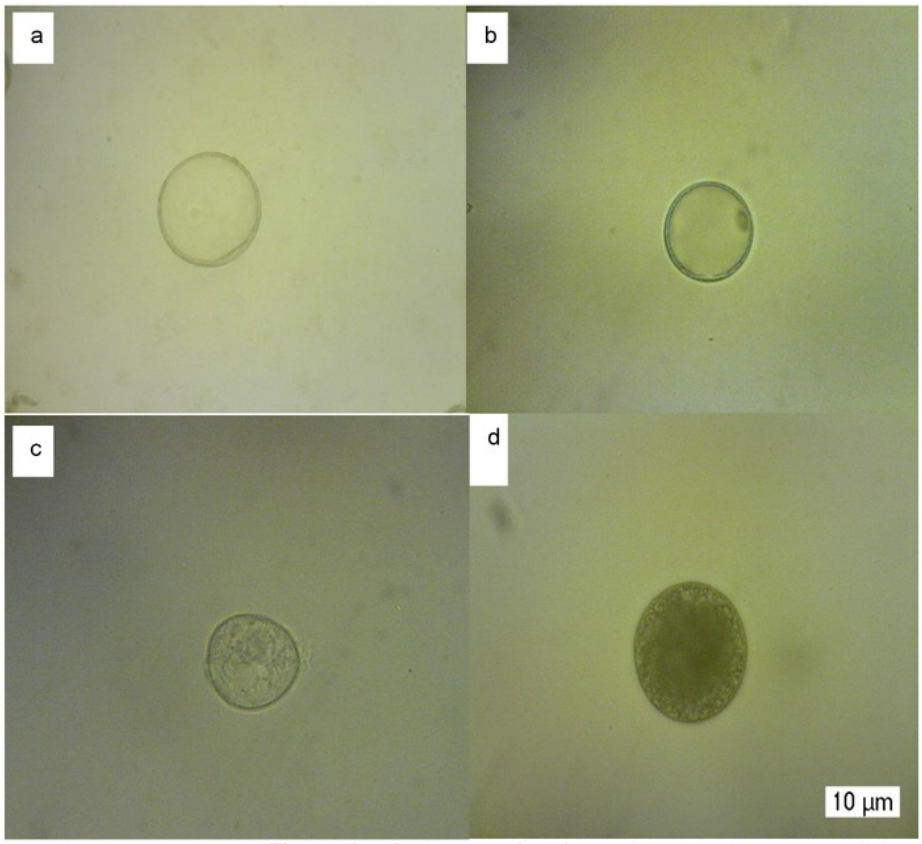

Figure 1. microspore development
(a. early uninucleat; b. uninucleat c. multinucleate; d. mature pollen)

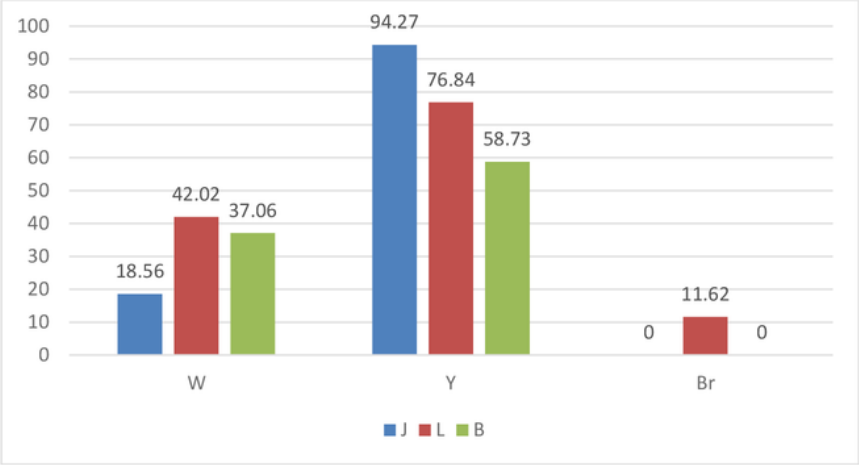

Figure 2. Percentage uninucleate stage of microspore
(anther colors W: White; Y: Yellow; Br: Brown, Sampling Location (J: Jember;L: Lumajang; B: Bondowoso)

Microspore stage obtained in this research is early uninukleat, uninukleat, multinucleate and pollen grain. Each stage has different form variations (Figure 1).

The purpose of this study is to obtain microspore stage that suitable for donor plant microspora culture. The percentage of uninukleat stages from lowest to highest can be seen in (figure.2)

In this study were determine four stage of microspore that is early uninukleat, uninukleat, multinucleate and pollen grain.. Every stage of microspore has different characteristic. Microspores were at the early-late uninucleate stage was responsive for microspore culture (Ibrahim et al., 2014). Determine at the right stage microspore is important tool for micropore culture It has been reported that the older stage of microspores may produce toxins and inhibitors during microspore culture which could reduce the efficiency of the microspore derived double haploid production. Microspore culture can induced or reprogrammed towards the sporophytic pathway to produce gametic-embryos (Appiah et al., 2013). The determination of this uninukleat stage for microspore is successful in other poacea families. For example the uninucleate stage has been found to be suitable for rice callus induction (Kinoshita et al., 2012).

The highest percentage of uninucleate stage was found in the yellow anther of 94.27% from Jember sampling area. Li (2003) stated that anther color is related to microspore development stage; therefore anther color can become a very important criterion for selection suitable buds to induction of embryogenetics. a qualitative character which is not easily influenced by both environment and genotype we can propose a view that anther color as criterion for selection suitable microspore for induction embryogenesis.

CONCLUSION

Each anther color has a different percentage of stages of microspores. besides that from three sampling location that is jember, lumajang and bondowoso, percentage of number of microspora stages obtained also vary. The highest percentage of uninucleate stage was found in the yellow anter of 94.27% from Jember sampling area. So that uninucleate stage we used for suitable development stage for microspore culture
.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

We would like to thank Ministry of Research and Technology, and Higher Education, Republic of Indonesia, via Pekerti Grant Scheme 2017.

AUTHOR CONTRIBUTIONS

SDA makes research design and wrote the journal manuscript. All authors contribute to the implementation of research. DNRS analyzes the research data. IAA and MRD review the manuscript journal. All authors read and approved

the final version.

REFERENCES

Ali, A., Iqbal, j and Naz, S. 2008. *An efficient protocol for large scale production of sugarcane through micropropagation*. Journal of Botany 40(1): 139-149

Appiah, R., Ankrah, N., Liu, W., Konzak, and Wettstein, D. Generation of Doubled Haploid Transgenic Wheat Lines by Microspore Transformation. Plos One 8(11).

Ferrie, A and Caswell, K. 2011. *Isolated microspore culture techniques and recent progress for haploid and doubled haploid plant production*. Plant Cell Tiss Organ Cult (104) :301–309

Morais L., Aquiar, M., Silva, P., Camara, T., Cursi, D., Junior, A., Chapola, R., Carneiro, M and Filho, J. 2015. *Breeding of Sugarcane*. Handbook of Plant Breeding Springer 9.

Goralski, G., Rozier, F., Matthys-Rhochon and Przywara, L. 2014. *Cytological Features Of Various Microspore Derivatives Appearing During Culture Of Isolated Maize Microspores*. Acta Biologica Cracoviensia Series Botanica 47(1): 75–83

Hinchee, M and Fitch, M. 1984. *Culture Of Isolated Microspore of Saccharum spontaneum)*. Journal of Plant Physiology 113:4.

Ibrahim, A., Kayat, F., Hussin, Z., Susanto, D and Arrifullah, M. 2014. Determination of Suitable Microspore Stage and Callus Induction from Anthers of Kenaf (Hibiscus cannabinus L.). Scientif World Journal 2014(5).

Islam, S., Ara, I., Tuteja, M and Subramaniam, S. 2013. *Efficient Microspore Isolation Methods for High Yield Embryoids and Regeneration in Rice (Oryza sativa L.)*. International Journal of Biological Science and Engineering 7 (12).

KInoshita, A., Okamoto, Y., Ishimura, S and Satake, T. 2012. Determination of Optimum Pollen Developmental Stage for Inducing Callus in Anther Culture of Rice. Breeding Research 2 (2):73-79

Li,X and Guan, C. 2003. Studies of microspore culture and doubled haploid breeding on rapeseed: plant regeneration from microspore derived embryos of F1 hybrids between Brassica napus and B.juncea. Proceeding IRC.

Mishra, V and Goswami, R. 2013. *Haploid Production in Higher Plant*. Ijcbs Review Paper 1(1).

Mishra, R and Rao, G. 2016. *In-vitro* Androgenesis in Rice: Advantages, Constraints and Future Prospects 23(2): 57-68

Moore, P and Botha, F. 2014. *Sugarcane Physiology Biochemistry and Functional Biology*. USA: John Wiley and Sons, Inc.

Moraes, A., Bered, F., Carvalho, F and Kaltchuk-Santos, E. 2008. Morphological markers for microspore developmental stage in maize. Brazilian Archives of Biology and Technology 51(5).

OGTR. 2013. *The Biology Of Saccharum spp. (Sugarcane)*. http://www.ogtr.gov.au. 26 April 2017.

Pratap, A., Gupta, S and Takahata, Y. 2009. Book Chapter Microsporogenesis and haploid breeding "Biology and Breeding of Crucifers. India: CRC Press.

Scagliusi, S. 2014. Establishing isolated microspore culture to produce doubled haploid plants in Brazilian wheat (Triticum aestivum L.). Australian Journal of Crop Science 8(6):887-894.

Suaib., Mangoendidjojo, W., Mirzawan, P and Indrianto, A. 2007. Proporsi microspore uninukleat pada empat klon tebu (Saccharum spp.). Berkala Penelitian Hayati 12(2):145-152.

Suaib, Woerjono, Mirzawan dan Indrianto, A. 2013. *Kultur Mikrospora, Alternatif, Peluang dan Prospek Perbaikan Genetik Pada Populasi Tanaman Tebu (Saccharum spp.)*.Berkala Penelitian Agronomi 2 (1):79 – 87.

Tarique, H., Mannan, M., Bhuiyan,m dan Rahaman, M. 2010. *Micropropagation of Sugarcane through leaf sheath culture*. Int. J. Sustain. Crop Prod. 5(2):13-15

USDA Foreign Agriculture Service. 2016. Indonesia Sugar Annual Report 2016. https://gain.fas.usda.gov. 1 Juni 2017. 3

Basic Study Microspore Development of Indonesian Sugarcane (Saccharum officinarum L.)

ORIGINALITY REPORT

12%	9%	7%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	screamnet.rsl.ru Internet Source	4%
2	nparc.cisti-icist.nrc-cnrc.gc.ca Internet Source	3%
3	Pratap, Aditya, S Gupta, and Y Takahata. "Microsporogenesis and Haploidy Breeding", Biology and Breeding of Crucifers, 2009. Publication	1%
4	zenodo.org Internet Source	1%
5	"Crop Improvement Under Adverse Conditions", Springer Nature America, Inc, 2013 Publication	1%
6	aip.scitation.org Internet Source	1%
7	Kruasuwan, Worarat, and Arinthip Thamchaipenet. "Diversity of Culturable Plant Growth-Promoting Bacterial Endophytes	1%

Associated with Sugarcane Roots and Their Effect of Growth by Co-Inoculation of Diazotrophs and Actinomycetes", Journal of Plant Growth Regulation, 2016.

Publication

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		