DEVELOPMENT OF MICROSPORE CULTURE TECHNOLOGY FOR SUGARCANE IN INDONESIA

Septarini Dian Anitasari[a], Dwi Nur Rhikmasari[a], Ida Ayu Astarini[b], Made Ria Defiani[b]
[a]Biologi Education, FP. MIPA IKIP PGRI Jember
[b]Biology Departement, FMIPA Udayana University
Correspondence: septarini@ikipjember.ac.i /085338123232

Abstract

Microspore culture is an efficient tissue culture technique for producing haploid on many industrial plants. Until now there has been no publication of the success of this technique in modern sugarcane cultivation. Optimization of microspores culture techniques is still not much developed in Indonesia. The research purpose is the production of haploid lines for the selection of the best varieties that can be used in sugarcane breeding. Microspore culture techniques used a pre-treatment temperature of 4^0C with different storage times on uninucleated sugarcane microspores before culture isolation. Data analysis using description from picture and table. The results showed that there was an effect on the embryogenesis of microspores after culture isolation. Each time the microspores are stored has a different effect on the number of embryos produced.

Keyword: sugarcane, microspore, uninucleate

Introduction

Sugarcane species (*Saccharum officinarum* Linn.) Is a potential plant of the poaceae family originating from tropical and sub-tropical regions (Anbanandan and Eswaran, 2018). Indonesia is a tropical country that has a lot of sugarcane. in various countries, sugarcane is widely used as bioenergy / bioethanol and mainly sugar production (Zhao and Li (2015).

Sugarcane is also a potential in the field of health pharmacy. Biotechnology of tissue culture has been known as a plant breeding technique that produces pure strains rapidly (Suslow *et al.,* (2002). One of the modern plant breeding techniques that has been widely applied to estate crops in the world is microspores, microspores are able to produce plants that are both haploid and double haploid, with pure line varieties, the selection of the best varieties is easier because completely homogeneous and homozygous in one generation (Al-Khayri *et al*., 2015).

In this study developed microspore culture in sugar cane plants in Indonesia. Breeding using microspore culture have actually success to several other plants in grass families have also been successful applied like rice (Islam *et al*., 2013), Wheat (Scagliusi, 2014), maize (Zheng *et al*., 2003), barley (Li and Devaux, 2003). However, in sugarcane there have been no reports of successful application of microspores that produce haploid plants on sugarcane. Earlier research reports that there is effect of media and hormone and temperature in microspores culture o produced proximate and callus forms (Hinchee and Fitch, 1984). Applied technology of microspore culture can produce embryo like microspore structure (Suaib et al., 2008). so microspore culture techniques we were developed with stress treatment to trigger embryogenesis in sugarcane plants to produce haploid plants so that they can be used as pure lines of sugarcane plants.

Methods and Materials

The first step is anther selection based on anther color, especially yellow to brown anther. The anther was stored in mannitol treatment 0.3 M solution in a period of 0,7,14,21 days before microspores culture. The microspores culture was begun with 200 anther which was pounded on mortar and stamfer slowly in a medium 0.3 M mannitol. the microspores have seen yellowish in the solution then filtered using a 100 µm filter. suspended filtration was centrifuged using 10 ml of 10 ml mannitol media with 4^0C cold temperature centrifugation at 750 rpm for 5 m. After that the resulting pellet was transferred in a 4 ml petri dish containing MS medium at a density of 3×10^4 microspores per petridish. Each petridish coated with parafilm and stored at 25^0C variations in room temperature 25^0C in dark conditions. data were observed after 90 days of isolation. And data were analyzed with Anova One-Way and Duncan Test.

Results

The conclusion of this study is the development of microspore culture techniques with stress treatment using mannitol 0.3M during different storage times successfully induced microspore embryogenesis with varying number of embryos.

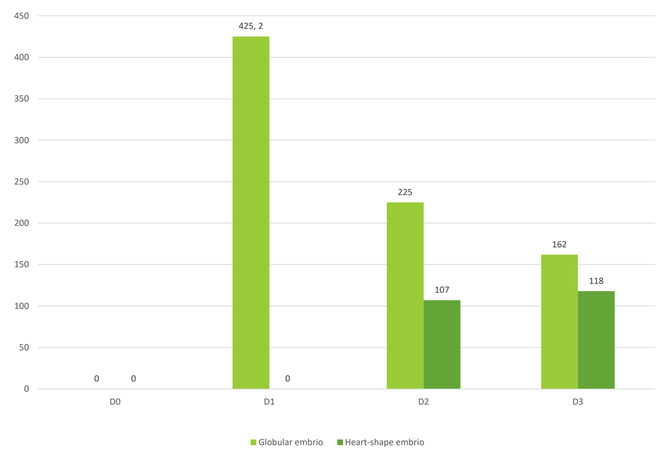

Figure 1. Embryonic Microspore Number from microspore culture *(D0 (Early Incubation),D1 (30 day), D2 (60 day), D3 (90 day)*

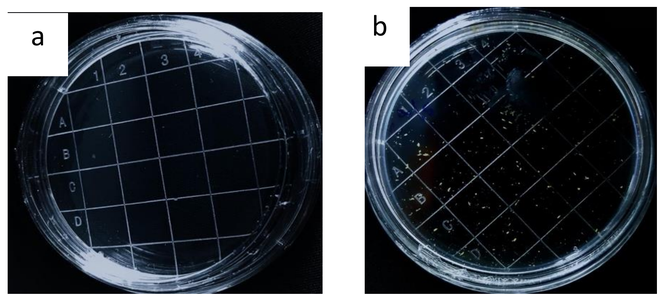

Figure 2. Embrio shape on petri microspore culture at early incubation (a) until 90 day culture (b)

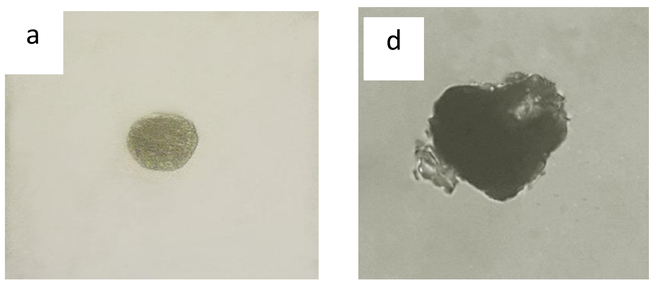

Figure 3. Microscopy shape at 100x microspore culture early incubation (a) until 90 day culture (b)

Discussion

Basically sugarcane breeding with microspores culture technology in Indonesia has not been developed much. so far there have been no reports of the success of this technique on sugar cane. so the development of this technique continues to be developed with various stress treatments. Microspores have the ability to develop into haploid plants using microspores by changing the life path to sporophytes by preventing the development of pollen (gametophyte pathways). The sporophyte process is carried out by giving stress treatment to microspores so that embryogenesis. The resulting embryos are embryos that are truly homozygous so that it is very easy to study plant breeding and the selection of the desired varieties (Touraev *et al*., 1997).

The results in this study were diverse embryos after isolation of microspore cultures. Stress treatment given during microspore isolation is effective in producing sugarcane embryos. The success of this technique is supported with influential such as the type of genotype donor plant, stress treatment, media used when isolating microspores, culture techniques, the amount and density of microspores in petri and the stages of microspores development used as donor plants (Chaar *et al*., 2014).

The process of embryogenesis in microspores culture is a special and unique system because microspores cells can be programmed specifically with stress treatment of the embryogenesis pathway. But the success of this technique is specific and not the same in every plant. Stress treatment can be given in the form of incubation temperature variations to trigger embryogenesis (Prem *et al*., 2012). This statement is in line with research by Suaib *et al*., (2013) which states that temperature treatment can trigger embryogenesis.

Microspora culture techniques in this study used pre-treatment of stress on the anther before isolation of the microspora culture. The selected Antera was stored in a 0.3 M mannitol solution for 7 days at 4^0C. In the study of Ayed *et al*., (2010) in the isolation of other family (wheat) poace, mannitol was effective in triggering microsporic embryogenesis. Isolation of sugarcane microspores in this study used two different variations of storage temperature to determine the appropriate temperature to produce high embryogenesis.

The selection of microspora culture techniques in this study was effective in producing sugarcane plant embryos. the next research stage is expected that the embryo that has been produced can become a haploid or double haploid plant so that it can be used as a pure strain for sugarcane in Indonesia.

Conclusions

The conclusion of this study is the development of microspore culture techniques with stress treatment treatment in the form of immersion in mannitol 0.3M during different storage times successfully induced microspore embryogenesis with varying number of embryos.

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Contact:

Septarini Dian Anitasari,S.Si., M.Si
IKIP PGRI JEMBER
Email: septarini@ikipjember.ac.id
Phone: 085338123232

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