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Submission date: 15-Oct-2019 07:54PM (UTC-0700)

Submission ID: 1193754470

File name: CHEK_PLAGIAT_1.pdf (646.28K)

Word count: 1997

Character count: 11062

IDENTIFICATION OF UNINUCLEATE STAGE FOR MICROSPORE CULTURE OF SUGARCANE "BL"

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ABSTRACT

Microspore culture is the latest in vitro plant breeding technology for the production of haploid and double haploid plants. The uninucleate stage of microspores plays a role in producing microspora embryogenesis. No research has been reported that succeeds in producing embryos through microspores in sugarcane. The purpose of this study was to identify the uninucleated microspora stage for preparation of microspores culture based on the color variation of the "BL" cane anther. The research method is carried out through microspore Stage observations under a microscope. Experiments were carried out according to a complete randomized design (CRD) and data were analyzed using the 5% t-test and followed by the Duncan 5% test using SPSS statistics. The number of nucleated microsporates was found in the yellow anther by 89.3%. The low percentage of the 7.2% uninucleated stage in white is considered to be the initial stage of the anther, while the next stage of the anther is 2.5%. Results of the t-test analysis showed a significant difference between the anther colors of the microspore uninucleate stage ($\alpha = 0.00$). Different anther colors give different percentages of microspore uninucleate stages. This study shows that there are four types of non-nucleated stages such early uninucleate stage, uninucleate stage, mid-uninucleate stage and late uninucleate stage.

Keyword: uninucleate, microspore culture, saccharum

INTRODUCTION

Sugar is one of the important commodities in the Indonesian economy. Sugar is not only consumed directly by people as a food additive, but is also used in the food and beverage industry (Lestari et al., 2016). Sugar is also an important source of food and bioenergy and is an important economic component in many countries, especially in the tropics and subtropics. Nearly 100 countries produce sugar cane on 22 million hectares of land, around 0.5% of the total world area used for agriculture. The development of tissue culture technology has an important role in the propagation of plants that require a short time and produce plants that are free of disease. so tissue culture is an important biotechnology in producing superior seed production. in other countries such as Australia, India and the Philippines have implemented this technology for commercial seed production by producing fast and quality varieties of plants.

(FAO, 2008).

Tissue culture technology that has been popular for a number of years is microspores culture. Microspora culture produces haploid plants and can directly become double haploids in a

relatively short time. New varieties produced in this method are superior seeds that can be produced in a short time compared to conventional methods. Microspora culture methods have been used successfully in many crop plants. Embryogenesis produced in large quantities and the processes used. It doesn't take years to produce new varieties(Kahrizi and Mirzaei, 2012).

Double Haploid plants are very important to produce genotypes that are fully homozygous. the use of homozygous plants is at the core of the plant breeding program in producing new varieties in a number of plant species. This method is effective for producing embryos in the development of pure genetic breeding lines in a single generation (Rahman and Jimenez, 2016)

Methods Microspora culture can produce both haploid and double haploid plants in one generation of plant breeding. there are several factors that influence the culture of microspores including genotypes, physiological conditions of plants, pre-treatment of anther, the composition of the media used for culture, conditions during culture isolation and the right stage of microsporal development as a culture donor (Gray, 2016).

Development stage of microspores is a must to be considered before microspora culture is carried out. This stage has an important role in the success of microspores culture to produce microspora embryogenesis. Only microspores have special characteristics, namely at certain stages of development that can be directed to the path of embryogenesis. The best stage for microspores to isolate microsporal culture is that of microspores which have stages around the first mitosis from uninucleated microspore to initial bicellular microspore (Altman and Hasegawa, 2012).

Even though developmental stage of microspore sugarcane has been reported (Suaib *et al.*, 2007). The need for the stage of development of microspores used in culture isolation is different in each plant variety. in principle microspores at the uninucleate stage can be induced into embryonic microspores by changing the plant development system. The process carried out is to change the direction that should be the gametophyte pre-program into embryonic reprogramming that leads to the development of sporophytic plants. But the important thing to remember is that even though the age of the anther is the same, checking the stages of microspora development must still be carried out because each variety has a different response to culture isolation. sometimes in the same anther, only a small amount can turn into an embryo (Silva, 2016). No reports on successfully microspore culture in sugarcane.so, the aim of this study was to identify uninucleate stage for microspore culture base on different color of anther sugarcane “BL”

Material and Method

This research using Sugarcane “BL” variety were collect from Jember. The material for the research consist of petri 3x3 mm, micropipette, light microscope, beaker glass, object glass, forceps, glass rod, mortar, stample. The method that using in this research is using The panicle that were harvested in the morning at 06.00 WIB. After that we wrapped on newspapers letter then stored in an ice box. The anther from panicles were collected in different color (white, yellow and brown) and subjected to cold pretreatment in water beaker glass at 4⁰C for 3 days in the dark conditions. The anthers were place in 100 ml of aquadest, squeezed using glassrod and then filtered through 100 µm filter paper to get the stage of microspore. Stage of microspore was examined under light microscope. The experiment was designed according to Completely Random Design (CRD) and the data were analyzed using t-test 5 % and followed by Duncan’s test 5% using SPSS Statistical Software package.

Result and discussion

Identification results of uninucleate stages for microspore culture from anther sugarcane “BL” were shown in Figure 1. Based on previous research below (Suaib *et al.*, 2007), percentage of microspores development in sugarcane shows that there is no consistency between panicles that have not been and have raised branches. Therefore, identification of uninucleate stage in this research using anther color parameter.

Anther color varied in each plant. The variety of color anter from bright yellow to purple color (Moore and Osgood, 1987). This study investigate young anther to old anther to identify microspore stage. The result of this study showed the number of uninucleate microspores mostly found in yellow anther (89,3%). Low percentage (7.2%) of uninucleat stage at white color were regarded as early stage anther. The later stage anther found only 2.5% at uninucleate stage because most of the microspore has developed into pollen grain (Figure 1).

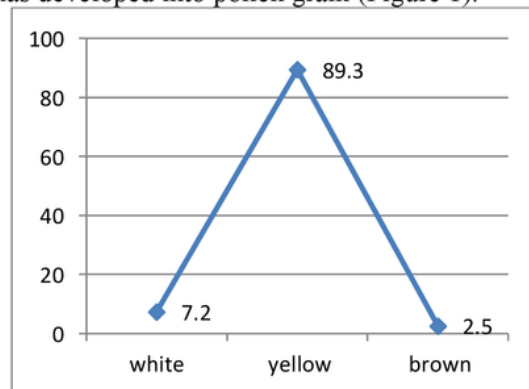


Figure 1. Percentage uninucleate stage sugarcane based on anther color

Results of analysis using T-test showed that the anther color of sugarcane have real different on uninucleate stage microspora, white with yellow anther color ($\alpha = 0.00$), white with brown ($\alpha = 0.011$) and yellow with brown ($\alpha = 0.00$). Different color of anther given different mean and percentage of uninucleate stage microspore (Table 1).

Table 1. Mean of Uninucleate stage microspore in each anther

Anther Color	Mean of Uninucleate stage
Yellow	343.67 ± 10.12^c
Brown	8.33 ± 4.16^a
White	23.33 ± 4.04^b

The highest successful of embryogenesis in microspore culture is related the stage of anther development (Gupta, 2016). Uninucleate stage is very important in microspore culture plant. Liu (2004) explained that microspores at the uni-nucleate stage and are single cells is the best stage for microspore culture. The basic study from the research, microspores are haploid cells that containing of the gametic number of chromosomes. Microspores can be initiated to form embryonic microspore and will be developed into haploid plants or double haploid plant using spontaneous program or chemical induced. Ibrahim *et al.* (2015) described that stage of microspore development is the most critical factor affecting haploid production. For many species, success is achieved only when anthers are collected during the uninucleate stage of pollen development. Cytological microscopy studies from this research show that there is various uninucleate stage can be found in sugarcane "BL" (Figure 2).

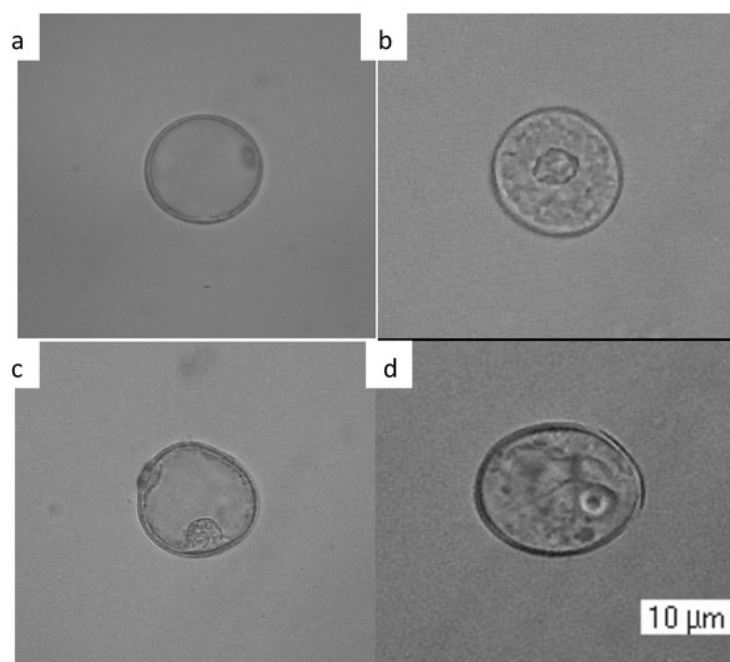


Figure 2. Various uninucleate microspore stage sugarcane "BL"
(a. Early uninucleate b. uninucleate c. mid uninucleate d. late uninucleate)

The stages of microspore in this research are characterized mainly by position of the nucleus. In most crops, the early uninucleate stage to mid uninucleate stage of microspores is the most suitable for embryogenesis response microspore culture (Datta, 2005). Results of this

study showed there are four types of uninucleate stage such as early uninucleate, uninucleate, mid uninucleate and late uninucleate. It is known in this study each type of microspore has its own characteristic.

Characteristic of early uninucleate stage sugarcane “BL” was nucleus located close by cell wall of microspore (Fig. 2a). The uninucleate stage (Fig. 2b) has spherical shape of cell with central-located nucleus (Suaib *et al.*, 2007). At the mid uninucleate stage (Fig. 2c), the microspore enlarge in size and the nucleus become smaller and more densely staining with the formation of a large central vacuole, the nucleus is pushed to the side opposite the germ pore, and during late uninucleate stage (Fig. 2d) the nucleus become enlarge and undergoes the first pollen grain mitosis (Bajaj, 2012).

Conclusion

Uninucleate stage of the microspores mostly found in yellow anther 89.3%. Low percentage early stage of uninucleate was white in color, at 7.2% and later stage anthers at 2.5%. Analysis of t-test showed a significant difference between anther color to uninucleate stage microspore. Different color of anther gave different percentage of uninucleate stage microspore. This study showed that there are four types of uninucleate stage such as early uninucleate, uninucleate, mid uninucleate and late uninucleate.

Acknowledgement

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

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