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Abstract: There have been no reports of microspore culture success in sugarcane crops. One factor that can influence the success of the culture is microspore viability. This study was conducted to find out viable microspores that are suitable for use as a culture donor plant. Pretreatment soaking anther in 3M mannitol solution and storage at 4^0C temperature can increase the viability of microspores. The microspore viability test used three staining dyes: iodine dyes, lactophenol blue and acetocarmine. Anova 5% analysis showed that each dye gives a different effect on the viability of microspores of sugar cane (sig. 0.00). The dye of lactophenol blue is an effective viability test technique in microspores culture.

Keywords: microspore culture, embryogenesis, Saccharum officinarum

1. Introduction

Microspores culture is the most popular method to produce a haploid plant that has a good technology compared with other techniques. microspores culture produce embryogenesis with haploid pure line and can be induced into double haploid in quick time (Segui-simaro and Nuez, 2008).

Microspore culture technology has been successfully applied to plant breeding programs such as brassica (Kalashnikova *et al*., 2011), wheat (Ayed *et al*., 2010), Chili (Yin *et al*., 2010) and etc.

Microspore culture technology sugar cane in Indonesia has not been developed. It is necessary to undergo several tests prior to microspores culture isolation. The initial test is used to see the viability of microspores that will be used as a microspore culture donor plant. one of the microspore viability tests is a staining technique using acetocarmine (Lyra *et al*., 2011), Lactophenol Blue and IKI (Melloni *et al*., 2013). This staining testing technique has been applied to several crops such as microspore of kenaf (Ibrahim *et al*., 2014), cucumber (Vizintin *et al*., 2004), *Jatropha curcas* L (Abdelgadir *et al*., 2012). The microspore viability test in this study will be used as a basis for determining viable microspore for culture donor plant.

2. Materials and Methods

Material Preparation

This study used Bululawang Sugarcane variety. Harvesting panicle still wrapped inside the petals of the flag leaves then cut the panicle at 2-3 segments below the root of the panicle. Harvesting using icebox then put into the incubator with 4^0C for 1 day. After stirring the panicles were soaked in two kinds of soluted 0,3 M mannitol solution and aquadest at 4^0C for 3 days.

Identification of Microspore Viability

The panicle is grouped according to yellow color. A total of 100 anthers were pounded using mortar and pounder which was given 10 ml aquadest solution. microspores were filtered using 100 µl and 10 µl filters. as much as one drop of the solution placed on top of each glass object in IKI solution, acetocarmine 1% and Laptopenol Blue then closed with glass cover and immediately conducted observations under the microscope. Cell viability was calculated by dividing the total number of cells colored by the total number of cells observed in one field of view multiplied by 100% (Winarto and Rahmawati, 2007).

The indicator of this method is that the viable microspore will reveal its essence, whereas the nonviable microspore does not provide a staining reaction or microspores seen without a nucleus. The generative nuclei are smaller in size and with a lighter color

absorption intensity, while the vegetative core is larger but the intensity of light absorption is less. The viability and status of microspore core cleavage were observed using a 100x magnification microscope.

3. Results and Discussion

Percentage Microspore viability is different in each staining method (Table 1). Anova 5% analysis showed that each dye had a different effect on the viability of microspores of sugarcane (sig. 0.00).

Table 1. Percentage Microspore Viabily (%)

No	Dyes	Aquadest		Mannitol	
		viabel	Non viabel	viabel	Non viabel
1	IKI	88.72	11.28	92.89	7.12
2	Laptopheno l Blue	51.29	48.70	60.84	39.15
3	Acetocarmi n	43.45	56.54	46.19	53.81

The response of staining on microspores can be seen in Figure 1. The staining method indicates that viable microspores can absorb dyes while non-viable cannot absorb dyes.

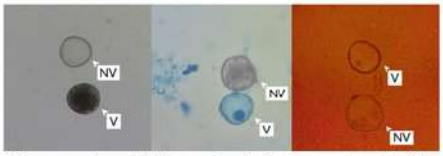


Figure 1. Effect Staining method for viability microspore a. IKI; b. Lactophenol; c. Acetocarmine (V: Viable; NV: Non-Viable)

Each staining technique produces a significant percentage difference in the viability of microspores. Comparison of microspore viability testing techniques is performed to produce accurate data in the determination of the viability of microspores. In addition, the percentage of viability of microspore depends on the ability of the pollen to respond to the given staining. This is because the viability of microspores is influenced by several factors such as temperature, microspore density, media used and incubation duration (Mariani and Bots, 2005)

The first microspore test was performed by pounding the anther on the aquadest solvent. After microspore screening is placed on the object glass and then given a dye solution. The observation of microspore viability in a light microscope showed that each dye had a different dye reaction on the cane microspores (Fig. 1). This is because each coloring method gives different and specific results in each species (Vizintin and Bohanec, 2004). Warid and Palupi (2009) also stated that there is no special standard for testing microspore viability so another technique is used to compare the percentage of microspore viability. one way is to compare the coloring technique. The comparison aims to

confirm the percentage of viability of microspores produced.

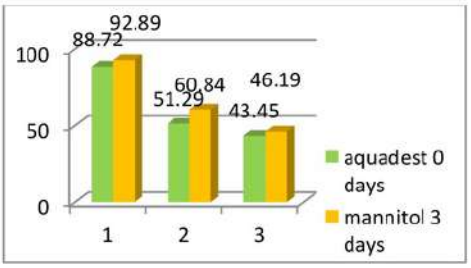
The percentage of microspore viability with aquadest solvent, the highest microspore viability was found in microspores stained with IKI of 88.72%. IKI staining is the most commonly used method of staining by cane breeders. The high value of viability of microspores in IKI dyes in this staining is based on the staining of starch present in the pollen grains. However, starch substances are not the only means of limiting tube germ tube, and for that reason, the validity of this method has been criticized by researchers (Melloni *et al*., 2013). The high value of viability using IKI shows that the high content of starch substances. starch substances indicate that the cell has entered the pollen grain / mature pollen phase. so it is not appropriately used for staining in microspore cultures.

Percentage via microspores via Lactophenol Blue is 51.29%. The viable microspore is characterized by a change of cell color to blue and non-viable microspore cannot absorb color so no color change occurs (Vizintin dan Bohanec, 2004).

In this study, lactophenol blue is effectively used as a dye in the viability test of microspores of sugarcane as preparation of microspore donor plants (figure 1). This is because the nucleus is stained perfectly (dark blue). The colored cell nucleus indicates that there is a viable uninucleate microspore that can be used as a microspore culture donor plant. Microspora viability test is used to know the percentage of cells that have the potential of embryogenesis so that it can respond to embryogenesis at microspore culture. In microspore culture, the uninucleate stage is an effective step in altering gametophytic pathways toward sporophytes (Wahidah, 2010). Dafni *et al.* (2000) also states that pollen is said to be viable if it has a percentage of pollen viability above 50%

Acetocarmine dye is widely used by researchers to test the viability of microspores in poacea such as wheat(Wang *et al*., 2015) and corn (Hosseini, 2015). In this study, the use of acetocarmine is less effective to determine the viable microspores. The percentage of microspores produced is 43.45%. the colored cells are also limited to microspore cell walls so that the cell nucleus cannot be ascertained the viability

Soaking the anther before the microspore viability test using mannitol at a low temperature of 4^0C can increase the viability of sugarcane microspores. This can be seen in the following figure (Figure 2).



In certain species, the use of mannitol may increase microspore viability (Neumann et al., 2008). In tobacco, low-temperature treatment may increase microspore viability before the culture is performed (Jain *et al.*, 2013). In the brassica plant, soaking the anther in the mannitol solution at a low temperature of 40C has a synergistic effect on the development of the microspore stage. In addition, the combined treatment resulted in higher plant regeneration rates in regenerated populations (Zeng *et al.*, 2015).

4. Conclusion

Anova 5% analysis showed that each dye gives a different effect on the viability of microspores of sugar cane (sig. 0.00). The dye of lactophenol blue is an effective viability test technique in microspores culture. The use of 3M mannitol solution and 40C temperature can increase the viability of microspores. The future project is comparing the viability test with pollen tube germination to know the correlation of viability standard technique.

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