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Growth and development of plant tissue culture and conventional seed source from several varieties sugar cane (*Saccharum officinarum* L.) on field

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Sugarcane (Saccharum officinarum L.) is an important source of commercial sugars. The use of sugar cane is almost 70% of world sugar production. The availability of high-yielding seeds cane in large quantities and relatively short time is expected to support the successful development of cultivation and improvement of product quality. Information on the superiority of tissue culture seeds compared to conventional seeds in Indonesia is limited. Therefore, this study was conducted to obtain primary data and compare the growth of tissue culture and conventional cane crops, in three varieties of Indonesia through morphological and physiological observations that occur in sugar cane cultivation in the field. This study was designed using Randomized Block Design. This study used two different seed source: tissue culture and conventional culture; with three cane varieties, PS 881, PS 862, and BL. The results of this study showed that plant seed source tissue culture planted on plantation showed superior characters including number of tillers, number of segments, resistance to mosaic disease, and vascular disease (Ratoon Stunting Disease). Thus, these results reveal that seed source from tissue culture is a realistic and better alternative as a substitute for conventional sugar cane as sugar cane in Indonesia.

Keywords: seed cane, Ratoon Stunting Disease, Saccharum officinarum, variety.

INTRODUCTION

Sugar cane is a globally strategic commodity for Indonesia because it has high economic value, especially as raw material of sugar (Sukmadjaja and Syakir 2014). No less than 420,000 ha of sugarcane-cultivation land throughout Indonesia and sugarcane production reaches an average of 60-70 tons / ha (Putra et al. 2013). National sugar production in 2015 is targeted at 2.95 million tons but its consumption reaches 5.77 million tons. With the government's plan to achieve selfsufficiency in sugar by 2018, the total area of sugarcane planting is estimated to reach 895 thousand ha and about 20% of the planting area is new sugarcane, so it takes 22.55 billion cane seeds. Needs of sugarcane seeds until now can not be fulfilled either by the government, as well as sugarcane seed breeder (Mulyono, 2011).

The availability of superior seeds cane in large quantities and within a relatively short time is expected to support the successful development of cultivation and improvement of product quality. Conventional propagation of plants is still limited by the plant ability to produce new seeds in large quantities and uniforms (Sukmadjaja and Syakir 2014), as well as longer propagation times due to the low rate of multiplication (Ali and Afghani, 2001). So the development of tissue culture technology is expected to be able to rapid propagation of disease-free planting material in the mass production of quality seeds on sugarcane.

The tissue culture technique or micropropagation is defined as the variation of somatic cultivation of cells, tissues, or controlled plant organs under in vitro conditions with the aim of producing a large number of hereditary plants (Udhutha et al. 2016), which are genetically identical to the parent plant, in a relatively short period of time compared with conventional propagation (Lal et al., 2014), does not require a large space, health and quality of seed is more assured (Ramgareeb et al. 2010). Experiments in 2009 mentioned that the research of the CoJ 80 var crop in India, planting sugar cane from tissue culture with 60 cm spacing compared to conventional sugar cane, showed significantly higher yield (Sandhu et al. 2009). Furthermore, in 2016, genotype crops research of NCo-334, the origin of conventional seed plant capable to produce 35064.9 of two eyes mules per hectare with 1: 13.98 propagation ratio compared to the tissue culture seed 852075.5 of two-eyes mules with 1: 4011 propagation ratio (Ibrahim et al., 2016). A multiplication rate of 7-8 times can be achieved from one planting to use in the next conventional method. This multiplication rate is slow and takes at least 4-5 years for the sugar mill to obtain new varieties that will be introduced in some areas extensively (Kaur and Shandu 2014). The experiments performed by Cavallaro et al. (2014) suggests that conventional propagation of Arundo donax L. can restrict the large-scale cultivation. because iťs time-consuming, substantial expenses and high efforts to establish plant crops with stalk cuttings that require accurate agronomic management.

Until now, the studies of tissue culture crops in the context of growth performance evaluation for local Indonesia varieties have not been documented. Some of the local superior Indonesian varieties that continue to be demanded are PS 881, PS 862, and BL varieties which is able to produce high rendemen. Therefore, this study was conducted to obtain primary data and compare the growth of tissue culture and conventional sugar cane in three Indonesia varieties through morphological and physiological observations that occur in sugar cane cultivation in the field

MATERIALS AND METHODS

This research was conducted at Bakalan area, Pasuruan, East Java. Seed testing to determine the infection of vascular disease was done in the Pest and Disease Laboratory, Indonesian Sugar Research Institute Pasuruan. Cultivating and observation conducted from July to December 2016.

This research was designed usina Randomized Block Design with factorial pattern. There are 4 combinations from 2 factors, namely seeds source and varieties. The first factor is seeds source, i.e. tissue culture seed source and conventional seed source. The second factor is the seeds varieties. We used PS 881, PS 862, and BL (Bulu Lawang) varieties. Each factor combination performed in a single experimental plot repeated 3 times. We used tissue culture and conventional seed source in single bud setts from sugarcane plant var. PS 881, PS 862, and BL (Bululawang). Each plot consists 10 lines of cultivation with 100 cm distance between rows. Side cultivation done as much as 2 rows left and right. The cultivation line for the research is 6 rows center. Observations were made on six rows in three plant in each research the plot. Conventional cane setts and tissue cultures seeds were planted with 6-meter-long cultivation lengths. Each line planted with 15 stalks of single bud setts. Cultivation with plant to plant spacing 40 cm and distance between cultivation rows is 100 cm. Plant growth was observed through stalk height, number of tillers, number of leaves, leaf area, stalk diameter, number of segments, and length of segment at 1-5 months after planting.

The chlorophyll index was measured by spectrophotometer with the addition of acetone (Stokes et al 2016). The taken leaf sample was the third leaf from the plant tip. Taken 0.1 g leaf then crushed by mortar and pestle. Crushed leaf added by 10 ml of 85% acetone, then filtered with filter paper while poured into the test tube to obtain a clear extract. Measurements were made by Spectrophotometer (Spectronic 21 D, Milton Roy brand) at 645 nm and 663 nm wavelength (Proklamaningsih et al., 2012). Calculation of chlorophyll used the following formula:

chlorophyll a = 0,0127 (OD 663) - 0,00269 (OD 645)

chlorophyll b = 0,0229 (OD 645) - 0,00468 (OD 663)

total of chlorophyll = 0,0202 (OD 645) - 0,00802 (OD 663)

Seed health test on sugar cane plant at age 5 months after planting. Seed health was observed by calculating the intensity of major disease of sugar cane (RSD and mosaic). ELISA test used to detect RSD vascular disease through in-vitro reaction between antigen and antibody to form antigen and antibody complex. The method is based on the conjugation between bacteria, antibodies and enzymes. By adding dye substrate it can be seen the conjugation formed (Fahmi 2012).Data obtained from the measurements of stalk height, stalk diameter, shoot number, leaf number, leaf area, length of segment, and number of sections were analyzed statistically using twoway analysis of variance (ANOVA) forwarded to the Tukey test.

RESULTS

Sugar Cane Growth from tissue culture and conventional seed source

Based on the ANOVA statistic test, the use of varieties and seed origin has significant effect (p <0.05) on the number of tillers, height of stalk. diameter of stalk, number of segments, length of segment, and leaf area (Table 1). Because ANOVA statistic test result is significant with value below 0,05 then continued with Tukey test. Sugarcane plants from different varieties showed varying ANOVA assay results in the growth response of shoot number, stalk height, stalk diameter, number of segments, length of segment, and leaf area (Table 2). Similarly, the two seed source showed statically significant variation across all measured response variables, except on the number of leaves, total chlorophyll, and leaf area (Table 3). The number of tillers in the BL variety was higher than that of PS 881, so BL was significantly different from PS 881. While the variety of PS 862 produced the number of buds which was not different from PS 881 and BL. In contrast, PS 862 has the highest stalk diameter value between two other varieties, PS 881 and BL. Differences in crop varieties have not shown significant differences in the stalk response variable, the number of segments, and the length of the segment. Leaf area on each varieties significantly different. With the highest leaf area on PS 881 (410,74 cm²), followed by PS 862 varieties of 361,41 cm² and BL of 302,22 cm².

The results obtained from the Tukey test (Table 4) showed that stalk height, stalk diameter, number of segments, length of segment, and leaf area of sugar cane from two different seed source have statically significant different values except in tillers number, number of leaves, and total chlorophyll. High value of stalk in plant PS 881, PS 862, BL from the tissue culture crop is bigger than PS 881 crop from conventional cane setts.

The stalk diameter of BL varieties from tissue culture crop is the smallest (2.59 cm). Table 4 shows that the diameter of BL varieties of tissue culture crop is significantly different from PS 881 conventional cane setts, PS 862 conventional and tissue culture crops. PS 881 varieties from tissue culture seed source has the largest leaf area and significantly different with PS 862 varieties from conventional seed source, BL tissue culture and conventional seed source. The leaf area of PS 862 varieties of tissue culture crops is significantly different from BL varieties of conventional and tissue culture crops. And the interaction between BL varieties with conventional seed origin has the smallest leaf area so significantly different from PS 881 conventional and tissue culture seed, as well as PS 862 varieties.

Seed Health from tissue culture and conventional seed source

The results showed that maize-infected sugar cane was PS 881 and BL varieties from conventional seed source, respectively 1.61% and 0.69%. Mosaic disease infection is caused by viruses. The virus has an arrangement that resembles a protein and becomes inactive due to various chemical and physical agents (eg heat). The mosaic disease spread worldwide. Various kinds of ticks can spread the disease from one plant to another. Another index of sugar cane infection that is observed is vascular disease RSD. Based on the observations it was indicated that the tissue culture seeds is not infected with RSD disease. While in the conventional seeds infected with RSD, as occurs in PS 881 varieties (Figure 1).

DISCUSSION

The number of tillers, stalk height, number of segments, and the length of the segment on culture tissue plant crop has a higher value than conventional plants. In contrast, the diameter of conventional plant stalks larger than the tissue culture plant. The higher plant stalk, smaller diameter of the plant stalk. This related to Hoy et al (2003) research that showed the tendency of tissue culture seeds in the first year is thinner and the diameter will increase rapidly in ratoon and next plant generation. High amount buds plant produce smaller diameter because the thickness of sugarcane directly adjusts the number of tillers per clumps (Ibrahim et al. 2016) and related to the cytokines effects (Sood et al. 2006).



Figure 1: Serological test results of RSD disease in sugar cane. Note: 1A, 1C positive control; 1B, 1D: negative control; tissue culture seed source 1st replication (1E: BL; 1F: PS 881; 1G: PS 862); tissue culture seed source 2nd replication (1H: BL; 2A: PS 881; 2B: PS 862); tissue culture seed source 3rd replication (2C: BL; 2D: PS 881; 2E: PS 862); conventional seed source 1st replication (3A: BL; 3B: PS 881; 3C: PS 862); conventional seed source 2nd replication (3G: BL; 3H: PS 881; 4A: PS 862), red code show infected RSD.

Table 1. ANOVA for comparison of tissue culture and conventional seed sources of sugarcane varieties.

Source of variations	Number of tillers	Number of leaves	Total of chlorophyll	Stalk height	Diameter of stalk	Leaf area	Number of segments	Length of segment
Varietiess	3,98*	44,88	0,000028	427,8*	0,29078*	17582,2*	3,3176*	2,48755*
Seed sources	5,07*	136,43	0,000054	1354,9*	0,23007*	1838,2*	12,4076*	7,55894*
Varieties*seed souces	0,03*	14,71	0,000000	468,2*	0,02702*	11,2*	0,8258*	0,07447*

Note: The symbol "*" indicates very highly significant difference

Table 2. Comparison o	f sugarcane genot	ypes based on Tukey	y test
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Varieties	Tukey Test									
	Number of tillers	Number of leaves	Total of chlorophyll	Stalk height	Diameter of stalk	Leaf area	Number of segments	Length of segment		
PS 881	4,25 b	53,88 a	0,0077801a	194,00 a	3,03 ab	1739,77a	11,86 a	12,36 a		
PS 862	4,50 ab	48,69 a	0,0109898a	190,27 a	3,19 a	1459,90b	12,25 ab	12,47 a		
BL	5,77 a	52,80 a	0,0118561a	177,87 a	2,75 b	1255,52 c	12,16 a	12,65 a		

Note: Numbers followed by the same letters in the same coloum are not significantly different at 5% Tukey test

Seed sources	Tukey Test								
	Number of tillers	Number of leaves	Total of chlorophyll	Stalk height	Diameter of stalk	Leaf area	Number of segments	Length of segment	
Tissue culture	5.37 a	54,54 a	0.0119384 a	196,06 a	2,88 b	1537,71 a	13,35 a	12,76 b	
conventional	4.31 b	49,04 a	0.0084789 a	178,70 b	3,10 a	1432,41 a	11,06 b	12,22 a	

Table 3. Comparison of Tissue culture and conventional seed sources based on Tukey test

Note: Numbers followed by the same letters in the same coloum are not significantly different at 5% Tukey test

Table 4. Comparisons of sugarcane varieties for different seed sources "B1" stands for tissue culture seed source while, "B2" indicates conventional seed source.

Varieties	Seed	Mean							
	sources	Number of tillers	Number of leaves	Total of chlorophyll	Stalk height	Diameter of stalk	Leaf area	Number of segments	Length of segment
PS 881	B1	4,74 a	58,17 a	0,0094961 a	196,74 a	2,88 ab	1783,37 a	13,08 a	12,60 a
	B2	3,76 a	49,60 a	0,0060641 a	159,00 b	3,18 a	1696,17 a	10,64 b	12,11 a
PS 862	B1	5,11 a	49,86 a	0,0129532 a	193,54 a	3,16 a	1529,72 ab	13,14 a	12,72 a
	B2	3,89 a	47,54 a	0,0090264 a	187,00 ab	3,23 a	1390,08 bc	11,35 ab	12,22 a
BL	B1	6,26 a	55,61 a	0,0133660 a	197,89 a	2,60 b	1300,05 bc	13,11 a	12,97 a
	B2	5,28 a	49,98 a	0,0103462 a	190,11 ab	2,91 ab	1210,99 c	11,20 ab	12,32 a

Note: Numbers followed by the same letters in the same coloum are not significantly different at 5% Turkey test

According to Fu and Wang (2011) internal factors are the genetic properties brought by plants as an originallity. In addition, the hormone also affected the tillering process. Naturally in the hormones has been produced by plant, such as auxin, cvtokines, and gibberellins, which influencing the regulation of the physiology of plants. The hormones is produced in an active meristematic tissue and then spreads through phloem vessels or parenchyma. The auxin induction can activate the proton pump (H +) on the plasma membrane and decrease the pH, thus being able to break the hydrogen bond between the cellulose fibers of the cell wall. Breaking up of hydrogen bonds facilitates the cell walls stretch easily and reduces pressure. The process makes the cell wall become loose. Low level pH is able to activate protease enzymes that degrade the proteins or polysaccharide constituents of the cell wall, so that the cells extend and expand (Campbell et al., 2008). The ratio between auxin and cytokinin also affects the process of plant growth and development. Apical meristem produced endogenous auxin that can suppress the growth of lateral shoots, or so-called apical dominance. According to Khuluq and Hamida (2014) auksin and cytokinin can provide inhibition effect of lateral shoot growth and can encourage the growth of lateral shoots.

Stalk size from tissue culture seed is higher than conventional seed source, both on PS 881, PS 862, and BL varieties. Tissue culture seed stalk height continues to increase from the age of 1-5 months. The tissue culture seed source from the young leaf roll explant have high meristematic characteristic, resulting in higher and more effective stalk growth than conventional seedlings. As mentioned by Ali et al. (2012) and Suhesti et al. (2015) cultures from young leaf roll were found to be a rapid, effective and reproducible procedure for in vitro culture methods in rapid clonal propagation of desired sugar cane genotype. On the diagram, appears that PS 881 from conventional seed has the lowest stalk height, this is due to infection of Ratoon Stunting Disease (Pawirosemadi 2011). The symptoms of RSD disease are most clearly seen in the middle segment of the sugarcane stalk and this symptom is present in some segments that sequence of sugarcane plants whose weight will be reduced, resulting in shorter stalk length than healthy plants. Such as on PS 881 varieties stalk from conventional seed sized 159 cm. Serological test results showed that there was only one that infected by vascular disease, ie PS 881 from conventional origin in number 1-3 repetition. In Indonesia, sugarcane cultivation use healthy seeds. The seeds can be obtained with 50°C hot water treatment for two hours. However, in this study there is no hot water treatment of all cultivation materials.Based on the observations indicated that the plant of tissue culture seeds is not infected with RSD disease.

According to Pawirosemadi (2011) the growth of sugar cane plant through 4 phases ie. germination, tillering, grand growth, maturation and ripening. At the grand growth phase, the diameter of the tissue culture seeds plant did not increase greatly. This occurs as a consequence of high number tillers formed (Sandhu et al., 2009). We obtained that more number of tillers, the smaller diameter of a plant stalk. As in PS 881 of tissue culture seeds plant that produced 5 tillers only has 2,88 cm diameter of stalk. Whereas in PS 881 of conventional seeds that produced 4 tillers has 3,18 cm diameter of stalk. The same is true of PS 862 and BL varietas. The same thing happened to PS 862 and BL varieties.

The number of leaf on the sugar cane is controlled by two factors: leaf formation rate and long life of each leaf. The early maturity sugarcane varieties are generally characterized by more number of leaves than low maturity varieties (Pawirosemadi 2011). This is in accordance to the results that early varieties of cooking such as PS 881 and PS 862 had more leaves than low maturity varieties, BL, both from tissue culture and conventional seeds. The number of leaves of PS 881 varieties from the tissue culture seeds was 58.17; PS 881 conventional seed was 49,59; PS 862 from tissue culture seeds was 49.85; and PS 862 conventional seed was 47,53. Furthermore, the

number of leaves on the BL variety of conventional tissue and conventional seeds were 55.6 and 49.98, respectively. Another factor that affects the great number of leaves is the origin of the seed. From the observation can be seen that the number of green leaves tissue culture seed is more than the conventional seed plants.

Chlorophyll total in the tissue culture seed plant is greater than the conventional seed plant. The amount of chlorophyll is accompanied by the growth of the number of leaves and leaf area. As in the PS 881 tissue culture seeds plant with amount of chlorophyll 0.009496 mg / g, with 1783.37 cm2 leaf area, compared to PS 881 from conventional seeds plant with 0.006064 mg / g amount of chlorophyll whose 1696, 17 cm2 leaf area. Similarly, occurs in other test variance, PS 862 and BL. This is similar to the results of research conducted by Proklaminingsih et al. (2012) leaf area high then the content of chlorophyll is also high and good growth. Similarly, that occurs in other varieties of plants (PS 862 and BL). This is related to Proklaminingsih et al. (2012) which states the greater the leaf area, the chlorophyll content is also high, and has good growth.

In relation to the assimilation properties, the total area of leaf surface has an important role, especially for photosynthesis process. The total area of the leaf surface depends on two factors: the number of leaves and the average surface area of each leaf. In addition, the total surface area of the leaves is also affected by the variety and environmental conditions. In the low maturity variety POJ 2883 has smaller total surface area of leaves per stalk than the early maturity sugar cane varieties (F 108). This is consistent with the results of the study that indicating the leaf area of BL varieties (low maturity), tissue culture and conventional seeds, is smaller, than the leaf area of PS 881 varieties (preliminary) from conventional and tissue culture seeds.

CONCLUSION

The results showed that tissue culture seeds that planted on plantation land yielded superior characters, including tillers number, number of segments, resistance to mosaic disease rate, and vascular disease (*Ratoon Stunting Disease*). Thus, these reveal that tissue culture seed can be a realistic and better alternative to replace the conventional seeds cane in Indonesia.

CONFLICT OF INTEREST

The present study was performed in absence of

any conflict of interest"

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AUTHOR CONTRIBUTIONS

SW designed the experiments. AMD performed the experiments, data analysis and also wrote the manuscript. YSWM and HP reviewed the manuscript. All authors read and approved the final version.

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