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Research Article
Effect Variety and Stratified Plantlet Nursery to the Growth Sugarcane (*Saccharum officinarum* L.) Propagated in Single Bud

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Abstract

Background: Studies was carried out to determine whether there is interaction effect between sugarcane varieties and stratified nurseries to the percentage of plantlet viability and its height. **Materials and Methods:** This study was conducted in Pernig and Mojokerto from June-August, 2015. The study method using a completely randomized factorial design consisting of two factors, namely the variety including Bululawang, VMC 76-16, Cokro and clone Columbia-2 while, the second factor is the stratified nurseries including Grandma's Plantlet Nursery (GPN), Mother's Plantlet Nursery (MPN) and Flat Plantlet Nursery (FPN). Thus, there were 12 treatment combinations and repeated three times. The bud-chips were planted in the trays with the size 4 cm during 3 months, every month data was collected for percentage of plantlet viability and its height. Data analyzed was done by Minitab 16 for ANOVA and LSD 5%. **Results:** The results showed that there is not interaction between stratified nursery and variety to the percentage of plantlet viability and plantlet height. Stratified nursery shows that GPN significantly produces percentage of plantlet viability and its height greater at Month After Sowing (MAS) while, MPN gives plantlet higher at the last 3 MAS. Bululawang gives percentage of plantlet viability significantly greater at 1 MAS only while, VMC 76-16 shows the plantlet higher at 1 and 2 MAS. Four varieties planted show the same response at 3 MAS to variables used. That means their responses to percentage of viability and plantlet height are the same. Based on this study, the use of single bud (bud-chip) technique based SOP able to produce percentage viability until 90%. **Conclusion:** It can be concluded that Columbia-2 is a promising introducing clone. On the other hand, superior bud-chips can be produced from both GPN or MPN and able to be used as the right solution.

Key words: Stratified nursery, bud-chips, sugarcane, growth

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Marketing Year (MY) 2013-2014, Indonesia is expected to produce 3,4 million metric tons (MMT) for 450.000 ha area harvested. Meanwhile in the same year, raw sugar imports are estimated to increase to 3.6 MMT due to demand of industrial food and beverage. Furthermore, in 2015-2016, Indonesia arranged to supply 7.5 MMT with total import 3.75 MMT (Wright *et al*., 2014). To reduce sugar import year by year, Sugarcane Development and Research Centre cooperates with PTPN X in Mojokerto, East Java adopted a new method to produce more effective and efficient sugarcane plant material.

The obtain ability of the superior sugarcane seed to farmers until today is still a major problem of the sugar industry in Indonesia. The availability of varieties with sufficient amount should be integrated with sugarcane growers. However, superior sugarcane seed provider (agencies and seed breeder) also faced difficulties and often suffered due to less demand of cane seeds. This situation is provoked by most of the sugar factory management which is not transparent and unfair manner regarding Commercial Cane Sugar (CCS). The sugarcane farmer is always disappointed due to CCS out of their expectation.

The conventional rules in Indonesia of producing the cane seed is started from Breeder Plantlet Nursery (BPN) to Grandma's Plantlet Nursery (GPN) to Mother's Plantlet Nursery (MPN) and Flat Plantlet Nursery (FPN) and finally planted at Gardens of Sugarcane Milled (GSM). The time needed every level of stratified nursery is 6-7 months caused by the multiplication of the seeds. Each hectare of MPN is only allowed to produce seeds for 7-10 ha. Moreover, the purity of seed cane in every level stratified plantlet nursery is different. Based on the availability of seed canes, then the number of seeds produced are limited. Conversely seed cane price per unity ahead of each level stratified plantlet nursery becomes expensive. In extreme conditions the availability of quality seeds is often unaffordable to farmers. Mostly sugarcane farmers only maintain ratoon to reduce the cost.

In conventional method, about 8-10 seed cane t ha^{-1} is used as planting material (Singh *et al*., 2014). One simple technology that is easy to implement is to multiply the seed cane in a single bud. This method could save about 80% by weight of the stalk as planting material (Jain *et al*., 2010). Furthermore, Budiarto (2013) reported that bud-chips followed by SOP can be used as an effective planting material. This study results showed 83% bud-chips viability, it was greater than other treatments. Jain *et al*. (2010) concluded that bud-chip could be one of the most viable and economical planting material in reducing the cost of sugarcane production. Furthermore, Kuri and Naik (2015) stated that bud-chip can be used as one of alternative ways to reduce the cost and also improve the quality of seed cane. Other advantages of bud-chip are less bulky, easily transportable and more economical seed material. By considering advantages of using bud-chip, so that is why bud-chip technology holds great promise in rapid multiplication of new cane varieties. Then, to generate the high viability of the plantlets propagated in a single bud, Standard Operational Procedure (SOP) must be applied. Some of the components that need to be considered to produce a high quality of single bud is quality seed, age seed, bud candidates, time for the process, the quality of the drill, the quality of Hot Water Treatment (HWT), the process of sterilization, hormone treatment, quality and the size of the pot trays, soil media and the quality of maintenance (Budi *et al*., 2013).

Sudiarso *et al*. (2014) reported that the conventional method showed Bululawang varieties better results in generating the number of tillers. Meanwhile, Budi *et al*. (2013) showed that Bululawang variety propagated in bud-chips produced plantlets more than conventional methods. According to Putri *et al*. (2013), bud-chip multiplication is largely determined by the composition of the media and the quality of cane seeds and the environment. Sudiarso *et al*. (2014) explains that the viability of bud-chip is also determined by the quality of candidates buds from the mother stock nursery. In another hand, Keethipala *et al*. (2001) found that 1 ha of MPN could produce bud-chips enough for 243 ha in 15-16 months compare to the conventional method which is enough only for 45 ha seed which take about 16-17 months.

However, sugarcane farmers in Indonesia until now use setts as planting material. Setts taken from the stem with 2-3 buds. Another way is to use the setts from top cutting. Ironically sugarcane seeds planted by a large part of the cane farmers. Mother stock nursery sources are not clear, even from plants cutting which cannot be counted how many times top cutting used as seed sources. So, finally it decreases sugarcane productivity per hectare significantly.

The purpose of this study was to investigate whether variety of sugarcane and stratified plantlet nurseries show interaction to the percentage of plantlet viability and plantlet height which propagated in single bud.

MATERIALS AND METHODS

The study material includes nursery parents (GPN, MPN and FPN) certified sugarcane varieties Bululawang, VMC 76-16, Cokro and Colombia-2 clone, a set of seed maker bud-chip include: BOR, HWT and tools as well as supported tools, pot tray size 4×9 cm, disinfectants, water, electricity,

home activities, nursery land, soil, organic matter, sand, greenhouse and black plastic as a cover nursery. Tools or materials support comprises: Guide books seedling production bud-chips, land nursery of seedlings bud-chips, soil, sand, organic fertilizer, ZA, tools planting and maintenance of plants (nozzles) complete, marker, transportation seedlings bud-chips, laptop, LCD, computers, printer, study, desk and stationery.

This study was conducted in Perning garden, Mojokerto and Jetis Gempokerep region sugar factory PTPN X from June-August, 2015. Activities were conducted in this study is to prepare varieties of certified cane seed in single bud (bud-chips). The study design used completely randomized design consisting of two factors: The first factor, the varieties include Bululawang, VMC 76-16, Columbia 2 and Cokro, the second factor is the level of the stratified parent nursery that consist of nurseries grandmother, nurseries mother and nurseries flat. The number of combination treatment is 12 and each treatment was repeated three times. Hence, the number of combinations of existing treatments is 36.

Each treatment combination was planted in a pot tray with the size of 4×9 cm with a round shape. This study requires 36 pot trays. Each pot tray consists of 60 pots. Thus, in this study there was 2160 pots. Placement combination replicates of each treatment were done randomly. Determination of plant samples each pot trays in each test were also done randomly. The data collection was done by observing the growth indicator of each treatment combination that includes percentage of plantlet viability and plantlet height at age 1, 2 and 3 months. Furthermore, the observed data analysed using LSD 5%.

RESULTS AND DISCUSSION

Results of analysis of variance (Table 1) showed there is non-significant interaction between treatment of stratified nursery and varieties on all variable observation used. Table 1 showed that stratified nursery is significantly affected by the two variables used including all the 3 months observed the percentage of plantlet viability and in 1 and 3 MAS for plantlet height. Meanwhile variety significantly affected to 1st MAS percentage of plantlet viability and it affected as well to 1st and 2nd MAS of plantlet height.

The effect of varieties and stratified nursery toward percentage of viability on the age 1, 2 and 3 months can be seen on Table 2. Further analysis, LSD 5% at Table 2 showed that Bululawang variety produced average percentage of plantlet viability greater than three others in the 1st month only. However, at the two last observations, there was the same percentage of plantlet viability of the four varieties used. In another side, GPN presents consistently percentage of plantlet viability higher than others until the last observation.

The absence of these differences at the last 2 months observed the percentage of plantlet viability indicated that all varieties on standard operating procedures are the same and then the result of each variety was able to optimize all of its potential in reaching the optimal percentage seedling viability. The process that occurs is present in the food reserves bud-chip sufficient material to enable and encourage the process of metabolism so, the first step all treatments tested have the same opportunity in the process of emergence of candidates shoots into shoots and roots. The process that occurs is to encourage a process that begins supporting the leaves cells inside the dome tip bud's candidates would split into meristematic layer and subsequent swelling buds at the end candidates. Furthermore, it widespread swelling and circling the tip area candidate buds. Once the neck leaves are formed then the cells in sub hypodermis become meristematic, buds and new shoots. Based on the results of this process, it took between 3-5 days so, during this time became very high humidity. Furthermore, the growing medium is crucial in the process of growth and development of the sugarcane crop seeds. Given the growing medium all the same treatment, so each treatment will be gripped by the limited media that pot tray measures in 4×9 cm. Proven ability to grow and develop the growth of sugarcane crop seeds for all treatments of all varieties tested at age 1 month there is a difference but at the age of 2 and 3 months analysis of variance showed no difference in the percentage of life. The big difference in the percentage of life is likely due to the variety of characters, their stress in

Table 1: Mean square of ANOVAs for percentage of plantlet viability and plantlet height during 1, 2 and 3 Months After Showing (MAS)

Source		Plantlet viability (%)			Plantlet height		
Month after planting	DF	1	2	3	1	2	3
Stratified nursery (S)	2	64.36**	59.71**	46.07**	7.13*	17.00	64.48**
Variety (V)	3	18.96**	12.29	13.15	19.94**	69.94**	22.53
Replicates	2	0.46	8.13	9.75	9.55**	9.84	47.98**
S×V	6	2.10	5.63	6.09	2.76	1.13	13.37
Error	22	3.91	7.81	6.44	1.52	10.97	8.91

**F-test 1% showed significantly difference

Table 2: Effect variety and stratified nursery on average of percentage of plantlet viability at 1, 2 and 3 Months After Sowing (MAS)

	Month After Sowing (MAS)		
Treatments	1	2	3
Stratified nursery (S)			
GPN	95.70[b]	94.10[b]	93.00[b]
MPN	92.40[a]	92.10[b]	91.10[a]
FPN	91.20[a]	89.70[a]	89.00[a]
LSD (5%)	1.61	2.28	2.07
Variety (V)			
Bululawang	98.00[b]	96.30	95.60
VMC 76-16	95.30[a]	94.90	93.90
Columbia-2	95.30[a]	93.00	91.30
Cokro	94.00[a]	92.30	91.30
LSD (5%)	1.86	ns	ns

Number in the same columns followed by the same alphabets mean non-significant difference at LSD 5%

particular the growing medium structure, permeability and water holding power. Moreover, when the age of 2 and 3 months of each variety showed the response to the viability measured.

Stratified parent nursery shows in Table 2 that Grandmother Plantlet Nursery (GPN) is very dominant in spite of the age of 2 months is not different from the others for plantlet height variable. This fact reinforced that life is essentially the percentage of seedlings greatly affected the quality of parent seed, growing medium, process of manufacture, maintenance and human resources. The results are consistent with the opinion of Budiarto (2013) which described that to accelerate germination of bud-chips, there are some factors that have to be considered, those are: The quality of bud-chip must be good, cutting the parent stalk after planting, regulating the distance of planting of each bud-chip, considering the availability of nutrients, fertilization, irrigation and drainage system.

Budi *et al*. (2013) also explained that the success percentage of survival of the seedlings propagated sugarcane plants in bud-chips can reach above 95%, when based on the implementation process and standard operating procedures established with regard to water management in particular nursery who conducted in the dry season. Galal (2016) strengths that single bud seed (bud-chip) which planted on tray statistically showed the higher results than conventional method toward indicator the percentage of the germination. It also proved that single bud seedling on tray able to reach the percentage of viability until 95%. In line with this, Jain (2011) explained that treat the single bud seed (bud-chip) by adding the plant growth regulator increases the germination process, root activity and the percentage of viability until 80% and also stated that bud-chip technique can help the sugarcane farmers and all of stakeholder for getting sugarcane seed with less risk and assured survival.

From the observation result of the average percentage of sugarcane seedling viability as shown in Table 2, it can be explained that the factor of stratified parent nursery significantly affects to the sugarcane plantlet viability at 1, 2 and 3 MAS. Grandmother's Plantlet Nursery (GPN) produced the greater number of seedling viability than others and also significantly different with others. Otherwise, at the observation 2 MAS, GPN is not significantly different with MPN toward the sugarcane plantlet viability. Then, the greatest average of sugarcane plantlet viability is 93.0% produced by GPN at 3 MAS.

This result is in line with Sudiarso *et al*. (2014) that stratified parent nursery, especially for grandmother plantlet nursery and mother plantlet nursery on Bululawang, PS 862, PS 881, Cokro, Colombia-1 and Columbia-2 which propagated in bud-chips and setts did not show significant difference toward percentage of plantlet viability. This fact described that the process and the implementation of producing sugarcane seed in bud-chips have been done based on Standard Operational Procedure (SOP) which able to increase the settlings viability. It is caused by bud-chips in sterile condition reserve food and by the limited media the eye bud will be growing in under pressure.

Goud (2011) stated that compare to the conventional method, seedling process by using single bud technique showed some advantages, they are: Able to save the cost of seed until 85%, the percentage of germination will be higher, increase the efficient of water usage and others. The use of single bud technique was the right solution to provide the availability of the superior sugarcane seed. Galal (2016) explained that single bud (bud-chip) is good technique to be developed and economically, it can decrease the cost of sugarcane cultivation. The study result of plantlet height can be seen on Table 3.

Furthermore, Table 3 presented average plantlet height (cm) in 1, 2 and 3 MAS. Bululawang and VMC 76-16 produced plant height higher significantly compare another two at the 1st MAS. However, there was non-significant difference of their plant height at the 3 months after sowing. Bululawang, VMC 76-16 and Cokro are superior sugarcane varieties in Indonesia. Meanwhile, the Columbia-2 is an introductive one. The result shows that Columbia-2 adapts to the current environment so that it has the same sapling height as well as percentage of plantlet viability compares to the others.

Columbia-2 clone finally able to grow and develop better by the metabolism of root system. The root system will eventually affect the growth of seeds and subsequent seedlings to reach optimal height. The process occurs due to the possibility of Columbia-2 clone more quickly adapted to

Table 3: Effect variety and stratified nursery on average of plantlet height (cm) at 1, 2 and 3 Months After Sowing (MAS)

	Month After Sowing (MAS)		
Treatments	1	2	3
Stratified nursery (S)			
GPN	12.70[b]	41.20	70.00[b]
MPN	11.60[a]	39.10	70.20[b]
FPN	11.20[a]	39.40	66.10[a]
LSD (5%)	1.01	ns	2.44
Variety (V)			
Bululawang	12.40[b]	38.80[a]	68.90
VMC 76-16	13.60[b]	43.90[b]	66.80
Columbia-2	10.60[a]	39.50[a]	70.60
Cokro	10.50[a]	37.40[a]	68.70
LSD (5%)	1.16	3.12	ns

Number in the same columns followed by the same alphabets means non-significant difference at LSD 5%

the environment so, the metabolic activity of the enzyme is more effective. There might also be a positive signal in the regulation of nitrogenize enzymes, phosphatases, acetyl-coenzyme A particularly IAA and calcium to the availability of nitrogen and energy. This enzyme activity greatly affected the availability of nutrients in the form of ions and cautions and the overall height will affect the growth of seedlings through meristematic cell elongation. This result was consistent to Budi (2011) that Columbia-2 clone verily has potential yield of over 15% as the additional information that the Colombia-2 clone showed its brix above 25% at 7th month after sowing. This fact showed that Columbia-2 clone is very active in metabolism process.

In general, the process that occurs is the leaves and stalk and stem segments derived from the meristem exists between differentiated tissues. In line with the course of time, the growth of protoplasm increases thus, further accelerate the formation of high sugarcane crop seeds. Due to the growing medium is limited pot tray, then each plant in a pot tray stresses, so that the process of tillering depressed. Not only that the correct irrigation period can be also considered as the one of factors to accelerate sugarcane growth. As Hagos *et al*. (2014) stated that applying first irrigation, in early times (five pre-planting irrigation and 1 day after planting) is strongly recommended for getting better sprouting rate, number of tillers and root establishment to sugarcane varieties expecting high cane and sugar yields in the tropical areas. Irrigation can be as one important aspect in plant growth, especially in dry land. Irrigation should consider the soil characteristic and also the balance of water soil. The row spacing also affected to the height of single bud seed. Cheema *et al*. (2002) and Khalid *et al*. (2015) proved that the row spacing 120 cm showed the higher of plant height than other row spacing 90 and 60 cm.

Another interesting result showed that GPN and MPN give plantlet high greater than FPN at the 3 MAS. It showed that the GPN and MPN can be a better place for growing bud-chips based on plantlet height. This study proved that the availability of certified superior sugarcane plant seeds can be accelerated by cutting phases of stratified Parent Nursery. Bud-chip seedlings approximately 3 months grew in the nursery before planting to the field. Bud-chips age of 3 months with the status nursery grandma directly cultivated from FPN without going through the parent nursery. Seed cane aged approximately 7 months with the status flat plantlet nursery could be distributed to growers of sugarcane as milled plantlet nursery in the form of setts or bud-chips according to the needs of sugarcane farmers. As the comparison, for the area that has same size, the stratified nursery by conventional technique during 28 months able to produce 10,000 ha of sugarcane mill nursery. However, if it used single bud (bud-chip) technique during 37 months able to produce 42.875 ha for sugarcane mill nursery. So, this used for keeping the purity of seed which will be planted in sugarcane mill nursery and also help to provide the availability of superior sugarcane seed. Budiarto (2013) strengthen that the best bud-chips should follow the SOP to have high viability. Based on Kumar and Suresh (2011) the use of bud-chip sugarcane showed the better result on the indicators of plant growth such as yields, the number of tiller, weight, plant height and others than conventional method result.

Acceleration stratified parent nursery is expected to be one solution to the accuracy of the availability and quality of seed cane varieties. Furthermore, it is expected to be realized in order to increase crop productivity of sugarcane per hectare. This strategy should be used as a policy to accelerate the accuracy of the availability and quality of certified seed cane. If acceleration between stratified nursery parent and propagated single bud is done then the resulting of sapling quality can be guaranteed, either purity, free of major diseases in particular and in faster time to meet requirement of certified quality seeds. Based on that in 1 ha nursery parent can meet the needs of 2 ha planted seedlings propagated in a single bud. Thus, the sugarcane plantation in East Java province which area of 220 ha can be completed in a maximum of 3 years.

There will be continue study related to the stratified nursery to produce and provide the availability of superior sugarcane seed which propagated by using single bud (bud-chip) based on SOP. Scientifically, this study result can help to accelerate the availability of superior sugarcane seed which became as the fundamental and crucial problem during long time.

CONCLUSION AND RECOMMENDATIONS

Based on the results of study and discussion can be concluded that there is non-significant interaction between variety and stratified parent nursery to percentage of plantlet viability and plantlet height. Columbia-2 could be a promising clone because based on this study result it has similar percentage of viability and its height to existing varieties. Moreover, the sugarcane plantlets from GPN show greater result than MPN and FPN in percentage of plantlet viability. However, it has similar height to MPN. That means whether GPN or MPN could be used as solution and alternative way for providing superior sugarcane seed for sugarcane mill nursery by using single bud (bud-chip) based SOP.

The availability of superior seed cane certified and appropriate varieties to meet the needs of quality and uniform seed cane could be obtained in less than 3 years. This can be done by selecting the superior seed cane certified propagated bud-chips, stratified parent nursery (elders) and propagated simultaneously bud-chip and setts based on farmer's preference.

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