Evaluation of the health and sugarcane seed production from the first ratoon cane at various harvest ages

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Abstract. Increasing the productivity of sugarcane can be done by using healthy and pure seeds. This study aimed to evaluate the health and production of sugarcane seed from ratoon sugarcane derived from tissue culture. The study was conducted in 2017 using a split-plot design, as the main plots were varieties BL and PSJK922; as subplots, the age of seed cane harvest was 5, 6, 7, and 8 months, repeated three times. Bud set from each variety was planted in 5 rows with a length of 8 m. Varieties did not significantly affect all variables of plant growth and seed germination. Seed harvesting age at plant cane (PC) and first ratoon (RC1) affected sugarcane growth except for the number of productive tillers at plant cane. The longer the harvesting age of sugarcane seeds, the higher the attack of the shoot and stem borer. In addition, in plants with seeds originating from the first ratoon, no Ratoon Stunting Disease (RSD) was found. Therefore, the first ratoon cane can be used as a source of healthy sugarcane seeds and produce high seed quality following Indonesian national standards for sugarcane seed production if supported by good management.

Key words: in vitro propagation, germination, ratoon stunting diseases

1. Introduction
One of the sugarcane propagation methods is by using the bud set from the 6-8 month-old plant cane. The supply of sugarcane seed is frequently constrained by the low propagation factor, which makes it difficult to meet the demand. Sugarcane propagation that has been done in irrigated land is around 1:7; meanwhile, propagation in dry land is around 1:5[1]. Pure and healthy seeds are the priority for successful propagation. Regarding these factors, seeds from plant cane frequently become a bottleneck for sugarcane development in local farmers. This matter might be caused by the quantity and quality of the seeds from the plant cane, which is not able to meet the seed demands.

The quality of sugarcane tissue cultured seeds is better and healthier than conventional seeds [2]. However, to date, there is no sufficient information for the quality of the first ratoon cane obtained from tissue culture, especially for the hardiness towards a systemic disease such as Ratoon Stunting Disease (RSD). The utilization of the first ratoon cane seeds obtained from tissue culture is expected to achieve a significant production and also maintain the health of the seeds. Increasing the tissue culture's productivity, purity, and healthiness first ratoon cane seed was done by using the single bud planting method. Single Bud Planting (SBP) seeds are considered superior compared to the conventional bud set seed as it makes the seed selection better. In addition, the seeding process of SBP is shorter (2-2.5 months) along with minimal nursery area and simultaneous sapling growth [3]. The number of saplings from a single bud planting seed is known to be more significant by using the
simultaneous sapling growth method[4],[5]. The usage of single bud planting seeds per hectare is less than the bud set seeds. Single bud planting seeds only need 15,000-20,000 (1.5-2 ton) seeds per hectare, while bud set seeds to require 7-8 ton seeds for planting a hectare ground [6]. The tissue culture first ratoon cane seeds that packed in a single bud planting are considered to add more value to the sugarcane seedling system. Based on this background, it is necessary to carry out a valuation of the health and sugarcane seed production from the first ratoon cane at various harvest ages.

2. Research methodology
2.1 Experiment procedure
The experiments were carried out at the Karangploso Experiment Field-Sweetener and Fiber Crop Research Institute, Malang, Indonesia, from January 2016 to December 2017. This study was conducted by employing the split-plot design, with BL and PSJK 922 varieties were used as the main plot, while the harvest age (5, 6, 7, 8 months) was used as the sub-plot. Replication of every treatment was done three times. In every repetition, each sugarcane clone was planted inside the plot, which consisted of 5 sections with 8 m width. The distance between each center was 100 cm; meanwhile, the planting distance was 40 cm. Materials used in this study were the first generation (G1) of the tissue culture single bud planting. Furthermore, a 75th days SBP saplings were planted by 40 cm distance to each other so that there were 100 plants per plot. Before planting, the sections inside the plot were treated by using a fertilizer with a dosage of 5 tons/hectare, which equals to 5.5 kg per section. The plants were then maintained according to the sugarcane cultivation standard [7].

2.2 Observation Variables
2.2.1 Plant growth: The observation was done on the plant cane and the first ratoon cane at the age of 5, 6, 7, and 8 months after the planting day. The observation included the number of plants in a one-meter section, plant height, trunk diameter, number of segments, and the length of segments. In addition, the terms productive sapling were used to describe the number of saplings with > 1 m trunk height. Observation for the segment length, diameter, and the number of the segments, were performed through sampling 20 plants from the 2nd, 3rd, and fourth sections, and the plants were marked for the next observation.

2.2.2 Plant pests and disease: Observation for the RSD was carried out one week before the harvest day. The observation of RSD was done visually to the five trunks per example section. Serological analysis was performed using ELISA in the Laboratory of Disease, Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI). Observation of shoot borer disease and trunk borer disease on the seed was also carried out one week before the harvest day.

2.2.3 Seed viability test: Seed viability tests were performed to examine the viability of the seed after the seed has been harvested. The test was performed by using a bud chip with Hot Water Treatment (HWT) at 52°C for 30 minutes [8]. Afterward, seeds were soaked in fungicide and growth hormone for 10 minutes. The bud chip sugarcane seeds were then sprouted in the sand media for 14 days at the greenhouse. The test was carried out on 50 seeds and repeated four times. The first observation was performed on the 7th day after the planting day by counting the normal seeds. The 2nd observation was then carried out on the 14th day after the planting day by counting the normal and abnormal seeds. Furthermore, the average of seed viability was then calculated from all repetitions. The percentage of seed viability test was then counted by using the following formula:

\[ \text{Seed viability} = \frac{\text{7th day’s after planting normal seed} + \text{14th day’s after planting normal seed}}{\text{total seed}} \times 100\% \]

2.3 Data analysis
Observational data were analyzed using Variant Analysis and continued with the Duncan test with a 5% P-value.
3. Results and discussions

3.1 Plant cane and first ratoon cane growth

The observed plant growth variables were the height and diameter of the trunk on the day of harvest (Table 1). The variant analysis result showed that seed varieties influence the height and diameter of sugarcane. The height and diameter of sugarcane plants are used as indicators to measure the environmental or treatment effects on the plants as both factors are the most visible factors that can be measured from the plants [9]. In addition, in sugarcane plants, the height is a reflection of the number of segments and their length. The segment length also describes the growth process in the meristematic, intercalary, or in apical meristem area. The higher the number of segments, the higher the number of bud sets. Besides, a larger diameter and higher number and of segments may also increase the fresh-biomass weight [10],[11].

Furthermore, variant analysis results also showed that the time of harvesting influenced the length and diameter of the segments. Trunk growth is an important phase for sugarcane because it will influence and determine the resultant bud sets [12]. The average length of the 5-month-old segments was longer than other observational ages. This might be caused by the sufficient water and nutritional supply, which helps the elongation phase. Meanwhile, at the 6, 7, and 8 months old, the water and nutritional supply had decreased, which also influenced the trunk elongation phase [13]. Our results also fit with previous reports [14], which stated that maximum growth of the trunk could be achieved by the optimum environmental factors, especially the water supply, optimum pH, and sufficient essential nutrients, which results in the optimum metabolic process inside the plants.

Furthermore, the length and diameter of the plant cane seed showed a higher average than the first ratoon cane seeds. Both BL and PSJK varieties have passed the Indonesia National Standard (in Indonesia called SNI) for sugar cane seed standard, which should have segments longer than 15 cm and a diameter larger than 2 cm. The standard length for sugarcane segments to be used as the potential seeds is segments with 15-20 cm length with more than 2 cm diameter [12],[15].

| Variety | Month After Planting (MAP) | Segment Length Average (cm) | Segment diameter Average (mm) | Seed Viability Average (%) |
|---------|----------------------------|-----------------------------|-------------------------------|-----------------------------|
|         |                            | PC RC1                       | PC RC1                        | PC RC1                      |
| BL      | 5                          | 24.05d 21.03bcd 22.23a 22.68b 84.33e 84.33de | 17.43a 18.25a 23.93c 19.80a 65.67a 71.00a |
|         | 6                          | 19.93b 23.03b 22.23a 21.30ab 84.33e 84.33de | 22.68d 19.45d 21.73a 21.38ab 87.00f 87.00f |
|         | 7                          | 20.20bc 21.88bc 23.18b 22.53b 79.33c 80.48c | 22.13cd 18.48cd 22.20a 22.00b 82.33de 86.00de |
|         | 8                          | 17.43a 18.25a 23.93c 19.80a 65.67a 71.00a | 18.90ab 19.25ab 22.90b 23.25b 82.00d 82.00d |
| PSJK    | 5                          | 22.68d 19.45d 21.73a 21.38ab 87.00f 87.00f | 16.93a 17.90a 23.10b 21.58ab 73.33b 74.33b |
|         | 6                          | 22.13cd 18.48cd 22.20a 22.00b 82.33de 86.00de | 18.90ab 19.25ab 22.90b 23.25b 82.00d 82.00d |
|         | 7                          | 18.90ab 19.25ab 22.90b 23.25b 82.00d 82.00d | 16.93a 17.90a 23.10b 21.58ab 73.33b 74.33b |
|         | 8                          | 17.43a 18.25a 23.93c 19.80a 65.67a 71.00a | 22.68d 19.45d 21.73a 21.38ab 87.00f 87.00f |
| Average | BL                         | 20.40 21.04 22.89 21.58 78.42 80.04 | 20.16 18.77 22.48 21.58 81.17 82.33 |
|         | PSJK                       | 20.16 18.77 22.48 21.58 81.17 82.33 | 20.40 21.04 22.89 21.58 78.42 80.04 |

Notes: Numbers followed by the same letter in the same column shows no significant difference by the 5% P-value on DMRT.

3.2 Sugar cane seed productivity

The seed productivity per land unit is determined by the ability of the seeds to produce productive saplings and also by counting the number of segments. The higher the number of productive saplings and the number of segments implies the higher number of sugarcane seeds. The productive sapling is the main factor in obtaining a high rate of seed productivity [16].
The result showed that plant variety has no effect on the number of productive saplings. On the 5 and 6 months-old first ratoon cane, the average number of the productive sapling showed no significant difference between BL and PSJK922 variety. The significant differences were obtained from the sapling on the 7 and 8 months-old plants. The number of productive saplings from the BL variety was more than PSJK922. First ratoon cane productive saplings were created optimally at the 7 and 8 months old. Moreover, it has been reported that the sugarcane sapling development usually happens on the 3 to 9 months old plants [17].

Furthermore, based on the variant analysis, the time of harvesting had no significant effect on the number of productive saplings per meter inside the section; on the contrary, the first ratoon cane saplings. The number of productive saplings for the first ratoon cane increased along with the time of harvesting. This experiment also showed that the sugarcane sapling from the plant cane indicated a simultaneous growth. In addition, the number of productive saplings from the 5 and 8 months harvested plant showed no significant difference. The growth of productive sapling from the single bud planting seed produces uniform sapling [16],[17]. A single bud planting seed that is planted at 2.5 months old or when having 5-6 leaves means that this seed has sprouted and entered the sapling development phase. Therefore, by using the single bud planting seed, it would produce uniform saplings. As a result, the bud set of every segment will grow simultaneously [16].

The time of harvesting had a significant effect on the first ratoon cane saplings. This was caused by the different bud set ages which developed from the first ratoon cane plants, resulting in different sapling development times. This result is supported by the previous studies [16],[18], which stated that the productive sapling development on the first ratoon cane occurred at eight months old.

Furthermore, the time of harvesting also had a significant effect on the number of segments. The longer time of harvesting, the higher the number of bud set segments. BL variety seed on the five months old generated bud set segments with an average of 7.88 segments, while PSJK variety only had 6.95 segments. In addition, in the eight months old, BL reached 16.83 segments, while PSJK only generated 6.95 segments.

Sugarcane seeds were harvested at the age of 6 to 8 months old by cutting the trunk that had a minimum of nine segments [14],[15]. Segments number 5 to 11 (around seven segments) were then used for a seedling. At the five-month-old, we found that the plant cane has seven bud sets, indicating that the five-month-old harvested plant could be used as a proper candidate for potential seeds. It is also supported by the length and diameter variable of the trunk. In addition, we found that the number of segments from the first ratoon cane showed no significant difference between plant cane from both varieties that we use.

Single bud planting seeds were obtained from the bud set, which was maintained at the nursery until the seed had 5-6 leaves or around 2.5-3 months-old would then proceed to be planted in the field. The single bud planting seeds were collected through several phases, such as selection of bud set, seed viability, and vigor. Besides, there was also a selection to detect the presence of the shoot and trunk borer using the HWT treatment to prevent and reduce the source of the sugarcane disease. HWT treatment was then followed by the treatment using growth hormone to stimulate the growth of the bud set. The number of bud sets from the single bud planting seed was greater compared to the bud set seed. Utilization of the single bud planting generates more productive sapling than the bud set seeds [7].

Furthermore, the productive saplings and segments per trunk can be used as the basis to do productivity taxation of the single bud planting seed. The productivity taxation of bud set is based on the number of productive saplings per meter section as well as the number of segments per trunk in one hectare. The productivity taxation was carried out by counting 90% of the area and 95% of the healthy bud set. Hence, the taxation result showed that the time of harvesting didn't have any significant effect on the number of the bud set. The longer the time of harvesting, the more bud set was spotted on the plant (Table 2). On the other hand, the seed viability that fit with the sugarcane seed standard was obtained from the 5th to 15th segments (around 11 segments). The number of seeds generated by the BL variety was greater than the PSJK922 variety. This result indicated that the
number of the seed was affected by the number of productive saplings and also the number of segments. The number of segments from the BL variety from the 1st-year-old plants was also higher than the PSJK922 variety.

### Table 2. Productive saplings, number of segments, and number of bud set from BL and PSJK922 varieties on different time of harvesting.

| Variety | Months After Planting (MAP) | Productive sapling average | Number of segments average | Number of bud set average ha⁻¹ |
|---------|-----------------------------|-----------------------------|---------------------------|-------------------------------|
|         |                             | PC  | RC1 | PC  | RC1 | PC  | RC1 | PC  | RC1 |
| BL      | 5                           | 10.75a | 7.00a | 7.88b | 9.23a | 724.270.5 | 552.415.5 |
|         | 6                           | 11.25a | 7.35a | 9.43c | 10.28a | 907.048.1 | 646.020.9 |
|         | 7                           | 10.50a | 10.93c | 13.05e | 12.33b | 987.525.0 | 1.027.967 |
|         | 8                           | 11.25a | 13.50d | 16.83g | 14.53c | 1.058.063 | 1.269.675 |
|         | 5                           | 11.75a | 7.43a | 6.95a | 9.28a | 698.214.4 | 589.525.9 |
|         | 6                           | 10.50a | 7.43a | 9.53c | 10.08b | 855.555.8 | 640.347.1 |
|         | 7                           | 10.75a | 9.30d | 12.63e | 12.30b | 1.011.038 | 874.665.0 |
|         | 8                           | 10.25a | 11.28c | 15.63f | 14.03e | 964.012.5 | 1.060.884 |
| Average | BL                          | 10.94 | 9.69  | 11.27 | 11.59 | 919.226.5 | 874.019.5 |
|         | PSJK                        | 10.81 | 9.30  | 11.18 | 11.42 | 882.205.0 | 791.355.5 |

Notes: Numbers followed by the same letter in the same column, shows no significant different by the 5% P-value on DMRT.

3.3 Seed viability test

The variant analysis result showed that seed age and variety have a significant effect on seed viability. The seed viability of PSJK 922 showed a higher rate compared to the BL variety. According to [18], BL variety tends to have a slower viability rate. Meanwhile, PSJK 922 has a medium viability rate [19]. Hence, the variety has a significant effect on seed viability. This result is also supported by [20], which also reported that the variety determines the viability of the seed.

On the other hand, a longer time of harvesting can decrease the seed viability, both on plant cane and also first ratoon cane. The 7 and 8 months-old harvested plant and first ratoon cane were able to generate 12-16 bud sets. Meanwhile, the seed viability tests from all segments from the BL and PSJK varieties using 7-8 months-old plants showed a percentage less than 80%. In addition, the utilization of 11 plant cane bud sets from 7-8 months-old BL variety was able to produce a viability rate around 79.33%, while from the first ratoon, cane produced 80.48%.

The viability of the sugarcane bud set has followed the gradient spraying trend where the shoot-tip has a higher viability rate compared to the lower-point in the bud set [20],[21]. Low viability of the bud set above the 13th segment might be caused by the covered bud set, which also made the bud set hardened, resulting in abnormal growth. As shown in Table 1 and Table 2, the number of segments and seed viability has a negative correlation with the seed viability. Older cane sugarcane seeds will result in a higher number of segments. Moreover, older sugarcane seeds are also associated with older bud sets, which then results in a decrease in viability rate. The recommended viability rate for sugarcane seeds is 80% [14],[15]. The slow-growth seed could cause a variant growth. According to [22],[23], the declining seed quality was occurred naturally and heavily relies on time, while the physiological decline caused by environmental factors, which also affected the seed viability and vigor. These factors will also affect the bud set, such as the dormant of the seed. The dormancy will decrease the viability rate. To overcome those problems, usually seed will be treated by HWT for 45 minutes and the addition of gibberellin 1.5 ml/l to the bud set. It is known to boost the growth of the seed [8].
3.4 Sugar cane seed health
In this section, the result shows that seed age positively affects the shoot borer percentage per plant. In BL and PSJK922 variety by the harvest age 5, 6, 7, and eighth months, it was affected significantly. The shoot borer percentage on the BL variety is higher than the PSJK922 variety. Meanwhile, the first ratoon cane seed also increased 21.86% for the BL variety and 28.97% on the PSJK922 variety compared to the plant cane (Table 3).

Table 3. Sugar cane seed health on different harvest age.

| Variety | Harvest age | Shoot borer average | Escalation (%) | Trunk borer average | Escalation (%) | RSD |
|---------|-------------|---------------------|----------------|---------------------|----------------|-----|
|         |             | PC                  | RC1            | PC                  | RC1            | PC  | RC1 |
| BL      | 5           | 2.43c               | 3.03d          | 24.69               | 1.45d          | 1.58cd | 8.96 | negative | negative |
|         | 6           | 2.50cd              | 3.08d          | 23.20               | 1.58e          | 1.68d  | 6.33  | negative | negative |
|         | 7           | 2.58d               | 3.10d          | 20.15               | 1.53de         | 1.60cd  | 4.57  | negative | negative |
|         | 8           | 2.58d               | 3.08d          | 19.38               | 1.73f          | 1.83e  | 5.78  | negative | negative |
| PSJK    | 5           | 1.73a               | 2.43ab         | 40.46               | 1.10a          | 1.30a  | 18.18 | negative | negative |
|         | 6           | 1.83a               | 2.33a          | 27.32               | 1.20b          | 1.38ab  | 15.00 | negative | negative |
|         | 7           | 2.03b               | 2.53bc         | 24.63               | 1.20b          | 1.48bc  | 23.33 | negative | negative |
|         | 8           | 2.13b               | 2.63c          | 23.47               | 1.30c          | 1.58cd  | 21.54 | negative | negative |
| Average | BL          | 2.52                | 3.07           | 21.86               | 1.57           | 1.67  | 6.41  |           |            |
|         | PSJK        | 1.93                | 2.63           | 28.97               | 1.20           | 1.58   | 19.51 |           |            |

Notes: Numbers followed by the same letter in the same column, shows no significant different by the 5% P-value on DMRT.

The variant analysis result shows that seed age has a significant effect on the trunk borer average. BL variety has a higher percentage than PSJK922. In addition, on the first ratoon cane seed, the percentage is increasing around 6.41% on BL, while 19.51% on PSJK922 compared to the plant cane (Table 3). Nonetheless, the percentage was no more than 5% for the shoot borer, while no more than 2% for the trunk borer [14], [15]. The result shows that the seed harvested at 5-8th months old is still below the maximum tolerance values for the health standard. In addition, the health of the seed can be improved by using single bud planting.

In addition, based on the longitudinal visual observation followed by the PCR analysis, there is no sign of the RSD symptoms on the first ratoon cane plant, both BL and PSJK 922 variety. Seed from the first ratoon cane tissue culture is suitable to be used on the field because the health and its productivity is not reduced, and the quality of the seed fits with the sugarcane seed standard.

4. Conclusion
The time of harvesting affects the seed productivity; nonetheless, it didn't affect the quality of the sugarcane seed. Seed viability is decreasing with the increase of time for harvesting. The first ratoon cane from the tissue culture is suitable to be used as seed by using 11 segments from the 5th to 15th segments. There are no RSD symptoms in the first ratoon cane obtained from the tissue culture.

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