FIELD PERFORMANCE OF SUGARCANE cv. PSJT 941 PUTATIVE MUTANT RESULTED FROM GAMMA RAY CO^{60}-IRRADIATION

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Abstract. A study has been carried out to evaluate the field performance of twenty clones of gamma ray Co^{60}-induced putative mutant of PSJT 941 Sugarcane cultivar in *PT Perkebunan Nusantara IX* field, Pemalang Indonesia. This research has been carried out to 1) evaluate the growth difference between mutant and wild type clones of PSJT 941 sugarcane cultivar; 2) determine the clone which shows the best field growth. This research has been carried out experimentally using 20 putative mutant clones produced from gamma ray Co^{60} irradiation and one wild type clone, with 5 replications. The variable observed was the sugarcane growth, with the parameters measured include number of siblings, plant height and stem diameter. The research results showed that the putative mutants showed highly significant differences in terms of number of siblings and plant heights, and significantly different in terms of stem diameter, compared to the wild type. Clone 941.1.7 was promising, which showed an average number of siblings of 5 siblings/plant, a plant height that was 28.8% shorter than the control plant, the largest average of stem diameter of 30.2 mm, a greater tolerance to drought, and has the potential to become an early maturing sugarcane. Further research is needed to re-confirm those putative mutants.

Keywords: Gamma Co^{60}, PSJT 941, putative mutant, sugarcane

A. Introduction

The development of the sugar industry in Indonesia plays an essential role in the country's economy and the fulfillment of people's basic needs. The demand for sugar has been increasing every year. Sugar imports data indicate that national sugar production has not been able to meet market needs. Data (BPS, 2021) shows that national sugar production has decreased from 2.36 million tonnes (2016) to 2.13 million tonnes (2020). Meanwhile, national sugar consumption in 2020 was 2.66 million tonnes. To fulfill the national sugar demand, the government has imported sugar. Sugar import has increased from 4.48 million tonnes (2017) to 5.53 million tonnes (2020).

Sugarcane (*Saccharum officinarum* L.) is one of the most important agricultural commodities. Sugarcane has a high economic value because it is a sugar-producing plant for staple households and industries (Sukmadjaja & Mulyana, 2011). The on-farm and off-farm factors can cause the decline in national sugar production. Some of the on-farm problems include a decrease in the area of sugarcane plantations, a shift in sugarcane cultivation to dry land/fields, the dominance of ratoon cane proportions compared to plant cane, the incompatibility of cultivated sugarcane varieties to their field, uncontrolled fertilization, and low silica (Si) content in sugarcane plantations. Meanwhile, off-farm problems include the declining interest of farmers in growing sugarcane due to less competitive income. Data from Central Java (BPS, 2020) showed a decrease in the Central Java Farmer Exchange Rate at the end of 2022 by 0.38 %. However, it is interesting to see that for the plantation sub-sector, sugarcane exchange rate has increased by 0.85 percent, which means that sugarcane sector has promising opportunities.

Efforts to expand agricultural land now has led to the utilization of marginal lands, such as saline soils (Rosmayati & Bayu, 2016; Tolib et al., 2017). The width of saline soils increases as the temperature and sea level rise caused by climate change and global warming. The area of marginal land in Indonesia is large and has the potential to be utilized; for example, the area of saline land in Indonesia is around 0.44 million hectares (Tolib et al., 2017), even a report (Dyah, 2017) showed that the area of dry land in Indonesia reaches 60.7 million hectares.

Sugarcane cultivar PSJT 941 is one of the superior sugarcane cultivars which is widely cultivated in Indonesia. This cultivar is resistant to fire wound and *blendok* diseases, and is resistant to both shoot and stem borers. This cultivar has a yield value of 9.01%-12.4%. PSJT 941 is a medium-ripeness sugarcane cultivar that can grow well on *grumosol* soil with climate type C2 (Indonesian Sugar Research Institute, 2014).

Developing new sugarcane cultivars through conventional methods is not prospective because it takes a long time and has a low success rate. Applying in vitro plant culture techniques to induce somaclonal variation is a potential method for obtaining new variations in sugarcane. Somaclonal variation can occur spontaneusly or induction such as through chemical induction using EMS, *Cholcisin*, or physical induction, using gamma rays and X-rays (Manchanda et al., 2018; Rai et al., 2011). The cause of somaclonal variation in plants may include modification of histone proteins, repetitive DNA's appearance, transposable elements' activation, and changes in DNA methylation patterns. Changes in DNA methylation can be detected through the occurrence of polymorphism in test plants (Francischini et al., 2017), which can be analyzed by Random Amplified Polymorphic DNA (RAPD) techniques (Patade et al., 2006; Rastogi et al., 2015; Suprasanna et al., 2007).

The results of our previous study found nine primers (consisting of OPB-08, OPB-10, OPE-02, OPD-01, OPK-04, OPL-02, OPN-11, OPA-02, and OPA-13) that can detect polymorphisms di sugarcane. Six clones of 941 sugarcane mutant were confirmed which included clones 941.1.1, 941.1.3, 941.1.7, 941.2.3, 941.2.9, 941.2.10) and two of them (941.2.3 and 941.2.10) showed interesting character differences. In addition, the other 14 putative mutants were also interesting to see their performance in the field. Therefore, this research was focused on field testing of those mutants and putative mutants.

B. Material and Methods

1. Plant Materials

Plant materials used were six mutants of PSJT 941 sugarcane, 14 putative mutants of PSJT 941 sugarcane resulting from radiation with Co^{60} gamma rays, and one wild-type clone, collections of Plant In Vitro Culture Laboratory Faculty ff Biology, Universitas Jenderal Soedirman. The preparation of planting material has been carried out by sowing the mutants and wild type sugarcane shoots at *PT Perkebunan Nusantara IX*.

2. Field Performance

This research has been conducted experimentally on the field of *PT Perkebunan Nusantara IX*, Pemalang, Indonesia, using a completely randomized block design (CRBD). The treatments included 20 sugarcane clones resulted from radiation with Co^{60} gamma rays consisting of: 6 mutants of PSJT 941 sugarcane (941.1.1; 941.1.3; 941.1.7; 941.2.3; 941.2.9; 941.2.10), 14 putative mutants of PSJT 941 sugarcane (941.1.2; 941.1. 4; 941.1.5; 941.1.6; 941.1.8; 941.1.9;

941.1.10; 941.2.1; 941.2.2; 941.2.4; 941.2.5; 941.2.6; 941.2.7; 941.2.8), and one wild type clone. Each treatment will be repeated 5 times, resulting in 105 experimental units. The variables measured were the growth sugarcane, with the parameters measured including the number of tillers, plant height, and stem diameter. Observations and measurements of research parameters were carried out every three months.

3. Data Analysis

The data obtained were analyzed using an Analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at a 95% confidence level using DSAASTAT VER 1.514 software.

C. Results And Discussion

The field performance evaluation of 20 putative mutant of sugarcane clones (PSJT 941) showed that all clones exhibited favorable growth. Among 20 sugarcane mutant clones and the wild types showed diverse growth, which indicated that there was variation caused by the irradiation. This study assessed three key growth parameters: the number of siblings, plant height, and stem diameter, which are critical indicators contributing to sugar yield. These has been reported by Akter et al., (2016); Ali et al., (2015); Majeed et al., (2018), that sugar production in sugarcane is strongly influenced by growth parameters, particularly the number of siblings, stem diameter, and plant height.

The results of the DMRT analysis for the mean number of siblings at three months (Figure 1) revealed that clone 941.1.5 exhibited the highest sibling number, with an average of 7.8 siblings per plant. This result was substantially higher than the wild-type control, which recorded an average of only five siblings per plant. Conversely, clone 941.1.2 produced the lowest sibling number of 2.8 siblings per plant, that was significantly different to both the other clones and the control.

The DMRT analysis of the number of siblings in sugarcane clones at six months (Figure 2) revealed a decline in the average sibling count across all tested sugarcane clones, including the control. The highest sibling counts were observed in clones 941.1.2 and 941.2.4, with an average of 5.6 siblings per plant. In contrast, clone 941.1.5 reduced the average sibling count to 4.4 siblings per plant. Clone 941.1.2 recorded the lowest sibling number, averaging 2.0 siblings per plant, representing a decrease of 0.8 siblings compared to prior measurements. A similar trend was observed in the control plants, where the sibling count dropped from 5 to 3 siblings per plant, indicating a reduction of 2 siblings per plant. The decline in sibling numbers across these sugarcane clones is attributed to the onset of the dry season during their vegetative growth stage. Prolonged drought conditions resulted in significant soil moisture depletion, leading to the death of several sibling shoots in the affected clones. Drought stress severely disrupts physiological processes in plants, leading to wilting, stunted growth, and, in extreme cases, mortality due to water deficit. Riajaya et al. (2020), reported that sustained drought conditions in agricultural environments drastically reduce soil water availability, resulting in extensive plant mortality, particularly in species which unable to tolerate prolonged periods of water stress. The observed reduction in sibling numbers is thus directly related to the adverse effects of drought-induced stress on plant survival and growth.

Plant height is one of the growth and production parameters in sugarcane cultivation. The sugarcane plant's growth strongly influences sugarcane's sugar content. Based on the DMRT sults, plant height at three months (Figure 3) and six months (Figure 4) shows that control plants have the highest average plant height which accounted for 154.8 cm and 199.8 cm, respectively. The shortest plant height is shown by sugarcane clone 941.2.1, with an average height of 66.8 cm at three months; this result is also consistent with the results at the age of six months, with an average height of 101.8 cm. The data also showed that the mutant and putative mutant sugarcane clones showed shorter plant height than the control plants. This is an expected result because the assembly of early maturing sugarcane is expected to have a shorter height with high sugar content. Short plant height also indicated the occurrence of random mutations that occur due to Co^{60} gamma irradiation. Suhesti et al. 2021; Yunita et al. (2020) reported that gamma irradiation can be random, so it can positively and negatively affect plant growth. The reduced growth and physiological responses of irradiated sugarcane shoots proved that radiation can cause physiological damage, gene mutations, or chromosomal mutations. Furthermore, Choi et al. (2021) showed that the use of gamma radiation can damage plant DNA; this DNA damage will result in the changes in both physical and physiological changes.

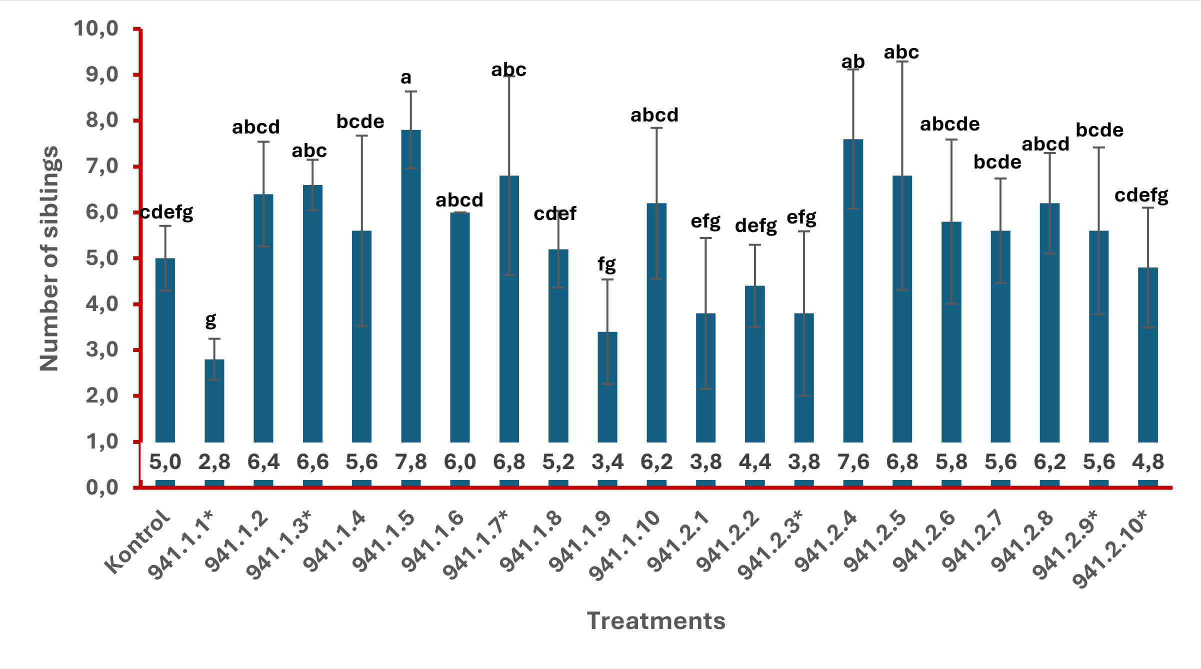

Figure 1. The results of DMRT on the average number of siblings of Sugarcane cv. PSJT 941 Putative Mutant Resulted from Gamma Ray Co^{60}-Irradiation three months after planting. Note: a,b—Means marked with different letters are statistically different at DMRT 95%.

The results of the DMRT test on the average stem diameter at the age of 3 months (Figure 5) showed that the putative mutant clone 941.2.7 was the clone that had the largest stem diameter of 30.2 mm. These results were not significantly different to mutant clones 941.1.1, 941.1.3, 941.1.7, and 941.2.3. Putative mutant sugarcane clones tended to have an increase in stem diameter. Sugarcane clones with the lowest stem diameter was 941.2.8 (21 mm), much smaller than that of the control in stem diameter (22.6 mm).

Furthermore, the observation of stem diameter at six months (Figure 6) showed the same trend as the observation at three months. Sugarcane clones that had the largest diameter were putative mutant clone 941.2.7 with a diameter of 30.8 mm. The results also showed that all mutant and putative mutant clones showed an increase in stem diameter. In comparison, the wild-type sugarcane (control) showed a low stem diameter (23.6 mm). Gamma radiation can stimulate the growth of primary branches stem diameter and induce axillary buds in plants, stimulate pod formation, and others; this can occur when applying low concentrations of gamma radiation (Ali et al., 2015; Anne & Lim, 2020).

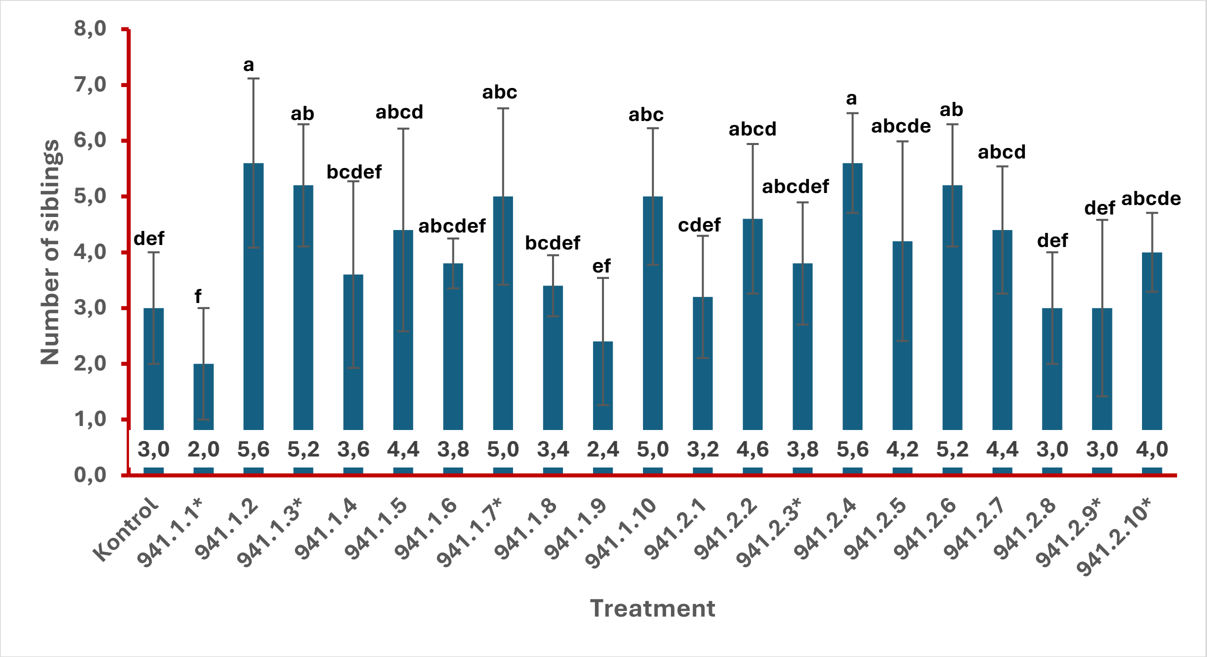

Figure *2*. The results of DMRT on the average number of siblings of Sugarcane cv. PSJT 941 Putative Mutant Resulted from Gamma Ray Co^{60}-Irradiation six months after planting. Note: a,b—Means marked with different letters are statistically different at DMRT 95%.

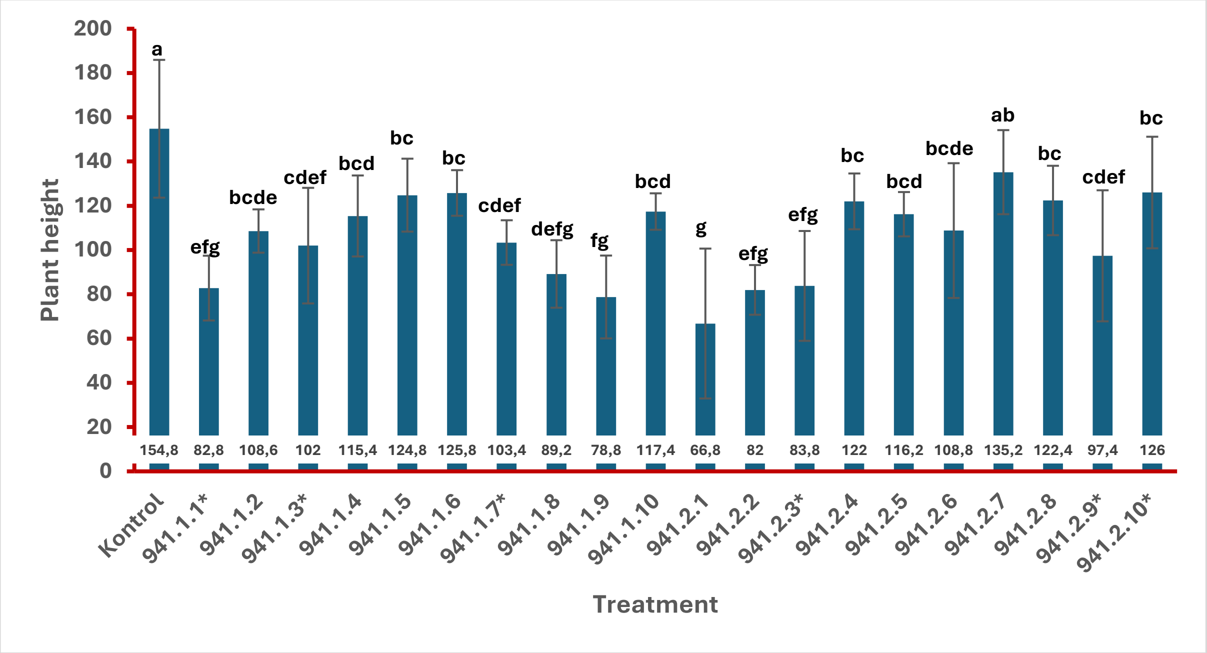

Figure 3. The results of DMRT on the average plant height of Sugarcane cv. PSJT 941 Putative Mutant Resulted from Gamma Ray Co^{60}-Irradiation three months after planting. Note: a,b—Means marked with different letters are statistically different at DMRT 95%

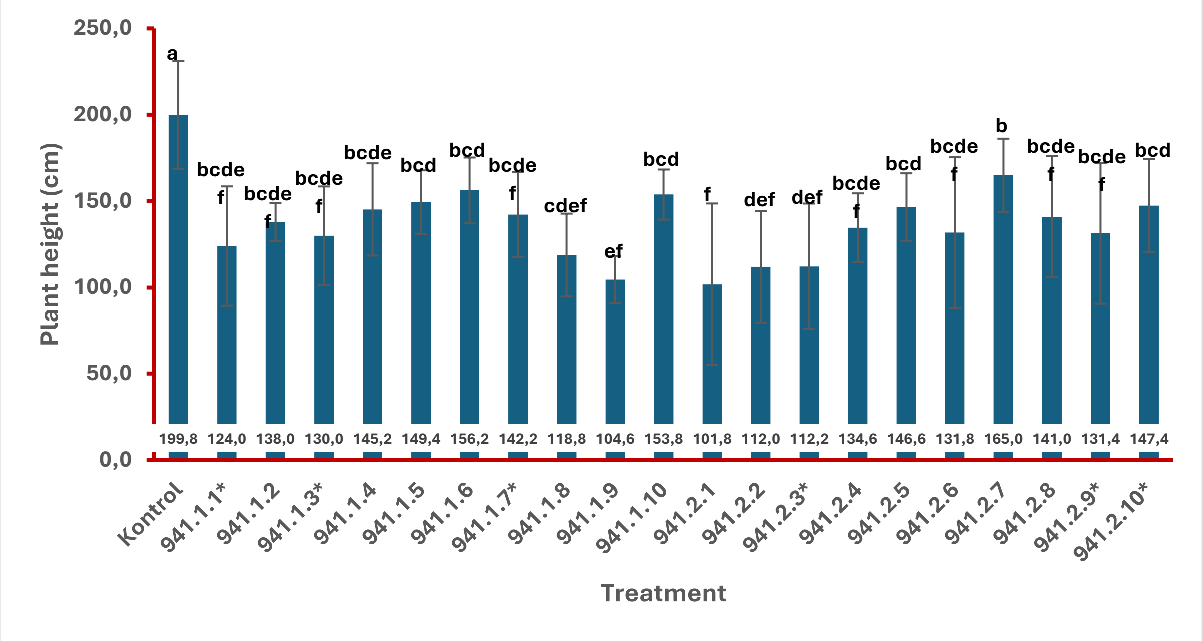

Figure 4. The results of DMRT on the average plant height of Sugarcane cv. PSJT 941 Putative Mutant Resulted from Gamma Ray Co^{60}-Irradiation six months after planting. Note: a,b—Means marked with different letters are statistically different at DMRT 95%

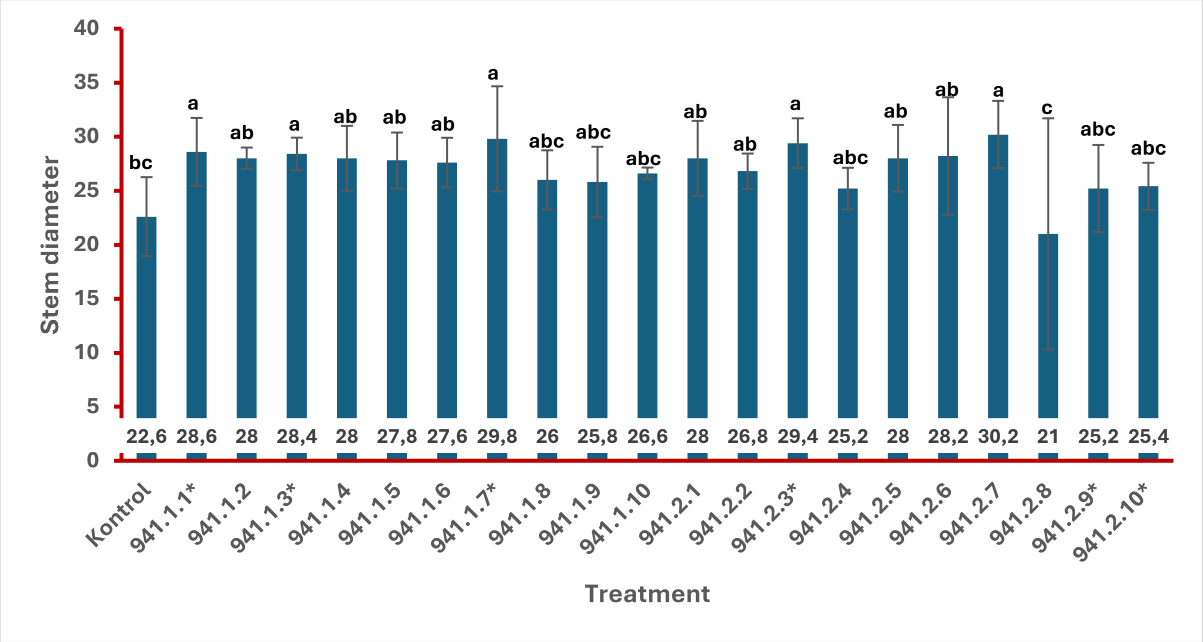

Figure 5. The results of DMRT on the average stem diameter of Sugarcane cv. PSJT 941 Putative Mutant Resulted from Gamma Ray Co^{60}-Irradiation three months after planting. Note: a,b—Means marked with different letters are statistically different at DMRT 95%

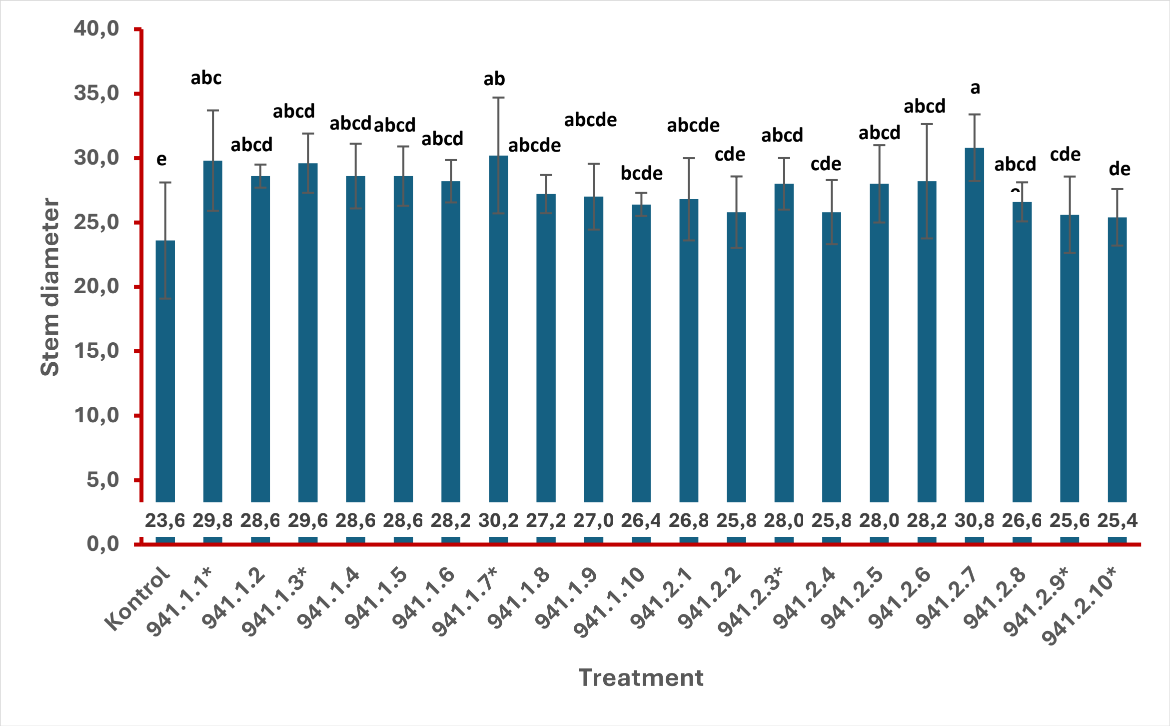

Figure 6. The results of DMRT on the average stem diameter of Sugarcane cv. PSJT 941 Putative Mutant Resulted from Gamma Ray Co^{60}-Irradiation six months after planting. Note: a,b—Means marked with different letters are statistically different at DMRT 95%

Based on the observations of all parameters, potential sugarcane clones had been identified. These potential clones had many siblings, shorter plant height, and larger stem diameter. Among the 21 sugarcane clones tested, clone 941.1.7 was promising. Sugarcane clone 941.1.7 has an average number of siblings of 5 siblings/plant, a plant height that was 28.8% shorter than the control plant, and the largest average of stem diameter of 30.2 mm. Sugarcane clone 941.1.7 can be assumed to have a greater tolerance to drought. In addition, clone 941.1.7 has the potential to become an early maturing sugarcane.

Changes in plant height in putative sugarcane can be caused by gamma radiation at specific dose which can cause the cell cycle process to stop at the G2 / M phase during cell division. Gamma radiation can cause damage to the entire genome (Gadakh et al., 2015; Nikam et al., 2014, 2015; Wi et al., 2007). Yasmeen et al. (2020) stated that mutation induction using gamma radiation in low dose can increase agronomic parameters such as plant height, brix content, number of tillers, and sucrose content in sugarcane cv. NIA-0819, NIA-98, and BL4. Hapsoro et al. (2018) dan Jan et al. (2012) stated that using gamma radiation either at low or high dose can lead to the increase or decrease of plant growth rate and biochemical or physiological processes of a plant. Furthermore, Piri et al., (2011) reported that high doses of gamma radiation may damage the ultrastructural organelles and subsequently affect plant phenotypes.

D. Conclusion

The research results showed that the putative mutants showed highly significant differences in terms of number of siblings and plant heights, and significantly different in terms of stem diameter, compared to the wild type. In addition, almost all putative mutants indicated better ideotype parameters such as greater number of siblings, shorter plant height, and bigger stem diameter. Clone 941.1.7 was promising, which showed an average number of siblings of 5 siblings/plant, a plant height that was 28.8% shorter than the control plant, the largest average of stem diameter of 30.2 mm, a greater tolerance to drought, and has the potential to become an early maturing sugarcane. Further research is needed to re-confirm those putative mutants.

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