Micropropagation of Ten Genotypes of Sugarcane Mutants on Media Containing BA and Thidiazuron

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Abstract. Mutation induction in sugarcane by gamma irradiation has produced 10 mutant clones. Each mutant clone needs to be reproduced before further release. Multiplication of mutant clones is done in-vitro to produce true-to-type mutant sugar cane seeds. Four in-vitro media containing BA (Benzylaminopurine) and Thidiazuron were designed to obtain the right media for each sugarcane mutant. The medium used was basic Murashige Skoog (MS) medium containing 100 mg/l PVP and 3% sucrose, with the addition of BA (1 and 3 mg/l) and thidiazuron (0 and 0.5 mg/l). The explants used were shoots from the ten genotypes of the sugarcane mutants, measuring ±0.5 cm. The study used a completely randomized design with observational variables: shoot height, number of shoots, number of roots, visual shoots and roots. The results showed that growth media containing 3 mg/l BA and 0.5 mg/l thidiazuron was the best medium for multiplication of mutant sugarcane. Growth regulator auxin NAA was more effective in inducing the formation of sugarcane roots. Clones K10 and K8 had the best shoot multiplication ability compared to other mutant clones.

Keywords: clonal propagation · *Saccharum officinarum* · multiplication · growth regulator

1 Introduction

Indonesia's population growth is very fast resulting in the need for sugar consumption is also increasing. Until now Indonesia has to import to meet national sugar consumption needs. On the other hand, Indonesia's sugar production has decreased due to global climate change which has caused weather anomalies that have resulted in a decrease in sugar cane yields. Climate change also causes the need for optimal dry months and wet months for sugarcane plants to become unavailable. Therefore, the assembling of new

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varieties to replace old varieties whose genetic potential has decreased must be carried out so that there is no decrease in yield which will have an impact on national sugarcane productivity.

The development of new varieties must be supported by good seed propagation technology. Until now, farmers still apply conventional propagation, namely through cuttings with 2–3 eyes. This method results in limited plant propagation, requires large areas of land, mother plants and a lot of labor, as well as obligate pathogen infections and systemic disease attacks that are difficult to avoid.

Propagation using in vitro techniques or tissue culture is one of the solutions in seed propagation of sugarcane plants because it can produce a lot of seeds, has the same genetics as the parent, does not require large areas of land, and is disease free, especially systemic diseases [1–3]. The application of in vitro culture techniques in seed multiplication from new superior varieties produced is useful for replacing old varieties with new varieties on a large scale. One of the important steps in shoot propagation through in vitro culture is multiplication. Several things affect the rate of shoot multiplication in vitro, namely the composition of the media, the type of growth regulator used, the type of explant, the size of the explant, and the density of the explant [4].

To increase the rate of shoot multiplication, it is necessary to modify the composition of the culture media by adding growth regulators (PGR). Cytokinin and auxin are commonly used PGR groups. Shoot formation is more influenced by cytokinins. Benzyl adenine (BA) is a synthetic PGR and has more stable and stronger properties than other types of cytokinin hormones.

Genotype is a determining factor in sugarcane in vitro culture. Sugarcane culture in vitro can provide different responses between genotypes [5]. [6] said that rapid clonal propagation of sugarcane planting materials depends on the genotype and the combination of plant growth regulators used. According to [7] the media used for in vitro multiplication of sugarcane shoots was MS + IBA 0.5 mg/l + BAP 0.3 mg/l, while [8] stated that 0.5 mg/l BAP media + 0.5 mg/l kinetin produced the highest number of shoots and leaves compared to the treatment without hormone or plant growth regulator. [9] Reported that the combination of 2.46 μM IBA + 1.33 μM BAP resulted in the formation of the most sugarcane shoots in PSJT 941 and Kidang Kencana varieties. In general, BA has a major influence on the development of explants, namely in the formation of shoots, shoot multiplication, and promoting cell division in plant metabolism to form the parts/organs needed. The aim of this study was to obtain the proper propagation protocol for each sugarcane mutant genotype.

2 Materials and Methods

2.1 Plant Material

This research was conducted at the Tissue Culture Laboratory of the Cell and Tissue Biology Research Group, Center for Research and Development of Biotechnology and Agricultural Genetic Resources, Bogor. The plant materials used were ten mutant genotypes of gamma-ray irradiated sugar cane, namely K1 to K10. The explants used were shoots from the ten genotypes of the sugarcane mutants, measuring ±0.5 cm.

2.2 Methods

Multiplication of shoots of sugarcane mutants was carried out using basal medium of Murashige Skoog (MS) containing 100 mg/l PVP and 3% sucrose, with the addition of BA (1 and 3 mg/l) and thidiazuron (0 and 0.5 mg/l). The explants were cut into pieces and planted in the media formulation.

The explants were incubated in a culture room with a temperature of 23–25 °C and a light intensity of 800–1000 lx for 16 h every day. Observations were made on the number of shoots, shoot height, and visual shoots. Shoots that have good morphology are transferred to the media to induce root formation. Rooting induction was carried out using ½ MS medium with the addition of synthetic auxin, namely IBA or NAA at a low concentration, ie 0.5 mg/l.

The bottles containing the shoots were placed in the culture rack using a TL lamp with an irradiation intensity of 1000 lx for 16 h a day. The experimental design used was a completely randomized design with 10 replications. Observations were made on the number of roots and visual roots.

2.3 Research Design and Data Analysis

The experiment was arranged using a completely randomized design (CRD) with two factors, namely the sugarcane mutant genotype and the media formulation. The treatment was repeated three times, one vial per repetition, and each replicate (bottle) consisted of three explants.

Observations were made when the explants were five weeks old after planting (5 WAP). The variables observed were: 1) the number of shoots per explant (the number of new shoots produced by the explant); and 2) shoot height (measured from the growing point to the tip of the longest leaf), 3) number of roots. Analysis of variance (ANOVA) was carried out to see whether or not there was a treatment effect on the observed variables and whether there was an interaction effect between factors. If the influence of a single factor is significant, then it is continued with the average difference test using the least significant difference test (LSD) at the 5% level. Observational data were tested with SAS software.

3 Results and Discussion

3.1 Plant Height and Shoots

The results of the statistical analysis showed that there were significant differences in the interactions between the mutant clones and the media used on the parameters of plant height and number of shoots (Tables 1 and 2). Table 1 show that the highest shoots were produced from K6 clones on MD3 media which were not significantly different from K5 clones on MD2 media, K5 clones on MD4 media and K6 clones on MD4 media, while the shortest shoots were obtained from K7 clones on MD4 media. It can be seen that clones K5 and K6 had the best plant height in all media formulations compared to the other clones. On the other hand clone K7 had the slowest elongated growth in all the media used. MD3 media is the medium that most supports plant elongation compared to

Table 1. Effect of mutant genotypes and growth media on shoot height

	Growth Media			
Mutant Clone	MD1	MD2	MD3	MD4
K1	2,07 bcdef	1,50 ghijk	2,10 bcdef	1,90 cdefghi
K2	1,43 hijkl	1,23 klmn	1,50 ghijk	1,80 defghij
K3	1,33 jklm	1,37 ijklm	2,0 bcdefg	1,80 defghij
K4	2,00 bcdefg	1,63 fghijk	2,33 abcd	1,93 cdefgh
K5	2,20 abcde	2,50 ab	2,43 abc	2,50 ab
K6	2,27 abcde	2,00 bcdefg	2,70 a	2,40 abc
K7	1,50 ghijk	0,93 lmn	0,87 mn	0,80 n
K8	1,40 hijklm	1,27 jklmn	1,77 efghijk	1,27 jklmn
K9	1,40 hijklm	1,27 jklmn	2,00 bcdefg	2,00 bcdefg
K10	2,27 abcde	2,23 abcde	2,00 bcdefg	1,90 cdefghi

other media, this shows that the use of BA at a concentration of 1 mg/l combined with Thidiazuron 0.5 mg/l is the best plant growth regulator combination to support plant height growth. However, if the BA concentration is increased to 3 mg/l, plant height decreased.

Means within a column having the same letter are not statistically significant (p = 0,05) according to Duncan's Multiple Range Test.

MD 1 = MS + BA 1 mg/l + sucrose 3%
MD 2 = MS + BA 3 mg/l + sucrose 3%
MD 3 = MS + BA 1 mg/l + Thidiazuron 0,05 mg/l + sucrose 3%
MD 4 = MS + BA 3 mg/l + Thidiazuron 0,05 mg/l + sucrose 3%

The composition of hormones in the growth media plays crucial part. For shoot development BAP and Kinetin play vital role [2].

The media formulation used in in vitro culture must support plant growth in order to obtain a large number of seedlings/planlets with good quality. The growth regulator used will greatly determine the success of plant micropropagation in in vitro culture. Statistical analysis showed that there was a significant interaction between the mutant clones and the growth media (Table 2). The highest number of shoots, namely 8.67, were produced by K1 clones on MD2 media, followed by K2 clones on MD2 media, K10 and K9 on MD4 media, whereas the lowest number of shoots were produced from K1 clones on MD3 media, K3 on MD4 media, K6 on MD2 media, and K7 on MD4 media. According to [10], sugarcane propagation uses conventional methods can produce 8–12 shoots per 6 months. The results of this shoot count indicate that propagation of sugarcane seedlings through tissue culture produces more shoots than conventional propagation.

Table 2. The effect of mutant genotypes and media on the number of shoots

Mutant clones	Media			
	MD1	MD2	MD3	MD4
K1	4,33 ghij	8,67 a	3,33 j	5,33 defghij
K2	4,67 fghij	8,33 ab	4,17 hij	6,87 abcde
K3	3,67 ij	5,33 defghij	6,00 cdefgh	3,33 j
K4	4,67 fghij	4,00 hij	5,33 defghij	7,00 abcde
K5	5,33 defghij	5,67 defghi	6,00 cdefgh	7,00 abcde
K6	4,67 fghij	3,33 j	4,00 hij	6,67 abcdef
K7	6,00 cdefgh	4,33 ghij	5,00 efghij	3,33 j
K8	7,00 abcde	6,67 abcdef	7,33 abcd	4,33 ghij
K9	4,00 hij	5,67 defghi	4,00 hij	8,0 abc
K10	5,33 defghij	6,33 bcdefg	7,00 abcde	8,33 ab

Means within a column having the same letter are not statistically significant (p = 0,05) according to Duncan's Multiple Range Test.

MD 1 = MS + BA 1 mg/l + sucrose 3%
MD 2 = MS + BA 3 mg/l + sucrose 3%
MD 3 = MS + BA 1 mg/l + Thidiazuron 0,05 mg/l + sucrose 3%
MD 4 = MS + BA 3 mg/l + Thidiazuron 0,05 mg/l + sucrose 3%

Clones K10 and K8 had the best shoot multiplication ability compared to the other clones, whereas K3 and K6 had the lowest shoot multiplication ability (Table 2). In general it was seen that MD4 media was the best medium for shoot multiplication (Fig. 1), this indicated that BA combined with thidiazuron could induce cell division to form new shoots, except for clones K7 and K8, where the use of MD4 media reduced the number of shoots produced.

Benzyl adenine (BA) and thidiazuron are synthetic cytokinins that are often used for plant propagation in in vitro culture. BA plays an active role in the formation and multiplication of shoots, while thidiazuron functions in stimulating the production of endogenous cytokinins and inhibiting the formation of cytokinin oxidase. BA and TDZ are two groups of cytokinins which do not work alone but interact with each other in directing the growth and development of explants. The use of 2,4 D and thidiazuron can induce embryogenesis in coffee plants [11]. In sugarcane lower NAA concentration in combination with BAP highly induced shoot regeneration [12].

The research results obtained showed that each clone had a different response to the same media formulation. This condition indicates that each mutant clone has changed its genetic characteristics even though they come from the same genetic background. Changes in genetic properties that occur can be caused by treatments given during culture, including gamma irradiation which causes changes in the DNA structure so that

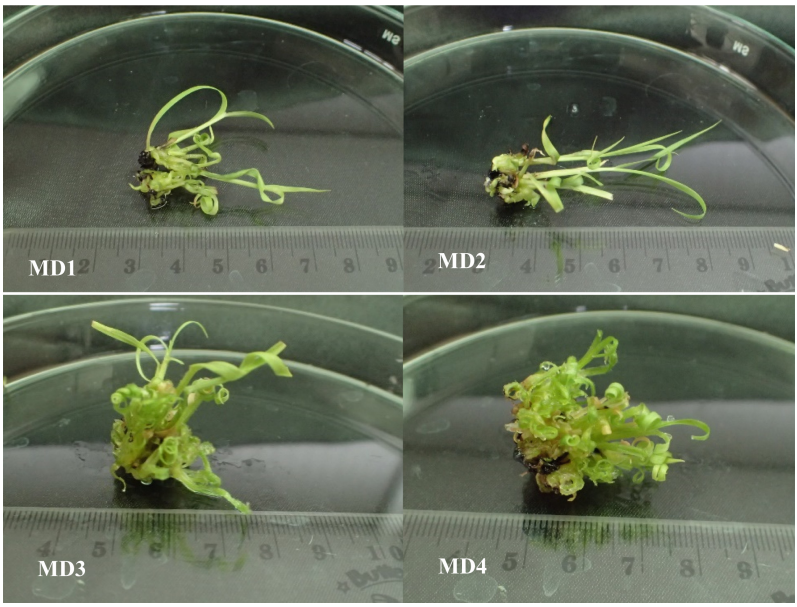

Fig. 1. Shoot multiplication of K2 mutans on MD1, MD2, MD3, and MD4 media. MD1 = MS + BA 1 mg/l + Sucrose 3%, MD2 = MS + BA 3 mg/l + Sucrose 3%, MD3 = MS + BA 1 mg/l + Thidiazuron 0,05 mg/l + Sucrose 3%, MD4 = MS + BA 3 mg/l + Thidiazuron 0,05 mg/l + Sucrose 3%

different gene expression is formed. The results obtained are in line with the statement of [13] that genotype affects the growth and development of plant species.

3.2 Root Induction

Rooting is one of the stages in plant propagation that determines the success of transferring plantlets from tissue culture to the greenhouse, this process is called acclimatization. Roots function as organs that absorb water and nutrients from the soil into plants, therefore good plant roots are needed to support plant growth in greenhouses and in the field.

The results of the statistical analysis showed that there was no interaction between the mutant clones and the media formulation used on the number of roots parameter, but significant differences were seen in each single factor (Fig. 2). The fastest root formation response was obtained from clone K6, which was 12 days after planting (DAP), with the highest number of roots produced, namely 8, which was significantly different from other mutant clones. On the other hand, clone K7 had the slowest root formation, because the number of roots produced was also the highest. a little (Table 3).

The use of auxin NAA growth regulator was more effective in inducing the formation of sugarcane roots (Table 4). The average number of roots formed on media with the addition of 0.5 mg/l NAA was 6.60, significantly different from the roots produced on media containing 0.5 mg/l IBA (Table 4). The results obtained are in line with the research of [14] where the addition of 2.5 mg/l NAA to MS media was able to stimulate

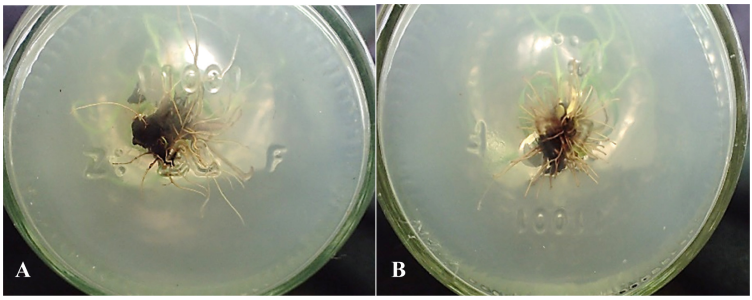

Fig. 2. Root performance of K5 mutant clones on added media IBA 0.5 mg/l (A) and NAA 0.5 mg/l (B)

root formation. While [15] observed that media supplemented by NAA 2 mg /l NAA produced faster and better rooting in all of the sugarcane genotypes tested. Best rooting of sugarcane generated from media containing NAA with various concentrations ranging from 0.2 mg/l to 2 mg/l [15–18].

Table 3. Time of root initiation and number of roots of ten sugarcane mutant clones

Mutant Clone	Root Initiation (HST)	Number of Root
K1	15	5,67 bc
K2	12	6,83 ab
K3	18	4,50 bc
K4	20	4,17 c
K5	15	5,50 bc
K6	12	8,00 a
K7	20	4,00 c
K8	18	4,17 c
K9	15	5,17 bc
K10	16	4,67 bc

Means within a column having the same letter are not statistically significant (p = 0,05) according to Duncan's Multiple Range Test.

Table 4. Number of roots in rooting induction media

Media	Number of Root	Root Visuals
1/2 MS IBA 0.5 mg/l	3,93 b	thin
1/2 MS NAA 0,5 mg/l	6,60 a	thick

4 Conclusion

The interaction between the mutant genotype and the media formulation had a significant effect on shoot height and number of shoots. The best multiplication medium for shoots of sugarcane mutants was media containing 3 mg/l BA and 0.5 mg/l thidiazuron. Sugarcane mutant roots grew better on media containing growth regulators NAA than IBA. Mutant clones K10 and K8 had the best shoot multiplication ability compared to other mutant clones.

References

1. Minarsih, H., I. Riyadi, Sumaryono, A. Budiani.: Mikropropagasi Tebu (*Saccharum officinarum.* L) Menggunakan Sistem Perendaman Sesaat. Jurnal Menara Perkebunan. 81 (1): 1-8. (2013)
2. Qazi, A., G.S. Nizamani, M.T. Khan, S. Yasmeen, S.K. Baloch, M. Ali, I.A. Khan, S. Ahmad, M.R. Nizamani and M.A. Siddiqui.: In-vitro management of phytohormones for micropropagation of sugarcane. Pakistan Journal of Agricultural Research, 33(1): 180-191. (2019)
3. Lal, N.: Micropropagated Plants as Alternative Planting Material to Sugarcane Setts. Indian J Biol; 8(1):27-30. (2021)
4. Mazri, M.A.: Effect of basal medium, explant size, and density on the in vitro proliferation and growth of date palm (*Phoenix dactilifera* L.) Cultivar "16 biss". Not. Sci. Biol. 5(3): 323-327. (2013)
5. Fiah, R.L., Taryono dan Toekijo.: Kemampuan regenerasi kalus empat klon tebu (*Saccharum officinarum* L.). Vegetalika. 3(1), pp. 91-101. (2014)
6. Yadav, S, T.E. Nagaraja, H.C. Lohithaswa, K.V. Shivakumar, Poonam Kumari Yadav, Poonam Yadav, Ganpat Louhar and Jagdish Yadav.: Genetic Response of Sugarcane (*Saccharum officinarum* L.) Genotypes to Varying Concentrations of Cytokinins for in vitro Shoot Multiplication. Int.J.Curr.Microbiol.App.Sci. 8(02): 1080-1088. (2019)
7. Azizi, A.A.A, I. Roostika, D. Efendi.: Multiplikasi tunas in vitro berdasarkan jenis eksplan pada enam genotipe tebu (*Saccharum officinarum* L.). Jurnal Littri. 23 (2): 90-97. (2017)
8. Praseptiana, C., S. Darmanti, E. Prihastanti.: Multiplikasi tunas tebu (*Saccharum officinarum* L Var. Bululawang) dengan perlakuan konsentrasi BAP dan Kinetin secara n vitro. Buletin Anatomi dan Fisiologi. 2 (2): 153-160. (2017).
9. Suhesti, S. Nurul, Khumaida, G.A. Wattimena, M. Syukur, A. Husni, E. Hadipoentyanti, R.R.S. Hartati. Induksi kalus dan regenerasi dua varietas tebu (*Saccharum officinarum L.)* secara in vitro. Jurnal Littri. 21 (2): 77-88. (2015)
10. Dyah, R.P, dan Parnidi.: Pengadaan benih tebu bermutu. *dalam* Bunga Rampai "Peningkatan Produktivitas Tebu untuk mempercepat Swasembaada Gula". Malang. Balit Tanaman Serat.: 33-54. (2015).
11. Ibrahima, M., R. Hartati, Rubiyoa, A. Purwito and Sudarsono.: Direct and indirect somatic embryogenesis on Arabica coffee (*Coffea arabica*). Indones. J. Agric. Sci. 14 (2): 79-86. (2013).
12. Ullah, M. and M.S. Khan.: Effects of carbon sources and growth regulators on the tissue culture of sugarcane. Sarhad Journal of Agriculture, 38(1): 312-321. (2022).
13. Ali S, Iqbal J and Khan M S.: Genotype independent in vitro regeneration system in elite varieties of sugarcane. Pakistan J. Bot. 426 3783-90.(2010)

14. Dinesh P, Thirunavukkarasu P, Saraniya A R and Ramanathan T.: In Vitro Studies of Sugarcane Variety Co-91017 Through Micropopagation of Shoot Tip Culture. Adv Plants Agric Res 26:263–68.(2015).
15. da Silva, J.A., Solis-Gracia, N., Jifon, J. et al.: Use of bioreactors for large-scale multiplication of sugarcane (*Saccharum* spp.), energy cane (*Saccharum* spp.), and related species. In Vitro Cell.Dev.Biol.-Plant 56, 366-376 (2020).
16. AL-Arady, H. J., Jasim, A. M., & Abbas, M. F.: Micropropagation of sugarcane *Saccharum officinarum* Plant via Organogensis callus in vitro. Iraqi Journal of Aquaculture, 17(2), 91-101. (2020)
17. Salokhe, S.: Development of an Efficient Protocol for Tissue Culture of Sugarcane. Plant Cell Biotechnology and Molecular Biology, 22(27-28), 9-21. (2021)
18. Iqbal, A., Khan, R.S., Khan, M.A. et al.: Efficient Regeneration in Sugarcane Using Thin Cell Layer (TCL) Culture System. Sugar Tech 25, 168-176 (2023).