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Analysis of Total DNA Quality and Quantity in Commercial Varieties of Sugarcane (*Saccharum officinarum* L.) at PT Gunung Madu Plantations

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Background: Sugarcane (*Saccharum officinarum* L.) is an agricultural commodity that plays a role in the production of granulated sugar, has economic value, and is a source of state income. There is a decline in sugarcane productivity due to the low number of superior sugarcane seeds. One way is to provide molecular markers for kinship studies. Kinship can be identified based on the similarity of characters due to genetic composition. **Objective:** This research aimed to analyze the quality and quantity of total DNA in commercial varieties at PT Gunung Madu Plantations (GMP). **Methods:** This research was conducted at the PCR Laboratory in June-August 2023. The plant material used was leaves from 12 commercial varieties that were 29 days old. Sugarcane leaf samples were isolated and extracted using the CTAB method. The DNA quality test used 1% agarose and the quantity test was carried out using a µDrop spectrophotometer at an absorption ratio of 260/280. **Result:** The research showed that DNA fragments had the thickest and brightest color in samples 2, 6, 8, 9, and 13. The DNA purity of the samples ranged from 1.80-1.93 with a concentration between 394-1400 µg/ µL. The lowest DNA concentration was in the GM 869 and the highest was in the GM 1834 variety. Both of DNA quantity and quality show that GM 1686, GM 1834, and GM 108 have high concentrations with thick and bright bands **Conclusion:** From the above findings, it can be concluded that GM 1686, GM 1834, and GM 108 varieties have high purity (1.87-1.93) and concentrations (1,332-1,429ng/ µL) with thick and bright bands.

Keywords: sugarcane, DNA quality analysis, DNA quantity analysis

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is listed as an agricultural commodity that contributes 75% of the production of granulated sugar and has economic value and is a source of income for the country (Dlamini, 2021). However, sugarcane cultivation is currently experiencing fluctuations, with 2022 showing a decline in productivity compared to 2019 and 2020 (Central Statistics Agency, 2021; Putra et al., 2024).

The decline in sugarcane productivity is caused by several aspects, including a reduction in sugarcane area, plant pest organisms, or the absence of superior seeds for drought-stress tolerant sugarcane cultivars (Meiriani et al., 2019). PT Gunung Madu Plantations (GMP) is a sugar industry that strives to increase sugar production by producing superior varieties (Windiyani et al., 2022; Mahfut et al., 2023). Efforts have been made to create drought-stress tolerant varieties, namely sugarcane breeding with molecular markers through DNA isolation (Mahfut et al., 2024a).

The first step of identifying molecular markers is DNA extraction by lysis cells, separating DNA, and precipitation (Mahfut et al., 2024b). These three stages aim to separate DNA from other components to obtain the highest DNA purity. DNA testing is carried out using quantity and quality tests. The quantity test uses a spectrophotometer calculated by comparing A260 nm and A280 nm. Quality test using agarose gel electrophoresis. This research aimed to analyze total DNA quantity and quality in commercial sugarcane varieties at PT GMP.

METHODS

Plant Materials

The materials used were 12 samples of commercial varieties sugarcane leaves aged 27 daysliquid nitrogen, cetyl trimethylammonium bromide (CTAB) buffer 3%, 2β-mercaptoethanol, proteinase-K, chloroform, isoamyl alcohol, sodium acetate, isopropanol, ethanol 70%, Tris-EDTA (TE) buffer, agarose, 1x Tris-Borate EDTA (TBE) buffer, gel red staining, loading dye, 1 kB marker, and distilled water.

DNA Isolation

The work procedure for implementing DNA isolation begins with taking samples of sugarcane leaves that are 27 days old. The sample was cleaned, weighed at 0.07 grams, cut into small pieces, and placed in a 2 mL centrifuge tube. Add sterile pellet and liquid nitrogen then grind with a tissue lyser for 3 x 60 seconds, ground with 800 µL/samplesof CTAB extraction buffer, then simultaneously added 10 µL 2β-mercaptoethanol, and 5 µL proteinase-K. The sample was homogenized using a vortex, then placed in a waterbath at 65^{o}C for 30 minutes instead of standard, and every 10 minutes it was slowly inverted. Next, it was centrifuged within 10 minutes, at 14,000 rpm, and a temperature of 25^{o}C. A stock solution of chloroform isoamyl alcohol (CI) was added to the tube in the amount of 1x the volume of the sample obtained. The sample was centrifuged at 4^{o}C for 5 minutes at 14,000 rpm, then the supernatant was separated and repeated twice. The sample was placed in the refrigerator for ± 30 minutes at a temperature of -20 ^{o}C. Added sodium

acetate and isopropanol then centrifuged for 5 minutes, temperature 4 ^{o}C, and 14,000 rpm to form a DNA pellet. The DNA pellet was cleaned using 500 µL of absolute alcohol, centrifuged for 5 minutes, temperature 4^{o}C, and speed 14,000 rpm which was carried out twice. The pellet drying process can be done by placing the tube upside down on a tissue for 24 hours at room temperature. Besides, can use a dry bath for 4 x 10 minutes at a temperature of 50^{o}C. DNA was dissolved using 40 µL of TE buffer with pH 8.0, for further analysis.

Quantitative and Qualitative DNA Analysis

The DNA yield was measured by using NanoDrop spectrophotometer at 260nm. DNA purity was determined by calculating the absorbance ratio A260/280. Polysaccharide contamination was assessed by calculating the absorbance ratio A260/230. For quality and yield assessments, electrophoresis was done for twelve DNA samples in 1% agarose gel. The working principle of the NanoDrop spectrophotometer is that pure DNA can absorb ultraviolet light due to the presence of purine and pyrimidine bases (Mollah et al., 2022).

DNA with a purity of ≥ 1.8 was subjected to qualitative testing using 1% agarose and 10 µL red staining gel. The agarose *well* is filled with 1 µL sample DNA, 1 µL loading dye, and 4 µL distilled water. The marker used is 1 kb. Electrophoresis was carried out at a voltage of 60 V for 105 minutes.

RESULTS AND DISCUSSION

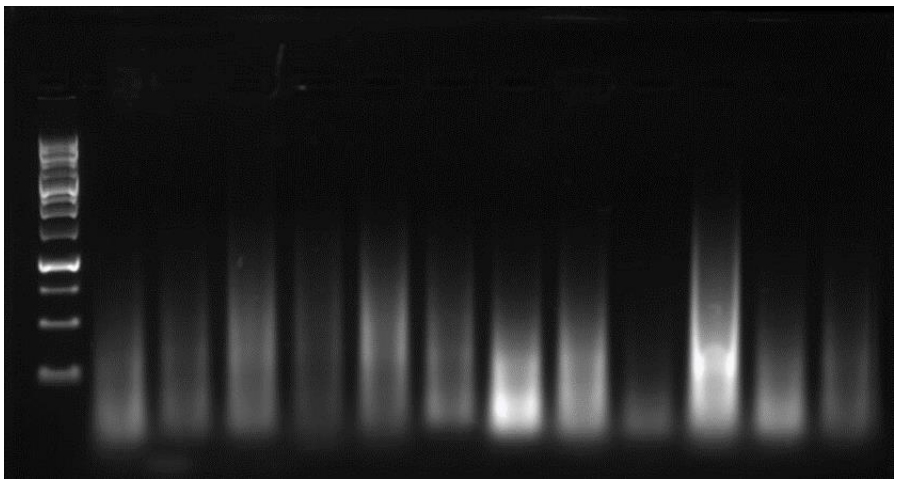
DNA isolation from commercial sugar cane plants in this study used the CTAB method. The samples used were young leaves because they have a soft texture and contain little fiber, so they are easy to grind to obtain DNA purity (Hikmatyar et al., 2015).

DNA quantity testing is carried out to determine the purity and concentration of DNA. DNA purity is calculated by comparing A_{260} and A_{280} and shows a value of 1.8-2.0. DNA purity <1.8 indicates protein and UV contamination, while DNA purity >2.0 indicates chloroform and phenol contamination (Aristya et al., 2020). The results of the DNA quantity test are shown in **Table 1** as follows.

Table 1. DNA Quantity Test with the NanoDrop Spectrophotometer

No	Variety	Purity (A260/A280)	DNA Consentration (ng/µL)
1	GMP 7	1.83	515.6
2	GP 11	1.84	655.7
3	SS 57	1.84	790.5
4	GM 047	1.84	753.2
5	GM 612	1.81	974.6
6	GM 1206	1.91	1222
7	GM 1686	1.87	1332.4
8	GM 1834	1.93	1429.6
9	GM 869	1.82	394.1
10	GM 108	1.88	1408.4
11	GM 253	1.82	648
12	GM 654	1.85	742.9

DNA purity in this study ranged from 1.8-1.9 with concentrations ranging from 394-1,400 ng/µL. The purity of extracted DNA was excellent, suggesting that the preparations were sufficiently free of proteins and polyphenolics/ polysaccharide compounds. The DNA with the lowest concentration was shown in the GM 1834 variety. The highest DNA concentrations were respectively in the GM 1686, GM 1834, and GM 108 varieties. One of the factors that influenced the low level of DNA purity was the sample grinding method. If the method of grinding the sample is not too strong, the other components will not be destroyed so soluble proteins and carbohydrates can still be found. Another factor that influences the low level of DNA purity is the way the DNA sample is taken in the tube (Iskandar et al., 2014). A DNA concentration that is too low will produce fragments that are very thin on the gel or even invisible visually, whereas a DNA concentration that is too high will cause the fragments to appear thick, making it difficult to differentiate between one fragment and another (Mahfut, 2020). Next, it was tested qualitatively using electrophoresis with 1% agarose gel as presented in Figure 1.



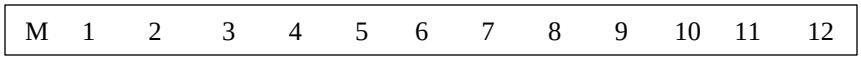


Figure 1. Qualitative DNA testing using electrophoresis; M= Marker (1 Kb); 1 = GMP 7; 2= GP 11; 3= SS 57; 4= GM 047; 5=GM 612; 6=GM 1206; 7= GM 1686; 8=GM 1834; 9=GM 869; 10=GM 108; 11=GM 253; 12=GM 654

The results of the DNA quality test showed that the GM 1686, GM 1834, and GM 108 varieties showed thick and bright bands. This is in accordance with the quantity test that has been carried out. High concentrations are indicated by thick DNA bands that collect or do not spread out. The presence of bonds between DNA molecules that are broken during the extraction process will show the shape of DNA bands that spread out (Roslim et al., 2020). This causes the DNA genome to be cut into parts and at a smaller size. This is caused by excessive physical movement during the DNA isolation process and can also be caused by the activity of the chemicals used (Yang *et al.,* 2021).

We proposed CTAB methods with slight modification for rapid and high yields of DNA for SNPs methods. Single Nucleotide Polymorphism (SNP) is a genomic variant at a single base position in DNA or a single letter where the genome varies from other genomes, in most cases by two alleles or a difference of one nucleotide base. These SNPs cause changes in amino acids in conserved protein areas, which are located in non-coding areas of the genome and synonymous SNP (sSNP) in coding areas which have no phenotypic impact but can provide markers that are useful for population genetic studies (Kurniawati et al., 2020). Moreover, this method is comparable to any other conventional method for isolation of DNA in terms of their speed because it requires around 5 hours up to the final DNA re-suspension in the fresh sample.

CONCLUSION

GM 1686, GM 1834, and GM 108 varieties have high purity (1.87-1.93) and concentration (1,332-1,429ng/ µL) with thick and bright bands.

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