Colchicine induced morphological variability in sugarcane

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Abstract. Mutation breeding is one of the tools in genetic improvement. The existence of morphological differences among the mutants and the original indicates genetic changes in putative mutants. The research aimed to determine morphological characters of sugarcane colchicine-induced putative mutants. The materials were 21 numbers of putative mutants derived from the PS 881 variety as a parent. Morphological traits were observed on a seven-month-old plantation of sugarcane mutants in January 2019 at the UPBUP screen houses. The descriptor list consisted of 34 morphological characters, including leaves, sheath, stalks, internodes, and buds. The results showed that putative mutants have a narrow diversity and separated into two large clusters with a dissimilarity level of 0.25 with PS 881. Cluster I consisted of 11 putative mutants (38B, 14A, 33A, 18A, 4B, 31A, 52A, 4A, 3A, 30A, and 6A) and cluster II consisted of 10 putative mutants (54A, 38A, 37A, 36B, 60A, 49A, 27A, 22B, 17A, and 9A). Several mutants have leaves with darker green color, larger stalk diameter, and longer internode than their parents. Further evaluation needs to be done to identify mutants with high production potential.

1. Introduction
Sugarcane breeding through mutation induction using colchicine has produced several putative mutants from varieties of origin PS 881 [1]. The use of mutation techniques with induction using colchicine is known to increase the genetic diversity of plants. This technique is mostly used to create new diversity with specific traits. Some morphological characters can indicate for mutants resulting from colchicine induction before analysis of the number of chromosomes in the moon orchid plant [2]. Observed morphological character studied its genetic distance. The coefficient of inaccuracy between putative mutants indicates a difference between mutants caused by differences in the observed character response. The higher the value of incompatibility between characters, the other kinship between characters. This increase in genetic diversity is the principal capital for plant breeders, especially sugarcane, to obtain new superior varieties according to their desired properties. The greater the changes, the more genetic diversity there is. High genetic diversity in germplasm will provide a high chance for genetic improvement of plants [3]. New traits formed by mutation induction are very diverse because mutations are random.

Changes in morphological character occurred in research [4] observed in the appearance of the greener orchid leaf color than the control. Identification becomes a critical stage that includes determining or tracing the origin of varieties differentiation [5], by looking at their morphological equations, testing the purity and characterization of varieties to classify a collection to be included in the germplasm collection [6]. The collection of germplasm is very important, it means the
characterization of morphology as the initial information needed in the search for superior character and diversity [7]. Therefore, selection is required to obtain new mutants according to the desired breeding purposes. To determine the genetic diversity due to induction treatment with colchicine, the identification of putative mutant sugarcane based on the morphological character in putative mutant sugarcane resulting from induction of colchicine mutation that has significantly increased genome size. This study aims to determine the diversity of morphological character of some putative mutant sugarcane resulting from the induction of chemical mutations with colchicine.

2. Materials and methods

The research was conducted in January 2019 (on a seven-month-old sugarcane mutant) at the Agricultural Superior Seed Management Unit (UPBUP) screen house, Indonesian Agency for Agricultural Research and Development. The observation materials used are the PS 881 cane variety and 21 putative cane mutants with a genome size that differs markedly from the original variety. The plants still in the early generation of tissue culture (M1V4). The putative mutants were 17A, 30A, 18A, 52A, 22B, 54A, 49A, 60A, 27A from colchicine 0.03 %, and 9A, 6A, 36B, 38A, 31A, 33A, 14A, 38B, 3A, 4B, 37A, 4A from colchicine 0.05 % that derived from previous research [1]. The observed putative mutant cane was seven months old after planting because it was stable [8] and compared to the variety of origin of PS 881.

Observations were made on 34 morphological characters using the modified Lamadji description [9], including six morphological marker characters on the leaf, nine characters on the sheath leaf, five characters on the internode, six characters on the stalk, and eight characters on the bud. Qualitative data analysis is done descriptively. Calculation of genetic distance between putative mutants done by grouping the hierarchy of similarities using the gower method, and clustering done by agglomerative method UPGMA (Unweighted Pair Group Method with Arithmetic Mean) with PBSTAT-CL 2.1 program.

3. Result and discussion

The results showed that morphological diversity was observed among putative mutants of sugarcane originated from variety PS 881 in some characters. These characters were as followed:

3.1. Leaf character

Observations of the leaves characters showed that there was a diversity or change on some characters of the cane putative mutant leaves to the original variety PS 881. The changes are found in the curved character of leaf blade erectness, the height of the auricle, the position of the auricle, leaf color, the color of dewlap, and leaf width (Table 1). All putative mutants and non-mutant PS 881 have their leaves blade erectness by less than half of the peak leaves, except for 30A mutants whose leaf blade erectness is equal to or greater than half the leaf strands of the peak. All putative mutants and PS 881 have auricle height three times shorter than the width. Except for mutant 9A whose auricle height is more than three times the width.

The position of the auricles of all putative mutants straight, changed from the original variety PS 881, whose position of the auricles is oblique. This is in accordance with the research [10], which states that the morphological marker character of auricles contributes significantly to the degree of similarity [11]. The color character is purple on dewlap for all putative mutants and PS 881, except mutant 60A whose dewlap is green. PS881 as the original variety has a dark green leaf color. Some mutants change their leaf colors to the green colors, which are 9A, 17A, 6A, 36B, 30A, 38A, 18A, 54A, 49A, 3A, 37A, and 4A. Some mutants turn to yellowish-green discoloration, there are 33A, 38B, and 4B. The width of the leaves in the original variety PS 881 is relatively narrow (<4 cm). Some putative cane mutants resulted from colchicine-induced mutations, have wider leaves (4-5 cm), namely 6A, 36B, 18A, 52A, 22B, 54A, 37A, 4A, and 27A.
Table 1. Morphological diversity based on leaf character for 21 putative mutants and non-mutant

| Morphological character | PS 881 | Putative mutants |
|-------------------------|--------|------------------|
|                         | 9A     | 17A  | 6A  | 36B | 30A | 38A | 18A | 52A | 31A | 33A | 22B | 54A | 14A | 38B | 49A | 3A | 4B | 37A | 4A | 60A | 27A |
| Leaf-blade erectness    |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| - < ½ from top          | √      | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    |
| - ≥ ½ from top          |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Auricle height          |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| - <3x long              | √      | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    |
| - ≥3x long              |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Auricle position        |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| - straight              | √      | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    |
| - oblique               |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Leaf color              |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| - dark green            | √      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| - green                 | √      | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    |
| - yellowish-green       |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Dewlap color            |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| - purple                | √      | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    |
| - green                 |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Leaf width              |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| - narrow                | √      | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    | √    |
| - middle                |        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

3.2. **Leaf sheath character**

Observations of the leaf sheath character showed that all putative mutants and PS 881 as original variety have hairs on the leaf sheath (Table 2). Varieties PS 881 and other putative mutants have the leaf sheath hairs area less than a quarter of the hairs width (narrower), except for putative mutants 9A, 17A, 6A, 36A, 22B, 54A, 38B, 49A, and 60A. All putative mutants and PS 881 have the same peak distance from the point of the hairiness area, except for the putative mutant 14A. The hair length character in PS 881 and the putative mutants is shorter than 2 mm, except the putative mutants 9A, 17A, 36B, 22B, 49A, 60A, and 27A, which are longer than 2 mm.

On PS 881 and seven putative mutants have an unciform hairiness position character, the other two putative mutants are straight, and 12 other putative mutants position their backfield feathers are dentoid. The density of the hairiness in 10 putative mutants and the origin varieties are categorized as rare (<75/cm²). While the other 11 putative mutants have a dense backfield (≥75/cm²). The hairiness character of the leaf sheath is present in all putative mutants and the PS 881 variety. The origin varieties (PS 881) and other putative mutants have an easily peel off the dry leaf sheath. Putative mutants 9A, 17A, 6A, 52A, 3A, and 4A have the middle feeling to peel off the dry leaf sheath. This sheath protects the stalk from high transpiration. The wax layer of the leaf sheath is slight for varieties PS 881 and 12 putative mutants, while the nine putative mutants have a medium wax coating on the leaf sheath. The leaf sheath color is almost entirely green, except for 38A, 52A, 22B, 49A, and 27A, which have purplish-green leaf sheath colors.
Table 2. Morphological diversity based on leaf sheath character for 21 putative mutants and non-mutant

| Morphological character | PS 881 | 9A | 17A | 6A | 36B | 30A | 38A | 18A | 32A | 31A | 33A | 22B | 54A | 14A | 38B | 49A | 3A | 4B | 37A | 4A | 60A | 27A |
|-------------------------|--------|----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|-----|----|-----|-----|
| Hairs height            |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - <¼ length             | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - ≥¼ length             | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| From point              |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - <1 cm                 | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| - ≥1 cm                 |         | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ |
| Length                  |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - <2 mm                 | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| - ≥2 mm                 |         | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ |
| Position                |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - straight              | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| - dentoid               |         | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ |
| - unciniform            | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| Density                |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - rare                  | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| (≥75/cm²)              |         | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ |
| - dense                 | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| Hairiness              |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - present              | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| Peel-off dry sheath     |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - middle               | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| - easy                 |         | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ |
| Waxiness               |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - few                  | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| - many                 |         | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ | ✓ ✓ |
| Colour                |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - purple green         | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |
| - green               | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ | ✓ ✓ ✓ ✓ |

3.3. Stalk character
Observations of the stem character show that the primordial character of the upper root of the bud peak does not change, as does the character category of the trunk diameter, and the character of the trunk crack (Table 3). The original variety PS 881 and all mutants do not have primordia roots over the top of the bud, the diameter of the stem is relatively tiny (<2.5 cm) and has no stem cracks. Characters that change in mutants compared to their original variety are in the character of the number of root primordia. Varieties PS 881 and most putative mutants have several root primordia on the stems of less than two rows, while putative mutants 54A and 4A have a two-to three-line number of root primordia.
Table 3. Morphological diversity based on stalk character for 21 putative mutants and non-mutant

| Morphological character | Putative mutants |
|-------------------------|------------------|
|                         | PS 881 | 9A | 17A | 6A | 36B | 30A | 38A | 18A | 52A | 31A | 33A | 22B | 64A | 14A | 38B | 49A | 3A | 4B | 37A | 4A | 60A | 27A |
| Number of rows of root primordia |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - <2 rows                | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| - 2-3 rows               | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| - >3 rows                | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| Root primordia on bud tip |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - absent                 | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| - present                | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| Colour                   |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - green                  | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| - yellowish-green        |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - greenish-yellow        |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - purple green           | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| Diameter                 |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - 2.5-3 cm               | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| - <2.5 cm                | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| Waxiness                 |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - thin                   | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| - middle                 |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - thick                  | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |
| Stalk cracks             |       |   |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - absent                 | √     | √ | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   |

Some putative mutants undergo stem discoloration from the purplish-green varieties of PS 881 to green (31A) and yellowish-green (6A, 36B, 4B, 37A, and 4A). There are variations in the character changes of medium stem wax layers on putative mutants 6A, 36B, 30A, 18A, 52A, 31A, 33A, 14A, 38B, 3A, 4A, and 60A, as well as thin stem wax layers on putative mutants 4B and 27A. Varieties PS 881 and putative mutants 9A, 17A, 38A, 22B, 54A, 49A, and 37A have thick layers of stem wax.

3.4. Internode character

The results of observations on the trunk segment character showed that the cross-sectional character of the trunk segment did not change. Varieties of origin PS 881 and all mutants have a round cross-section (Table 4). Characters that have been changed on putative mutants compared to their original variety are in the character of the arrangement of segments, the shape of the segment, the length of the segment, and the groove of the bud. PS 881 variety has a straight segment arrangement, the shape of the kelos segment, the length of the segment is relatively short (<13 cm), and the bud grooves are on some segments.
Table 4. Morphological diversity based on internode character for 21 putative mutants and non-mutant

| Morphological character | Putative mutants |
|-------------------------|------------------|
|                         | PS 881 | 9A | 17A | 6A | 36B | 30A | 38A | 18A | 52A | 31A | 33A | 22B | 54A | 14A | 38B | 49A | 3A | 4B | 37A | 4A | 60A | 27A |
| Alignment               |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - straight              | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - zigzag                |        | ✓  |     | ✓  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cross-section shape     |        | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - round                 | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - flat                  |        | ✓  |     | ✓  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Internode shape         |        | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - cylindrical           | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - konis                |        | ✓  |     | ✓  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| - curved               | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - barrel               |        | ✓  |     | ✓  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Length                 |        | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - >15 cm               | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - 13-15 cm             | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - <13 cm               | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| Bud groove             |        | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - absent               | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - half present         | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |
| - all bud              | ✓      | ✓  | ✓   | ✓  | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   | ✓   |

Some putative mutants have morphological change of alignment, which are in the zigzag arrangements at 9A, 18A, 31A, 38A, and 4B. The cylindrical segment forms of all putative mutants change, except for 33A, 49A, and 60A, which are in the form of loss. The length of the segment is classified as long (> 15 cm) in the putative mutant 49A and is classified as medium size (13-15 cm) at 36B and 37A. Bud grooves also change in some putative mutants. Some putative mutants do not have bud grooves i.e., mutants 6A, 30A, 38A, 52A, 33A, 54A, 14A, and 49A, while putative mutants 18A, 22B, 38B have bud grooves on all segments.

3.5. Bud character

Observations on the diversity of bud characters in 21 cane putative mutants and varieties of origin of PS 881 can be seen in Table 5. Observations of the bud character showed that the character of the base position of the wing of the bud in the origin variety PS 881 and the putative mutant does not change, which is above the middle of the bud. Similarly, the character of the wing size of the bud is equally broad, the presence of basal edge hair of the bud, the position of the bud position on the former lead smelter, and the shape of the buds are round (Table 5). Characters that change in putative mutants compared to their original variety are in the character of the edge shape of the wings of the buds. The PS 881 variety and most putative mutants have a flat bud wing edge shape, while the putative mutants 38A and 18A have a jagged bud wing edge character. Most putative mutants also have a central character growing at a position above the middle of the bud, but in putative mutants, 38A and 54A have a central position growing below or middle of the bud. Furthermore, the PS 881 variety in the character of the bud does not have crested hair, but all the putative mutants have crested hair.
Table 5. Morphological diversity based on bud character for 21 putative mutants and non-mutant

| Morphological character | PS 881 | 9A | 17A | 6A | 36B | 30A | 38A | 18A | 52A | 31A | 33A | 22B | 54A | 14A | 38B | 49A | 3A | 4B | 37A | 4A | 60A | 27A |
|-------------------------|--------|----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|-----|----|-----|-----|
| **Wings top**           |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - middle                | √      | √  | √   | √  | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √   | √  | √  | √   | √  | √   | √   |
| - another               |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| **Wings size**          |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - absent                |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - same broad            |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - narrow base           |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| **Wing shape**          |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - flat                  |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - uneven                |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| **Prominence**          |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - under middle bud      |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - up middle bud         |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| **Base hair**           |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - absent                |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - present               |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| **Hair on top**         |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - absent                |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - present               |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| **Bud base position on leaf scar** |    |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - on tip                |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - above                 |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| **Bud shape**           |        |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - ovate                 |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - oval                  |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |
| - roundish              |         |    |     |    |     |     |     |     |     |     |     |     |     |     |     |     |    |    |     |    |     |     |

3.6. Cluster analysis of morphological characters on sugarcane putative mutants

Cluster analysis of 34 morphological marker characters showed some similarities among 21 putative mutants to the original PS 881 variety. Characters that have similarities include: curved leaves (< 1/2 of the top), the height of the leaf ears (less than three times wide), the color of the triangle of the leaves (purple), the height of the backfield feather area (≥ 1 cm), the entity of the edge field (there), the cross-section of the stem segment (round), the primordia of the upper root of the bud (none), the diameter of the stem (< 2.5 cm), and no cracking of the stem, the position of the base of the bud wing above the middle of the bud, the size of the bud wings are the same width, the position of the buds on the former leaf smelt, and the shape of the rounded buds. A swarm containing genotypes tends to have a high degree of similarity. This means that the genetic distance is the smallest [12]. The results of the grouping analysis based on 34 characters of sugarcane morphological markers in 21 mutant putative sugarcane and varieties of origin PS 881 are presented in Figure 1.

The observed putative mutants are divided into two groups at a coefficient of 0.25. Group I consists of 11 cane putative mutants that bear similarities to the origin varieties PS 881, namely putative mutants 38B, 14A, 33A, 18A, 4B, 31A, 52A, 4A, 3A, 30A, and 6A. Group II consists of 10 sugarcane putative mutants that bear similarities, namely 54A, 38A, 37A, 36B, 60A, 49A, 27A, 22B, 17A, and 9A. The degree of similarity between putative mutants is great. This is thought to be due to the origin of plants still the early generation of tissue culture colchicine mutation induction (M1V4) tested outside the
greenhouse. The phenotype expression displayed is still almost uniform. Genetic diversity is influenced by differences in environmental conditions, ways of reproduction, and natural selection [13]. However, the environment affects the phenotypic variation on qualitative morphological properties [14].

![Dendrogram of morphological characters from 21 putative mutants and parent](image)

In the early generation of 21 cane putative mutants, there was no morphological diversity, in the sense that it is still similar to its original variety, PS 881. These putative mutant morphological similarities are in leaf morphological markers (D1-D6), leaf shedding (P1-P9), segments (R1-R5), stems (B1-B6), and buds (M1-M8). All morphological characters used in this experiment are highly inherited traits [9]. Although it can change due to environmental influences, morphological markers are still used in sugarcane research institutes because morphological characters are stable enough to identify varieties [15]. The results of an analysis of 21 cane putative mutants and varieties of origin of PS 881 showed similar qualitative morphological properties. This may be due to the genetic diversity between mutants is narrow based on morphological characters [10]. Although, the genome sizes are diverse in range for sugarcane mutants that derived from colchicine-induced [16]. The morphological differences described are not too different according to the cluster analysis, but it is hoped that there will be a character that changes and has a major effect on the yield components that support the direction of production. Because the mutation using colchicine changes at the genome level [17, 18]. Further evaluation needs to be done to identify mutants with morphological character that correlated with high production potential.

4. Conclusion
The grouping of 34 morphological marker characters shows the character similarities of the 21 putative mutants to the original varieties (PS 881). It is divided into two groups with a coefficient of 0.25. This value determine the diversity of morphological character of sugarcane putative mutant resulting from colchicine-induced.

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