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AGRONOMIC CHARACTERISTICS OF SUGARCANE CULTIVAR GMP3 MUTANTS INDUCED THROUGH COLCHICINE

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SUMMARY

In sugarcane (*Saccharum officinarum* L.) crops for further improvement, one of the breeding efforts can continue through colchicine-induced mutations. Previously, PT GMP had developed through breeding GMP3 cultivars by mutation induction using colchicine. Nevertheless, no studies have investigated how colchicine affects agronomic traits. This pertinent research complements the results of previous work, namely, observing the anatomical characteristics of stomata. The presented study sought to determine the agronomic traits of sugarcane cultivar GMP3 breeding through colchicine induction. The study perceived a descriptive analysis of the agronomic features of nine-month-old mutants of sugarcane cultivar GMP3, carried out in 2021 at the Gunung Madu Plantations, Lampung, Indonesia. The assessment compared the treatment means, with the data further analyzed through clusters and PCA analyses using MVSP software. The results revealed that 21 mutants of the sugarcane cultivar GMP3 had considerable genetic diversity, such as medium-sized leaf width, dark green leaf color, cylindrical internode shape, no dorsal plane hairs, leaf shape with branches at the edges of the leaves, medium internode length (>13 cm), and stem diameter (2.5–3.0 cm). The phenetic analysis showed the degree of relationship between the control and 21 mutants of the cultivar GMP3, with similarity indices ranging from 0.70 to 1.00, confirming the similarity of agronomic traits. The principal component analysis (PCA) indicated the relationship between the control and cultivar GMP3 mutants for qualitative traits with an eigenvalue (>0.20), revealing that 13 agronomic traits played a considerable role in cluster grouping. These findings can become a basis for future research on colchicine-mutated sugarcane.

Keywords: Sugarcane (*Saccharum officinarum* L.), cultivar GMP3, colchicine mutation, agronomic traits, cluster and principal component analyses

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Key findings: The 21 colchicine mutants of cultivar GMP3 revealed considerable diversity in agronomic traits, i.e., medium-sized leaf width, dark green leaf color, leaf shape with branches, no dorsal hairs, cylindrical segment shape, medium segment length (>13 cm), and medium-sized stem diameter (2.5–3.0 cm). The results obtained can benefit a basis for future sugarcane breeding through colchicine mutation.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an indigenous grass in tropical and subtropical regions worldwide (Ram *et al.*, 2022). Sugarcane is a monocot of perennial nature with a maximum plant height of six meters (Chandran *et al.,* 2023). The stem produces the sugar and has the highest sucrose concentration. Additionally, sugarcane with low fiber content facilitates the processing and extraction of sucrose (Kar *et al*., 2023). Sugarcane domestication and use started around 8000 BC in New Guinea and later reached Southeast Asia and India. In the present era, sugarcane crops are the prime supplier of sugar and biofuels (Weksanthia *et al.*, 2021). Being a valuable crop, it is also beneficial in manufacturing ethanol and paper (Gonçalves *et al.,* 2024).

The world's sugar consumption has risen due to considerable population growth (Verma *et al.,* 2020). Nevertheless, the rise in sugar consumption has not coincided with increased sugar production due to various factors (Kandhro *et al.*, 2021; Dwivedi *et al.*, 2023). One of the key causes is the sugar source, as the sugarcane crop is a highly perishable one that various environmental factors can affect, including pests, diseases (Krishna *et al*., 2023; Gonçalves *et al.*, 2024), and extreme weather conditions (Sanghera *et al.,* 2022). These make it hard to cultivate sugarcane regularly with high yields to meet the world's expanding sugar demand (Kuldeep *et al*., 2019). Additionally, growing labor and energy expenses have contributed to the enhanced cost of sugar production. Therefore, it is harder for sugar manufacturers to compete with less expensive sweeteners like corn syrup (Palachai *et al*., 2019). Sugarcane production in 2017–2021 reached 2.1–2.3 t/ha, yielding 7%–8% sugar (Central Bureau of Statistics, 2021).

PT Gunung Madu Plantations (PT GMP) is one of the sugarcane plantation industries in Indonesia searching for ways to enhance sugar production by assembling superior sugarcane cultivars (Hapsoro *et al*., 2015). PT GMP offers various commercial sugarcane cultivars, and 30% of the total area cultivates GMP3, the most dominant cultivar. However, field data revealed that the cultivar GMP3 has small stems with narrow leaf widths and low sugar yield (Windiyani *et al.,* 2022; Mahfut *et al*., 2023b). Based on this, improving plant characteristics through breeding can get the desired traits using numerous techniques, including genetic engineering (Ullah *et al*., 2013; Viana *et al*., 2019; Tabasum *et al*., 2010), hybridization, and selective breeding can achieve this goal (Chandran *et al.,* 2023; Kar *et al.*, 2023). Plant breeding techniques can also be advantageous in the sugarcane industry to develop cultivars with increased sugar content (Riajaya *et al.,* 2022), tolerance to pests and diseases (Mahfut, 2020, 2021, Mahfut *et al*., 2015, 2020a, b, c, 2023c, Gonçalves *et al.,* 2024), and improved environmental adaptability (Windiyani *et al.,* 2022; Mahfut *et al*., 2023a, d).

Mutation through inducing colchicine is one plant breeding technique that can help enhance sugarcane productivity (Wiangwiset *et al*., 2023). According to Yasmeen *et al.* (2020), compound colchicine interferes with mitotic division, the process by which cells better divide and replicate efficiently. Such mutation may lead to emerging novel features like higher sugar content and enhanced natural resistance to pests and diseases (Weksanthia *et al.,* 2021; Krishna *et al*., 2023). Numerous studies on colchicine-induced sugarcane mutants have materialized, which experienced utmost modifications in their agronomic traits, such as stem length, internode number and length, sugar content, and productivity (Nadeem *et al*., 2020; Mangrio *et al*., 2022;

Wibisono *et al.,* 2022; Chandran *et al.,* 2023). However, findings from Krishna *et al.* (2023) reported a decline in plant height and leaf length.

PT GMP has induced colchicine mutation in the sugarcane cultivar GMP3; however, the mutation impact received no further investigation nor any data recorded on the agronomic traits in sugarcane mutants bred through colchicine induction. Any investigation has not focused on how colchicine affects the agronomic attributes of sugar cane at PT GMP. Our previous study (Mahfut *et al.*, 2023b) showed that sugarcane cultivar GMP3 mutants had Graminae-type stomata. The diversity in the stomata was high due to variations in the stomata size between the cultivars GMP3 mutants and their control. Their findings also indicated that stomata were excellent taxonomic evidence for identifying and analyzing the sugarcane mutants induced by colchicine (Mahfut *et al*., 2021; Clark *et al.*, 2022). The presented study sought to determine the agronomic traits in the breeding of sugarcane cultivar GMP3 mutants through colchicine induction. The result of this research is to provide alternative seeds for superior sugarcane varieties that can increase sugarcane productivity and meet the world's sugar needs.

MATERIALS AND METHODS

Plant materials

The studied plant materials in 2021 at the PT Gunung Madu Plantations, Lampung, Indonesia, comprised 21 mutants of sugarcane cultivar GMP3. All the mutant samples, i.e., M1, M2, M3, M4, M5, M7, M8, M9, M10, M11, M12, M13, M15, M16, M17, M18, M19, M21, M22, M23, and M24 came from the collection of PT GMP. Observations based on the agronomic traits attained documenting sugarcane cultivar GMP3 mutants at nine months old.

Agronomic traits observation and analysis

Data collection by direct observations occurred in the field. The field data recording on 33 parameters included leaves, leaf midribs, stems, segments, and bud eyes, following the method of Windiyani *et al*. (2022) (Table 1). The descriptive analysis of agronomic traits made comparisons among them. Then, agronomic facts analyzed kinship, with qualitative characterization by scoring from the descriptive data to the binary data. The analysis by grouping the mutants had descriptions through a dendrogram using the Multivariate Statistical Package (MVSP) software version 3.2. The cluster analysis used the Unweighted Pair-Group with Arithmetic Average (UPGMA) method for genetic distances. Furthermore, the principal component analysis (PCA) helped determine the traits that influence the grouping between the mutants.

RESULTS AND DISCUSSION

Sample collection

The results of the observations on agronomic traits showed that the sugarcane cultivar GMP3 mutants had many similar traits as the control, and only a few traits were different. These seven different agronomic traits in various GMP3 mutants were stem diameter, leaf midrib, shape, width, color, internode shape, and internode length. The variations in the seven agronomic traits between the mother cultivar GMP3 (control) and GMP3 mutants are available in Figure 1.

Stem characteristic

The observations showed considerable variations in several stem traits of the sugarcane cultivar GMP3 mutants. The variation appeared in the stem diameter.

Table 1. Agronomic traits and scoring of the sugarcane cultivar GMP3 and GMP3 mutants.

Code	Characteristics	Characteristics' Score
D1	Leaf curvature	1: < 1/3 leaf ; 2: 1/3-1/2 leaf
D2	Leaf width	1: Narrow (<4cm), 2: Medium (4-5 cm), 3: Wide (>5 cm)
D3	Leaf color	1: Dark green, 2: Green, 3: Yellowish
D4	Leaf triangle color	1: purple. 2: green
D5	Leaf ears	1: No ears, 2: Ear length 1 times the width (weak)
D6	Leaf ear position	1: upright. 2: oblique
P1	Back fur position	1: down, 2: upright
P2	Width of dorsal hair area	1: <1/4 width of leaf midrib, 2: =/> width of midrib
P3	Peak distance of dorsal hair area	1: <1cm, 2: =/>1cm
P4	Dorsal hair density	1: rare, 2: dense
P5	Leaf-sheath detachment	1: hard, 2: easy
P6	Leaf-sheath color	1: white, 2: green
R1	Segment arrangement	1: straight, 2: zigzag
R2	Segment shape	1: Cylindrical, 2: barrel, 3: spherical, 4: conical, 5: reverse conical, 6: convex concave
R3	Section cross-section	1: Round, 2 : flat
R4	Internode length (cm)	1: long (>15cm), 2: medium (13-15cm), 3: short (<13cm)
R5	Root ring	1: above the eye, 2: not above the eye
R6	Number of root points	1: <2 rows, 2: 2- 3 rows, 3: >3rows
R7	Eye grooves	1: None, 2: present in some ruses, 3: present in all segments
B1	Rod color	1: Green, 2: Yellow, 3: Yellowish green, 4: Purplish green, 5: Reddish green
B2	Wax coating	1: Thick , 2: thin
B3	Stem diameter	1: Large (>3cm), 2: medium (2.5-3cm), 3: small (<2.5cm)
B4	Bar cracks	1: None, 2: one
B5	The nature of the cork rods	1: None, 2: available
B6	Hole in stem	1: Yes, 2: absent
M1	Eye position	1: On the leaf midrib, 2: on the leaf midrib
M2	Eye shape	1: Round, 2: Long round, 3: ovate
M3	Crested hair	1: none, 2: present
M4	Eye wing edge	1: no wings, 2: equal in width
M5	Eye wing shape	1: Toothed, 2: flat
M6	Growth center	1: Under/in the middle of the eye. 2: above in the middle of the eye
M7	Basal fringe hair	1: none, 2: present
F1	Flowering	1: rare/sporadic (<5%), 2: moderate (5-15%), 3: heavy (>15%)

Figure 1. Differences in agronomic traits between the sugarcane cultivar GMP3 and GMP3 mutants.

Cultivar GMP3 has medium-sized stem diameters (2.5–3.0 cm), while some of the GMP3 mutants have large stems with increased diameters (>3 cm). This variation in the said trait was visible, particularly in the cultivar GMP3 M1 mutant.

Leaf characteristic

The outcomes indicated variations in leaf traits of the cultivar GMP3 mutants. These variations were apparent in leaf width, color, and shape. The mother cultivar GMP3 had a narrow leaf width (<4 cm), while some of the GMP3 mutants had a medium leaf width (>4 cm). This type of variation was evident in the cultivar GMP3 M13 mutant. The leaf color of the cultivar GMP3 was light green, while the GMP3 mutant had a dark green leaf color. This variation was distinct in the GMP3 M1 mutant. Several GMP3 mutants have different leaf shapes with branches on the edges of the leaves, and these leaf variations were noticeable in the cultivar GMP3 M22 and M19 mutants.

Leaf-midrib trait

The observations about the leaf sheath also displayed variations for the leaf midribs in the sugarcane cultivar GMP3 mutants. These variations emerged in the width of the fur on the back. The mother cultivar GMP3 had a dorsal hairline (<1/4) at the width of the leaf midrib, whereas the GMP3 mutant did not have a dorsal hairline. This variation in the dorsal hairline occurred in the cultivar GMP3 M13 mutant.

Internode characteristic

The results showed differences in the internodes of the cultivar GMP3 mutants. These variations were visible, particularly in the length and shape of the internodes. The mother cultivar GMP3 had a short internode length (<13 cm), while several GMP3 mutants owned a medium internode length (>13 cm). This variation in the internode length was particular in the cultivar GMP3 M13 mutant. The cultivar GMP3 has a sloping internode shape, and the GMP3 mutant has a cylindrical shape. This variation in the internode shape manifested in the GMP3 M1 mutant.

According to Nadeem *et al*. (2020), sugarcane stalks often consist of numerous parts and have a cylindrical shape. Khan *et al.* (2022) also reported the results of induced sugarcane mutants with cylindrical internodes with numerous zigzag alignments and longer internodes (15.5 cm) than controls. It was also evident in this study that, similar to the GMP mutant, the mutant sugarcane exhibited comparatively longer segments with clearly visible internal sponge pith lines. The pith can cause an increase in the sucrose content of sugarcanes (Mangrio *et al.*, 2022).

Phenetic analysis

The relevant study used a similarity index calculation to determine the degree of kinship between the 21 GMP3 mutants and the mother cultivar GMP3. The similarity index between the cultivar GMP3 and its 21 mutants ranged from 0.70 to 1.00 (Figure 2). The cultivar GMP3 mutants with a similarity index of 1.00 showed absolutely the same agronomic traits (Table 2).

Based on the cluster analysis, it was notable that the cultivar GMP3 and the 21 GMP3 mutants can be in two large clusters, namely, Cluster A and Cluster B. Cluster A has a similarity index value of 0.70 consisting only of cultivar GMP3, and it has a separate and dominant branching type. Cluster B has a similarity index of 0.78 comprising 21 mutant genotypes, i.e., M17, M24, M4, M8, M21, M16, M10, M9, M23, M12, M7, M13, M18, M15, M5, M11, M2, M3, M19, M22, and M1. Cluster B subdividing comprised five sub-clusters, viz., sub-cluster I consisting of M17, M24, M4, M8, and M21. Sub-cluster II consists of M16, M10, M9, M23, M12, and M7, while sub-cluster-III consists of only a single branch, namely, M13. The sub-cluster IV consists of mutants, i.e., M18, M15, M5, M11, M2, M3, M19, and M22, while the sub-cluster V consists of the single branch, M1.

Table 2. Similarity index (%) for the agronomic traits among the sugarcane cultivar GMP3 and GMP3 mutants.

Populations	GMP3	M17	M1	M18	M16	M4	M10	M7	M15	M9	M8	M13	M21	M5	M24	M23	M12	M11	M2	M3	M19	M22
GMP3	1																					
M17	0.69	1																				
M1	0.79	0.71	1																			
M18	0.75	0.86	0.71	1																		
M16	0.76	0.78	0.78	0.81	1																	
M4	0.72	0.90	0.80	0.78	0.90	1																
M10	0.63	0.93	0.75	0.84	0.97	0.87	1															
M7	0.82	0.87	0.71	0.75	0.87	0.84	0.90	1														
M15	0.69	0.84	0.74	0.96	0.84	0.81	0.87	0.78	1													
M9	0.65	0.81	0.78	0.81	0.93	0.84	0.97	0.87	0.84	1												
M8	0.70	0.84	0.80	0.72	0.90	0.87	0.87	0.84	0.75	0.84	1											
M13	0.68	0.93	0.73	0.71	0.78	0.86	0.81	0.83	0.75	0.78	0.80	1										
M21	0.76	0.86	0.77	0.75	0.93	0.90	0.90	0.87	0.78	0.87	0.90	0.78	1									
M5	0.71	0.90	0.74	0.90	0.84	0.75	0.87	0.78	0.93	0.90	0.75	0.69	0.78	1								
M24	0.69	0.75	0.86	0.78	0.90	0.93	0.87	0.84	0.81	0.84	0.93	0.86	0.90	0.75	1							
M23	0.74	1	0.72	0.81	0.93	0.84	0.97	0.93	0.84	0.93	0.84	0.78	0.87	0.84	0.84	1						
M12	0.76	0.84	0.71	0.75	0.93	0.90	0.90	0.87	0.78	0.87	0.84	0.78	0.87	0.78	0.84	0.93	1					
M11	0.77	0.84	0.68	0.90	0.84	0.75	0.87	0.78	0.93	0.84	0.81	0.69	0.78	0.93	0.75	0.84	0.78	1				
M2	0.71	0.75	0.75	0.90	0.90	0.81	0.93	0.84	0.93	0.90	0.81	0.75	0.84	0.93	0.81	0.90	0.84	0.93	1			
M3	0.76	0.81	0.68	0.83	0.84	0.75	0.87	0.78	0.87	0.84	0.81	0.69	0.78	0.87	0.75	0.84	0.78	0.93	0.93	1		
M19	0.76	0.75	0.74	0.90	0.84	0.81	0.87	0.78	0.93	0.84	0.81	0.75	0.78	0.87	0.81	0.84	0.78	0.93	0.93	0.93	1	
M22	0.76	0.81	0.74	0.90	0.84	0.81	0.87	0.78	0.93	0.84	0.81	0.75	0.78	0.87	0.81	0.84	0.78	0.93	0.93	0.93	1	1

The results of this study were consistent with previous research by Mahfut *et al*. (2023b), which also showed a variation in stomata features across controls in all GMP3 mutants. The analysis's findings reached two primary clusters: Cluster A, which has 18 accesses and a similarity index of 0.25, made up of M7, M9, M5, M24, M18, M8, M11, M4, M16, M17, M2, M22, M21, M19, M15, M13, and M1. M7, M9, and M5 belonged to sub-cluster I, and M24, M18, M8, M11, M4, M16, M17, M2, M22, M21, M19, M15, M13, M13, and M12 made up sub-cluster II. Meanwhile, Cluster B has four accessions with a similarity score (0.30): GMP3, M23, M10, and M3. Sub-cluster I was M23 and sub-cluster II was GMP3, M10, and M3. Mutations in distinct responses in sugarcane varieties result in differences in mutant sizes (Mahfut *et al*., 2023b), such as the width of the stomata opening and the stomata's length, number, and density, which, in turn, cause cluster separation in the analysis of morphological characteristics, including agronomic ones (Nadeem *et al.*, 2020).

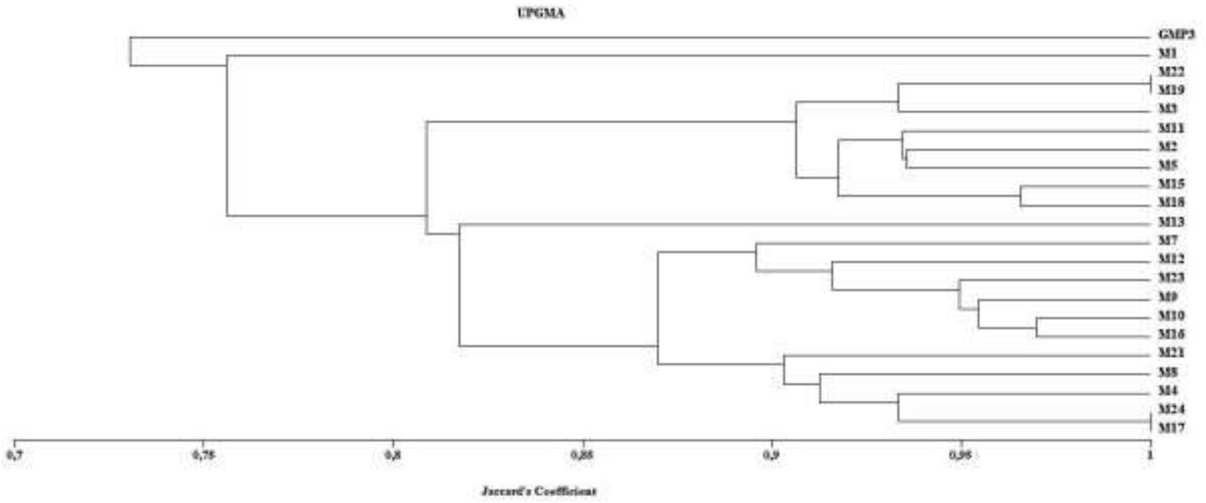

Figure 2. Dendrogram of the phenetic relationship among the sugarcane cultivar GMP3 and GMP3 mutants using UPGMA cluster analysis based on Jaccard's Coefficient.

Based on the similarity index value for all agronomic traits, the most closely related accession is cluster A (M16, M10, M18, and M15), with a similarity index value (0.98). Based on the field data, it was familiar that the four accessions had very similar characteristics and were difficult to distinguish. It indicates the possibility of species similarity in the collection (Windiyani *et al.,* 2022). Based on the cluster analysis, variations in the agronomic traits of the cultivar GMP3 mutant surfaced for the leaf traits, i.e., medium-sized leaf width and dark green leaf color. The leaf midrib traits also did not have dorsal hairs. A large stem diameter was also visible, and the bud eye had an oval eye shape and an eye groove on some segments.

A higher similarity index means that the phenetic analysis results between samples were becoming more similar, and vice versa (Windiyani *et al.,* 2022). A group's degree of relatedness can be valid using the similarity coefficient. Kinship and genetic distance were associated, as the coefficient of similarity shows. The similarity coefficient value increases with increasing kinship between individuals and decreases with increasing genetic distance (Mahfut *et al.*, 2023b). The results of this study indicate that GMP3 mutations and GMP3 variations were comparable, which accounts for their close relationship. Verma *et al*. (2020) state that the difference between the objects under comparison was better when the similarity index was higher. It suggests a close relationship between some GMP3 variety mutations (Yasmeen *et al*., 2020).

Principal component analysis (PCA)

In the presented study, PCA analysis employed the MVSP software version 3.2. The PCA analysis showed the relationship between the mother cultivar GMP3 and the cultivar GMP3 mutants for agronomic traits, grouping these mutant populations (Figure 3). The population groups comprised three clusters. First is cluster-I, consisting of the GMP3 cultivar separated by a crack characteristic (B4) and dorsal hair density (P4). Cluster II consists of GMP3 mutants, viz., M2, M3, M5, M11, M15, and M18 based on the traits of segment length (R4), wax layer (B2), and eye groove (R7). Cluster III contained mutants M1, M4, M7, M12, M13, M16, M21, M23, and M24 based on the parameters, i.e., leaf blade curvature (D1), leaf color (D3), segment shape (R2), and eye position (M1). The main component I has the strongest variable value compared to the main component II.

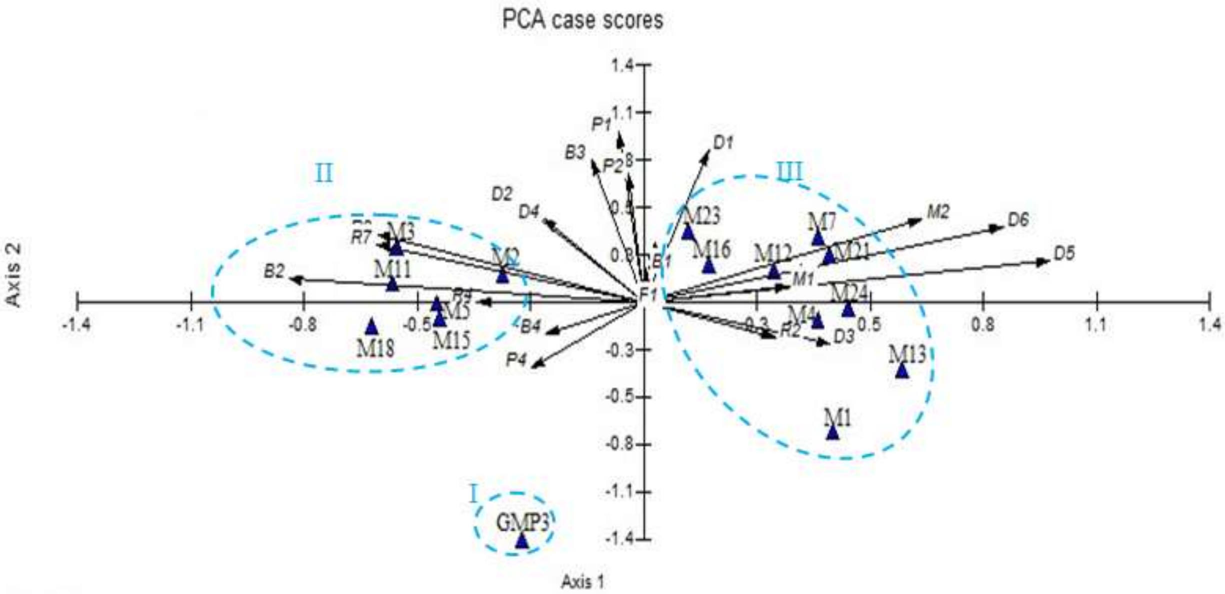

Figure 3. Principal Component Analysis (PCA) of the sugarcane cultivar GMP3 and GMP3 mutants. Note: Axis 1: Principal Coordinate 1, Axis 2: Principal Coordinate 2.

Mahfut *et al.* (2023b) studied the differences in stomatal characteristics between GMP3 mutants and controls. The results indicated that cluster I, which includes GMP3, M7, M9, M10, M17, and M23, is distinctive of variations in stomata length and aperture width. The M2, M8, M11, M16, M22, and M24 were apparent in Cluster II. These clusters' arrangement depended on three characteristics: stomatal density, index, and number.

The PCA result showed an eigenvalue indicating the percentage contribution value of each grouping (Windiyani *et al*., 2022). An eigenvalue (> 0.20) indicated the trait with the most vital role in cluster grouping (Mahfut *et al*., 2023b), and the total variation on axis 1 contributes 21.9% to the variation of the 33 agronomic traits used, with an eigenvalue of 4.163. Meanwhile, the total variation on axis II was 17.9%, with an eigenvalue of 3.420. The eigenvalue size showed the influence of each characteristic, which can be seen from the short length of the formed projection (Windiyani *et al*., 2022). Wibisono *et al.* (2022) stated a resemblance between the results of cluster analysis and PCA. Both analyses can separate several botanically similar species and are a widely used technique for discovering cluster structures in numeric taxonomy (Mahfut *et al*., 2023b). In addition to the cluster grouping pattern (I, II, and III), similarities in various traits played a considerable role in separating samples between cluster analysis and principal component analysis (Table 3).

Based on the scatter diagram, 13 traits are vital in grouping the sugarcane cultivar GMP3 mutants (Figure 3 and Table 2). The 13 traits, i.e., curved leaf blade (D1), leaf width (D2), leaf color (D3), leaf triangular color (D4), leaf ears (D5), position of leaf ears (D6), position of dorsal hairs (P1), the width of the dorsal hair area (P2), the distance from the crest of the dorsal hair area (P3), the stem diameter (B3), the wax layer (B2), the eye groove (R7), and the shape of the eye (M2), together can separate all the samples on the PC1 and PC2. According to Nadeem *et al*. (2020), the length and direction of the arrows indicated the agronomic characteristics mainly influencing the grouping. Arrows pointing to a particular group indicate the most influential agronomic quality. The extent of the arrow was directly proportional to the agronomic characteristic (Sanghera *et al*., 2022).

Table 3. Eigenvalues, percentage of variance, and character loading on the first two principal components.

Characteristic's code	Characteristic's name	Characteristic loading	
		PC1	PC2
D1	Leaf curvature	0.072	0.409
D2	Leaf width	-0.141	0.272
D3	Leaf color	0.208	-0.111
D4	Leaf triangle color	-0.112	0.224
D5	Leaf ears	0.455	0.109
D6	Leaf ear position	0.403	0.202
P1	Back fur position	-0.029	0.459
P2	Width of dorsal hair area	-0.017	0.346
P3	Peak distance of dorsal hair area	-0.299	0.183
P4	Dorsal hair density	-0.126	-0.176
P5	Leaf-sheath detachment	0	0
P6	Leaf-sheath color	0	0
R1	Segment arrangement	0	0
R2	Segment shape	0.147	-0.096
R3	Section cross-section	0	0
R4	Internode length (cm)	-0.186	0.002
R5	Root ring	0	0
R6	Number of root points	0	0
R7	Eye grooves	-0.299	0.154
B1	Rod color	0.003	0.093
B2	Wax coating	-0.398	0.063
B3	Stem diameter	-0.059	0.381
B4	Bar cracks	-0.11	-0.083
B5	The nature of the cork rods	0	0
B6	Hole in stem	0	0
M1	Eye position	0.162	0.041
M2	Eye shape	0.312	0.223
M3	Crested hair	0	0
M4	Eye wing edge	0	0
M5	Eye wing shape	0	0
M6	Growth center	0	0
M7	Basal fringe hair	0	0
F1	Flowering	0	0
	Eigen value	4.163	3.420
	Percentage	21.912	17.999
	Cum. Percentage	21.912	17.999

Based on the agronomic traits, it was notable that cultivar GMP3 mutants received effects only from some parts of the qualitative features. It followed Mahfut *et al*. (2023b) that the sensitivity of each plant species to colchicine application will be different even on the part of the treated plant. Phenetic analysis of the cultivar GMP3 and 21 GMP3 mutants proceeded through two methods, namely, cluster analysis and principal component analysis. Cluster analysis proved beneficial in summarizing the data by grouping objects based on specific characteristics in common among the objects studied, and principal component analysis was advantageous in knowing the traits that play a vital role in grouping (Chandran *et al.*, 2023). The kinship relationship between the plant species analysis can determine how dissimilar they are by calculating the correlation coefficient, similarity index, taxonomic distance, and group analysis values (Windiyani *et al.,* 2022). Overall, these measurement methods aim to determine the similarities between plant types based on the number of traits (Avivi *et al.,* 2019).

The greater the genetic similarity value among the sugarcane cultivar GMP3 mutants, the closer the genetic distance among the mutants. Genetic distance with a high value indicates that most of the clone genomes were identical, as caused by a lack of parental diversity and clones similar to their offspring (Chandran *et al.,* 2023). Differences in agronomic traits between mutants and control could be due to mutations caused by the colchicine application, giving rise to new individuals different from their parents and causing genetic diversity (Yasmeen *et al.,* 2020; Sukmawati *et al*., 2021). According to Chandran *et al.* (2023), chromosomal mutation can result in variations in plant properties. Mangrio *et al.* (2022) reported that one of the effects on agronomic traits was due to colchicine treatment, leading to an emerging new trait. PCA is an analysis used to determine the grouping of an individual in a population based on the agronomic qualities involved in the main components. According to Mahfut *et al.* (2023b), there was similarity between the cluster analysis and principal component analysis. These analyses can commonly identify the cluster structures in numerical taxonomy and can distinguish several closely related genotypes.

CONCLUSIONS

In the sugarcane cultivar GMP3 mutants, the diversity was evident for agronomic traits, i.e., medium leaf width, dark green leaf color, branched leaf shape, no back hairs, cylindrical segment shape, medium segment length (>13 cm), and medium stem diameter (2.5–3.0 cm). The phenetic analysis showed the degree of relationship between the mother cultivar GMP3 (control) and 21 GMP3 mutants, with a similarity index ranging from 0.70 to 1.00, which confirmed that the agronomic traits were similar. Based on the cluster analysis, the cultivars GMP3 and GMP3 21 mutants reached two large cluster groupings with similarity index values of 0.70 and 0.98. The principal component analysis (PCA) showed the relationship between the cultivar GMP3 and GMP3 21 mutants for qualitative traits, with an eigenvalue (>0.20) revealing that 33 with 13 agronomic attributes are most vital in cluster grouping.

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