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Phenotypic stability of mutant sugarcane (*Saccharum officinarum* L.) tolerant to drought stress based on variations in morphological traits at the greenhouse scale

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Abstract One of the problems of sugarcane (*Saccharum officinarum* L.) cultivation due to unpredictable weather is stunted growth, which leads to decrease productivity. Efforts to enhance sugarcane productivity under drought stress can be achieved through plant breeding via mutation induction. Changes in phenotypic stability in mutants lead to morphological alterations that subsequently increase adaptability to water deficit stress. Previous research has resulted in 11 superior mutant sugarcane clones produced through colchicine induction from 4 drought-tolerant sugarcane varieties, namely GMP6, RGM047, RGM1183, and RGM186, at PT Gunung Madu Plantations (GMP). The identification and evaluation of the stability of five morphological traits of leaves and leaf sheaths need to be conducted on these 11 superior drought-resistant sugarcane clones in a greenhouse setting. All clones exhibited diversity in the morphological traits of leaf color and the ease of leaf sheath detachment, with a significance level of 0.05. A dendrogram from the phylogenetic relationship analysis formed three main clusters based on similarity in correlation coefficients, similarity index, taxonomic distance, and cluster analysis. Meanwhile, Principal Component Analysis of the five morphological traits formed two clusters, showing the total variation on two axes, with axis 1 accounting for 90.21% (eigenvalue 5.84) and axis 2 for 4.42% (eigenvalue 0.28), totaling 94.64% for both axes. The clustering was based on traits that strongly contributed to the grouping, namely leaf width, leaf blade curvature, leaf color, leaf sheath traits, and leaf sheath color.

Keywords: Drought stress, Greenhouse scale, Morphology traits, Phenotypic stability, Sugarcane mutant

Introduction

National sugarcane production of *Saccharum officinarum* L.) has decreased by 4% in the last five years (Mahfut *et al*., 2023a; 2023b; 2023c;

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2023d). The main cause of low sugar production is the lack of superior sugarcane cultivars or seedlings (Hailu *et al*., 2021), including those that are resistant to drought stress (Mangena and Mushadu, 2023). Plant breeding through mutation induction is one of the efforts to produce superior seeds (Chandran *et al*., 2023). The impact of genotype changes on mutant crop buds leads to morphological changes (Khan *et al*., 2022) which further improve adaptability to drought stress and increase sugar productivity.

The first change seen due to drought conditions in sugarcane is the morphological character of the leaves (Windiyani *et al*., 2022). Drought can have a detrimental effect on growth, such as reducing the number of saplings and narrowing the leaf area (Seleiman *et al*., 2021). However, the effect of colchicine induction is able to increase the stem height and the number of green leaves, but also reduce the number of internodes (Manzoor *et al*., 2019; Maurya and Bahadur, 2022). So that colchicine induction can be an alternative to develop superior seedlings that are resistant to drought stress.

Research on the development of superior varieties of cholchisin-induced sugarcane is still rarely carried out in Indonesia. Mahfut *et al*. (2023b) have induced 0.1% and 0.2% colchicine mutations in one of the ungul varieties at PT Gunung Madu Plantations (GMP), namely GMP3 and produced 21 mutant clones. Identification of superior mutant cones can be carried out based on morphological (Siraree *et al*., 2018; Rae *et al*., 2013; Bakker *et al*., 1999), agronomic (Pires *et al*., 2024; Desalegn *et al*., 2024), anatomical (Babu *et al*., 2022; Singh *et al*., 2025), physiological, and molecular (Rodrigues *et al*., 2021).

Mahfut *et al*. (2023b) reported that the results of anatomical characterization in 21 GMP3 mutants showed variation in stomata size in a similarity index of 0.20 indicating a rather high level. Furthermore, Pires *et al*. (2024) reported that agronomic characterization results showed a variety of characteristics, including medium-sized leaf width, dark green leaf color, branching leaves, lack of dorsal hairs, cylindrical segment shape, medium segment length, and medium-sized stem diameter. Meanwhile, the results of molecular characterization (Rodrigues *et al*., 2021) showed that the results of PCR-RAPD ampfification using 5 informative primers produced 35-60 DNA bands with 28 polymorphic bands and a similarity index of 0.47-1.00. This proves that 21 GMP3 mutants are superior varieties of sugarcane from plant breeding which can then be used in the provision of superior seeds to increase sugarcane productivity.

The previous research produced 11 superior mutant sugarcane clones from 4 varieties, namely GMP6, RGM047, RGM1183, and RGM186, through colchicine induction at PT GMP. This study was conducted to identify and evaluate leaf morphological variation in 11 superior sugarcane clones resistant to drought stress on a greenhouse scale.

Materials and methods

Plants material

The research was conducted from June to October 2023 in the greenhouse of PT GMP. The plant material used consisted of a control and 11 superior mutant clones, namely clones 36, 63, 44, 160, 162, 200, 202, 218, 226, 293, and 296, all of which were 3 months old. The observation samples were sugarcane leaves with three replicates.

Observation of morphological traits

The observation of morphological traits followed the procedures established by PT GMP. The morphological traits observed included leaves and leaf sheaths. Leaf characteristics consisted of leaf blade curvature, leaf width, and leaf color, while leaf sheath characteristics included the ease of leaf sheath detachment and leaf sheath color.

Data analysis

Data analysis was conducted following the methods of Windiyani *et al*. (2022) and Vijayakumar *et al*. (2015), using Multivariate Analysis of Variance (MANOVA) at a 5% significance level, with further testing by LSD at 5%. The analysis of genetic relationships was conducted using morphological data characterized both quantitatively and qualitatively, by converting quantitative data into binary data, followed by cluster analysis of the relationships among clones. The similarity operational taxonomic unit (OTU's) group was calculated using Gower's general similarity coefficient based on the scoring results. Cluster analysis was represented in the form of a dendrogram using the Multivariate Statistical Package (MVSP) version 3.2 software and the Unweighted Pair-Group with Arithmetic Average (UPGMA) to determine genetic distances for cluster analysis, as well as Principal Component Analysis (PCA) to determine the quantitative influence on clustering.

Results

Observation of morphological traits

The observation of morphological traits in several superior sugarcane clones, namely clones 36, 63, 44, 260, 162, 200, 202, 226, 218, 293, and 296 in the greenhouse, revealed variations in all leaf traits under drought stress treatments at 100% concentration (Table 1) and 0% concentration (Table 2). The leaf traits under drought stress treatments at 0% and 100% concentration showed variations in leaf blade curvature, leaf width, and leaf color.

Table 1. Genetic variation in morphological traits based on leaf characteristics in 11 sugarcane clones at 100% concentration

Morphological traits	Clone										
	Clone 36	Clone 63	KCone 44	Clone 160	Clone 162	Clone 200	Clone 202	Clone 226	Clone 218	Clone 293	Clone 296
Leaf leaf arch (D1). 0 = <1/3 leaf; 1 = 1/3 - 1/2 leaf; 2 = 1/2 - 2/3 leaf	0	0	0	0	0	1	1	1	0	0	1
Leaf width (D2). 0 = narrow (<4cm); 1 = medium (4-5cm); 2 = width (>5cm)	0	0	0	0	0	0	0	0	0	0	0
Leaf color (D3). 0 = dark green; 1 = green; 2 = yellowish green	2	2	2	2	1	1	2	1	2	1	1

Table 2. Genetic variation in morphological traits based on leaf characteristics in 11 sugarcane clones at 0% concentration

Morphological traits	Clone										
	Clone 36	Clone 63	KCone 44	Clone 160	Clone 162	Clone 200	Clone 202	Clone 226	Clone 218	Clone 293	Clone 296
Leaf leaf arch (D1). 0 = <1/3 leaf; 1 = 1/3 - 1/2 leaf; 2 = 1/2 - 2/3 leaf	0	0	0	0	0	1	1	1	0	0	1
Leaf width (D2). 0 = narrow (<4cm); 1 = medium (4-5cm); 2 = width (>5cm)	0	0	0	0	0	0	0	0	0	0	0
Leaf color (D3). 0 = dark green; 1 = green; 2 = yellowish green	2	2	2	2	1	1	2	2	2	2	2

The observations of leaf sheath characters, such as its detachment and color, also showed variations under drought stress treatments at 100% and 0% concentrations (Table 3 and 4).

Table 3. Genetic variation in morphological traits based on leaf sheath characteristics in 11 sugarcane clones at 100% concentration

Morphological traits	Clone										
	Clone 36	Clone 63	KCone 44	Clone 160	Clone 162	Clone 200	Clone 202	Clone 226	Clone 218	Clone 293	Clone 296
Shedding of leaf sheath (P5). 0 = difficult; 1 = moderate; 2 = easy	0	0	0	1	1	0	0	0	1	2	0
Color of leaf sheath (P6). 0 = white; 1 = purple; 2 = green	1	1	1	1	1	2	1	1	1	2	2

Table 4. Genetic variation in morphological traits based on leaf sheath characteristics in 11 sugarcane clones at 0% concentration

Morphological traits	Clone										
	Clone 36	Clone 63	KCone 44	Clone 160	Clone 162	Clone 200	Clone 202	Clone 226	Clone 218	Clone 293	Clone 296
Shedding of leaf sheath (P5). 0 = difficult; 1 = moderate; 2 = easy	0	0	0	0	0	0	0	0	0	0	0
Color of leaf sheath (P6). 0 = white; 1 = purple; 2 = green	1	1	1	1	1	1	1	1	2	2	2

Phenetic relationship analysis

The phenetic analysis of 11 mutant clones was conducted using two methods: cluster analysis and principal component analysis. Cluster analysis was performed using the Multi Variate Statistical Package (MVSP) version 3.1 with the UPGMA algorithm for dendrogram reconstruction. The analysis resulted in the formation of three clusters: Cluster I (clone 63), Cluster II (clone 296 0%, clone 200 0%, clone 226 0%, clone 202 0%, clone 63 0%, 293 0%, clone 162 0%, clone 160 0%, clone 296 100%, clone 218 0%, clone 218 100%, clone 202 100%, clone 200 100%, clone 160 100%, clone 44 0%, and clone 36 0%), and Cluster III (clone 293 100%, clone 226 100%, clone 162 100%, clone 44 100%, and clone 36 100% (Figure 1).

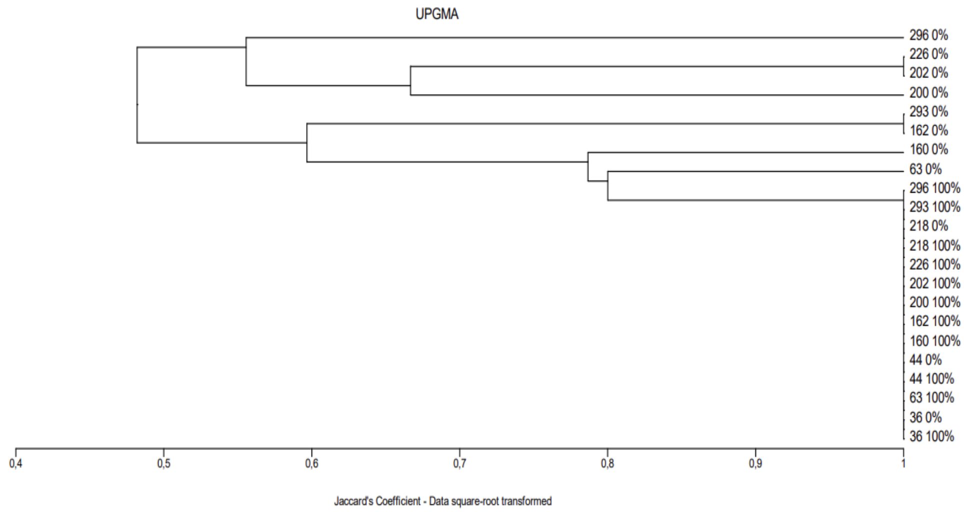

Figure 1. Dendogram of phenetic relationship of 11 superior sugarcane clones of PT GMP based on morphological traits

Principal component analysis

PCA is an analysis used to determine the clustering of individuals within a population based on the morphological traits involved in the principal components. The results of PCA using MVSP software version 3.2 showed that there is a differentiation between clones treated with 100% and 0% based on morphological traits (Figure 2). The clustering is divided into two clusters: Cluster I consist of (clone 36 0%, clone 63 0%, clone 44 0%, clone 160 0%, clone 162 0%, clone 200 0%, clone 202 0%, clone 226 0%, clone 218 0%, clone 293 0%, and clone 296 0%). Cluster II consists of (clone 36 100%, clone 63 100%, clone 44 100%, clone 160 100%, clone 162 100%, clone 200 100%, clone 202 100%, clone 218 100%, clone 226 100%, clone 293 100%, and clone 296 100%).

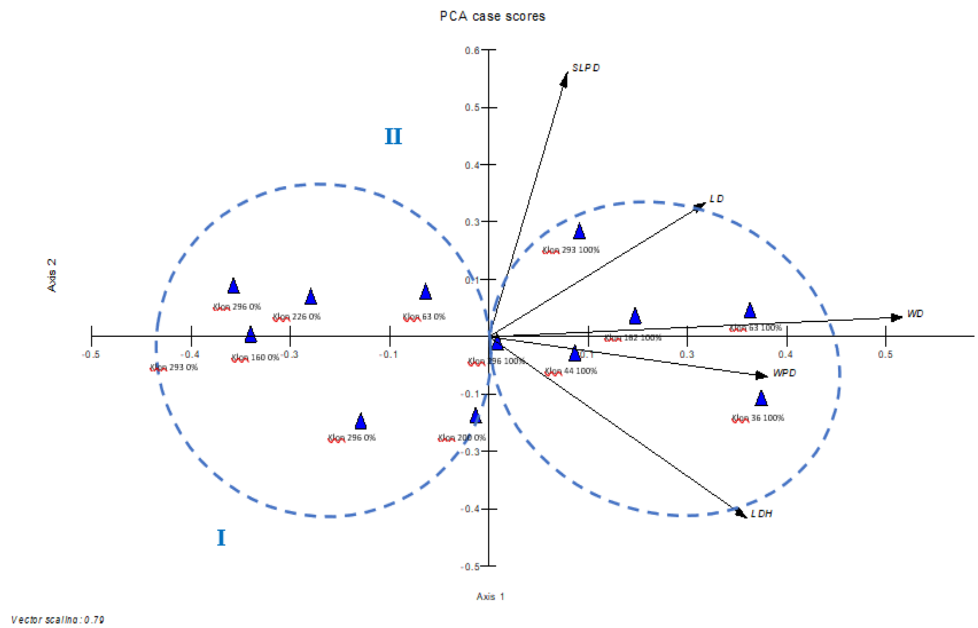

Figure 2. Principal component analysis of phenetic relationship of 11 superior sugarcane clones of PT GMP based on morphological traits

The PCA results indicate that the samples are clustered into two groups. This clustering is based on leaf width, leaf blade curvature, leaf color, sheath detachment trait, and sheath color. Principal component II has the strongest variable values compared to principal component I. The morphological traits that contribute to the clustering include leaf blade curvature, leaf width, leaf color, sheath detachment trait (dry), and sheath color. The morphological traits involved in the accession clustering are presented in Table 5.

Table 5. Genetic variation in morphological traits based on leaf sheath characteristics in 11 sugarcane clones at 0% concentration

Code	Traits	Axis 1	Axis 2
LHD	Leaf Blade Curve	0,374	-0,512
LD	Leaf Width	0,502	-0,322
WD	Leaf Color	0,426	-0,407
SLPD	Leaf Sheath Abscission	0,385	0,676
WPD	Leaf Sheath Color	0,528	-0,109
	Eigen values	5,842	0,287
	Percentage	90,215	4,428
	Cum. Percentage	90,215	94,643

Discussion

The comparison of morphological variations in superior mutant sugarcane under drought stress treatments at 100% and 0% concentrations shows only slight differences. At the 100% concentration, the length of leaf blade curvature was longer in clones 200, 202, 226, and 296 compared to the 0% concentration. Conversely, at the 0% concentration, clones 218 and 293 exhibited longer leaf blade curvature than at the 100% concentration. Saleem *et al*. (2023) reported that at a concentration of 100%, a greater length of leaf blade curvature is observed in the mutant sugarcane clone compared to the 0% concentration. Khan *et al*. (2022) stated that the increased length of leaf blade curvature in this mutant sugarcane clone is highly significant and serves as a key characteristic in determining an elite mutant clone because it influences the outer leaf structure, which plays a role in photosynthesis. The leaf blade also contains veins composed of vascular bundles that may be surrounded by bundle sheath cells.

In contrast, the leaf width character across all superior mutant clones under 100% and 0% concentration treatments showed the same narrow result of less than 4 cm. This occurred as a response to water deficiency conditions, which led to inhibited growth, including limited weekly increases in leaf width. Seleiman *et al*. (2021) reported that during drought stress, the plant's water content decreases, resulting in reduced turgor pressure and inhibited growth. Cell enlargement and elongation are disrupted, causing a reduction in leaf area and number (Abbas *et al*., 2023).

Regarding leaf color, most mutant clones exhibited a yellowish-green hue. However, clones 226, 293, and 296 showed a yellowish-green color at the 0% concentration, while at the 100% concentration, they appeared green. Hu *et al.* (2023) reported that drought stress, in addition to causing a reduction in leaf development and premature leaf senescence, can also damage leaf pigments and decrease the rate of photosynthesis. The results of this study indicate that the superior mutant clone is capable of maintaining chlorophyll integrity, thus ensuring that the photosynthesis rate remains unaffected and sugarcane productivity increases.

Drought stress treatments induced differences in the leaf sheath characteristics of clones 160, 162, and 218, changing from difficult (0) to moderate (1), while clone 293 became easy (2). The results of this study align with Que *et al*. (2024), who found that the superior mutant sugarcane clone exhibited leaf sheath characteristics that made them easier to detach. Khan *et al*.

(2022) also explained that drought stress is a factor that hinders sugar production, leading to an increase in the number of tillers through shoots by accelerating the detachment of leaf sheaths.

On the other hand, leaf sheath color characteristics showed differences in clones 200 and 218. Clone 200 exhibited a purple leaf sheath color at 0% concentration, which changed to green at 100% concentration, while clone 218 showed the opposite. This indicates that the superior mutant sugarcane clone maintains its leaf pigments despite the color change. Ebrahimi *et al*. (2023) explained that chlorophyll pigments, which give the green color, and anthocyanin pigments, which give the purple color, can both be used in the process of photosynthesis, respectively.

Cluster analysis is useful for summarizing data by grouping samples based on certain shared characteristics, whereas principal component analysis helps identify the characters that play a role in the grouping (Alaida and Aldhebiani, 2022). According to Alves *et al*. (2022), the UPGMA method is commonly used in taxonomic studies because of its stable classification, where all characters have equal weight, making it more accurate in depicting the relationships between different cultivar samples. Principal component analysis was conducted to create a scatter plot diagram using the Euclidean biplot type. This analysis determines the role of each character in the grouping (Mahfut *et al*., 2021a; 2021b).

The phenetic relationships between plant types can be analyzed to determine the extent of their dissimilarity by calculating correlation coefficients, similarity indices, taxonomic distances, and using cluster analysis (Yeshitila *et al*., 2024; Cidade *et al*., 2033). In general, all these measurement methods aim to determine the similarity between different plant species being compared based on a set of characteristics (Ibrahim *et al*., 2024).

The phenotypic stability of 11 superior mutant sugarcane clones potentially tolerant to drought stress, based on variations in morphological traits at the greenhouse scale, showed variations in leaf color and sheath detachment traits across all clones. The dendrogram of phenetic relationship analysis formed two main clusters, while principal component analysis (PCA) of five morphological traits leaf and sheath revealed a total variation on two axes: axis 1 with 90.21% and an eigenvalue of 5.84, and axis 2 with 4.42% and an eigenvalue of 0.28, resulting in a combined percentage of 94.64%.

Elumalai *et al*. (2023) and Siraree *et al*. (2017) reported that the cluster grouping represents non-correlated groups that might be useful for future breeding. The clustering is thought to be due to differences in the male and female parents of each superior sugarcane cultivar. The morphological traits formed are suspected to be due to similarities in the female parent. The clustering pattern based on the dendrogram and scatter plot shows the clustering of sugarcane clones based on morphological traits. Mutant sugarcane clones that share many similar characteristics will have a higher similarity value, causing them to cluster together in the same group (Park *et al*., 2024).

Based on the results of the principal component analysis (PCA) of five morphological characters, Axis1 accounted for 90.21% of the total variation with an eigenvalue of 5.84, while Axis2 accounted for 4.42% with an eigenvalue of 0.28. The combined percentage of the two axes represents 94.64% of the variation from the five characters. PCA was also utilized to identify the morphological characters that significantly contribute to group formation. The contribution of each character to the grouping is represented by the eigenvalues, which indicate the influence of each character (Deku *et al*., 2022) and can be observed from the length of the projections formed (Elhaik, 2022).

The genetic relationship among cultivars can be determined based on the dendrogram and the morphological character similarity matrix values; the higher the similarity coefficient between species, the closer the genetic relationship (Alhaithloul *et al*., 2024). The grouping pattern observed in the dendrogram, further supported by PCA, shows the distinguishing character values that cause individuals to separate (Table 5). The grouping pattern formed is almost identical to the clustering results on the dendrogram. Based on the scatter plot, Cluster II is formed due to the similarity in leaf and leaf sheath characteristics.

Pires *et al*. (2024) stated that there is congruence between the results of cluster analysis and principal component analysis. Both analyses are commonly used methods for recognizing cluster structure in numerical taxonomy and can differentiate closely related plants (Sukmawati *et al*., 2021). The grouping pattern based on the dendrogram and scatter plot demonstrates the clustering of sugarcane cultivars based on the similarity of agronomic characters. Furthermore, the results of this study can be used to determine the stability of leaf morphological characters in all superior mutant clones.

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