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Clustering and Principal Component Analysis of Genetic Diversity in Elite Sugarcane Clones Over Pooled Crop Cycles

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ABSTRACT

The experiment was conducted from January, 2021 to December, 2023 (3 years) at the Regional Agricultural Research Station (RARS), Anakapalle, Andhra Pradesh, India, to evaluate the genetic diversity among 25 elite sugarcane clones for cane yield and juice quality traits. The experiment followed a randomized block design with three replications across three crop cycles, and pooled data were used for analysis. A total of eight quantitative and qualitative traits were recorded for phenotyping. K-means clustering effectively classified the genotypes into two distinct clusters, with Cluster 1 comprising 16 genotypes and Cluster 2 containing 9 genotypes. The first four principal components (PCs) were retained in the Principal Component Analysis (PCA) based on eigenvalues (≥ 1), explaining 87.52% of the total variation. PC1 accounted for 42.5% of the variation, followed by PC2 (17.61%), PC3 (14.58%), and PC4 (12.82%). Pearson correlation coefficient showed nine significant positive correlations and one significant negative correlation among the assessed traits. These findings provided valuable insights into genetic diversity, facilitating the selection of superior parental lines for sugarcane breeding programs.

KEYWORDS: Correlation, K means, PCA, sugarcane

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1. INTRODUCTION

Sugarcane (Saccharum spp.) was a tall, perennial grass grown mainly in tropical and subtropical regions for its sweet juice, which was processed into sugar. It served as a key source of sucrose and was also used for ethanol, biofuels, and animal feed (Tolera et al., 2023). Optimal growth occurred in warm climates with abundant water and fertile soil. The phylogenetic history of sugarcane involved complex hybridization, mainly between Saccharum officinarum and Saccharum spontaneum, driven by natural selection and human domestication, which resulted in a highly polyploid and genetically diverse lineage (Tena et al., 2023).

Developing high-yielding sugarcane clones that were adaptable to various agroclimatic conditions was considered crucial for enhancing plantation and ratoon productivity, ultimately increasing farmers' income (Tabassum et al., 2023). However, a significant gap existed between sugarcane demand and supply due to climate change, long crop durations, and the vulnerability of existing clones to stresses (Abu-Ellail et al., 2023). These challenges contributed to a decline in production over the past decade. To address this, systematic breeding programs were initiated to create superior clones with improved yield and juice quality (Rakesh et al., 2023). The success of these initiatives relied on genetic diversity within the germplasm, as increased variability enhanced the potential for developing high-yielding varieties (Singh et al., 2019).

Molecular markers were more precise than morphological and biochemical markers for assessing genetic diversity in sugarcane populations (Tawadare et al., 2019). However, morphological markers remained indispensable, as they directly reflected trait expression under field conditions and captured genetic variation within a species' genome (Janrao et al., 2019). By integrating both quantitative and qualitative traits, morphological markers helped differentiate genetic variation from phenotypic variation, making them invaluable in identifying, selecting, and utilizing superior clones in sugarcane breeding programs (Vinu et al., 2022).

Genetic diversity studies provided critical insights into the relationships among individuals within a population, identifying trait associations that contributed to overall diversity (Rao and Chaturvedi, 2022; Rao et al., 2024). This information was essential for selecting genotypes with desirable characteristics for breeding programs. Furthermore, understanding genetic diversity facilitated hybridization efforts by enabling breeders to select genetically distinct parents, thereby maximizing heterosis and improving crop performance (Alemu et al., 2022). Ultimately, such studies played a pivotal role in crop improvement, leading to the development of high-yielding, stress-tolerant, and resilient cultivars suited to varying environmental conditions.

K-means clustering was an unsupervised machine learning algorithm that grouped genotypes based on genetic similarity by minimizing intra-cluster variance, aiding in the identification of genetic diversity patterns (Barth et al., 2022). Principal component analysis (PCA) reduced the dimensionality of genetic data while preserving significant variation, transforming correlated variables into uncorrelated principal components for clearer visualization of population structure (Syakur et al., 2018). The first few principal components explained most genetic variation, helping breeders identify key traits for selection. Cluster analysis, combining K-means and PCA, further refined genotype classification by grouping individuals based on genetic distances, enhancing breeding programs and crop improvement (Jarwar et al., 2019).

The present study aimed to evaluate 25 sugarcane clones from the early maturity cluster across three growing seasons. The pooled data from these evaluations were analyzed to assess genetic diversity, with the objective of identifying highly heterotic combination clones suitable for further hybridization programs. This approach facilitated the selection of genetically diverse and superior parental lines, enhancing the efficiency of breeding programs aimed at developing high-yielding and resilient sugarcane varieties.

2. MATERIALS AND METHODS

2.1. Planting materials and experimental location

Nineteen sugarcane clones from the early maturity cluster were selected (Table 1) and evaluated alongside six standard checks (83V 15, Co86249, 2000A 225, 2003A 255, 20003 V46, and 87A298) across three growing seasons. Among these clones, five were developed by the Regional Agricultural Research Station (RARS), Anakapalle; six by the Sugarcane Research Station, Vuyyuru; and eight by the Agricultural Research Station, Perumallapalli. The trials were conducted at RARS, Anakapalle (17.6896° N, 83.0024° E), with the first plant and ratoon cycles spanning from January, 2021 to December, 2023 (2 years) and the second plant crop from January, 2022 to December, 2023 (one year). A Randomized Block Design (RBD) with three replications was implemented to ensure statistical reliability. Three-budded sets were used for planting, while the ratoon crop was established from stubble following the harvest of the first plant crop. Each genotype was planted in six rows, with each row measuring six meters in length and spaced 90 cm apart. Standard agronomic practices were uniformly followed across all crop cycles (Kamat et al., 2023). To maintain consistency in data collection and analysis, the three eksali crops were harvested between 300 and 320 days after planting.

Table 1: List of the clones used in experiment

Sl. No.	Genotype	Developed by
1.	2009A 252	ANGRAU-RARS, Anakapalle
2.	2005T 50	ANGRAU-ARS, Perumallapalli
3.	2011T 88	ANGRAU-ARS, Perumallapalli
4.	2012T 58	ANGRAU-ARS, Perumallapalli
5.	2009V 89	ANGRAU- SRS, Vuyyuru
6.	2010V 146	ANGRAU- SRS, Vuyyuru
7.	2008V 257	ANGRAU- SRS, Vuyyuru
8.	2011A 67	ANGRAU-RARS, Anakapalle
9.	2010V32	ANGRAU- SRS, Vuyyuru
10.	2007V127	ANGRAU- SRS, Vuyyuru
11.	2010A229	ANGRAU-RARS, Anakapalle
12.	2011A262	ANGRAU-RARS, Anakapalle
13.	2009T5	ANGRAU-ARS, Perumallapalli
14.	2006T3	ANGRAU-ARS, Perumallapalli
15.	2009V127	ANGRAU- SRS, Vuyyuru
16.	2009A107	ANGRAU-RARS, Anakapalle
17.	2005T16	ANGRAU-ARS, Perumallapalli
18.	2009T10	ANGRAU-ARS, Perumallapalli
19.	2005T121	ANGRAU-ARS, Perumallapalli
20.	2003A255	ANGRAU-RARS, Anakapalle
21.	20003V46 (C)	ANGRAU- SRS, Vuyyuru
22.	87A298 (C)	ANGRAU-RARS, Anakapalle
23.	83V 15 (C)	ANGRAU- SRS, Vuyyuru
24.	Co86249 (C)	ICAR- SBI, Coimbatore
25.	2000A 225 (C)	ANGRAU-RARS, Anakapalle

2.2. Cataloging the phenotypic data

Cane yield was determined by harvesting the middle four rows of each plot and converting the total yield to a per-hectare basis. The number of millable canes (NMC) was manually counted within the net plot area and expressed as thousands per hectare ('000/ha). Stalk length and girth were measured from ten randomly selected samples per clone, and their averages were recorded. For quality assessment, Brix (%), fiber (%), and sucrose (%) were estimated at the 10th month of crop growth using five randomly selected canes per clone. A Brix refractometer and a sucrolyser were employed for precise measurements. CCS yield (t/ha), CCS percentage, and fiber (%) were estimated using the formula described by Nair et al. (1999):

2.3. Statistical analysis

Data from plant and ratoon trials were collected across three crop cycles and analyzed statistically. Bartlett's test was applied to evaluate the homogeneity of error variances across the three experiments using OPSTAT, an open-source statistical software. The non-significant results indicated uniform error variances, which allowed data from all three crop cycles to be pooled for further analysis. Genetic variance was estimated from the pooled dataset using OPSTAT software. K-means clustering and Principal Component Analysis (PCA) were performed using OPSTAT to assess genetic diversity among the evaluated sugarcane clones. The graphical representations of K-means clustering and PCA were generated using R-Studio, employing the "factoMineR" (Le et al., 2008) and "factoextra" packages (Irnawati et al., 2021) to enhance data interpretation and visualization.

3. RESULTS AND DISCUSSION

3.1. Analysis of variance

The pooled analysis of variance for yield and juice quality traits, as presented in Table 2, demonstrated significant effects of genotype, seasons, and genotype-by-season interactions for all evaluated traits, except for stalk length and the number of millable canes, where genotypic differences were not statistically significant. These results aligned with the findings of Kumar et al. (2023), Yadawad et al. (2023), and Vinu et al. (2024), who reported similar trends for cane yield, CCS yield, and sucrose content.

3.2. K-means

The optimal number of clusters (K) for the sugarcane population was determined to be two using the silhouette method (Figure 1). K-means clustering effectively grouped the genotypes into two distinct clusters without overlap. Cluster 1 comprised 16 genotypes, while Cluster 2 contained 9 genotypes (Figure 2). Cluster 1 exhibited superior performance for key agronomic and juice quality traits, as indicated by the 5% confidence interval. The genotypes within this cluster demonstrated higher cane yield, sucrose (%), cane length, CCS yield, and the number

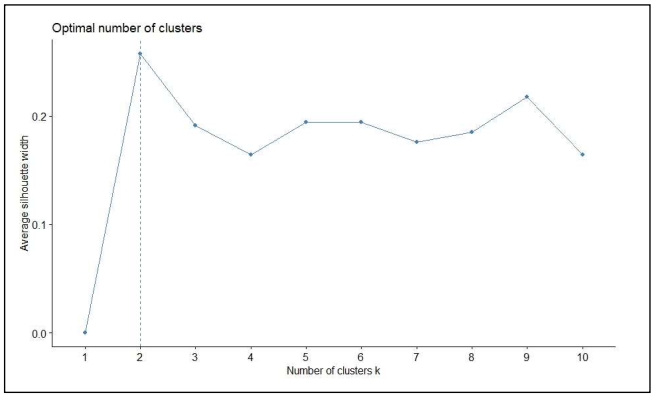

Figure 1: Determination of optimal number of cluster based sillhoutte method

Table 2: Combined pooled analysis of variance for eight characters in 25 sugarcane genotypes across three seasons

Source	Df	MSq	Pr (>F)	MSq	Pr (>F)	MSq	Pr (>F)	MSq	Pr (>F)
Location	2	55.29	0	211717. 4	0	3420.92	0	815.64	0
Genotype	24	19.92	0	259.49	0.41	3.93	0.96	23.38	0
Rep in Loca	6	3.77	0	223.7	0	1.15	0.34	2.29	0.04
Loc×Gen	48	4.99	0	242.56	0	7.7	0	8.42	0
Error	144	1		1		1		1	

Source	MSq	Pr (>F)	MSq	Pr (>F)	MSq	Pr (>F)	Mean Sq	Pr (>F)
Location	132.57	0	26.08	0	903.43	0	4486.746	0
Genotype	6.25	0	4.68	0	5.32	0	14.373	0
Rep in Loca	21.18	0	14.07	0	1.94	0.08	157.569	0
Loc X Gen	1.65	0.01	1.73	0	1.97	0	2.247	0
Error	1		0.74		1		1	

of millable canes. These traits were critical for commercial sugarcane production, highlighting the economic potential of genotypes within Cluster 1. On the other hand, Cluster 2 contained genotypes that showed higher fiber content (%) and CCS (%). While these clones were not as productive in terms of overall cane yield, their superior fiber and CCS content proved beneficial for specific industrial applications, such as biofuel production or enhanced sugar recovery efficiency. Similar findings were reported by Barth et al. (2022) in strawberry, where 194 genotypes were grouped into two clusters without overlapping each other. The classification of genotypes within each cluster based on K-means clustering was presented in Table 3, while the mean performance of the two clusters was illustrated in Table 4. The clear distinction between clusters suggested that genetic variability within the population was well structured, and cluster-based selection could be utilized for breeding programs aimed at improving specific traits (Barth et al., 2022).

3.3. Principal component analysis

Principal Component Analysis (PCA) was a powerful statistical tool that helped identify traits contributing most to variability within the sugarcane population. In the present study, eight principal components (PCs) were extracted, corresponding to the number of traits evaluated. PCs with eigenvalues less than 1 were considered less informative than the original traits and were not retained for further interpretation (Mehareb et al., 2023). Among the eight PCs, the first four accounted for 87.52% of the total variation, indicating their significant role in explaining trait diversity within the population (Table 5, Figure 3). These findings aligned with previous reports by Alemu et al. (2022) and Tesfa et al. (2024), which also identified four main components contributing 82.36% and 81.44% of the variation, respectively.

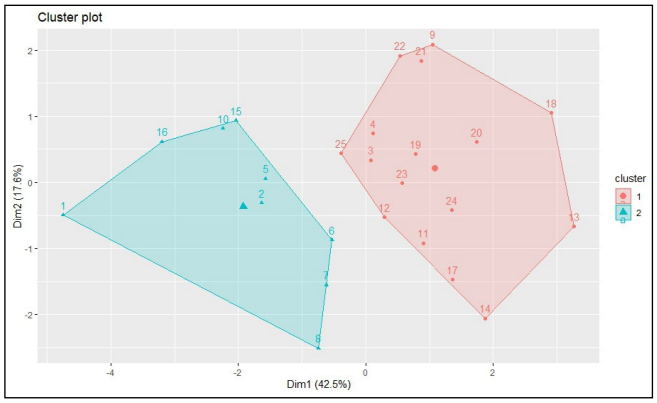

Figure 2: K means cluster grouped the 25 clones into two clusters (clone number see the table 1)

Table 3: List of clones grouping into two clusters

Cluster	No. of clones	List of clones
Cluster 1	9	2009A 252, 2009A107, 2007V127, 2009V127, 2005T 50, 2009V 89, 2010V 146, 2008V 257 and 2011A 67
Cluster 2	16	2011T 88, 2012T 58, 2010V 32, 2010A 229, 2011A 262, 2009T 5, 2006T 3, 2005T 16, 2009T 10, 2005T 121, 2003A 255 (C), 20003V 46 (C), 87A 298 (C), 83V 15 (C), Co86249 (C), and 2000A 225 (C).

The first principal component (PC1) had an eigenvalue of 3.40, explaining 42.50% of the total variability. Traits such as sucrose content (0.423), cane yield (0.463), commercial cane sugar (CCS%) (0.308), and CCS yield (0.507) exhibited high positive loadings (Table 6), indicating that PC1 represented the yield potential of each clone. According to Tesfa et al. (2020), factor loadings exceeding ±0.3 were considered significant, further supporting the contribution

Table 4: Cluster means across eight cane yield and juice quality traits

Cluster No.	Centres							
	1	2	3	4	5	6	7	8
1.	86.081	272.82	2.548	19.281	83.872	13.703	11.475	13.846
2.	82.543	237.504	2.407	19.183	76.962	13.784	10.628	14.167

of these traits. Similar findings were reported by Barreto et al. (2021), where cane yield and CCS yield were the dominant factors under PC1.

The second principal component (PC2) had an eigenvalue of 1.40, accounting for 17.61% of the total variation. This result was consistent with Sally et al. (2021), who reported 20.09% variation explained by PC2. Traits with distinctly

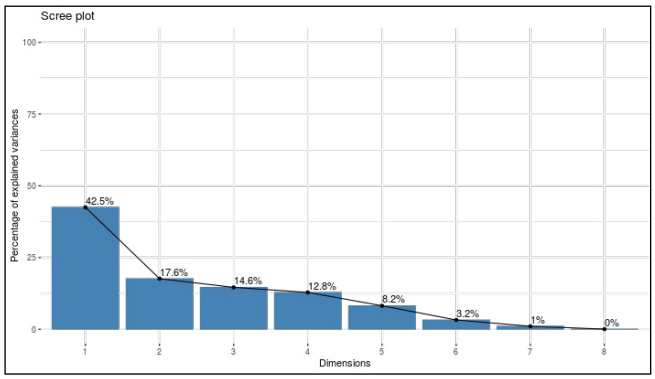

Figure 3: Screen plot constructed for eight principal components, showing contributions of PCs in variability

high positive factor loadings (Table 6) in this component were number of millable canes (0.35) and stalk length (0.415), highlighting their importance in determining plant architecture and productivity. Similar findings were reported by Vinu et al. (2022) for cane yield and stalk length.

Together, PC1 and PC2 explained 60.11% of the total variability, emphasizing a strong correlation among the studied traits. The third principal component (PC3) had an eigenvalue of 1.16, contributing 14.58% of the

Table 5: Eigenvalues of eight principal compounds

Principal compounds	Eigenvalue	Percentage of variance	Cumulative percentage of variance
PC1	3.4	42.5	42.5
PC2	1.409	17.611	60.111
PC3	1.167	14.587	74.699
PC4	1.026	12.828	87.527
PC5	0.654	8.179	95.705
PC6	0.258	3.228	98.934
PC7	0.082	1.027	99.96
PC8	0.003	0.04	100

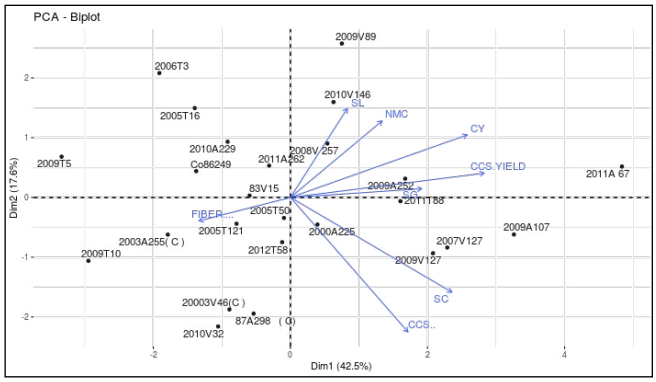

Figure 4: Biplot graph illustrating trait loadings, genotype distribution patterns, and genotype-trait relationships

variation. This component was mainly influenced by number of millable canes (-0.659), fiber content (-0.645), and sucrose content (0.322), indicating its relevance to biomass and quality traits. PC4 had an eigenvalue of 1.02, explaining 12.82% of the variation. Key traits in this component included stalk length (0.746) and stalk girth (0.419), confirming their role in plant vigor. These results aligned with Tolera et al. (2023), who also observed similar associations for stalk length and girth in the fourth PC. Overall, the results demonstrated that the first four PCs captured the majority of trait variability, with PC1 and PC2 being particularly significant in defining yield-related and structural traits. This analysis provided valuable insights into trait interrelationships, aiding in the selection of superior sugarcane genotypes for breeding programs.

The PCA biplot provided a comprehensive visualization of the relationships among traits and sugarcane clones, illustrating both the strength of trait contributions to the principal components and their intercorrelations. This graphical representation helped distinguish genotypes based on their trait performance, aiding in selection decisions. Figure 4 presented a PCA biplot based on eight quantitative and qualitative morphological traits, effectively highlighting their interrelationships. The plot distinctly separated cane yield (high PC1 score) from quality traits (high PC2 score), indicating that these trait groups contributed differently to genetic variation. The cosine angle between vectors further clarified these relationships: acute angles (<90°) represented positive correlations, right angles (90°) indicated no correlation, and angles close to 180° signified negative correlations.

It was evident from the biplot that fiber content (%)

Table 6: Loadings (Eigenvectors) of correlation matrix among eight cane yield and juice quality traits

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
No. of millable canes	0.24	0.357	-0.659	-0.107	0.201	0.547	0.168	-0.033
Stalk length	0.149	0.415	-0.032	0.746	0.355	-0.306	-0.161	0.043
Stalk girth	0.344	0.041	0.322	0.419	-0.595	0.494	0.045	-0.034
Sucrose (%)	0.423	-0.442	-0.15	0.161	0.064	-0.287	0.699	-0.054
Cane yield (t ha^{-1})	0.463	0.292	0.038	-0.313	-0.164	-0.33	-0.187	-0.656
CCS (%)	0.308	-0.627	-0.144	0.116	0.267	0.2	-0.593	-0.124
CCS yield (t ha^{-1})	0.507	0.113	-0.023	-0.272	-0.144	-0.22	-0.197	0.74
Fiber (%)	-0.239	-0.109	-0.645	0.21	-0.598	-0.287	-0.175	-0.011

was positioned on the opposite axis from cane yield, stalk length, CCS yield, number of millable canes, and sucrose (%), reflecting a negative correlation between fiber content and these traits. This suggested that improving fiber content might negatively impact yield-related traits, posing a challenge in simultaneous genetic improvement. Additionally, traits such as sucrose (%) and CCS (%) formed an acute angle (<90°), indicating a strong positive correlation. This implied that selecting for higher sucrose content would also enhance CCS (%), improving overall sugar recovery. The close clustering of traits further supported their positive associations, suggesting that improving one trait was likely to result in improvements in related traits, thereby increasing breeding efficiency.

This biplot also highlighted specific genotypic performance. Genotype 2011A 67 exhibited the highest mean values for

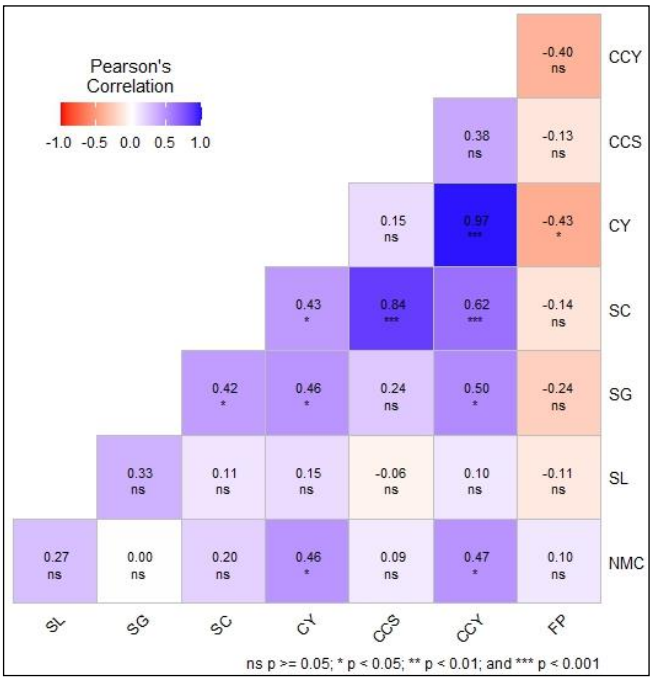

Figure 5: Pearson's phenotypic correlations among yield and quality traits evaluated in 25 elite sugarcane clones

cane yield and CCS yield, making it a promising candidate for high-yielding sugarcane breeding programs. Meanwhile, clone 2009V 127 showed the highest mean values for sucrose content and CCS (%), indicating its potential for improving sugar quality traits. These genotypes, being divergent from others due to their extreme values for key traits, represented valuable genetic material for sugarcane crop improvement programs. Overall, the PCA biplot provided crucial insights into trait relationships and genotype performance, facilitating targeted breeding strategies for yield and quality improvement in sugarcane.

Pearson correlation revealed nine significant positive correlations and one significant negative correlation among the eight yield and quality traits assessed (Figure 5), with p-values of <0.05, <0.01, and <0.001. Strong phenotypic correlations were observed between cane yield and CCS yield (0.97***), CCS (%) and sucrose (%) (0.84***), and CCS yield and sucrose (%) (0.62***). Moderate correlations were recorded between CCS yield and stalk girth (0.50*), CCS yield and the number of millable canes (0.47*), cane yield and sucrose (%) (0.42*), cane yield and stalk girth (0.46*), and cane yield and the number of millable canes (0.46*).

4. CONCLUSION

The clones 2011A 67 and 2009A107 showed higher cane and sugar yield than standard varieties, indicating strong genetic potential for hybridization. PCA analysis identified cane yield, CCS yield, stalk length, and sucrose (%) as key traits that contributed to variation, highlighting their importance in selecting promising genotypes for breeding programs aimed at enhancing sugarcane productivity.

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