Genetic Diversity, Population Structure, and Marker-Trait Association Analysis in Sugarcane (*Saccharum* spp. L.) Genotypes of Ethiopia

Melaku Tesfa Oljira

A Dissertation Submitted to:
The Department of Applied Biology
College of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy in Applied Biology (Specialization in Plant Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

May 2025

Adama, Ethiopia

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Approval Page

We hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the dissertation entitled "Genetic Diversity, Population Structure, and Marker-Trait Association Analysis in Sugarcane (*Saccharum* spp. L.) Genotypes of Ethiopia" by Melaku Tesfa Oljira.

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We, the undersigned, members of the Board of Examiners of the dissertation open defense by Melaku Tesfa Oljira have read and evaluated the dissertation entitled "Genetic Diversity, Population Structure, and Marker-Trait Association Analysis in Sugarcane (*Saccharum* spp. L.) Genotypes of Ethiopia" and examined the candidate during open defense. This is, therefore, to certify that the dissertation is accepted for partial fulfillment of the requirement of the degree of Doctor of Philosophy in Plant Biotechnology.

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Declaration

I hereby declare that this Dissertation entitled “Genetic Diversity, Population Structure, and Marker-Trait Association Analysis in Sugarcane (*Saccharum* spp. L.) Genotypes of Ethiopia” is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials used for this thesis have been duly acknowledged through appropriate citations.

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Recommendation

We, the supervisor(s) of this dissertation, hereby certify that we have read and revised the dissertation entitled "Genetic Diversity, Population Structure, and Marker-Trait Association Analysis in Sugarcane (*Saccharum* spp. L.) Genotypes of Ethiopia" prepared under our guidance by Melaku Tesfa Oljira submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Biotechnology. Therefore, we recommend the submission of the dissertation to the department for further review and defense.

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PUBLICATIONS DURING CANDIDATURE

Melaku Tesfa, Esayas Tena, Mulugeta Kebede (2023). Characterization and morphological diversity of sugarcane (*Saccharum officinarum L.*) genotypes based on descriptors traits. Journal of crop improvement. https://doi.org/10.1080/15427528.2023.2277473.

Melaku Tesfa, Esayas Tena, Mulugeta Kebede (2024). Multivariate analysis of genetic diversity among sugarcane clones (*Saccharum* spp.). Scientifica, Volume 2024, Article ID 4002024, https://doi.org/10.1155/sci5/4002024.

Melaku Tesfa, Esayas Tena, Mulugeta Kebede . Genetic variability, heritability, and genetic advance in sugarcane (*Saccharum* spp. hybrid) genotypes. Wiley, Agronomy Journal. Under review

Melaku Tesfa, Esayas Tena, Mulugeta Kebede. Correlation and path coefficient analysis in sugarcane (*Saccharum* spp. hybrid) genotypes. Agricultural Science Journal. Under review

Melaku Tesfa, Tilahun Mekonnen, Esayas Tena, Mulugeta Kebede, Kassahun Tesfaye. Genetic diversity and population structure of sugarcane (*Saccharum* spp.) genotypes: as revealed by SSR molecular markers. Elsevier, Crop Design Journal. Under review

ACKNOWLEDGEMENT

First and foremost, I would like to thank My Lord Jesus Christ for His love, health, strength, and protection throughout my PhD studies. His abundant blessings upon my family and me make everything possible.

I am proud and pleased to express my profound respect and gratitude to my advisors, Dr. Esayas Tena and Dr. Mulugeta Kebede, for their meticulous support and scientific guidance throughout the study period. I am also grateful for their supervision and guidance from research planning to final completion. I am deeply indebted for their constructive and valuable comments while preparing articles and the dissertation.

My heartfelt gratitude goes to Applied Biology Department, Adama Science and Technology University, for admitting me to the PhD study programme and for providing the necessary support. I am grateful to my former institute, the Ethiopian Sugar Corporation for allowing me to pursue the doctoral programme and partially fund my dissertation research. I would like to thank Fincha Sugar Factory for providing me with logistics to transport the experimental materials. I am very thankful to Ethiopian Sugar Corporation; Research Directorate for providing me vehicle during my trips to experimental sites. My sincere gratitude also goes to Wonji/Shoa Sugar Factory for providing me with agricultural input for the field experiments.

I would like to thank Fincha Research Station field assistants and researchers Negero Taye, Alemayehu Gadissa, Brihanu Fedessa, and Diribu Tesfaye for supervising seed cane preparation activities. I am grateful to all Kessem Research Station field workers, including Ali Hamza, Yebirzaf Belay, Lema Eka, and Yimer Derese, for their assistance with data collection and experimental field management. I am grateful to all Kessem research station laboratory technicians, Yohanis Garuma, Demitu Tibebu, and Kifilu Degefa for their assistance with cane sample analysis.

I would like to thank Metehara Research Centre Technical and Field Assistants Tesfaye Diriba and Nuredin Ahmed for their assistance with data collection, and experimental field management. I would like to thank all Metehara Research Center laboratory technicians, Kebede Feyera, late Tolessa, and late Kebebush Yadeta, for their help in cane sample analysis. I would like to express my heartfelt gratitude to Ato Abera Degefa, former Metehara Research Centre manager, for his assistance with various management aspects

of the study, as well as his hospitality during my stay in his home while conducting my research at the Metehara site. I am profoundly grateful to Ato Tilahun Tadesse, central laboratory head, for his keen support of materials and consumables while I was executing laboratory activities.

My heartfelt appreciation also goes to Institute of Biotechnology, Addis Ababa University for allowing me to do the molecular marker part of my study in their plant genetics laboratory and for providing me with consumables to use in the laboratory. I would also acknowledge Temesgen Dagnaw for his generous and unreserved technical assistance during lab work of this study. I am extremely grateful to Professor Kassahun Tesfaye, who genuinely allowed me to do the molecular part of my project in the plant genetics laboratory of the Department of Microbial Sciences, College of Natural and Computational Sciences, Addis Ababa University and to use consumables found in this laboratory without any limitations. I could not accomplish my research without his honorable support. I would like to express my heartfelt respect and gratitude to Dr Tilahun Mekonnen for providing laboratory reagents, PCR components, gel components, and unreserved technical advice and supervision in the laboratory. I would like to thank Professor Tileye Feyissa, Professor Tesfaye Sisay, and Dr Addis Simachew for their permission to provide laboratory equipment and consumables. I thank Mr. Belay Tolera and Dr Genet Atsbeha for their assistance with my field and laboratory data analysis.

I would like to express my deep gratitude to my instructors, PhD classmates, and colleagues for their encouragement and insightful scientific discussions. Your support and friendship have made my academic journey more enjoyable and fulfilling.

I would like to express my heartfelt admiration and gratitude to my sympathetic wife, Lemlem Misgana, for her extraordinary sacrifice in full-time care for our children (Milkyas and Heran). *Baby* and *Mita*, thank you for making me laugh after every difficult day. I am grateful to my sisters, brothers and relatives for their unwavering care, encouragement, and support throughout my academic career. Finally, I would like to express my gratitude to my father, late Tesfa Oljira, and my mother, late Bogalech Niguse, for their unreserved support, encouragement, and advice that have kept my spirit and motivation high throughout my educational journey.

CONTENT

LIST OF TABLES

TABLE PAGE

LIST OF APPENDIX TABLES

LIST OF FIGURES

FIGURE PAGE

ACRONYMS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AM	Association Mapping
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
CIMMYT	International Maize and Wheat Improvement Center
CSA	Central Statistics Agency
CTAB	Cetyl Tri-methyl Ammonium Bromide
CV	Coefficient of Variations
DArT	Diversity Array Technology
DUS	Diversity, Uniformity, and Stability
EDTA	Ethylene Diamine Tetra Acetic Acid
ESC	Ethiopian Sugar Corporation
EST	Expressed Sequence Tag
FAOSTAT	Food and Agricultural Office Statistics
GA	Genetic Advance
GAM	Genetic Advance as Percent of Mean
GBS	Genotyping by Sequencing
GCV	Genotypic Coefficient of Variation
GD	Genetic Distance
GLM	General Linear Mode
GTP	Growth and Transformation Plan
GWAS	Genome Wide Association Study
H	Nei's Genetic Diversity
H^2	Heritability
HVA	Handles Vereeniging Amsterdam
I	Shannon Information Index
ISO	International Sugar Organization
ISSR	Inter Simple Sequence Repeat
K	Kinship Relationship
LD	Linkage Disequilibrium
M.A.S.L	Meter above Sea Level
MAS	Marker-Assisted Selection
MCMC	Markov Chain Mont Carol
MLM	Mixed Linear Model
MTA	Marker Trait Association
PAGE	Polyacrylamide Gel Electrophoresis
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCV	Phenotypic Coefficient of Variation
PIC	Polymorphic Information Content
PVP	PolyVinyl PolyPyrrolidone

Q	Population Structure
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragmented Length Polymorphism
SAS	Statistical Analysis System
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
Ta	Annealing Temperature
TAE	Tris Acetate EDTA
TASSEL	Trait Analysis by ASSociation, Evolution and Linkage
TBE	Tris Borate EDTA
TEMED	N,N,N-Tetramethyl Ethylene Diamine
TRAP	Targeted Region Amplified Polymorphism
TVD	Top Visible Dewlap
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UPOV	International Union for the Protection of New Varieties

ABSTRACT

Sugarcane (Saccharum spp. hybrid) is the primary sugar and bioenergy crop in tropical and subtropical regions, including Ethiopia. However, its production and productivity have been severely hampered mainly due to a lack of improved varieties. Analyzing sugarcane germplasm genetic diversity and population structure provides valuable genetic information that breeders can use in improvement programs. In Ethiopian sugarcane germplasm collections, many genotypes remain phenotypically and molecularly uncharacterized. Additionally, no studies on marker-trait associations for Ethiopian sugarcane germplasm have been reported. Therefore, the present study aimed to investigate the genetic diversity, population structure, and association mapping of major agronomic traits of sugarcane genotypes of Ethiopia. Field experiments were conducted at Metehara and Kessem Sugar Estates between 2021 and 2022, involving 144 sugarcane genotypes. The experiment was laid out as a partially balanced lattice design with two replications. The data collected on 16 qualitative traits, 16 quantitative traits and 20 SSR markers was subjected to different statistical analysis. With regard to qualitative morphological diversity, the results indicated that the highest Shannon diversity index (H') was recorded for stalk corky patches while the lowest has been recorded for stalk growth crack. The H' pooled across qualitative traits by country of collection ranged from 0.00 for Mauritius and Brazil to 0.83 for India, with a mean of 0.62, implying there was high phenotypic variability within the Indian population and among the overall studied populations. The genotypes were grouped into four distinct clusters based on qualitative traits. The analysis of variance revealed significant differences ($P < 0.01$) among genotypes for all quantitative traits. PCA result showed the first four principal components (PCs) contributed to 81.44% of the overall variation. Multivariate cluster analysis grouped the genotypes into five distinct clusters based on genetic distance. Highly significant positive genotypic and phenotypic association ($P < 0.01$) was revealed between cane yield and traits, including sprouting percentage, tiller number, millable cane number, stalk height, single cane weight, and internode number. GenotypeCP6023, CP70/321, Mex57/197, D41/46, CP971944, B657/150, and B49-244 were selected based on Smith-Hazel selection index. The genotypic and phenotypic path coefficients showed a significant positive direct effect of the number of millable canes (1.174 and 0.75936) and single cane weight (1.067 and 0.49977) on cane yield. The study found high genotypic variability, high heritability, and high genetic advance for the number of tillers, millable canes, and cane yield, indicating that these traits can serve as selection criteria. Sugarcane genotypes CP6023, CP70/321, Mex57/197, D41/46, CP971944, B657/150, and B49-244 were selected for their higher mean performance of sugar yield than standard check and commercial varieties. Molecular analysis revealed a high level of genetic diversity in the markers, with an average of 1.9 numbers of alleles, effective alleles of 1.59, Shannon's information index of 0.35, Nei's gene diversity of 0.48, and polymorphic percentage loci of 88.59%, polymorphic information content of 0.64, and genetic differentiation of 0.37. Among the study populations, India had the most observed alleles (1.86), effective alleles (1.60), Shannon's information index (0.49), Nei's gene diversity (0.34), and unexpected heterozygosity (0.34). Cluster analysis using the UPGMA method showed four main clusters. The population structure analysis also identified six subpopulations within the genotype sets. AMOVA analysis elucidated that 96% and 4% of the total variability were within and among populations, respectively. The principal coordinate analysis grouped the genotypes into four clusters in conformity with cluster analysis. Similarly, ten of the SSR markers UGSM665, UGSM667, UGSM681,

SMC1852LA, SMC286CS, SMC477CG, SMC36BUQ, SOMS168, SOGL41, and P89, showed high discriminatory power, with polymorphic information content values above average. General linear model and mixed linear model analysis showed that marker alleles UGSM665-8, SMC36BUQ-21, and P-142-7 were significantly associated ($p < 0.001$) with the number of millable canes, single cane weight, and leaf width traits, respectively. In this study, considerable phenotypic and genotypic genetic diversity was observed among the studied genotypes. In general, the results from this diversity and marker-trait association study based on qualitative and quantitative traits and SSR markers will be useful for breeders in selecting the best parental combinations for starting effective breeding populations and in marker-assisted breeding. Therefore, the current study will contribute to paving reliable ways for future sugarcane breeding programs in the country.

Keywords: Molecular markers, Multivariate analysis, Qualitative trait, Quantitative trait, Simple sequence repeat

CHAPTER ONE
1. INTRODUCTION

Sugarcane (*Saccharum* spp. hybrid) is a perennial monocot crop native to tropical regions. It belongs to the genus *Saccharum* and the family Poaceae, which comprises many essential monocotyledonous crop species such as maize, rice, wheat, sorghum, and barley (Poeaim et al., 2017). *Saccharum* has six distinct species, including *Saccharum officinarum* (2n = 8x = 80), *Saccharum robustum* (2n = 8x = 60-80), *Saccharum spontaneum* (2n = 10x = 40-128), *Saccharum barberi* (2n = 81-124), *Saccharum sinense* (2n = 111-120), and *Saccharum edule* (2n = 60, 70, and 80) (Moore et al., 2014). Sugarcane genetics is among the most complex in the plant kingdom due to its high ploidy levels (5x-16x), aneuploidy, and heterozygosity (Moore et al., 2014; Thirugnanasambandam et al., 2018). High ploidy level results in a total genome size of around 10 GB, which is ten times larger than the sorghum genome (Debibakas et al., 2014). *S. officinarum* is the most widely cultivated species because it stores more sucrose in ripened internodes (Pereira et al., 2017). Modern sugarcane cultivars are hybrids created by crossing *S. officinarum* (Nobel cane) and *S. spontaneum* (wild cane) species, followed by numerous backcrosses to *S. officinarum* (Hapsoro et al., 2015).

Sugarcane is the primary source of sugar and bioenergy, accounting for 60% of ethanol and 80% of white sugar production globally (Yang et al., 2020). It is currently grown commercially in over 120 countries between latitudes 30°N and 30°S worldwide. Brazil, India, China, Thailand, and Pakistan are the world's top five sugarcane producers in 2023 (Arora, 2024) while, South Africa is the leading sugarcane producing country in Africa (Thibane et al., 2023). In 2021, 1,859,390,044.30 tonnes of sugarcane were harvested from 26,349,551 hectares, with an average yield of 70.57 tonnes per hectare worldwide. While African total production in 2021 was 94,281,939.52 tonnes harvested from 1,578,685 hectares at a yield of 59.72 t/ha, Ethiopia harvested 1,169,527.4 tonnes of sugarcane from 26,462 ha at an average yield of 44.20 t/ha (FAOSTAT, 2024).

Sugarcane was introduced into Ethiopia from India during the Axumite Kingdom from the first to fourth centuries (Kobishanov, 1981; Munro-Hay, 1991), and commercial production began in 1951 at the Wonji/Shoa sugar mill by the Handles Vereeniging Amsterdam (H.V.A.), Netherlands' company (Esayas et al., 2018). Since then, sugarcane has been the sole raw material used for sugar production in the country's sugar processing

industries. The sugar industry plays a vital role in Ethiopia's socioeconomic development. It produces sugar, bioenergy, and various other products. Byproducts from sugar processing are utilized as animal feed and organic fertilizer. The industry also generates electricity through cogeneration, which supplements the national power grid. Additionally, it contributes to foreign currency earnings, creates numerous job opportunities, and stimulates investment in other sectors of the economy (Esayas et al., 2018).

Currently, sugarcane is produced by nine sugar estates in the country, with annual sugar and ethanol production of 400,000 tonnes and 25,388 m^3 (Esayas et al., 2018; USDA-FAS, 2024), respectively. With this production, Ethiopia ranks 48^{th} in sugar production globally, in 2021 (Reporterlinker, 2022). However, this production could not satisfy domestic consumption, contributing only 62% of the country's annual sugar demand, which is about 650,000 tonnes annually. Therefore, to fill this gap, the government imports 200 to 300 thousand tonnes of sugar annually, spending a high amount of foreign currency (USDA-FAS, 2019; ESC, 2020). Consequently, Ethiopia's current per capita sugar consumption is low and estimated to be 10 kg, even below the African average (18.2 kg), while the world average per capita consumption is 26.7 kg (ESIG, 2023; USDA-FAS, 2023). Despite this, the country is endowed with favorable climate for sugarcane and has enormous production potential and market opportunities. There is 1.4 million ha of land suitable for sugarcane production, hugely available trainable and productive manpower, and vast market outlets. These conducive environments would make the country attractive for private investors to play a part in the production and processing of sugarcane (ESIG, 2023).

On the other hand, the decline in sugarcane yield has become the most pressing issue in Ethiopian sugarcane cultivation. Tesfaye (2021) found that sugarcane yields at Finchaa, Wonji/Shoa, and Metehara Sugar Estates decreased by an average of 26.63%, 48.63%, and 49.03% between 1969 and 2008. In 2021, the average annual productivity of seven sugar estates was around 44.2 tonnes per hectare (FAOSTAT, 2024). Although Ethiopia's sugarcane productivity is still at par with many countries, there is a gap between the potential yield and the current achieved yield. Productivity declines can be attributed to a combination of poor management systems and a lack of innovative and improved technologies, the major one being lack of improved varieties. To alleviate the existing production challenges, Ethiopian cane production must employ advanced technologies

such as planting high-yielding varieties that suit to various agro ecologies and tolerant to biotic and abiotic stresses (Esayas et al., 2018).

The Ethiopian sugar industry has historically been relying on sugarcane varieties introduced from various breeding stations worldwide. These introduced varieties are not well-suited to the country's diverse agroecology, leading to the cultivation of only a few less productive varieties across the sugar estates that are deteriorating with long period of cultivation. To address this issue and increase sugarcane production and productivity, Ethiopian sugar industry plans to establish a sugarcane variety improvement facility and launch a full-fledged sugarcane breeding programme to develop improved and well-suited varieties. The International Society of Sugarcane Technologists (ISSCT) estimates that improvements in sugarcane varieties account for up to 60% of sugarcane productivity (Chen et al., 2011). Similarly, Seema et al. (2020) reported a threefold increase in global sugarcane yields over the last five decades, which they attributed to genetic improvements. Variety improvement is regarded as critical for increasing productivity through a breeding programme.

There are many sugarcane genotypes introduced to Ethiopia from many source countries as sugarcane setts and also in the form of sugarcane seed (fuzz). Furthermore, the Ethiopian sugarcane landraces have been collected and preserved (Esayas et al., 2018). An essential first step in any varietal development programme is to develop a germplasm with sufficient genetic variability (Cobb et al., 2019). Thus, the available germplasm should be characterized to determine the magnitude and distribution of genetic diversity and establish population structure among genotypes for strategic conservation and effective breeding (Adebabay et al., 2017). Accurately assessing genetic diversity and population structure is critical in sugarcane breeding. It helps to select desirable genotypes, identify diverse parents, and introduce desirable genes into the existing genetic base (Xiong et al., 2022).

Previous local studies reported sugarcane genetic diversity of Ethiopian genotypes using qualitative and quantitative morphological markers (Esayas et al., 2016ab; Mebrahtom et al., 2016; and Belay et al., 2023ab). There are also studies on genetic diversity and population structure of the local sugarcane germplasm using molecular markers like SSR and ISSR (Esayas et al., 2014, 2016c; Adebabay et al., 2017). However, these previous studies didn’t exhaustively include the genotypes and the molecular studies don’t represent

some introduced commercial varieties. Although their findings revealed the presence of diversity among the genotypes under consideration, they may not adequately estimate the existing variability in the germplasm. The available germplasm should be characterized with more number of informative molecular markers. DNA-based advanced molecular approaches have been recommended to complement morphological characterization for estimating genetic diversity and population structures in sugarcane populations (Marconi et al., 2011; You et al., 2016).

Morphological markers are few and usually affected by the environment and may not give a complete information on the genetic constitution of plant species (Singh et al., 2018a). In contrast to morphological markers, molecular markers are unaffected by environmental factors. Molecular markers are also polymorphic, abundant, reproducible, and inheritable according to Mendelian principles (Amiteye et al., 2021). Thus, it reflects the actual level of genetic variation with high stability and polymorphism (Poeaim et al., 2017; Wu et al., 2018). It gives a more accurate estimate of such variation than phenotypic or pedigree information (Quddus et al., 2019). Over the last decade, genetic diversity and population structure among *Saccharum* species have been assessed using molecular marker techniques. For instance, Singh et al. (2020) (SSRs), Singh et al. (2017a) (RAPDs), Padmanabhan and Hemaprabha (2018) (STMS), Devarumath et al. (2012) (ISSR), Singh et al. (2017b) (TRAP), and Poeaim et al. (2017) (AFLP) were among the many. Previous research has reported genetic diversity and population structure on some Ethiopian sugarcane genotypes. For example, Esayas et al. (2014) and Esayas et al. (2016c) used SSR markers, while Adebabay et al. (2017) used ISSR markers in their studies. Aside from that, only a few exotics and landraces were studied, while the Ethiopian Sugar Industry (ESI) has a vast collection of introduced genotypes and landraces. As a result, additional molecular genetic studies with polymorphic markers are required for the remaining germplasm.

In addition to the genetic diversity and population structure, linkage disequilibrium (LD)-based marker trait association is a powerful tool in enhancing the efficiency of marker-assisted selection. Marker trait association (MTA) is an essential approach for dissecting complex traits and identifying QTLs underlying trait variations in plants for marker-assisted breeding toward developing improved sugarcane cultivars. Recently, LD based marker trait association has become a popular alternative to bi-parental QTL mapping for identifying marker agronomic trait association in plant populations (Thomson et al., 2014;

Patel et al., 2015). Published results suggest that marker trait association is a valuable additional tool in the search to detect novel genes or quantitative trait loci for important agronomic characteristics (Gouy et al., 2015; Ukoskit et al., 2018).

Marker trait association has a high resolution as it uses recombination events that have occurred over an extended period, while bi-parental QTL mapping uses a limited number of recombination events; only two alleles at any given locus can be studied (Nguyen et al., 2020). Besides, marker trait association has several advantages, such as exploring unrelated individuals in natural diversity panels, potentially identifying multiple alleles, no need for expensive and tedious biparental populations, time saving and cost-effective, the possibility of exploiting historically measured trait data, using unknown pedigree, and can accommodate a large number of accessions.

Marker trait association has been exploited successfully to identify genomic regions contributing to agronomic traits at a great resolution based on linkage disequilibrium in sugarcane (Banerjee et al., 2015; Gouy et al., 2015; Racedo et al., 2016; Singh et al., 2016b). These studies have been conducted widely using different molecular markers such as AFLP (Coutinho et al., 2021), SSR (Bilal et al., 2015; Ukoskit et al., 2018), EST-SSR (Ukoskit et al., 2018), DArT (Debibakas et al., 2014), TRAP (Racedo et al., 2016). Despite a few emerging studies, research on marker-trait associations in Ethiopian sugarcane germplasm remains limited, particularly those integrating both morphological and molecular approaches. In spite of the development of advanced DNA sequence-based single nucleotide polymorphism (SNP), SSR markers are still a marker of choice in resource-limited laboratories where modern technology is not affordable. It is one of the most effective markers for genetic diversity, population structure analysis, and association mapping because of its large quantity, low dosage, co-dominant nature, reliability, and multi-allelic detection (Ali et al., 2019). As a result, SSR markers are markers of choice to investigate genetic diversity, population structure, and marker-trait association for sugarcane genotypes in Ethiopia for efficient crop improvement through breeding**.**

1.1. Statement of the Problem

Since its establishment, Ethiopia's sugar industry has relied on importing sugarcane varieties from abroad to meet the varietal requirements for sugar production. Over 1000 varieties have been imported and preserved in germplasm conservation gardens. Besides, more than 300 sugarcane landraces have been collected from all geographic regions of

Ethiopia, contributing to the broadening of the germplasm genetic base (ESC, 2021). The presence of considerable genetic resources per se should not be a final goal in breeding programs. To determine the diversity and population structure of the germplasm, these genetic resources should be characterised phenotypically (qualitatively and quantitatively) and molecularly. Furthermore, identifying markers associated with major agronomic traits is crucial in marker-assisted selection. These enable the breeders to select divergent parents for hybridization, to develop improved and well adapted varieties.

Researchers previously used morphological markers to study the genetic diversity and population structure of exotic and landrace germplasm (Esayas et al., 2016ab; Mebrahtom et al., 2016; Belay et al., 2023a). However, morphological trait analysis should be combined with molecular data to identify highly divergent parental genotypes suitable for crossing. Only a few molecular genetic studies have investigated the genetic diversity of exotic and landrace sugarcane materials, as well as population structure using SSRs (Esayas et al., 2014, 2016c) and ISSRs (Adebabay et al., 2017). Thus, most of the genotypes were left uncharacterized at the phenotype and molecular level and limited marker-trait association studies for Ethiopian sugarcane germplasm have been reported. As a result, more number of studies on genetic diversity in the available germplasm, population structure, and marker-trait association are critical for understanding the amount and distribution of genetic diversity in the available sugarcane germplasm and for future marker-assisted selection**.** Therefore, the present study was conducted to achieve the following objectives:

1.2. Objectives

1.2.1. General objective

- To assess the genetic diversity, population structure, and identify marker trait associations of major agronomic traits in Ethiopian sugarcane (*Saccharum* spp. L.) genotypes using morphological and SSR molecular markers.

1.2.2. Specific objectives

- To analyze the genetic diversity of sugarcane genotypes using qualitative and quantitative morphological markers
- To estimate genetic variance, heritability, and genetic advance in sugarcane (*Saccharum* spp. hybrid) genotypes

- To determine the correlation and path coefficient among yield and yield components in sugarcane (*Saccharum* spp. hybrid) genotypes

- To investigate the genetic diversity and population structure of sugarcane genotypes using SSR molecular markers

- To identify SSR markers that are associated with quantitative traits of sugarcane

1.3. Significance of the Study

Although the Ethiopian sugar industry has a large collection of exotic sugarcane genotypes and landraces, significant gaps exist in generating useful information with regard to their diversity, identification, and strategic conservation. Furthermore, the current classical breeding system may be more laborious, time-consuming, and costly. Several studies have been conducted on germplasm morphological genetic diversity, while limited studies have been conducted on molecular diversity. The present study has significant implications for sugarcane improvement for the following reasons:

- ❖ It allows breeders to select best parent material to develop varieties with desirable traits

- ❖ It contributes to the creation of a genetic marker database for use in marker-assisted selection.

- ❖ It identifies specific molecular markers associated with various important traits

- ❖ It helps in the conservation of sugarcane genetic resource for future breeding efforts

- ❖ It paves the way for more advanced genomic research to reveal more complex trait architecture

CHAPTER TWO
2. LITERATURE REVIEW

2.1. Origin and Distribution of Sugarcane

The origins of *Saccharum officinarum* are closely associated with human activity because it is a cultivated or a garden species not found in the wild (Sreenivasan et al., 1987). *S. officinarum* is thought to have originated and evolved in New Guinea, where it has been grown as a garden crop since 8000 B.C (Babu et al., 2022). Canes were traditionally used for archery, fencing, and house construction in the past and may have been selected perhaps with the help of animals like pigs or rats drawn to sweeter individual plants (Daniels & Roach, 1987). Along migration routes, sugarcane cultivation extended to China, India, and the Pacific islands. Around 500 B.C., it arrived in the Mediterranean. It was introduced to West Africa before Morocco, Egypt, Syria, Crete (Greece island), and Sicily, which were the primary producers until the fifteenth century (Babu et al., 2022).

The Portuguese and Spaniards introduced sugarcane cultivation to the New World in the sixteenth century. By the 17th century, it had spread throughout the Caribbean and Latin America, including Brazil, Mexico, and Peru. *S. officinarum* was grown scientifically in Mauritius, Reunion, Australia, Fiji, and South Africa before the Western movement (Irvine, 1977). Sugarcane plantations were established in South American, Caribbean, Pacific Island, and Indian Ocean countries during the 18th century. Today, the crop is grown in tropical and subtropical regions of more than 120 countries between latitudes 36.7°N and 31.0°S, with Brazil, India, Thailand, China, Pakistan, Mexico, Columbia, Indonesia, the United States, and Guatemala being the top ten countries in terms of production (WDA, 2022).

2.2. Taxonomy of Sugarcane

As per the taxonomic classification, sugarcane is classified under the genus *Saccharum* L., which is conventionally grouped with the tribe Andropogoneae within the Poaceae family. This tribe also comprises tropical and subtropical grasses, including sorghum and *Zea mays* (maize or corn). This tribe is further divided into groups, such as Saccharinae (Welker et al., 2014). Cultivated plants are more challenging to taxonomy because of their high mutation rate and extensive hybridization, which result in significant diversity in their variation patterns (López-Caamal et al., 2014). A similar situation arises in the taxonomy

of sugarcane, where plants belonging to five different genera combine to form the closely related interbreeding group known as the "*Saccharum* complex." According to Moore et al. (2014), the *Saccharum* complex consists of *Saccharum*, *Erianthus*, *Miscanthus*, *Narenga*, and *Sclerostachya* genera. These genera are distinguished by high levels of polyploidy and usually uneven chromosomal numbers (aneuploidy), which complicate taxonomy and lead to numerous revisions of the relationship between different taxa (Piperidis et al., 2020).

The *Saccharum* complex's genera have been the subject of more recent molecular investigation, which has led to proposals that the taxonomy should be reformed because several of the divisions seem to be polyphyletic (Hodkinson et al., 2002). Six primary species were thought to make up the *Saccharum* genus: four cultivated species (*S. officinarum* (2n = 80, x = 10), *S. barberi* (2n=111-120), *S. sinense* (2n=81-124), *and S. edule* (2n = 60-80), two wild species (*S. spontaneum* (2n = 40–128, x = 8) and *S. robustum* (2n = 60, 80) (Moore et al., 2014). According to Cheavegatti-Gianotto (2011), the name *S. officinarum* L. was coined by Carolus Linnaeus in 1752.

2.3. Ecology of Sugarcane

Due to its tropical origins, sugarcane is only cultivated in warm climates. However, it can withstand various tropical and subtropical temperatures and grow anywhere from 37°N in Southern Spain to 31°S in the Republic of South Africa (Srivastava & Rai, 2012) and altitudes up to 1500 masl or a little more. Sugarcane is grown under a wide range of rainfall, sun exposure, and temperature conditions. Around 20 to 30 °C is ideal for sugarcane sprouts; severe cold inhibits bud sprouting. A temperature of about 30 °C is optimal for tillering; below 20 °C causes it to slow down and eventually decrease (Santos & Diola, 2015). High light intensity and prolonged light exposure promote tillering. Variables, including water availability, spacing, manuring, weed management, etc., also greatly influence the tillering rate. Sugarcane grows best at an average mean temperature range of 24-32 ^{o}C. Temperatures below 5 ^{o}C and above 35 ^{o}C inhibit their growth, even in tolerant cultivars (Srivastava & Rai, 2012).

The crop performs well in tropical regions, receiving 600-1200 mm of annual rainfall (Malik & Hussnain, 2020). Rainfall during the active growth phase promotes fast growth, stalk elongation, and internode development. In general, two different types of climate are needed for sugarcane: (i) a lengthy, warm growing season with enough rainfall and

irrigation, long days with lots of sunshine, and higher relative humidity for quick growth and dry matter accumulation; (ii) a two- to three-month-long ripening season with warm days, clear skies and cooler nights and relatively dry weather without rainfall for buildup of sugar. Heavy rain is undesirable during ripening period since it lowers the juice's sugar concentration. Regarding the soil requirement for sugarcane cultivation, fertile, deep, well-drained and well-aerated loamy soils are ideal for sugarcane growth (Verheye, 2010). It can also thrive productively on lighter soils with sufficient irrigation and proper drainage systems and heavy clay soils supplied with organic matter. However, this crop is not suited for soils that are acidic, alkaline, or saline (Srivastava & Rai, 2012).

2.4. Biology of Sugarcane

Sugarcane is a crop of stout, jointed, fibrous stalks with sugar. It is a perennial crop with tillers at the bottom reaching 3-4 m in length and around 5 cm in diameter (Iqbal & Iqbal, 2014). This C4 plant exhibits remarkable efficiency in storing solar energy and converting it into chemical energy that can be harvested in sucrose and biomass output (Ahmad & Ming, 2024). The crop is cultivated primarily for its ability to accumulate a considerable amount (18–19%) of sucrose in its mature stem (Wu & Altpeter, 2015; Ferreira et al., 2017). Four general phases of sugarcane plant growth can be distinguished: the germination phase, the tillering phase, the grand growth phase, and the maturity and ripening phase. The first phase covers a period of planting up to the completion of germination of buds, which depends upon field conditions and lasts for 4-5 weeks. The tillering phase is the stage at which side shoots emerge from the axillary buds of an existing stalk to form additional cane stalks. The grand growth phase is the cane formation and elongation period, which may take 270-300 days. The last maturity phase and ripening for early mature varieties prevails for about 90 days. In this phase, rapid accumulation of sugar as well as conversion of simple sugar to cane sugar takes place (Srivastava & Rai, 2012).

2.5. Mode of Reproduction

Sugarcane reproduces mainly through vegetative propagation. It can also reproduce sexually through true seed but is less common in commercial cultivation. Sugarcane is used to produce planting material by taking advantage of its asexual reproduction capabilities. Sandhu et al. (2023) stated that asexual reproduction occurs in nodal buds attached to setts by rhizomes or stools. Cuttings from the stalk are the asexual method

used in commercial sugarcane propagation. These cuttings, which often contain three buds (eyes), are referred to as "seedcane" or "cane setts." They are called "seed canes" to differentiate them from actual, sexually reproduced seeds (Srivastava & Rai, 2012). A variety stays genetically stable and true to type when it is propagated using cuttings.

Sexual reproduction of sugarcane occurs through true seed, often called fluff or fuzz due to the presence of soft hairs. Sexual reproduction from true seed is primarily used for breeding purposes. Before 19 century, sugarcane was not known to be sexually reproducing, probably because it was not a valuable commodity (Pierre et al, 2014). Sugarcane flowering is a multi-step, complex process that photoperiods influence (Wickramasinghe et al., 2024). Temperature, day length, and other environmental factors influence flowering time. Certain varieties may flower profusely in their natural habitats but produce less when transplanted to other areas (Scarpari, 2015). Sugarcane is a cross-pollinating species despite its low selfing rate. Seven polycrosses experiments showed selfing frequencies ranging from 0 % to 45 % (Tew & Pan, 2010).

Sugarcane pollen is small and dispersed by wind. Under unfavorable environmental conditions (26^{o}C and 67% relative humidity), it rapidly desiccates following dehiscence, having a half-life of only 12 minutes, and becomes non-viable after 35 minutes (Leite do et al., 2013). Therefore, viable pollen is anticipated not to go very far throughout the field. Sugarcane pollen can be kept at 4^{o}C centigrade, and 90-100% relative humidity can still be somewhat viable (Moore and Nuss, 1987) for up to 14 days.

2.6. **Economic Importance of Sugarcane**

Sugarcane is one of the world's most valuable commercial economic crops (Srivastava & Rai, 2012). It is grown in approximately 120 countries in tropical and subtropical regions worldwide. Though its primary purpose is to produce white commercial sugar, it has recently received increased attention for its potential to produce ethanol, a green fuel (Singh et al., 2013; Crystian et al., 2018). This crop is expected to produce approximately 80% of the world's white sugar (ISO, 2020) and more than 35% of its alcohol (Parida et al., 2009). The crop also has much potential as a multipurpose crop. The green top can be used as cattle feed and fodder, filter cake as fertilizer, and bagasse as a raw material for paper, paperboard products, hardboard, second-generation ethanol, and cogeneration of electricity. In addition, the remaining debris from the field is used as low-quality cattle feed and mulch. Furthermore, it significantly contributes to the country's socioeconomic

growth by meeting domestic demand for alcohol and sugar, increasing foreign exchange earnings, creating significant job opportunities, and generating revenue (Santos, 2009).

2.7. Sugarcane in Ethiopia

According to Kobishanov (1981) and Munro-Hay (1991), sugarcane was introduced to Ethiopia from the first to the fourth centuries during the Axumite Kingdom. Since that time, smallholder farmers have cultivated sugarcane in various regions of the country, in their farms and homesteads. In the 2020/21 report by the Central Statistics Agency (CSA), it was noted that sugarcane was produced by 2913127 households across approximately 33,370 hectares, yielding an estimated total of 1,345,431.89 tons (CSA, 2021) from smallholding farmers. However, the production from smallholders is typically not utilized for industrial purposes; instead, it is primarily used for household consumption (such as chewing), making local sweeteners, animal feed, generating income through sales, constructing fences, and as firewood for cooking (Esayas et al., 2018). In some areas of Muslim communities, it is also used to prepare a local drink called 'Keribo.' To date, numerous local sugarcane landraces have been grown in different regions of Ethiopia by smallholder farmers. Recently, more than 300 local landraces have been collected from these sugarcane-growing areas and preserved in a conservation garden, where they are currently undergoing study for potential use in breeding programs and commercial production.

On the commercial side, sugarcane production began in 1951 on 5,000 hectares of land at Wonji, established by a Dutch company known as United N.V. Handles Vereeniging Amsterdam (HVA). This was followed by the Metehara and Fincha sugarcane plantations. Since the sugarcane produced by these plantations could not meet the growing demand for sugar in Ethiopia, the government implemented strategies to increase sugarcane production. This included new project development and the expansion of existing sugar factories under the previous 10-year Growth and Transformation Plan (GTP I & II). Currently, commercial sugarcane is cultivated at nine sugar factories, which include: Wonji Sugar Factory (16000 ha), Metehara Sugar Factory (10230 ha), Fincha Sugar Factory (21000 ha), Kessem Sugar Factory (2,823 ha), Beles Sugar Factory (13667 ha), Omo Kuraz-I Sugar Factory (9,519 ha), Omo Kuraz-II Sugar Factory (4,500 ha), Omo Kuraz-III Sugar Factory (2,300 ha), and Arjo Dedessa Sugar Factory (4,450 ha)

(FDREMF & ESC, 2019). Currently, these sugar factories have an average sugarcane productivity of 44.2 ton/ha in 2021(FAOSTAT, 2024).

Sugarcane plays a significant role in Ethiopia's economy. It is Ethiopia's main sugar crop, accounting for 100% of total sugar production. It contributes to national income through the production of sugar, molasses, and biofuel (ethanol), which are crucial for domestic consumption and export markets. The industry also provides both direct and indirect employment opportunities for many people. The establishment of sugarcane production is often linked to improvements in infrastructure in remote areas, such as irrigation systems, roads, and energy generation facilities. Additionally, sugarcane production in Ethiopia enhances food security by reducing the need for sugar imports and promotes the development of other industrial sectors, including beverages, food, pharmaceuticals, and alcohol factories (Ambachew & Firehun, 2010; ESC, 2017; Esayas et al., 2018).

Despite Ethiopia's favorable climate, suitable soil, over 1.4 million hectares of irrigable land, trainable and productive labor, and ideal geographical conditions for export market, several constraints hinder the sugar industry from reaching its full potential. The primary challenges include the use of low-yielding varieties, limited access to technology, insufficient irrigation water, poor field management practices, disease and pest prevalence, and a lack of agricultural inputs. Notably, the absence of improved varieties that can withstand biotic and abiotic stress significantly impacts commercial sugarcane plantations (Alemayehu et al., 2024). Ming et al. (2006) indicated that improved varieties can contribute to a 50–95% increase in global agricultural output.

Since its inception, the Ethiopian sugar industry has depended on importing exotic varieties for sugarcane production. These introduced varieties must be evaluated based on location and season (number of ratoons) to identify those with broad adaptability. More than twenty outstanding varieties have been released for commercial production over several years, boosting a mean potential cane yield of 100-240 tons per hectare, an estimated recoverable sugar percentage of 12.5%, and a sugar yield of 12.5 to 30 tons per hectare. Recently, two promising varieties—Keyi Shenkora and Tafach Shenkora—were released for commercial production from landrace collections, with a mean cane yield potential of 201-214 tons per hectare, an estimated recoverable sugar of 12.5%, and sugar yields ranging from 25.1 to 26.8 tons per hectare (Esayas et al., 2020).

Currently, the sugarcane germplasm of the Ethiopian sugar industry includes more than 1000 exotic varieties and 300 landraces (ESC, 2021). From this collection, a few genotypes have been evaluated using morphological and molecular markers, leading to the selection of some well-performing varieties for commercial production in various agro ecological zones of the country's commercial sugarcane growing regions. However, many of the imported varieties have not adapted well to Ethiopia's diverse agro-ecological conditions, leading to only a limited number of genotypes being cultivated. Therefore, it is essential to establish a breeding station focused on developing improved varieties that are resistant to biotic and abiotic stresses and adapted to the country's different agroecologies. This effort should involve classical hybridization programs utilizing all necessary biotechnological tools as part of the Ethiopian sugar industry's breeding program.

2.8. Morphological Markers

Morphological markers are based on the phenotypic traits discovered in the field or from field specimens. In general, they represent genetic diversity manifested as variations in phenotypic traits, such as the presence or absence of specific phenotypic traits, like leaf shape, hairiness, and the relative variability in plant height and color (Gökbayrak et al., 2010). Morphological characteristics are a type of traditional marker used in germplasm management. These markers are useful for diversity research because the marker's inheritance can be visually monitored. They effectively determine plant agronomic value and taxonomy classification, particularly when the traits are highly heritable (Yoseph et al., 2005). These morphological markers are readily available, do not require specialized equipment, are quick and simple to score, and can be studied at a reasonable cost (Nadeem et al., 2017).

Certain morphological characteristics are genetically based and unaffected by external influences, making them resistant to change (Piscitelli, 1994 as cited in Khalid et al., 2016). These are strongly inherited and can be called constant markers. Conversely, other indicators are more easily altered by their environment, either slightly or significantly. Environmental factors typically influence quantitative traits, such as size, numbers, color, etc. Furthermore, these markers have some disadvantages, such as low polymorphism, low heritability, late expression, sensitivity to developmental stage, labour-intensive, time-consuming, and necessitating the assistance of a species expert (Yoseph et al., 2005; Kesawat & Das, 2010), which may affect the estimation of genetic relationships. The use

of environment-neutral molecular markers, which focus directly on variations caused by genes or genetic material, has recently significantly overcome the limitations of morphological markers (Varshney et al., 2006).

2.8.1. Qualitative and quantitative morphological markers

Despite the similar appearance of sugarcane cultivars, their morphological characteristics vary widely (Chidambaram & Sivasubramaniam, 2017). Morphological markers have historically been used to identify and differentiate sugarcane varieties. Taxonomists and plant breeders use morphological markers to characterize and categorize sugarcane varieties into related groups, although molecular markers have recently become more widely used. The International Union for the Protection of New Varieties (UPOV) conducts the diversity, uniformity, and stability (DUS) test of sugarcane varieties using morphological descriptions of varieties, (Yu & Chung, 2021). The shapes of the vegetative organ are examples of stable traits that are more useful in distinguishing sugarcane variety (Windiyani et al., 2022). Khan et al. (2017a) identified several qualitative character forms for sugarcane, which are classified as discrete, continuous, and combination characters known as standard descriptors. Some discrete morphological markers include auricle shape at the edge of the leaf sheath, ligule shape at the leaf-sheath joint, stool habit, unexposed stem color, ivory markers on internodes, bud shape, bud cushion, and leaf color. Continuous morphological characteristics include the color of the exposed stem, the node swelling, the wax bloom on the internode, the color of the growth ring, the color of the leaf, the color of the leaf sheath, the color of the leaf sheath spines, the color of the dewlap, and the flowering. Some characters, however, were combined, such as internode alignment, internode shape, pith inside the stem, growth cracks in the internodes, number of rows of root eyes at the node, root eye arrangement, bud size, bud groove, leaf carriage, leaf sheath spines, and leaf sheath clasping (Khan et al., 2017).

Sugarcane morphological quantitative traits are quantifiable morphological attributes that differ between sugarcane cultivars. These characteristics are crucial for agricultural practices and breeding programs. Some of the critical sugarcane morphological quantitative traits are sprouting percentage, tiller number, millable cane number, single cane weight, stalk height, stalk diameter, leaf length, leaf width, leaf area, Brix%, purity%, sucrose%, pol%, cane yield, and sugar yield. These traits frequently fluctuate within a population and are influenced by both environmental variables and numerous genes

(polygenic inheritance). Sugarcane breeders and researchers use morphological characteristics to identify varieties, construct phylogenetic trees (relationships), and develop selection criteria for desirable features that are economically significant.

2.8.2. Morphological markers based genetic diversity

Diversity refers to the degree of variation within or among species. Genetic diversity is a prerequisite for hybridization in crop development programmes. Thus, selecting rich and genetically varied parents is essential to the success of the sugarcane breeding effort. Numerous techniques, including morphological data, pedigree data, and agronomic variables, have made it easier to analyze genetic diversity in sugarcane (Cordeiro et al., 2003; Brasileiro et al., 2014). Assessing the type and degree of genetic variability will aid in selecting better parents for hybridization. However, environmental factors have a significant impact on the effectiveness of morphological markers in determining the true level of variability within sugarcane germplasm (Sing et al., 2011). The information gathered from diversity analysis is helpful when selecting diverse sugarcane parents for crosses and making selections to maximize heterosis.

Assessing the phenotypic and genetic variability of the germplasm, cultivars, or advanced breeding materials available in their breeding programs fascinates plant breeders. Several statistical techniques are applied for genetic diversity analysis depending on the data set being used. Using agronomic and morphological data, multivariate statistical analysis techniques, including principal component analysis (PCA), D^2 analysis, and cluster analysis, are frequently used to evaluate the genetic diversity of sugarcane (Mujahid et al., 2001; Esayas et al., 2016ab; Shahzad et al., 2016). Another strategy is quantifying the degree of genetic diversity in attributes to clarify genotype differences within a population and support breeding efforts.

In its germplasm conservation garden, the Ethiopian sugar industry has preserved more than 1000 exotic sugarcane varieties and more than 300 sugarcane landrace collections (ESC, 2021). Among these genetic materials, a few have been evaluated by Ethiopian researchers to study their genetic diversity. For instance, Shitahun et al. (2017) assessed 11 introduced Cuban genotypes using univariate genetic analysis based on nine quantitative traits. They found highly significant differences among the genotypes. In another study, Shitahun et al. (2022) conducted a multivariate analysis of 22 exotic genotypes, considering ten quantitative traits. Their cluster analysis identified five distinct groups, and

principal component analysis revealed that three principal components explained 75.63% of the total variation observed. Esayas et al. (2016b) studied 400 genotypes using 21 quantitative traits and reported significant differences among the genotypes. They identified six principal components that explained 79.26% of the total variation in the material tested. Their cluster analysis categorized all genotypes into 20 major distinct clusters based on the similarity coefficients. In a multivariate analysis, Belay et al. (2023a) determined the genetic diversity of 196 genotypes using 13 agromorphological and five biochemical traits. The results showed that the sugarcane genotypes were grouped into five clusters based on agromorphological traits and into two clusters based on biochemical traits**.**

In another study, Belay et al. (2024) performed a univariate genetic diversity analysis and reported highly significant differences among the genotypes for all traits examined. Additionally, Feyissa and Zinaw (2014) noted significant variability among the studied genotypes regarding internode length, plant height, stalk diameter, millable cane, cane yield (ha/month), and sucrose percentage at a significance level of $p < 0.01$. They also found significant variability in sugar yield (ha/month) at a significance level of $p < 0.05$.

In addition to quantitative trait based genetic diversity analysis, qualitative morphological traits had also been used to study genetic diversity and population structure in Ethiopian sugarcane genotypes. Accordingly, Esayas et al. (2018) studied genetic diversity of 196 sugarcane landraces and 15 exotic genotypes using 16 qualitative morphological traits. They reported high (0.80) Shannon–Weaver diversity index among the genotypes considered. In another study Esayas et al. (2016) that the Shannon-Weaver diversity index (*H'*) for all sample genotypes ranged from 0.43 for (stalk corky crack) to 0.98 (degree of internode alignment) with a mean of 0.87. In addition, Belay et al. (2020) studied 196 sugarcane genotype using 16 qualitative traits and reported Shannon-Weaver diversity index ranged from 0.08 to 0.94, with a mean of value 0.65, indicating the presence of a wide range of qualitative morphological trait variability among the sugarcane genotypes.

Although the research discussed above will significantly contribute to the improvement of sugarcane varieties within the breeding program, it is still insufficient compared to the overall germplasm collections that need to be evaluated for selecting diverse core parents for hybridization. Additionally, some studies have utilized univariate analysis and included only a few genotypes to estimate variability among them. Therefore, it is

recommended to conduct a study that considers multiple genotypes and quantitative traits using multivariate analysis to enhance the effectiveness and efficiency of the breeding program**.**

2.8.3. Heritability and genetic advance

Heritability is an index of the transmission of characters from parents to their offspring. It is generally expressed in percentages. The estimation of heritability helps the plant breeder select elite genotypes. It also measures the degree of resemblance between relatives and the correspondence between phenotype and breeding value. Genetic advance is an estimate of how much improvement or gain can be anticipated in a particular trait within a population while selecting individuals based on that trait. Genetic advance is mainly relying on factors such as selection intensity, genetic variability, and heritability. Knowledge of heritability estimates and genetic advances is more helpful in predicting gain under selection than heritability estimates alone (Shoba et al., 2009). High heritability and high genetic advance values of sugarcane traits imply that they are influenced more by genes than by the environment and can be considerably improved through selective breeding due to significant amount of genetic variation that can pass to next generation**.**

Previous studies have found heritability in various sugarcane characteristics (Ghura, 2005; Khaled & Ahmed, 2008; Alam et al., 2017; Béhou & Péné, 2020). Chaudhary (2001) found that cane weight (84%), millable cane number (88%), and stalk diameter (85%) are highly heritable traits. Esayas et al. (2016e) found high broad-sense heritability (h^2) for millable cane number (0.624), stalk height (0.624), single cane weight (0.672), stalk diameter (0.730), and poll percentage (0.608). Belay et al. (2023b) reported high heritability and genetic advance as a percentage of the mean for number of sprouted buds, number of tillers, and cane yield. Similarly, high heritability coupled with high genetic advance as percent mean was reported for sugar yield per hectare per month by Dereje (2018). Furthermore, high heritability with high genetic advance as per cent of mean was found in sugar yield at harvest (Kumar et al., 2018). In general, high heritability and genetic advance allow breeders to precisely select desired phenotypic variation with in a population**.**

2.8.4. Associations of major quantitative trait

Correlation analysis is critical for plant breeders to determine the relationship between yield and other variables. According to Krishna & Kamat (2017), it provides information about the strength of genetic associations between traits. For example, it can demonstrate how various cane and quality characteristics relate to yield and sugar content. Superior genotypes are selected from various breeding populations using estimates of the correlations between yield components (Esayas et al., 2016d). According to Tyagi and Lal (2007), correlation coefficient analysis estimates the correlation between the characters and offers breeders some guidance on selecting the best parents. Numerous researchers have demonstrated a correlation between sugarcane genotypes (Esayas et al., 2016d; Swamy et al., 2016; Pandya & Patel, 2017; Parihar, 2020). According to Agrawal and Kumar (2018), cane diameter showed the strongest positive correlation with cane yield at the phenotypic level, followed by plant height, number of millable canes, single cane weight, and germination rate. Parihar (2020) evaluated thirty-five sugarcane genotypes for a quantitative trait. The results showed that germination, the number of tillers, the number of millable canes (NMC), cane height (m), cane thickness (cm), and cane weight (kg) were all positively related to cane yield and sugar output. Esayas et al. (2016e) also found low to strong genetic correlations between cane and sugar yield, with genotypic correlation values ranging from -0.005 to 0.884 for cane yield and 0.027 to 0.999 for sugar yield

Likewise, Feyissa et al. (2014) found a favourable correlation between cane yield and millable cane number ($r = 0.57$), internode length, cane diameter ($r = 0.31$), and number of internodes ($r = 0.27$), as well as single cane height ($r = 0.50$). Plant height and sucrose percentage were also significantly positively associated with internode length. In contrast, millable cane weight ($r = -0.26$) and sucrose percentage ($r = -0.43$) showed a negative and highly significant correlation. Esayas et al. (2016d) discovered a significant positive correlation ($P < 0.01$) between cane yield and millable cane number ($r = 0.832$), single cane weight ($r = 0.528$), stalk height ($r = 0.517$), and sugar yield ($r = 0.987$). Furthermore, tiller count and cane diameter had a significant positive correlation ($P = 0.05$) with cane yield. Early researcher reported that genetic relationships were generally greater than phenotypic correlations (Esayas et al., 2016d), implying that genetic factor play a great role in determining the trait than no-genetic factor or environment.

2.8.5. Path coefficient analysis

Before selecting the parental material for crossing, a breeder must understand how other qualities impact a particular character. Direct and indirect impacts must be distinguished since correlation coefficients can occasionally be deceptive. A path coefficient analysis can segment trait correlations into components based on the direct and indirect effects of independent characteristics on the dependent character (Tyagi & Lal, 2007; Swamy et al., 2016). Numerous studies have been published on path coefficient analysis for genotypes in sugarcane (Esayas et al., 2016d; Swamy et al., 2016; Pandya & Patel, 2017; Parihar, 2020). Agrawal & Kumar (2018) performed path coefficient analyses on sixteen sugarcane genotypes. The results showed that germination percentages, the number of millable canes, single cane weight, and stalk diameter positively affected cane production.

Another study that used path coefficient analysis for quantitative traits found that the number of tillers, number of millable canes (NMC), cane height, cane diameter, cane weight, juice weight, CCS percent, and juice extraction percent all had a significant direct effect on cane yield. Feyissa et al. (2014) also found that millable cane number (0.812) had the most significant beneficial direct impact on cane yield, with single cane weight (0.682) and pol percent (0.550) following closely behind. On cane yield, however, stalk diameter and Brix percentage had significant adverse direct effects and positive indirect effects through single cane weight. Genotypes should be selected according to millable cane number, single cane weight, and pol percent to obtain high cane and sugar yields. According to Al-Sayed et al.'s (2012) path analysis, the number of cane stalks had the greatest direct and indirect effects on sugar yield, followed by sucrose percent and stalk weight. A similar study found that millable cane number (0.812) had the greatest positive direct effect on cane yield, followed by single cane weight (0.682) and pol percent (0.550). Nonetheless, single cane weight had an indirect positive effect on cane yield, whereas stalk diameter and Brix percent had significant negative direct effects (Esayas et al., 2016d). They also discovered that selecting genotypes based on the number of millable canes, weight of a single cane, and pol% resulted in increased cane and sugar yield.

2.8.6. Cluster analysis

Cluster analysis is a multivariate statistical method that organizes genotypes or accessions into homogeneous clusters based on shared traits. It is also known as clustering, taxonomy analysis, or segmentation analysis. Because genotypes are separated into groups or

clusters, there will be a highly significant association between members of the same cluster and a modest degree of linkage between members of other clusters. Euclidean distance and similarity level matrix are used in the cluster analysis. When observations start to be categorized into clusters, similarity measures are first computed between observations and between clusters. The primary output of cluster is a dendrogram, a graphical representation of classifications that depicts how clusters form.

Several studies used cluster analysis to classify sugarcane genotypes. Karpagam and Alarmelu (2017) examined 120 sugarcane genotypes for qualitative traits, categorizing hybrids into seven clusters. The most significant distance between clusters was recorded between clusters IV and VI, and then clusters I and III, III and VII, and VI and VII. Mebrahtom et al. (2016) investigated genetic divergence in 49 sugarcane genotypes using cluster analysis for 12 yield-contributing characteristics, discovering nine clusters. Using cluster analysis, Shahzad et al. (2016) studied 60 sugarcane genotypes and found they could be grouped into seven categories. Similarly, Arrey and Mih (2016) used five landraces from western Cameroon to conduct cluster analysis. The multivariate analysis showed that the five landraces formed three clusters: one included NBFAg53, two (SBK36 and SMU58), and two (NBFPC48 and SNC16) in the second cluster. Using cluster analysis Tocher's approach to evaluate 400 sugarcane genotypes on 21 quantitative features, Esayas et al. (2016d) found that the genotypes could be categorized into 19 main clusters and one singleton. Of these clusters, the genotypes in clusters one, two, and three were 136, 120, and 80, respectively**.**

Mahalanobis (1936) recommended the powerful multivariate analysis technique known as D^2 analysis. It is sometimes referred to as "Mahalanobis D^2 statistics" and is a tool for determining the genetic diversity of the available germplasm. By measuring the force of differentiation at the intra- and inter-cluster levels, this approach aids in selecting genetically distinct parents for use in hybridization programs. Along with calculating the proportionate contribution of each component character to the overall divergence, the statistics also quantify the degree of diversification. One of the most promising methods for determining genetic divergence within a population group is applying multivariate measures and the statistical distance concept (Esayas et al., 20l6b). Numerous researchers in the sugarcane crop have suggested using Mahalanobis D^2 statistics to assess net divergence in breeding. Bisht et al. (2017) used Mahalanobis D^2 statistics to analyze 36 sugarcane genotypes and found that the genotypes could be divided into 11 groups. In

order to determine the inter- and intra-cluster divergence, Esayas et al. (2016b) analyzed 400 sugarcane genotypes on 21 quantitative traits using Mahalanobis' D^2 statistics. According to the cluster analysis, the intra-cluster D^2 values ranged from 2.16 to 10.60, while the inter-cluster values ranged from 7.24-5864.

2.8.7. Principal component analysis

A multivariate data reduction technique called principal component analysis (PCA) aims to preserve as much of the variation seen in the data set as possible while shedding light on the relationship between two or more traits. According to Thapa et al. (2009), PCA effectively identifies the best genotypes using quantitative and qualitative data. PCA transforms multi-correlated variables into a new set of uncorrelated variables for future research. These additional variables are just linear combinations of the old ones. This is based on developing eigenvalues and mutually independent eigenvectors, or principal components, arranged according to decreasing variance sizes. These components provide scattered data plots ideal for analyzing correlation and underlying variability (Khodadadi et al., 2011).

Researchers have investigated and reported PCA of quantitative traits in sugarcane (Arrey & Mih, 2016; Ahmed & Gardezi, 2017a; Khan et al., 2019). Gemin et al. (2006) discovered three principal components in *Saccharum spontaneum*, which accounted for 82.47% of the total variance. In another study, PCA reduced twenty-two qualitative attributes to nine PCs with a cumulative variance of 62.40% and an eigenvalue >1 (Karpagam & Alarmelu, 2017). Likewise, principal component analysis (PCA) characterized 60 sugarcane genotypes. The analysis resulted in seven principal components (PCs) with eigenvalues greater than one and 72.1% genotype variability (Shahzad et al., 2016). After analyzing the PCA of morphological and quality traits, Aamer et al. (2018) found that the first four PCs explained almost 85% of the variation in the data. Similarly, Esayas et al. (2016b) used principal component analysis to evaluate 400 sugarcane genotypes on 21 quantitative traits. They found that six principal components accounted for 79.26% of the overall variation in the tested materials. Furthermore, Tahir et al. (2013) discovered that two principal components accounted for 88% of the variation observed in the breeding material.

2.9. Molecular Markers

Molecular markers are distinct DNA segments that correspond to specific chromosomal regions, which can be detected and its inheritance can be monitored reliably. As a result researchers and breeders are adopting this marker as valuable tool for a variety of purposes. According to Varshney et al. (2007) and Jiang et al. (2013), these markers are widely used for crop management and improvement, conservation of endangered species, identification of close relatives, systematics and molecular phylogeny discovery, and genetic diversity assessment. They are based on natural variations in nucleotide sequences caused by insertions, deletions, and substitutions (Redelings et al., 2024). Because DNA markers can cover the entire genome, they are widely distributed and highly abundant. These markers are persistent and unaffected by environmental factors, epistatic effects, or developmental stages. Furthermore, they allow for individual selection as early as the seedling stage and can be detected in all tissues at all stages of development (Soriano et al., 2006). Molecular markers provide accurate substitute tools that are time-saving and cover various areas of sugarcane taxonomy.

There are numerous molecular markers available, each with advantages and disadvantages. However, the following desirable characteristics are expected in an ideal molecular marker. 1) highly polymorphic nature 2) Codominant inheritance 3) A frequent occurrence in the genome 4) Selective neutral behaviours 5) Easy access; 6) High reproducibility 7) Easy data exchange between laboratories (Kumar et al., 2009). Unfortunately, no single molecular marker outperforms the others across all parameters (Ruvolo et al., 2013). As a result, the techniques used will be determined by the study's goal, the sensitivity level of the marker system, available funds, expertise, and facilities (Yoseph et al., 2005). According to Collard & Mackill (2008), the most effective molecular markers for identifying genotypic varieties should be relatively simple and inexpensive to deploy, and they should evolve quickly enough to change within populations.

Molecular markers can be codominant, meaning they can detect the allelic difference, or dominant, meaning they cannot tell the difference between two genes when they are heterozygous. From hybridization-based restriction fragment length polymorphism (RFLP) to polymerase chain reaction (PCR)-based randomly amplified polymorphic DNA (RAPD; Welsh & McClelland, (1990); amplified fragment length polymorphism (AFLP;

Vos et al., 1995); inter-simple sequence repeat (ISSR) (Zietkiewicz et al.,1994) and simple sequence repeat (SSR; Litt et al., 1989); sequence-based high throughput single nucleotide polymorphism (SNP) (Wakeley et al., 2001); and most recently, ultra-high-throughput genotyping by sequencing (GBS) (Elshire et al., 2013) has been established for plant genetic analysis. All of these marker technologies have continued to evolve. The majorities of PCR-based methods only requires small amounts of DNA and are simple to use. They can also reveal an almost infinite number of markers.

2.9.1. Molecular markers used to study sugarcane

In the development of sugarcane varieties, selecting parents for crossing necessitates a thorough understanding of breeding procedures as well as meticulous germplasm characterization and evaluation. Since DNA markers are reliable, plentiful, polymorphic, and unaffected by environmental factors, they can be valuable tools in this process (Amiteye, 2021). More recent genetic markers were created in the wake of isozymes, morphology, and cell markers. Sugarcane breeding increasingly uses molecular markers to study and modulate significant agronomic traits. These markers have been used in marker-assisted selection (MAS), genetic mapping, cultivar identification, quantitative trait loci (QTL) mapping, paternity analysis, and sugarcane genetic diversity research (Ahmad et al., 2018).

The development of molecular markers and associated detection methodologies forms the foundation for molecular marker-assisted breeding, which speed up the breeding program. DNA markers, such as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR), and single nucleotide polymorphisms (SNPs), are used in quantitative trait locus (QTL) mapping.

Several studies have previously identified QTLs related to various sugarcane traits, including disease resistance (such as resistance to mosaic virus and orange rust), tillering ability, ratooning ability, sugar content, and resistance to leaf blight. These findings have facilitated marker-assisted selection in breeding programs aimed at improving these traits in sugarcane cultivars. For instance, Lu et al. (2023) identified seven QTLs associated with mosaic resistance on the SNP genetic map. Similarly, Yang et al. (2018) detected three QTLs that control orange rust resistance in sugarcane (qORR109, qORR4, and qORR102).

In another QTL analysis, Wang et al. (2023) mapped a total of 26 QTLs related to tillering ability and 11 QTLs associated with ratooning ability on two high-quality genetic maps derived from a 100K SNP chip. Additionally, Wang et al. (2022) revealed six QTLs (qSLB-1 to qSLB-6) responsible for sugar leaf blight (SLB) resistance through their QTL analysis. Using an extensive genetic map constructed with over 1,000 AFLP and SSR markers, Aitken et al. (2006) identified 37 QTLs related to brix and pol percent. Costa et al. (2016) detected a total of 25 QTLs: six for stalk diameter (SD), five for stalk weight (SW), four for stalk height (SH), five for fiber content, two for sucrose percentage (Pol), and three for brix percent. Finally, Anusonpornpurm et al. (2008) identified thirty putative QTLs related to stalk diameter, tillering, and fiber content from 180 AFLP simplex markers.

2.9.1.1. SSR marker analysis in sugarcane

Simple sequence repeats (SSRs), the smallest class of simple repetition, are sometimes referred to as short tandem repeats (STRs), simple sequence length polymorphisms (SSLPs), or microsatellites. SSRs are sequence blocks of DNA motifs ranging from one to six base pairs repeated five to fifty times. These motifs are surrounded by sequences that are typically unique to an organism's genome but remain conserved across species (Pan et al., 2007). SSRs are categorised as mono-, di-, tri-, tetra-, penta-, or hexa-SSRs based on the number of repeated base pairs. Perfect, imperfect, and compound SSRs are distinguished by their perfect repetitions, interruptions with new nucleotides, and two or more tandem motifs (Selkoe & Toonen, 2006).

SSRs, also known as microsatellite markers, are considered to be among the best PCR-based markers for a variety of uses in sugarcane genetics research and breeding because they are co-dominant, multi-allelic, highly informative, occur in high relative abundance and uniform distribution across the genome, and are repeatable in experiments (Ali et al., 2019). Since closely related sugarcane species share a high degree of sequence conservation, successful amplification is possible (Andru et al., 2011). SSR markers are valuable tools that provide high-level genetic information and are used to study sugarcane genetic diversity and population structure (Liu et al., 2018; Ali et al., 2019), variety identification (Pan et al., 2016), genetic map (Marconi et al., 2011; Andru et al., 2011), genetic association (Racedo et al., 2016; Ukoskit et al., 2018), paternity analysis (Santos et al., 2014), evolutionary relationship among sugarcane species (Brown et al., 2007),

marker-assisted selection (Pan et al., 1999), etc. Furthermore, fluorescence-labeled SSR markers combined with high-performance capillary electrophoresis (HPCE) for polyploid sugarcane genotyping have improved accuracy and detection power (Fu et al., 2016; Ali et al., 2017).

EST-SSRs provided biological evidence for transcript isoforms in sugarcane, together with expected and newly discovered genes, which greatly advanced the field of gene identification. The genetic data of species with complex genomes, such as sugarcane, can also be accessed by ESTs (Vettore et al., 2003). Including 237,954 EST sequences in 43,141 clusters, the sugarcane Expressed Sequence Tag Project (SUCEST) is the most comprehensive dbEST for sugarcane (Vettore et al., 2001). More than 5000 unique SSRs have been found in scientific studies (Oliveira et al., 2009) utilizing the SUCEST database (sugarcane ESTs; Vettore et al., 2003). Two hundred twenty one (221) SSR markers have also been created by the International Sugarcane Microsatellite Consortium (ISMC) using sugarcane genomic DNA sequences as a basis (Cordeiro et al., 2000).

2.9.2. Molecular genetic diversity studies

A successful breeding programme is based on an understanding of genetic diversity, which allows for the crossing of different parents to introduce desired traits into any genotype. Screening genotypes for desirable traits is critical for producing improved genotypes, as diversity lends itself to the use of various characters in breeding programmes. Earlier sugarcane breeders use anatomical and morphological characteristics to determine how different varieties are related. Several molecular techniques have been used in recent years to study genetic diversity in sugarcane cultivars and progenitor species. Molecular markers are an effective tool for measuring genetic diversity through genetic traits. Molecular markers allow us to evaluate genetic variation at the DNA level. Several studies have shown that molecular markers, which provide a more precise estimate of genetic diversity, are commonly used to characterize *Saccharum* complex germplasm (Aitken et al., 2005; Mary et al., 2006).

Various molecular markers have been developed and widely used to increase our understanding of sugarcane diversity (Singh et al., 2020), including RFLPs (Lu et al.,1994; Besse et al.,1997), RAPDs (Poeaim et al., 2017; Singh et al., 2017a), AFLPs (Lao et al., 2008; Poeaim et al., 2017), ISSRs (Devarumath et al., 2012; Oliveira et al., 2017), SSRs

(Esayas et al., 2016c; Khalid et al., 2019), SRAP (Chang et al., 2002; Yu et al., 2019), TRAP (Que et al., 2009; Singh et al., 2017b), DArT and SNP (Bello et al., 2019).

2.9.2.1. SSR genetic diversity studies

SSR markers, with their hypervariability, high levels of polymorphism, and codominant inheritance (Agarwal et al., 2008), are frequently used among molecular marker techniques in sugarcane to estimate genetic variation at the molecular level (Sing et al., 2020). This variation results from variations in the number of repeat motifs in a locus due to replication slippage and/or unequal crossing-over during meiosis. According to Hemaprabha et al. (2005, 2013), microsatellite markers are highly specific. They can differentiate between even closely related germplasm lines, making them an effective tool for analysing diversity and improving plant breeding techniques.

Several studies have demonstrated the widespread use of microsatellite markers, which provide a more precise estimate of genetic variation, in the characterization of *Saccharum* complex germplasm. Padmanabhan and Hemaprabha (2018) used 20 pairs of SSR primers to study the genetic diversity and population structure of 133 sugarcane hybrid derivatives, finding a high level of heterozygosity. After applying 30 primer sets to 17 sugarcane accessions, Smiullah et al. (2013) discovered 62 DNA fragments with an average of 2.14 bands per primer and genetic similarities ranging from 62.90 to 90.30% on sugarcane. Cordeiro et al. (2001) used 21 primer sets to investigate the genetic diversity of five sugarcane genotypes and discovered 17 pairs of primers that were polymorphic. However, the SSR-identified cultivars had a low PIC value (or degree of polymorphism) (0.23).

Banumathi et al. (2010) used 40 SSR primer pairs to assess genetic diversity in 48 clones (20 from rice and 20 from sugarcane). Khalid et al. (2019) used 16 exotic sugarcane genotypes to study genetic diversity based on 46 microsatellite markers. The studied genotypes showed genetic similarity ranging from 71 to 93%. Twenty-one highly polymorphic SSR markers were employed by Hameed et al. (2012) to analyze the genetic diversity and DNA fingerprinting of twenty sugarcane cultivars. They found 144 alleles, with a mean of 6.8 and 3–11 alleles per marker. Esayas et al. (2014) investigated newly introduced sugarcane accessions in Ethiopia, using 22 SSR markers to amplify 260 alleles and determine population linkages and differentiation.

2.9.3. Population genetic structure in sugarcane

Population structure refers to the non-random distribution of genotypes; that is, mating does not occur randomly in a structured population. In general, population structure refers to the presence of varying degrees of genetic relatedness among a sample's subgroups. It results from the unequal distribution of alleles among populations of different ancestry. Population structure correction is critical when collecting genotypes, especially in a breeding programme where genetic interactions between breeding lines are highly variable (Sukumaran & Yu, 2014). Understanding population structure is required for developing effective plans to improve crop genetic resource collection, administration, and preservation. Principal component analysis (PCA) and clustering-based approaches are frequently used to infer population structure, which is crucial for population genetics and association research (Meisner & Albrechtsen, 2018). Admixture proportion inference is another common method used for this purpose (Pritchard et al., 2000). STRUCTURE (Pritchard et al., 2000) is a widely used Bayesian approach for estimating population structure. In sugarcane population structure research (Esayas et al., 2016c; Ali et al., 2019; Wu et al., 2019), SSR markers were used to divide the population into subpopulations.

Wu et al. (2019) classified 150 accessions into two subpopulations (Pop1 and Pop2) using population structure analysis, principal coordinate analysis (PCoA), and phylogenetic analysis. Whereas accessions native to China were concentrated in Pop2, Pop1 had the most clones. With the assistance of STRUCTURE software, Singh et al. (2020) conducted a population structure analysis and showed that the 139 sugarcane accessions utilized in the study were classified into three subpopulations (SG1, SG2, and SG3). In another study, Padmanabhan & Hemaprabha (2018) also discovered a population structure consisting of 133 elite types and five subpopulations. Sing et al. (2020) identified three subpopulations within the 139 sugarcane accessions used in their population structure study. In additional research, 96 lignin and sugar metabolism genotypes obtained using TRAP candidate genes were organized into two and three subpopulations, respectively (Junior et al., 2020). Five optimal groups of germplasm were identified by structure analysis of sixty-three sugarcane hybrids utilizing 107 SSRs and 136 AFLPs (Inés & Jorge, 2015).

2.9.4. Linkage disequilibrium in sugarcane

Linkage disequilibrium (LD) refers to the non-random association of alleles at different loci within the genome. It is a critical population parameter in genetic studies and plays a

significant role in association mapping (AM) of crops. LD aids in locating genes associated with desirable agronomic traits by utilizing phenotypic information collected during selection. Due to its breeding history, which includes a strong foundation bottleneck and low levels of meiosis (resulting in low recombination) since the initial interspecific crosses, sugarcane exhibits significant levels of LD (Aitken et al., 2006).

The presence of high LD in sugarcane offers several benefits for association mapping, including:

1. Easing the identification of associations between genetic markers and traits of interest.
2. Increasing the power to detect these associations.
3. Reducing the number of markers required for AM applications; fewer markers can capture most of the genetic variation.
4. Decreasing the complexity and cost of genotyping.
5. Enhancing our understanding of sugarcane's population structure and genetic diversity (Barreto et al., 2019).

Currently, LD-based techniques are the most effective for identifying marker-trait associations (MTAs) in crops like sugarcane, where linkage mapping is challenging due to complications arising from polyploidy, inbreeding depression, and dominant marker annotation (Banerjee et al., 2015). Additionally, LD mapping has the advantage of utilizing naturally occurring variation and historical recombination, meaning it does not require mapping populations like recombinant inbred lines or backcross populations, nor does it depend on pedigree information. Several studies have estimated linkage disequilibrium (r^2) for the association mapping of valuable agronomic traits in sugarcane (Banerjee et al., 2015; Bilal et al., 2015; Siraree et al., 2017; Chun-Yan et al., 2024). Rosa et al. (2013) analyzed LD patterns in a subset of 80 sugarcane genotypes from the Brazilian variety panel using 100 SSR and 21 AFLP primers. Raboin et al. (2008) examined LD in sugarcane using AFLP markers and 72 modern cultivars, reporting that LD between closely linked markers steadily decreases within a 0–30 cM window. Similarly, Jannoo et al. (1999) studied linkage disequilibrium in 59 cultivars representing the most significant commercial clones from Mauritius using thirty-eight RFLP probes. Furthermore, Barreto et al. (2019) detected linkage disequilibrium in 134 sugarcane accessions using 100 SSR markers and five yield components.

2.9.5. Marker trait association in sugarcane

According to Wang et al. (2014), the initial stage in marker-assisted selection is the identification of marker-trait relationships. In plant genetics, marker trait association is the method most frequently employed to analyze complicated traits and pinpoint the genes causing trait changes (Wang et al., 2014). It is a different strategy that does not need multiple generations of offspring and the formation of biparental crossovers. According to Buntjer et al. (2005), marker trait association is a statistical method for determining the relationships between genotypes using molecular markers and phenotypes of different variables in reference germplasm sets. It is discovered that using marker trait association rather than traditional QTL mapping provides numerous advantages. These include (i) incorporating the entire recombination event in a crop species' evolutionary history (Myles et al., 2009). (ii) Less time is required; (iii) less expensive; and (iv) a larger number of alleles can be sampled (Zhu et al., 2008).

The aneuploid nature of sugarcane complicates the linkage mapping of quantitative trait loci (QTL) that govern agronomic variables of interest. Given the substantial linkage disequilibrium (LD) in sugarcane, association studies may be a more effective way to find molecular markers linked to particular attributes. Based on Debibakas et al. (2014), the substantial linkage disequilibrium can be explained by the small number of clones utilized in the first hybridization and the very short generations between modern cultivars. Yig et al. (2019) suggest that the high degree of LD in sugarcane suggests that a high marker density may not be necessary to complete GWAS. Recombination events in biparental populations are rare and can expand the size of linkage blocks (Gouy et al., 2015). According to Barabaschi et al. (2016), the quality of the phenotypic data, sample size, genetic architecture, and heritability of the trait under investigation all affect the power of discovering significant marker-trait relationships. Marker-trait association studies have been conducted in sugarcane using markers such as SSRs (Barreto et al., 2019), ESTs (Wei et al., 2006; Gouy et al., 2015; Singh et al., 2016a), AFLPs (Wei et al., 2006; Debibakas et al., 2014; Gouy et al., 2015), TRAPs (Racedo et al., 2016), DArT (Debibakas et al., 2014; Gouy et al., 2015; Racedo et al., 2016), and SNP (Fickett et al., 2018).

The widespread use of sugarcane genome-wide association mapping studies has facilitated the identification of marker-trait associations (MTAs) in genetically diverse plants. These

studies have focused on several traits, such as resistance to smut, root rot fungi, and leaf scald diseases (Wei et al., 2006), resistance to yellow leaf virus (Debibakas et al., 2014), sugar yield, bagasse content, and disease resistance (Guoy et al., 2015), sucrose and yield attributes (Banerjee et al., 2015), red rot disease (Singh et al., 2016b), cane yield and sugar content (Racedo et al., 2016), morphological yield trait (Siraree et al., 2017), and Pol% and sugar yield (Ukoskit et al., 2018).

In marker–trait association studies, General Linear Models (GLM) and Mixed Linear Model (MLM) are statistical method used to determine the relationship between genetic markers and phenotypic traits. GLM is a basic model for studying associations, while MLM is a more advanced model that considered population structure and kinship effects. MLM helps reduce both false positives and false negatives compared to simpler models like GLM. So, MLM can detect true associations than GLM (Kaler et al., 2020).

CHAPTER THREE
3. MATERIALS AND METHODS

3.1. Plant Materials

The study involved 144 sugarcane genotypes, which included 122 introduced exotic genotypes, 10 local landraces, and 12 commercial cultivars. Of these, genotypes NCo334 and B52/298 were considered as commercial standard (check variety) in the study. Genotypes were obtained from a field germplasm conservation garden at the Fincha Sugar Estate Research Center. The introduced genotypes were imported from various countries in different time periods (Table 1). The genotypes used in the study were selected from the available germplasm pool using a stratified random sampling method that considered countries of origin, time of introduction, and the landraces geographic location of collection. The origin of the genotypes used as criteria to group into populations. All the details on the name of the genotypes, place of origin, and genotype code are described in Table 1, and Figure 1.

Figure 1. Distribution map of the global collected sugarcane genotypes. The 144 genotypes were obtained from different countries around the world. Each red dot represents the sugarcane genotypes of the source countries. ArcGIS was used to locate genotype sources based on latitude and longitude of the origins (Esri, 2023).

3.2. Description of Experimental Sites

The field study was conducted at Metahara and Kesem Sugar Estates from April 2021 to May 2022. Metahara Sugar Estate is located in Fentale district, East Shoa Zone, Oromia region, 200 kilometers southeast of Addis Ababa, between a longitude of 39^{o}52'E and a latitude of 8'51'N, with an altitude of 950 meters above sea level (m.a.s.l). The region experiences average annual rainfall of 600mm and an annual temperature of 17.36°C to 32.97°C. The soil of the test field was described as vertisol (clay-textured) (BookerTate, 2009). The furrow irrigation system was used to grow the sugarcane genotypes of the experiment.

The Kessem Sugar Estate is located in the Afar Regional State, Zone Three, and Awash Fentale and Dullecha districts. It is located 250 kilometers from Addis Ababa, at longitude 40^{o}30'E and latitude 9^{o}26'N, and has an elevation of 820 meters above sea level. The area receives an average of 569 mm of rainfall annually, with the lowest and highest temperatures of 18.9°C and 34.2°C, respectively. The soil of the trial field was described as vertisol (clay texture) (WWDSE, 2005). The furrow irrigation system was employed to grow the sugarcane genotypes of the experiment.

3.3. Experiment Design and Layout

The experiment was laid out as 12 x 12 partially balanced lattice design with two replications. Each experimental plot had four furrows of five meters in length each and 1.45 meters between the furrows constituting an area of 29 m^2. The distance between replicates and adjacent plots was 2.9 and 1.5 meters, respectively. An equal number (17 setts) of three-budded setts from each genotype were planted end-to-end within the furrows. The necessary observations were collected from the two middle furrows, and the two rows on either side of the plot were used as boundary rows to protect the middle furrows. Conventional cultural activities were implemented regularly during the experiment according to the standard operating procedures of the respective sugar estates (SCRDC, 2016ab).

Table 1. Details of 144 sugarcane genotypes used in the study and their codes, and origin

Code	Genotype	Origin	Intr. /coll. Year	Code	Genotype	Origin	Intr./coll. year	Code	Genotype	Origin	Intr./coll. year
129	N-14	S. Africa	1980	2	Co-740	India	1962	42	CP61/39	USA	unknown
9	N53/216	S. Africa	1974	61	Co-1148	India	1987	37	CP70/321	USA	1983
1	N52/219	S. Africa	1974	69	Co-419	India	1954	27	CPCL02962	USA	2013
76	N6	S. Africa	1983	68	Co-680	India	1963	36	CP96-1252	USA	2013
130	V422	Ethiopia	2010	16	Co-976	India	1965	107	CP52/86	USA	unknown
127	V 46	Ethiopia	2010	8	Co-945	India	1970	131	C85-102	Cuba	2012
118	V7	Ethiopia	2010	104	B61-113	Barbados	1983	126	C89-147	Cuba	2012
119	V188	Ethiopia	2010	102	B49-388	Barbados	1974	109	C88-356	Cuba	2012
114	V139	Ethiopia	2010	52	B41/211	Barbados	1970	141	C9226	Cuba	2012
144	V43	Ethiopia	2010	60	B50/210	Barbados	1974	143	C32-368	Cuba	2012
75	V153	Ethiopia	2010	58	B657/150	Barbados	unknown	133	C88556	Cuba	2012
83	V189	Ethiopia	2010	56	B49-244	Barbados	1974	80	C98-128	Cuba	2012
86	V203	Ethiopia	2010	53	B35/69	Barbados	1983	78	C92-514	Cuba	2012
73	V141	Ethiopia	2010	50	B52/298	Barbados	1965	17	C86/165	Cuba	2003
128	VMC9660	Philippines	2012	59	B54/90	Barbados	1974	18	C90-501	Cuba	2003
112	VMC9647	Philippines	2012	45	B57/36	Barbados	1974	4	C86/56	Cuba	2003
111	VMC9662	Philippines	2012	40	B41-227	Barbados	1957	47	C132-81	Cuba	2003
139	VMC9655	Philippines	2012	48	B59/212	Barbados	1974	72	C1051-73	Cuba	2003
77	VMC95243	Philippines	2012	67	B59/250	Barbados	unknown	70	C86/12	Cuba	2003
79	VMC9222	Philippines	2012	65	B46/81	Barbados	unknown	87	C8751	Cuba	2012
89	PSR97105	Philippines	2012	7	B52-107	Barbados	1974	94	C90-530	Cuba	2012
115	PSR9784	Philippines	2012	12	B58-230	Barbados	1970	123	FG05-424	France	2012
90	VMC95252	Philippines	2012	13	B60/163	Barbados	1974	124	FG06-806	France	2012
30	VMC96-134	Philippines	2013	24	B78/505	Barbados	2003	122	FG04-420	France	2012
26	PSR97-092	Philippines	2011	21	B80-250	Barbados	2003	116	FG06-747	France	2012
121	2-555	Sudan	2012	23	B60-349	Barbados	unknown	125	FG05-155	France	2013
142	2-888	Sudan	2012	11	B51/410	Barbados	1983	92	FG04-275	France	2012
137	2-999	Sudan	2012	10	B49/06	Barbados	1974	6	FG03-520	France	2011
136	2-444	Sudan	2012	41	DB377/60	Barbados	1974	28	FG04-187	France	2011
140	2-333	Sudan	2012	19	DB386/60	Barbados	1974	32	FG04-466	France	2011
85	2-222	Sudan	2012	66	DB414/60	Barbados	1974	82	FG06-750	France	2012
93	2-111	Sudan	2012	71	DB288/57	Barbados	unknown	35	FG05-336	France	2013
96	2-777	Sudan	2012	98	D41/46	Guyana	1974	33	FG06-695	France	2011
44	NCo-334	India	1962	3	D42/58	Guyana	1974	34	FG06-114	France	2013
99	NCo-310	India	1953	138	Q-217	Australia	2013	31	FG05-425	France	2013

Table 1. Continued

Code	Genotype	Origin	Intr. /coll. year	Code	Genotype	Origin	Intr./coll. year	Code	Genotype	Origin	Intr./coll. year
106	NCo-376	India	1956	135	Q-200	Australia	2013	113	FG06-755	France	2012
134	Co-0238	India	2013	117	CP971944	USA	2012	120	FG04-896	France	2012
84	Co-622082	India	1960	132	CP001527	USA	2012	74	FG04-641	France	2012
105	Co-810	India	1974	110	CP001301	USA	2012	81	FG04-798	France	2012
101	Co-779	India	unknown	95	CP001302	USA	2012	97	FG06-119	France	2013
5	CoK-30	India	1970	51	CP71/400	USA	unknown	29	MPT97-035	Thailand	2013
100	Co-449	India	1957	49	CP6023	USA	1974	25	MPT96-273	Thailand	2013
57	Co-1230	India	1974	64	CP53/18	USA	1974	20	M202/46	Mauritius	unknown
54	Co-678	India	1960	62	CP36/11	USA	unknown	103	Mex54/245	Mexico	1970
43	Co-1007	India	1974	22	CP72-2083	USA	1983	38	Mex57/197	Mexico	1970
46	Co-421	India	1954	15	CP47/193	USA	unknown	63	SP70-1284	Brazil	2003
39	Co-967	India	1974	91	CP041566	USA	2012	108	PR1013	Puerto Rico	1970
14	Co-1001	India	1970	88	CP971989	USA	2012	55	PR1007	Puerto Rico	1970

3.4. Morphological Characterization

3.4.1. Qualitative morphological characterization

The morphological traits were assessed and characterized on ten cane stalks selected per genotype. The observations were recorded on 16 qualitative morphological characters at ten months when varieties showed distinct morphological characteristics (Table 2). The characters were evaluated and scored based on sugarcane descriptors (qualitative characters) given by USDA-ARS (GRIN, 2004). Characters were chosen based on ease of observation, availability, and usefulness in classifying and identifying genotypes.

Table 2. Descriptors, descriptor states and score codes used for characterization of sugarcane germplasm

Descriptor	Abbreviation	Score code and descriptor state
Presence or absence of bud cushion	BUDCUSHION	Present (1) and absent (2)
Relative degree of bud extension	BUDEXTEND	Below growth ring (1), touching (2), and extending above growth ring (3)
Relative bud shape	BUDSHAPE	Tall deltoid (1), short deltoid (2) ovate (3), narrow ovate (4), ovate with broad wing tip (5), ovate with emarginated basal wing (6), rhomboid (7), pentagonal (8), squarish pentagonal (9), round (10), round with central germpore (11), obovate (12), and rectangular (13)
Canopy structure	CANOPY	Open erect (1), open tip droopy (2), open semi-droopy (3), open droopy (4),compact erect (5), compact tip droopy (6), fan erect (7), and fan tip droopy (8)
Relative shape of dewlap	DEWLAPSHAP	Deltoid (1), squarish (2), squarish deltoid (4), ascending (5), descending (6), flaring (7), tall (8), narrow (9), subcrescent (10), and double crescent (11)
Relative plant erectness	ERECT	Prostrate (1), (4–8), and erect (9)
Relative internode alignment	INALIGN	Straight (1), slightly zigzag (2), and zigzag (3)
Relative internode shape	INSHAPE	Cylindrical (1), conoidal (2), obconoidal (3), tumescent (4), Bobbin-shaped (5), and concave convex (6)
Color of the leaves	LEAFCOLOR	Green (1), light green (2), purple(3), light purple(4), pink(5), light pink(6), and greenish yellow (7)
Type of auricle	AURICLEOUT	Absent (1), transitional (2), dentoid (3), deltoid (4), falcate (5), unciform (6), calcariform (7), short lanceolate (8), and long lanceolate (9)

Table 2. Continued

Descriptor	Abbreviation	Score code and descriptor state
Relative shape of ligule	LIGSHAPE	Orbicular-crescent (1), flat-crescent (2), crescent with broad lozenge (3), crescent with narrow lozenge (4), crescent with lozenge (5), broad-crescent (6), deltoid (7), linear-crescent (8), broad subarcuate (9) and inverted crescent (11)
P/absence of stalk corky cracks	STALKCORKC	Present (1) and absent (2)
Presence or absence of stalk corky patchs	STALKCORKP	Present (1) and absent (2)
Presence or absence of stalk growth cracks	STALKCRACK	Present (1) and absent (2)
Color of the exposed rind	RINDCOLE	Green (1), light green (2), dark green (3), yellow (4), light yellow (5), dark yellow (6), light purple (8), dark purple (9), brown (10), light brown (11), dark brown (12), yellowish green(13), greenish yellow (14), brownish green (15), brownish yellow (16), and brownish purple (17)
Presence or absence of bud groove	BUDGROOVE	Absent (1) and present (6)

3.4.2. Quantitative morphological characterization

Data on sixteen quantitative morphological traits were collected at different development phases of the sugarcane, including sprouting percentage (%), number of tillers, number of millable canes, stalk height (cm), stalk diameter (cm), leaf area (cm^2), leaf width (cm), leaf length (cm), number of internodes, single cane weight (kg), cane yield (t ha^{-1}), sugar yield (t ha^{-1}), Brix%, pol%, purity%, and sucrose%. Except for sprouting percentage and number of tillers, all data were collected during time of harvest. A brief description of each trait considered is presented in Table 3.

Sprouting percentage: The number of sprout shoot was counted from the two middle data rows at one and a half months after planting (MAP). The count data was converted to a percentage by dividing the total number of sprouts by the total number of buds planted in the two middle furrows of each plot and multiplied by 100.

Number of tillers: A tiller count was performed by counting all shoots raised from the underground buds of primary shoots in the plot's middle two data rows 120 days after planting (DAP). The total number of tillers counted on a plot basis was extrapolated to a hectare basis.

Number of millable cane: The total number of millable canes was recorded at harvest (13 MAP) from the two middle data rows of each plot and then extrapolated to a hectare basis.

Single cane weight (kg): The weight of twenty millable stalks were randomly taken from the middle two furrows of each plot at harvest then averaged.

Ten stalks were randomly resampled from the twenty pre-collected samples from each genotype plot to determine stalk height (cm), stalk diameter (cm), and the number of internodes. These samples were also used in the laboratory to analyse sugarcane quality using an indirect method (Kassa, 2010).

Stalk height (cm)*:* The height of ten stalk samples was measured from the bottom of the stalk to the top visible dewlap (TVD) with a tape metre and then averaged.

Stalk diameter (cm): The diameter of ten randomly selected stalks was measured with a Vernier calliper at three points on the stalk, at the top, middle, and bottom, and the average was calculated.

Number of internodes: The number of internodes on ten stalks was counted and averaged by dividing the total counted internodes by the number of stalks.

Leaf width (cm): The width of the leaf (cm) was measured at three points (bottom, middle, and upper part) of the third leaf of five randomly selected stalks using a tape meter at harvest and then averaged.

Leaf length (cm): Leaf length was measured from the bottom to the upper tip of the third leaf flag (the leaf that emerged at the top visible dewlap (TVD)) for five randomly selected stalks at harvest with a tape meter and then averaged.

Leaf area (cm^2): The leaf area was computed as the product of the average width (cm) and average length (cm) of the third leaf and calculated according to Sinclair et al. (2004) as follows:

$$LA = LL*LW*0.72 \quad [1]$$

Cane yield ($t\ ha^{-1}$): Cane yield was calculated by multiplying the number of millable cane stalks per hectare by average single cane weight at harvest.

$$\text{Cane yield } (t\ ha^{-1}) = \text{Number of millable cane/ hectare} * \text{single cane weight} \quad [2]$$

Brix percent: To determine cane quality, ten resampled stalks from each genotype were crushed separately using a Jeffco machine and pressed with a hydraulic press to extract

cane juice. Brix percent was estimated with a digital refractometer. The composite of ten stalk juices was first filtered and then cooled to 20 ^{o}C. The instrument was first calibrated to zero using distilled water. Then, one or two drops of the filtered sample were loaded onto the prismatic plate of the Brix meter. Eventually, the displayed number was read and recorded. The reading was then corrected using the temperature correction table and reported as corrected Brix % (Kassa, 2010).

Pol percent*:* Hundred milliliters of extracted juice was poured into a 250 mL beaker, and 0.5 g of basic lead acetate was added to precipitate all fat and protein. The juice was agitated for a few minutes and filtered in a funnel using filter paper No. 90 to get clear juice. The first 25 mL of filtered juice was discarded, leaving the remaining filtrate in the beaker. The 200-mm pol tubes were then rinsed three times and filled with a clear juice free of air bubbles. Later, the tubes were loaded into a polarimeter chamber set to 20 ^{o}C, and the chamber was closed. The number displayed on the screen was recorded as a percentage of Pol (Pol reading). The main Pol was computed by multiplying the Pol reading by the Pol factor (Table reading) (Kassa, 2010).

$$Main\ Pol = Pol\ Reading\ x\ Pol\ Factor \quad [3]$$

Purity percent*:* The ratio of pol and Brix was computed using the Winter Carp indirect method of cane juice and fibre analysis (Kassa, 2010) using the following formula:

$$\text{Purity\%} = \frac{\text{Pol\%}}{\text{Brix\%}} \text{x} 100 \quad [4]$$

Sucrose percent cane (%): was calculated using the Winter Carp indirect method of cane juice analysis (Kassa, 2010).

Sucrose percent cane = [pol% − (Brix% - pol %) non-sugar factor) cane factor] [5]

Estimated sugar yield (t ha^{-1}): was calculated as the product of the cane yield per hectare and the sucrose % cane (Kassa, 2010) using the following formula:

ESY (t/ha) = Cane yield (t/ha) x Sucrose percent cane [6]

Table 3: Description of quantitative morphological traits recorded in the 144 sugarcane genotypes

Traits	Code	Description of traits
Sprouting percentage	SP	Proportion of sett bud that produced new primary shoot
Tiller count/hectare	TCHA	Shoots arise from underground buds of primery shoot
Millable canes/hectare	MCHA	Number of matured canes ready to be harvested for processing
Stalk diameter (cm)	SD	Diameter of cane stalk measured at top, middle, and bottom
Stalk height(cm)	SH	Length of millable stalk measured from bottom to the TVD
No. of internodes (count)	NI	Count of total internodes per cane stalk and then averaged
Single cane weight (kg)	SCW	Weight of cane sample taken from the trial plot and averaged
Leaf length (cm)	LL	Linear distance from base to upper tip of the third leaf flag
Leaf width (cm)	LW	Width of the third leaf flag at top visible dewlap
Leaf area (cm^2)	LA	Area of the third leaf flag at top visible dewlap
Brix percent	Brix %	Brix is the overall soluble solids in the juice
Pol percent	Pol %	Pol is the sugar present in the juice
Purity percent	Purity%	Amount of sucrose present in the juice relative to the impurities
Sucrose percent	Sucrose	Amount of sugar recovered from the cane.
Cane yield (t/ha)	CYHA	The weight of cane yielded per hectare of land
Sugar yield (t/ha)	SYHA	Amount of white sugar yielded per hectare of land

3.5. Molecular Characterization

3.5.1. Plant materials

Leaf tissue samples from all 144 genotypes were collected from a field experiment conducted at Metahara Sugar Estate for a diversity study. Leaf tissue samples were placed in a zip-lock bag with silica gel in a 1:10 ratio for drying. The dried samples were then kept with the silica gel at room temperature until DNA extraction.

3.5.2. DNA Extraction

Total genomic DNA was extracted from the leaf tissue of all 144 sugarcane genotypes using the Cetyltrimethylammonium bromide (CTAB) method, as described by Doyle and Doyle (1987), with minor modifications to the procedure. The quality and quantity of the DNA were assessed using agarose gel electrophoresis (1% w/v) and a Nanodrop-2000 spectrophotometer (Thermo Scientific), respectively. Samples that exhibited faint, highly

smeared, or no bands were re-extracted to ensure that all samples had clear bands for further analysis. The genomic DNA was then normalized to a working concentration of 100 ng/µl using 0.1 M Tris-EDTA buffers for PCR, specifically for SSR analysis. Both the concentrated DNA stock and the working DNA solution were stored at -20°C.

3.5.3. Genotyping

Twenty-five simple sequence repeat markers (SSRs) that showed high polymorphism in previous studies (Singh et al., 2010; Esayas et al., 2016b; Ahmed & Gardazi, 2017a) were chosen for this study. The PCR conditions for each SSR marker were optimized. Briefly, the PCR conditions for a single SSR were optimized by PCR amplification at three different annealing temperatures using three sugarcane genotypes of different origins. Then, the amplicons were checked using 1% agarose gel electrophoresis. The optimum annealing temperature that provides the most apparent band was identified. Based on the optimization results, 20 pairs of polymorphic SSR primers with clear, scorable bands were selected and used in the present study.

Polymerase chain reactions (PCR) were carried out in a 10µL reaction volume consisting of 5µL of 2x Taq plus master mix with blue dye, 3.5µL of nuclease-free water, 0.5µL of 0.2 pmol/µl each forward and reverse primer, and 0.5µL of DNA template normalized to 100ng/µL. PCR was performed using the thermocycler (Biometra, Germany) with the following thermal cycling conditions: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30s; annealing at 50 to 58 °C, depending on markers (Table 2), for 30s; and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min and held at 4 °C.

3.5.4. Polyacrylamide gel electrophoresis and silver staining

The amplified PCR fragments were resolved on an 8% polyacrylamide gel (PAGE). The gel matrix was prepared as stated by McGookin (1988), containing 10.7 ml of polyacrylamide: acrylamide-bisacrylamide (29:1), 5 ml of 1 × Tris-Boric acid EDTA (TBE) buffer, 600 µl of 10 % ammonium persulfate, 33 µl of N,N,N-tetramethyl ethylene diamine (TEMED), and 21.3 ml of sterile dH_2O. A tank buffer of 1 × TBE (100 mM Tris-HCl, 83 mM boric acid, and 1 mM EDTA-Na_2 at pH 8.0) was used. Eight microliters of PCR product containing blue loading dye were electrophoresed at a constant power of 120 V, 18 W, and 35 milliamps for 4.30 h in a vertical electrophoresis system (BioRad,

China). A 50- and 100-bp DNA ladder was employed as a marker to estimate the size of the PCR fragments and alleles.

The gel was silver-stained with minor modifications (Liu et al., 2017) after electrophoresis was completed. The gel was removed from the electrophoresis assembly and washed with 500 ml of distilled water for 2 min, then impregnated in 500 ml of 0.75 g AgNO3 for 5 min with gentle manual shaking. Extra silver nitrate was removed by washing the gel with 500 ml of dH2O for 1 minute. After that, the gel was incubated in a 500-ml developer solution containing 10g NaOH and 750 µL formaldehyde (HCHO, 37 %) with gentle shaking until the bands were visible (approximately 30 min). The gel was then rinsed with 500 ml of dH_2O for 1 minute. Finally, the resolved DNA fragments (bands) were visualized in a gel documentation system (BioRad®, China). Gel images were captured and saved for molecular marker analysis using Image Lab software version 6.1 (BioRad, 2020). All laboratory analyses were performed at Plant Genetics Laboratory of Department of Microbial Sciences and Genetics, Addis Ababa University.

3.6. Data Analysis

3.6.1. Qualitative morphological data analysis

The phenotypic frequency, distribution, and the Shannon-Weaver diversity index, H', of the 16 qualitative morphological characters were calculated in the Microsoft Excel program (MS Corporation, 2018) using a formula developed by Shannon and Weaver (1948), which is given as:

$$H' = -\sum_{i=1}^{n} p_i * \ln p_i \quad [7]$$

Where n is the number of phenotypic classes for a character, and Pi is the relative frequency in the ith class of the jth trait. H' was estimated for each trait, group of traits, and country group. To keep H' value within the range of 0 to 1, each value was divided by its maximum value, ln(n). By grouping different characters across the locations (countries), the additive properties of H were used to evaluate the diversity of locations and characters within the population. The average diversity of H' over j traits was estimated as:

$$H' = \frac{H_i}{j} \quad [8]$$

H_i represents the total amount of diversity when considering all j traits together. The dissimilarity coefficient was calculated in all the pairwise comparisons of the sample germplasm to group the genotypes. The average linkage method with the unweighted pair group method with arithmetic average (UPGMA) was used to perform hierarchical cluster analysis in R (R Core Team, 2020). Before cluster analysis, the morphological trait frequencies were standardized to a mean of zero and a variance of one using the R language. The inter-cluster distance was calculated using Minitab statistical software version 17 (2000).

3.6.2. Quantitative morphological data analysis

3.6.2.1. Analysis of variance

To test the variation between genotypes, the analysis of variance (ANOVA) was computed for observations collected for each quantitative trait using a partially balanced lattice design with the general linear model (Proc GLM) procedure in SAS software version 9.2 (SAS Institute, 2009). Before ANOVA analysis, the Shapiro-Wilk method in Minitab software version 17 (2000) were used to test the normality of data. Mean separation for traits with a significant mean difference was performed using Tukey's Studentized Range Test (HSD) at a 5% probability level. The traits' mean, range, and range units were computed using the Variability package in R software (R Core Team, 2023). To combine the recorded data from the two experimental locations, the homogeneity of the error variance was checked using the F-max method (Hartley, 1950). The ratio of the largest mean square of error (MSE) to the smaller mean square of error was computed as follows:

$$\text{F ratio} = \frac{\text{Large MSE}}{\text{Small MSE}} \qquad [9]$$

If the larger error mean square was not three times larger than the smaller error mean square, the error variance was taken as homogeneous. The ANOVA model of the combined data was then expressed as:

$$Yuij = \mu + \pi u + \beta j + \tau i + (\pi\tau)\, ui + \varepsilon uij \qquad [10]$$

Where, Yuij is the response to the i^{th} treatment in the block j in the u^{th} environment, μ is the general mean, πu is the effect of the u^{th} environment, τi is the effect of the i^{th} treatment, βj is the effect of the jth block, $(\pi\tau)$ ui is the interaction effect of the u^{th} environment and the i^{th} treatment and euij is the random error component associated with the observation

yuij, and is assumed to be normal and independent distributed with mean 0 and variance σ^2e.

3.6.2.2. Estimate of variance components

The variation between the genotypes was determined using range, mean, standard error, phenotypic and genotypic variance, and coefficient of variation. The variance component results evaluated the phenotypic and genotypic coefficients of variation, heritability, and genetic advances. All variance components were estimated using the variability packages in R software (R Core Team, 2023), with the formulas listed below.

3.6.2.3. Phenotype and genotype variability

The phenotypic and genotypic variances of each trait were calculated using Burton and Devane's (1953) formula for a partially balanced lattice design variance analysis.

$$\sigma^2 g = \frac{Msg - Msgl}{rl} \quad [11]$$

$$\sigma^2 e = Mse \quad [12]$$

$$\sigma^2 gl = \frac{MSgl - MSe}{r} \quad [13]$$

$$\sigma^2 p = \sigma 2g + \frac{\sigma 2gl}{L} + \frac{\sigma 2e}{rL} \quad [14]$$

Where σ^2g, σ^2p, and σ^2e were genotypic variances, phenotypic variance, and environmental variance; Msg and Mse were the mean squares for the genotypes and error in the analysis of variance, respectively, and r was the number of replications. The phenotypic variance was computed as the genotypic and environmental variance sum.

3.6.2.4. Coefficient of variation

The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were computed according to Johnson et al. (1955).

$$GCV = \frac{\sqrt{\sigma 2g}}{\bar{X}} \times 100 \quad [15]$$

$$PCV = \frac{\sqrt{\sigma 2p}}{\bar{X}} \times 100 \quad [16]$$

Where GCV and PCV were the genotypic coefficients of variation and phenotypic coefficients of variation, respectively, σ2g was genotypic variance, and σ2p was phenotypic variance. It was the mean value of a particular trait. The PCV and GCV values were ranked low, medium, and high, with 0 to 10%, 10 to 20%, and 20% and above, respectively (Shivasubramanian & Menon, 1973).

3.6.2.5. Estimation of heritability

Heritability (H^2) is a reliable measure of how traits are passed from parents to their progenies. In a broad sense, for mean values, heritability was the ratio of genotypic variance to phenotypic variance computed following the formula described by Allard (1960). It was calculated taking into consideration two replicates and two locations.

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100 = \frac{\sigma 2g}{\sigma 2g + \frac{\sigma 2gl}{L} + \frac{\sigma 2e}{rL}} \quad [17]$$

Where, $\sigma 2g$, σ_p^2, and H^2 = genotypic variance, phenotypic variance, and heritability in broad sense, respectively. Heritability values were categorized as low (0–30%), moderate (30–60%), and high (60% and above) according to Robinson et al. (1949).

3.6.2.6. Estimation of expected genetic advance

Genetic advance assesses how much gain you may attain from phenotypic selection for a trait. The formula presented by Nair et al. (1998) was used to calculate genetic advances:

$$GA = h^2 b \cdot K \cdot \sigma p \quad [18]$$

Where, GA = expected genetic advance, H^2 = heritability in a broad sense, σp = the phenotypic standard deviation, K = the selection differential value which was 2.06 at 5% selection intensity.

Genetic advance as percent of mean computed as follows:

$$GAM\ (\%) = \frac{GA}{Gm} \times 100 \quad [19]$$

Where, GA = genetic advance, Gm = general mean of population. Genetic advance as per cent mean was categorized as low (0-10%), moderate (10-20%) and high (20 and above) as given by Johnson et al. (1955).

3.6.2.7. Correlation and path coefficient analysis

Phenotypic and genotypic correlations were estimated using the "phen_cor" and "geno_cor" functions of the "Metan" package in R software version 4.2.3 (R Core Team, 2023). Multicollinearity diagnosis was also undertaken using the "Metan" package and the "path_coeff" function of R statistical software version 4.2.3 (R Core Team 2023). The data were further subjected to path coefficient analysis using the "path_coeff" functions of the 'Metan' package of R version 4.2.3 (R Core Team, 2023), which carries out path analysis according to the method of Dewey & Lu (1959). In this analysis, cane yield was kept as the dependent factor, with the other traits as independent (causal) factors. Finally, RindSel version 3.0 (Pacheco et al., 2016), developed by CIMMYT-BSU, was used to perform path index analysis based on genotypic path coefficients.

3.6.2.7.1. Estimation of correlation coefficient

The phenotypic and genotypic correlation coefficients between yield and yield component traits were computed as described (Singh and Chaudhary, 1985).

$$\text{Genotypic correlation} = \frac{\text{Cov}_{(g)1.2}}{\sqrt{\sigma^2(g)_1 \sigma^2(g)_2}} \quad [20]$$

$\text{Cov}_{(g)1.2}$ is covariance of genotype between the traits X and Y, $\sigma^{2(g)1}$ is genotypic variance of the trait X_1, and $\sigma^{2(g)2}$ is genotypic variance of the trait X_2

$$\text{Phenotypic correlation} = \frac{\text{Cov}_{(p)1.2}}{\sqrt{\sigma^2(p)_1 \sigma^2(p)_2}} \quad [21]$$

$\text{Cov}_{(p)\ 1.2}$ is covariance of phenotype between the traits X and Y, $\sigma^{2\ (g)1}$ is phenotypic variance of the trait X_1, and $\sigma^{2\ (g)2}$ is phenotypic variance of the trait X_2.

3.6.2.7.2. Estimation of path coefficient

Path coefficient analysis was also conducted for the estimation of the direct and indirect effects of various yield component traits on yield. The path coefficient was calculated as described by Dewey and Lu (1959).

$$r_{ij} = P_{ij} + \sum r_{ik} \cdot P_{jk} \quad [22]$$

Where, rij denotes the relationship between independent traits i and the dependent traits j as determined by the phenotypic and genetic correlation coefficients. Pij represents the components of the direct influences of the independent trait i on the dependent trait j as estimated by the path coefficients. Prik. Pjk is the summation of components of indirect influences of a particular independent trait i on a specific dependent trait j through the rest of the independent traits k. The contribution of the rest unidentified traits were estimated as the residual as given by:

$$PR = \sqrt{(1 - \sum p_{ij.} r_{ij})} \quad [23]$$

Path coefficient level was described as very high (>1.0), high (0.30 – 0.99), moderate (0.2 – 0.29), low (0.1 – 0.19), and negligible (0.00 – 0.09) (Lenka and Mishra, 1973).

3.6.2.8. Cluster Analysis

Cluster analysis was carried out after standardizing the means of all quantitative traits to unit variance and mean zero. Mean standardization was performed with the scale function of the *Stats* package in R software version 4.3.2. (R Core Team, 2023), according to (Ruiz et al., 1997), to avoid the effect due to the scale divergence. The *dis* function of the *Stats* package (R Core Team 2023) was used to generate the Euclidean distance matrix based on the previously standardized or scaled mean value. The number of clusters was determined using the *NbClust* function of the *Stats* package (Charrad et al., 2014) in the R software. Hierarchical clustering was performed via the unweighted pair group mean average (UPGMA) (Sneath & Sokal 1973) and *hcust* function of clustering package (Maechler et al., 2019) in R software. Genetic distances computed by cluster analysis were utilized to construct a dendrogram with the *fviz_dend* function of the *facto-extra* package (Kassambara & Mundt, 2020) in R software, which represents the relationships of the genotypes. Inter-and intra-divergence among clusters was also estimated with the *dist* function of *Stats* package in the R software (R Core Team, 2023).

3.6.2.9. Principal component analysis

Principal Component Analysis (PCA) is an essential data analysis tool for estimating the contribution of each trait to the overall observed variations. Data on standardized traits were further utilized for PCA with the *prcomp* function of the *Stats* package in the R software version 4.2.3 (R Core Team, 2023) to explore the relationship between

genotypes. Principal component biplot analysis and graph construction were performed with the *fviz_pca_biplot* of the *factoextra* package (Kassambara & Mundt, 2020) in the R software.

3.6.3. Molecular data analysis

3.6.3.1. Data scoring

Gel images were scored manually, creating a binary matrix containing clear, unambiguous, and reproducible DNA bands. In this matrix, '1' denotes the presence of a band, while '0' implies the absence of bands in all 144 genotypes analyzed. Although SSR markers are usually co-dominant, the polyploid nature of the sugarcane genome complicates the separation of heterozygotes and homozygotes alleles; thus, each marker used in the present study was treated as a dominant marker. The fragmented size of the amplicons generated by the SSR markers was determined based on a standard molecular weight marker (50 and 100 bp DNA) using Biorad Image Lab software version 6.1 (Biorad, 2020). Data obtained from 20 SSR markers were used to determine sugarcane genotypes' genetic diversity and population structure, and marker trait association.

3.6.3.2. Statistical data analysis

In this study, the absence and presence of SSR markers for each genotype were scored using a binary matrix. The polymorphism information content (PIC) was evaluated by applying the equation PIC = 1 – ΣP2ij, in which Pij is the frequency of the j^{th} allele for the i^{th} locus, summed across all the locus alleles. POPGEN version 1.32 was used to estimate different genetic parameters such as the effective number of alleles per locus (Ne), observed number of alleles per locus (Na), Shannon information index (I), heterozygosity (Ht) and Nei's genetic diversity (H), genetic diversity within a population (Hs), percentage of polymorphic loci, gene flow (Nm = 0.5 (1- Gst)/Gst), and population differentiation coefficients (Gst) (Yeh et al., 1999). Nei (1978) genetic distance, molecular variance analysis (AMOVA), principal coordinate analysis (PCoA), genetic differentiation tests (PhiPT), and p-values were calculated using GenAlEx software version 6.5 (Peakall & Smouse, 2012). Mega version 11.0.13 software (Tamura et al., 2021) performed cluster analysis using similarity matrices. The dendrogram was created using the unweighted pair-group method with an arithmetic average (UPGMA) through the same software. The

robustness of the node of the dendrogram tree was assessed using 1000 bootstrap replicates.

The population structure and admixture patterns were estimated using the STRUCTURE ver.2.3.4 software (Pritchard et al., 2000) to identify the subpopulations in the genotype panel using a Bayesian model-based clustering algorithm. The project run time was set at 100,000 burn-in times, followed by 250,000 Markov Chain Monte Carlo (MCMC) replications. The individual K estimate was run three times with a K value ranging from 1 to 10 numbers. The optimal number of sub-populations (ΔK) was calculated following the simulation method of Evanno and Regnaut (2005) using the web-based POPHELPER v1.0.10 (Francis, 2017), resulting in a clear peak at the optimal K value. The bar plot for optimal K was developed using structure software (Pritchard et al., 2000).

LD between SSR markers (loci) was determined using Tassel software version 5.0, and represented by the statistical coefficient of determination (R^2) (Ukoskit et al., 2018). Alleles with frequencies less than 0.05 were not included in the LD calculation. The LD-values between all pairs of SSR loci were plotted as triangle LD plots to estimate the general view of LD patterns.

The marker-trait association analysis between the molecular and phenotype data was carried out through the General Linear Model (GLM) and Mixed Linear Model (MLM), taking into accounts the Q-matrix from STRUCTURE software and Kinship in TASSEL 5.0. In comparison to the general linear model (GLM), MLM (K+Q) can reduce spurious associations caused by population structure (Bradbury et al., 2007). Markers with a minimum frequency of less than 5% alleles were excluded. The kinship matrix was obtained based on all the SSR markers using TASSEL 5.0 (Ritland, 1996). The correlation coefficient (R^2) was used to assess the phenotypic variation caused by each marker-trait association. The statistical model used for identifying SSR markers associated with traits was as follows:

$$Yklmnf = \mu + k + ML + Am\ (ML)\ Kn + \varepsilon kmn \qquad [24]$$

Where Yklmnf is the phenotypic observation, μ is the general mean, k is the fixed effect of a k^{th} subpopulation of the population structure (Q matrix), ML is the fixed effect of the L^{th} marker, Am (ML), Kn is the random effect of the m^{th} genotype nested in the L^{th} marker associated with the n^{th} kinship coefficient, and εkmn is the error.

CHAPTER FOUR
4. RESULTS AND DISCUSSIONS

4.1. Qualitative Morphological Diversity

4.1.1. Frequencies of phenotypic traits

A descriptive statistical analysis was conducted to clarify the overall frequency distribution and the variability in qualitative morphological traits found across and within the region. The overall frequency distribution of 16 sugarcane qualitative traits was presented in Table 4 while, Table 5 presents the frequency distribution for 16 qualitative traits of genotypes categorized by region. All the qualitative traits demonstrated significant differences in their distribution and their degree of variation. Additionally, it was observed that the frequency and distribution of each qualitative morphological traits differed across various locations. The traits are organized by grouping similar traits to improve readability and reduce redundancy.

Bud cushion, bud extension, bud shape, and bud groove: From a total of 144 genotypes studied, 63.9% of the genotypes were found with bud cushion, whereas the rest 36.1% were without bud cushion (Table 4). In contrast with the present finding Esayas et al. (2016a) reported that out of 400 sugarcane genotypes studied, 75% of them had no bud cushion. Belay et al. (2022) also revealed that among the 196 sugarcane genotypes evaluated, 68.37% of the genotypes were without bud cushion, which differed from the present finding. The bud cushion trait showed a polymorphic pattern across all locations except for Brazil, Mauritius, and Mexico; they were monomorphic (Table 5). The latter result may be because only a few genotypes were considered from these countries. The presence of bud cushion was found at a higher frequency in all locations except in the USA and, Philippines. Furthermore, all the Guyana genotypes had no bud cushion, on contrary Belay et al. (2022) revealed absence of bud cushion for genotypes from Cuba, India, and Puerto Rico. In this study, a significant proportion (90%) of the Ethiopian genotypes (local landraces) contained bud cushion traits (Table 5). Esayas et al. (2016a) reported the highest frequency of this trait in sugarcane genotypes from Ethiopia, in conformity with the current result. In consistent with current result Esayas et al. (2018) reported that a great proportion (70%) of sugarcane landraces from Ethiopia exhibited no bud cushion.

Regarding the degree of bud extension (BUDEXTEND), significant proportions (61.8%) of the genotypes contained extending buds above the growth ring, followed by growth ring touching phenotypic class (36.1%) and below the growth ring (2.1%) (Table 4). Similar result was reported by Esayas et al. (2018). In consistent with present finding, Belay et al. (2022) reported 59.18%, 20.92% and 19.90% for, buds touching, above, and below the growth ring, respectively. In contrary, Altoveros et al. (2003) reported that bud tip position of most genotypes (46.75%) was below the growth ring. The phenotypic classes of degree of bud extension were observed in genotypes from all locations with different proportions except Brazil, Philippines and Mauritius, which are monomorphic. However, bud extension below growth ring class was expressed only in the Indian and Philippine genotypes with a lower proportion, 11.1% and 9.1%, respectively. Seventy percent (70%) of Ethiopian landraces exhibited bud extending above growth ring phenotypic class (Table 5). Esayas et al. (2018) reported that most of the Ethiopian local landraces (53%) expressed bud extending above growth ring phenotypic class, in conformity with the current result.

Out of the 144 genotypes evaluated, larger proportion of genotypes (23.6%) exhibited an ovate bud shape, followed by 22.2% of the genotypes showed a squarish pentagonal phenotypic class (Table 4). In line with the present result, Alarmelu and Hemaprabha (2022) revealed that ovate bud shape was a dominant phenotype in the studied genotypes. In disagreement with findings of the present study, Esayas et al. (2016a, 2018), Khalid et al. (2016), and Belay et al. (2022) found that tall deltoid, triangular pointed, and round bud shapes were dominant bud shape phenotypic classes, respectively. Evaluation of this character revealed that bud shape had a polymorphic distribution in genotypes from all locations except for genotypes from Brazil and Mauritius (monomorphic). In this study, ovate bud shape exhibited the highest proportion in landraces of Ethiopia (50%) and Sudan population (62.5%). Contrary to current findings Esayas et al. (2018) revealed that local accessions in most regions had tall deltoid bud shape. The present result also showed that genotypes from Thailand exclusively exhibited a pentagonal bud shape. On the other hand, obovate and rectangular bud shapes had the lowest distribution across the locations; they were recorded solely in genotypes from India and the Philippines, with less frequency (5.6% and 9.1%, respectively) (Table 5).

Concerning bud groove qualitative trait, most of the sugarcane genotypes (77.8%) had bud grooves (BUDGROOVE), while 22.2% had no bud groove in their stalk. Contrary to the

present finding, Belay et al. (2022) reported that only 10.20% of the genotypes possessed bud groove traits. Esayas et al. (2016a) reported a high frequency of bud grooves in sugarcane genotypes, which agrees with the present result. The genotypes from all over the locations had a significant bud groove. Eighty percent (80%) of local landraces in Ethiopia were found to have bud grooves. This indicates the limited variability of this trait within and across locations among the sugarcane genotypes. Inconsistent with the present result, Belay et al. (2022) found the minor frequency (3.5%) of bud groove trait in Ethiopian local landraces.

Canopy structure and relative plant erectness: The genotypes varied greatly in the canopy structure (CANOPY) trait. Genotypes with open semi-droopy genotypes making up highest the proportion (32.6%) while genotypes with open erect, fan erect, and compact erect had the lowest proportion, each proportionate 6.3%. Esayas et al. (2016a), Karpagam and Alarmelu (2017), Esayas et al. (2018) and Belay et al. (2022) found that the most common phenotypic trait in sugarcane was compact tip-droopy canopy structure, which is in contrast to the results of the current study. However, the genotypes from Brazil and Mauritius and Puerto Rico only showed compact tip droopy and open semi-droopy phenotypic classes, respectively (Table 5), suggesting that there was no variation in this trait among the genotypes of sugarcane from these regions.

From the five phenotypic classes of plant erectness traits, the majority (40%) of genotypes had semi-erect (70°) stalks; with a smaller percentage (0.7%) having erect orientation (90°) (Table 4). This result is in contrary to findings by Esayas et al. (2018). Belay et al. (2022) revealed that 54.08% and 25.51% of 196 sugarcane genotypes exhibited semi-erect and erect growth habits, respectively, in conformity with the present finding. According to Farooq (1989), erect cane attitude is an essential quality trait that makes the cane variety suitable for mechanical farming. For relative plant erectness, the phenotypic classes were polymorphic in some locations. On the other hand, the erect phenotypic class was less frequently distributed across the locations, exclusively recorded for the germplasms of Cuba and South Africa at 6.3% and 42.9%, respectively. Ethiopian landraces were semi-erect (grade 8) and relatively erect (grade 7) in equal proportion.

Table 4**.** Overall frequency distribution of observed qualitative traits in 144 sugarcane genotypes

S/No	Character	Observed phenotypic class	Scale	Number of genotype	Frequency (%)
1	BUDCUSHION	Present	1	92	63.9
		Absent	2	52	36.1
2	BUD EXTEND	Below growth ring	1	3	2.1
		Touching	2	52	36.1
		Extending above growth ring	3	89	61.8
3	BUDSHAPE	Tall deltoid	1	15	10.4
		Ovate	3	34	23.6
		Narrow ovate	4	4	2.8
		Ovate with emarginate basal wing	6	4	2.8
		Rhomboid	7	7	4.9
		Pentagonal	8	28	19.4
		Squarish pentagonal	9	32	22.2
		Round	10	16	11.1
		Round with central germ pore	11	1	0.7
		Obovate	12	1	0.7
		Rectangular	13	2	1.4
4	CANOPY	Open erect	1	9	6.3
		Open tip droopy	2	24	16.7
		Open semi droopy	3	47	32.6
		Open droopy	4	18	12.5
		Compact erect	5	9	6.3
		Compact tip droopy	6	19	13.2
		Fan erect	7	9	6.3
		Fan tip droopy	8	9	6.3
5	DEWLAPSHAPE	Squarish	2	33	22.9
		Squarish deltoid	4	68	47.2
		Ascending	5	2	1.4
		Descending	6	20	13.9
		Flaring	7	4	2.8
		Tall	8	1	0.7
		Narrow	9	1	0.7
		Subcrescent	10	2	1.4
		Double crescent	11	3	2.0
6	ERECT	Prostrate(1) –erect (9)	5	10	6.9
		Prostrate(1) –erect (9)	6	30	20.8
		Prostrate(1) –erect (9)	7	58	40.3
		Prostrate(1) –erect (9)	8	45	31.3
		Erect	9	1	0.7
7	INALIGN	Straight	1	18	12.5
		Slightly zig zag	2	116	80.6
		Zig zag	3	10	6.9
8	INSHAPE	Cylindrical	1	41	28.5
		Conoidal	2	37	19.8
		Obconoidal	3	1	0.7
		Tumescent	4	19	13.2
		Bobbin shaped	5	3	2.1
		Concave convex	6	43	29.9
9	LEAFCOLOR	Green	1	94	65.3
		Light green	2	40	27.8
		Greenish yellow	7	10	6.9
10	AURICLEOUT	Transitional	2	64	44.4
		Deltoid	4	5	3.5
		Falcate	5	5	3.5
		Unciform	6	1	0.7

Table 4. Continued

S/No	Character	Observed phenotypic class	Scale	Number of genotype	Frequency (%)
		Calcariform	7	1	0.7
		Short lanceolate	8	29	20.1
		Long lanceolate	9	39	27.1
11	LIGSHAPE	Orbicular-crescent	1	5	3.5
		Crescent with broad lozenge	3	6	4.2
		Crescent with narrow lozenge	4	2	1.4
		Crescent with lozenge	5	84	58.3
		Broad-crescent	6	31	21.5
		Linear-crescent	8	1	0.7
		Broad subarcuate	9	9	6.3
		Narrow subarcuate	10	5	3.5
		Arcuate	12	1	0.7
12	STALKCORKC	Present	1	28	19.4
		Absent	2	116	80.6
13	STALKCORKP	Present	1	62	43.6
		Absent	2	82	56.9
14	STALKCRACK	Absent	1	128	88.9
		Present	6	16	11.1
15	RINDCOLE	Green	1	9	6.3
		Light green	2	17	11.8
		Dark green	3	2	1.4
		Yellow	4	1	0.7
		Light yellow	5	2	1.4
		Dark yellow	6	1	0.7
		Purple	7	14	9.7
		Brown	8	25	17.4
		Light brown	9	4	2.8
		Dark brown	10	7	4.9
		Yellowish green	11	6	4.2
		Greenish yellow	12	4	2.8
		Brownish green	13	20	13.9
		Brownish yellow	14	15	10.4
		Brownish purple	15	11	7.4
		Brownish yellow	16	3	2.1
		Brownish purple	17	3	2.1
16	BUDGROOVE	Absent	1	32	22.2
		Present	6	112	77.8

Color of the leaves and relative color of exposed rind: The color of the leaves (LEAFCOLOR) varied from green (65.3%), light green (27.8%), to greenish-yellow (6.9%) (Table 4). In conformity with the present result, Belay et al. (2022) found that 89.80% of the genotypes expressed green color while 10.2% exhibited light green leaf color. Abubakar et al. (2013) reported findings conforming to the present result. Among the three phenotypic classes, the green color was exhibited in the highest proportion in all locations except in the genotypes from Guyana, which expressed solely light green color (monomorphic). Compared to the green color, the light green colour was distributed fairly evenly across the locations. The greenish-yellow phenotypic class was observed in genotypes from three locations, namely the USA, France, and Barbados, in an equal

distribution. Green and light green phenotypic classes were found in 90% and 10% proportion in the Ethiopian landraces, respectively (Table 5). This shows that the sugarcane genotypes studied had limited variability for the leaf color qualitative trait, implying this trait is less powerful and useful for characterization and diversity studies of sugarcane. The high frequency of green leaf color may be due to the long term selection strategy being implemented to improve green leaf color feature, since, high rate of photosynthesis substantially associate with green leaf than other leaf colors.

The color of the exposed rind (RINDCOLE) was the most diverse qualitative trait observed among the genotypes. From 17 exposed rind color phenotypic classes, brown color occurred in the highest proportion (17.4%) of genotypes, followed by brownish green (13.9%) and light green (11.8%). Khalid et al. (2016) reported different sugarcane rind colors among the genotypes considered. The phenotypic classes in this trait exhibited polymorphism distribution in all locations (Table 5). Windiyani et al. (2022) and Ekpélikpézé et al. (2016) also noted the most significant variation in exposed rind color among all the morphological features.

Relative internode alignment and internode shape: From the three internode alignment phenotypic classes, high frequency (80.6%) was observed by the slightly zigzag phenotypic class, followed by straight (12.5%) and zigzag (6.9%) phenotypic class (Table 4). In agreement with the current result, Esayas et al (2018) reported that 44% of the sample germplasm were slightly zigzag. It was noticed that a slightly zigzag phenotypic class remarkably exhibited at the highest frequency in genotypes from all the regions, followed by straight internode alignment, implying the presence of variability for this character. The zigzag phenotypic class was poorly distributed across the locations and found only in the germplasm from India (5.6%), France (21%), Barbados (11.5%), and Puerto Rico (50%) (Table 5). Similarly, all genotypes in the Sudan region exclusively possessed straight internode alignment, while Ethiopian landraces had straight and slightly zigzag classes with 40% and 60% frequency, respectively. It is suggested that straight or nearly straight (slightly zigzag) internode alignment of cane stalks is a very essential character for mechanized farming and postharvest handling in sugarcane (Farooq, 1989).

Relative internode shape (INSHAPE) was another diverse trait among the genotypes, in which concave-convex shape showed a relatively high frequency (29.9%), followed by cylindrical (28.5%), and conoidal (19.4%) (Table 4). Esayas et al. (2016a) reported

concave convex shapes as the most frequent, which is similar to this result. In respect to the internode shape trait; the cylindrical shape was the most commonly distributed class across the regions, followed by the concave convex internode shape. An obconoidal shape was found only in Ethiopian landraces. On the other hand, the concave-convex phenotypic class had a great proportion (60%) in the Ethiopian region (Table 5). Similar result was reported by Esayas et al. (2018).

Type of Auricle, Relative Shape of Ligule, and Relative Shape of Dewlap: For the auricle type, 27.1% of the genotypes had long lanceolate auricles, while significant proportion (44.4%) genotypes had a transitional auricle type (Table 4). Esayas et al. (2016a) discovered short and long lanceolate outer auricle types as the predominant class of this trait, in consistent with the current study's findings. In this study, the evaluation result indicated that the transitional type of auricle was the most widely distributed phenotypic class across the regions, followed by the long lanceolate class. On the contrary, unciform and calcariform auricle types were found to reside solely in one region. The unciform auricle was observed solely in Cuban genotypes, while calcariform in the Philippines alone. Significant proportions (60%) of Ethiopian landraces have a transitional type of auricle (Table 5).

As for the shape of the ligule (LIGSHAPE), the highest frequency (58.3%) was recorded by that of a crescent with a lozenge character, followed by a broad crescent (21.5%) (Table 4). Esayas et al. (2018) and Belay et al. (2022) also found a crescent with a lozenge ligule shape in the highest frequency (27%) and (29.08%), respectively in agreement with the current result. Altoveros et al. (2003) and Esayas et al. (2016a) also reported findings similar to the current study's. Among the phenotypic classes of ligule shape, crescent with lozenge was predominant in almost all regions except Guyana, Brazil, and Mauritius. Linear-crescent and arcuate ligule shapes had the most minor frequency (0.7%) each, indicating low genetic diversity in populations for these traits. Arcuate ligule, phenotypic class occurred only in Barbados, and the linear crescent shape was observed only in Barbados and Brazil population. The transitional phenotypic class was predominantly recorded (90%) in the Ethiopian local landraces (Table 5).

In the case of the relative shape of a dewlap (DEWLAPSHAP), 47.2%, 22.9%, and 13.9% of the genotypes exhibited squarish, squarish deltoid, and descending dewlap shapes, respectively (Table 4). Consistent with the present result, Belay et al. (2022) revealed a

high frequency of squarish deltoids (31.12%), followed by descending shapes (27.55%) in the genotypes under consideration. On the other hand, squarish deltoid dewlap shape was the most widely distributed phenotypic class, with the highest proportion across the locations. Tall, narrow, and subcrescent classes were rare phenotypes found solely in genotypes from one location: tall class in India, narrow class in France, and subcrescent class in Barbados (Table 5). This indicates that the sugarcane genotypes considered for this trait have a high degree of variability in the dewlap shape traits.

Stalk Corky Cracks, Corky Patch, and Growth Cracks: Wide variation was observed for the stalk corky crack (STALKCORKC) traits. Only 19.4% of genotypes showed corky cracks on the stalk, while 80.6% had no this characteristic (Table 4). In supporting the current result, Belay et al. (2022) reported that the majority of the genotypes (98.98%) were considered to lack corky cracks in the stalk. Genotypes of all the regions predominantly had no stalk corky cracks. For instance, eighty per cent (80%) of the Ethiopian local landraces had no this traits (Table 5). A similar result was reported by Esayas et al. (2018). Furthermore, all genotypes from Sudan, Guyana, Australia, Thailand, Brazil, and Mauritius lacked stalk corky cracks (Table 5).

In examining the qualitative traits of stalk corky patches among the characterized genotypes, it was found that 62 genotypes (43.6%) exhibited corky patches on their stalks, while the remaining 82 genotypes (56.4%) were devoid of this trait. These findings contrast with those of Belay et al. (2022), who reported that 92.5% of the genotypes studied showed no corky patches. Both phenotypic classes of this trait were widely distributed across locations, with the notable exception that all genotypes from Guyana, Australia, Thailand, and Mauritius lacked this trait. A higher proportion of genotypes without corky patches came from Sudan (62%), the USA (52.9%), Cuba (68.7%), France (68.4%), the Philippines (72.4%), South Africa (57.1%), Guyana (100%), Australia (100%), and Thailand (100%). Conversely, a significant number of genotypes from Ethiopia (80%) and Barbados (57.7%) displayed corky patches on their stalks. Additionally, genotypes from Puerto Rico and Mexico showed equal proportions of both phenotypic classes. Consistent with the current findings, Esayas et al. (2018) reported that 81% of Ethiopia's landrace genotypes exhibited stalk corky patches.

Of the 144 evaluated sugarcane genotypes, 88.9% were devoid of stalk growth cracks (STALKCRACK) (Table 4). In agreement with the current result, Belay et al. (2022)

revealed that only 10 of 196 genotypes (5.10%) had stalk growth cracks. Esayas et al. (2018) found that 75% of the germplasm had no stalk growth cracks, in conformity with the present finding. In the current study, most genotypes across locations had no stalk growth crack traits. This indicates that the trait was expressed at low frequencies in genotypes from a specific location. This indicates limited variability among the tested materials related to this character. In this study, all genotypes from the Philippines, Guyana, Australia, Thailand, Brazil, and Mauritius lacked corky growth cracks on the stalks (Table 5).

In general, this study revealed a wide distribution of phenotypic classes of traits evaluated, which indicates the presence of different races and combinations of races in sugarcane genotypes of different regions worldwide. This variability of genotypes based on morphological markers can aid sugarcane breeders in identifying the uniqueness of genotypes, maintaining genetic diversity and for property rights by plant breeders. Differences in qualitative morphological traits of various sugarcane varieties have been reported (Akhtar et al., 2006; Arrey & Mih, 2016; Esayas et al., 2016a; Khan et al., 2017; Karpagam & Alarmelu, 2017; Alarmelu & Hemaprabha, 2022; Belay et al., 2022). The present study also showed that various phenotypic classes of some traits were distributed commonly in all regions, suggests genetic stability, widespread genetic variability across different populations. This could be due to natural selection and adaptation, gene flow or genetic drift, polygenic inheritance, and environmental influence. According to, Erskine and Williams (1980) traits with high heritability and location stability can be used as genetic markers in variety identification and classification.

Previous researchers have reported that morphological traits showed nearly the same result with some molecular markers, like RAPDs in common bean (*Phaseolus vulgaris* L.) (Johns et al., 1997) and wild rose (*Rosa acicularis*) (Debener et al., 1996) crops. Thus, qualitative morphological traits can efficiently distinguish among genotypes (UPOV, 2005; Perera et al., 2012), eliminate duplicates, used to select valuable germplasm, and assess diversity (Kalyan et al., 2017). Piscitelli (1994) also revealed that among the morphological qualitative traits, wax, color, shapes and structure of the aerial organ are very important as environmental fluctuations do not influence them. Shrestha and Bharti (2021) reported that the bud shape was the most outstanding organographic characteristic of the sugarcane stalk.

Table 5. Frequency distribution of qualitative morphological traits across and within the region or country of origin

		Country															
Trait	Scale	Eth	Sud	USA	Cub	Ind	Fra	Phi	Bar	Dem	Aus	Tha	S.Af	Bra	PRi	Mau	Mex
BUDCUSHION	1	90	87.5	47	68.8	77.8	53	36.4	61.5	0	50	50	85.7	100	50	100	100
	2	10	12.5	53	31.2	22.2	47	63.6	38.5	100	50	50	14.3	0	50	0	0
BUD EXTEND	1	0	0	0	0	11.1	0	9.1	0	0	0	0	0	0	0	0	0
	2	30	25	29.4	37.5	44.4	53	63.6	19.2	0	50	50	28.6	0	0	100	50
	3	70	75	70.6	62.5	44.4	47	27.3	80.8	100	50	50	71.4	100	100	0	50
BUDSHAPE	1	20	0	17.6	0	22.2	15.8	0	7.7	0	0	0	14.3	0	50	0	0
	3	50	62.5	35.3	12.5	11.1	5.3	9.1	38.5	50	0	0	14.3	0	0	0	0
	4	0	0	0	0	5.6	0	0	3.8	0	0	0	0	100	0	0	0
	6	0	0	0	0	0	0	18.9	0	0	0	0	14.3	0	0	0	0
	7	10	12.5	11.8	6.3	5.6	5.3	0	0	0	0	0	0	0	0	0	0
	8	10	12.5	17.6	37.5	22.2	21	9.1	11.5	50	50	100	0	0	0	0	50
	9	10	0	0	25	16.7	26.3	27.3	30.8	0	50	0	57.1	0	50	100	50
	10	0	12.5	17.6	12.5	5.6	26.3	27.3	3.8	0	0	0	0	0	0	0	0
	11	0	0	0	6.3	5.6	0	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	5.6	0	0	0	0	0	0	0	0	0	0	0
	13	0	0	0	0	0	0	9.1	0	0	0	0	0	0	0	0	0
CANOPY	1	10	12.5	0	6.3	5.6	5.3	0	0	50	50	0	28.6	0	0	0	0
	2	30	12.5	5.9	6.3	38.9	15.8	9.1	23.1	0	0	0	14.2	0	0	0	0
	3	30	25	35.3	37.5	52.2	31.6	36.4	38.5	0	50	0	28.6	0	100	0	0
	4	0	25	23.5	6.3	0	21	27.3	3.8	0	0	0	28.6	0	0	0	50
	5	0	12.5	5.9	6.3	5.6	10.5	9.1	7.7	0	0	0	0	0	0	0	0
	6	0	12.5	17.6	18.8	11.1	10.5	0	15.4	50	0	0	0	100	0	100	50
	7	20	0	5.9	12.5	5.6	5.3	9.1	0	0	0	50	0	0	0	0	0
	8	10	0	5.9	6.3	5.6	0	9.1	11.5	0	0	50	0	0	0	0	0
DEWLAPSHAPE	2	10	75	17.6	25	18.8	5.3	36.4	23.1	0	0	50	28.6	0	50	0	50
	4	50	12.5	47.1	62.5	43.8	57.9	54.5	38.5	50	100	50	57.1	100	0	100	0
	5	0	0	5.9	0	0	5.3	0	0	0	0	0	0	0	0	0	0
	6	20	12.5	17.6	6.3	18.8	10.5	9.1	15.4	0	0	0	14.3	0	50	0	50
	7	0	0	5.9	6.3	5.6	5.3	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	5.6	0	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	5.3	0	0	0	0	0	0	0	0	0	0
	10	10	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0
	11	10	0	5.9	0	5.6	10.5	0	23.1	0	0	0	0	0	0	0	0
ERECT	5	0	0	0	12.5	18.8	5.3	0	11.5	0	0	0	0	0	50	0	0
	6	0	25	29.4	6.3	50	10.5	27.3	19.2	0	50	0	0	0	0	100	50
	7	50	50	35.3	43.8	31.3	42.1	36.4	46.2	100	0	0	14.3	0	50	0	50
	8	50	25	35.3	31.3	11.1	42.1	36.4	4.8	0	50	100	42.9	100	0	0	0
	9	0	0	0	6.3	0	0	0	0	0	0	0	42.9	0	0	0	0
INALIGN	1	40	100	5.9	18.8	11.1	5.3	9.1	7.7	50	50	50	14.3	0	0	0	0
	2	60	0	94.1	81.3	83.3	73.7	90.9	76.9	50	50	50	85.7	100	50	100	100
	3	0	0	0	0	5.6	21	0	11.5	0	0	0	0	0	50	0	0
INSHAPE	1	30	37.5	17.6	50	27.8	10.5	9.1	30.8	100	0	100	28.6	0	50	0	50
	2	10	37.5	52.9	18.8	22.2	42.1	27.3	19.2	0	0	0	14.3	0	0	0	0
	3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5. Continued

Traits	Scale	Eth	Sud	USA	Cub	Ind	Fra	Phi	Bar	Dem	Aus	Tha	S.Af	Bra	PRi	Mau	Mex
	4	0	0	0	18.8	16.7	5.3	9.1	23.1	0	0	0	28.6	100	0	100	0
	5	0	0	0	0	5.6	0	9.1	3.8	0	0	0	0	0	0	0	0
	6	40	25	29.4	12.5	27.8	42.1	45.5	23.1	0	100	0	28.6	0	50	0	50
	1	90	37.5	64.7	81.3	55.6	57.9	63.6	69.2	100	0	100	14.3	100	100	100	50
LEAFCOLOR	2	10	62.5	23.5	18.8)	44.4	31.6	36.4	19.2	0	100	0	85.7	0	0	0	50
	7	0	0	11.8	0	0	10.5	0	11.5	0	0	0	0	0	0	0	0
	2	60	25	58.8	56.3	27.8	42.1	54.5	34.6	50	0	50	42.8	0	100	100	50
	4	0	0	0	6.3	5.6	5.3	0	3.8	50	0	0	0	0	0	0	0
	5	0	12.5	5.9	6.3	0	5.3	0	3.8	0	0	0	0	0	0	0	0
AURICLEOUT	6	0	0	0	6.3	0	0	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	9.1	0	0	0	0	0	0	0	0	0
	8	20	37.5	23.5	6.3	5.6	21.1	9.1	26.9	0	100	0	42.8	100	0	0	0
	9	20	25	11.8	18.8	61	26.3	27.3	30.8	0	0	50	14.3	0	0	0	50
	1	10	0	5.9	6.3	0	0	0	3.8	0	0	0	14.3	0	0	0	0
	3	0	0	0	0	0	10.5	0	0	50	50	0	0	0	0	0	0
	4	0	0	0	6.3	0	0	0	0	0	0	0	0	0	0	100	0
LIGSHAPE	5	90	75	41.2	37.5	72.2	57.9	63.6	46.2	0	50	100	71.4	0	100	0	100
	6	0	25	47	18.8	27.8	15.9	9.1	26.9	0	0	0	14.3	0	0	0	0
	8	0	0	0	0	0	0	9.1	0	0	0	0	0	100	0	0	0
	9	0	0	0	25	0	10.5	9.1	3.8	50	0	0	0	0	0	0	0
	10	0	0	5.9	6.3	0	5.3	9.1	3.8	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	3.8	0	0	0	0	0	0	0	0
STALKCORKC	1	20	0	23.5	6.3	22.2	21.1	9.1	26.9	0	0	0	28.6	0	50	0	50
	2	80	100	76.5	93.7	77.8	78.9	90.9	73.1	100	100	100	71.4	100	50	100	50
STALKCORKP	1	80	37.5	47.1	31.3	44.4	31.6	27.3	57.7	0	0	0	42.9	100	50	0	50
	2	20	62.5	52.9	68.7	55.6	68.4	72.7	42.3	100	100	100	57.1	0	50	100	50
STALKCRACK	1	90	87.5	82.4	93.7	88.9	78.9	100	92.3	100	100	100	85.7	100	50	100	50
	6	10	12.5	17.6	6.3	11.1	21.1	0	7.7	0	0	0	14.3	0	50	0	50
	1	0	0	5.9	12.5	11.1	0	0	11.5	0	0	50	0	0	0	0	0
	2	0	0	17.6	0	11.1	21.1	18.2	11.5	50	0	0	14.3	0	50	0	0
	3	0	0	0	0	11.1	0	0	0	0	0	0	0	0	0	0	0
	4	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RINDCOLE	5	0	0	0	0	0	5.3	9.1	0	0	0	0	0	0	0	0	50
	6	0	0	0	0	0	5.	0	0	0	0	0	0	0	0	0	0
	7	30	12.5	5.9	12.5	5.6	5.3	27.3	7.7	0	0	0	0	0	0	0	0
	8	30	0	17.6	37.5	22	10.5	18.2	7.7	50	0	0	14.3	0	0	100	0
	9	0	25	0	0	0	5.3	0	3.8	0	0	0	0	0	0	0	0
	10	10	12.5	0	0	5.6	5.3	9.1	3.8	0	0	0	0	100	0	0	0
	11	0	12.5	5.9	6.3	5.6	0	0	3.8	0	0	0	0	0	50	0	0
	12	0	0	5.9	0	0	5.3	9.1	3.8	0	0	0	0	0	0	0	0
	13	10	25	17.6	12.5	5.6	5.3	9.1	15.4	0	0	0	42.8	0	0	0	0
	14	0	0	5.9	12.5	5.6	21.1	0	11.5	0	100	50	14.3	0	0	0	0
	15	10	0	11.8	6.3	5.6	5.3	0	11.5	0	0	0	14.3	0	0	0	0
	16	0	12.5	5.9	0	0	0	0	3.8	0	0	0	0	0	0	0	50
	17	0	0	0	0	0	5.3	0	3.8	0	0	0	0	0	0	0	0
BUDGROOVE	1	20	25	11.8	31.3	38.9	10.2	27.3	19.2	0	50	0	28.6	0	50	0	50
	6	80	75	88.2	68.7	61.1	89.5	72.7	80.8	100	50	100	71.4	100	50	100	50

Shrestha and Bharti (2021) reported that the bud shape was the most outstanding organographic characteristic of the sugarcane stalk. In other study, Srinivasan et al. (2022) also elucidated that dewlap shape, ligule, and sheath auricles were outstanding value descriptors for identifying diverse sugarcane genotypes. The implications of the present study findings for sugarcane genetic material collection, identification, conservation and maintenance are immense.

On the other hand, the study indicates that the shape of the genotypes could be utilized either directly or indirectly as a selection tool for yield and mechanical cultivation in sugarcane. The straight cane stalk or erect growth habit is the characteristic that makes sugarcane varieties the most suitable for mechanical cultivation and post-harvest handling (Farooq, 1989). Canes expressing a divergent angle of more than 60 degrees from erectness are similarly considered inappropriate for mechanization (Cuenya and Mariotti, 1984). Abubakar et al. (2013) observed that more light is intercepted by green leaves of cane genotypes during photosynthesis, and they are associated with higher yield.

4.1.2. Phenotypic diversity index

The Shannon diversity index (H') was calculated to compare phenotypic diversity among genotypes and locations (countries). The extent of phenotypic diversity estimates and their partitioning within and between countries are shown in Table 6 & 7. A diversity index value of "1" characterizes the highest diversity, while an index value of "0" indicates that there is no variation in the distribution of the phenotypic class of a given trait.

The Shannon-Weaver diversity index values for each trait were calculated from the entire dataset and presented in Table 6. In this study, the 16 traits differed in their distribution and extent of variation. The computed Shannon-Weaver indices (H′) of individual qualitative morphological traits ranged from 0.50 for stalk growth crack to 0.99 for stalk corky patches, with an overall mean of 0.76 (Table 6), indicating a high level of diversity of the morphological traits used for characterization, reflecting most of the germplasm was very diverse when it comes to this type of traits. Esayas et al. (2018) reported estimates of the diversity index for individual traits ranged from 0.49 for stalk corky cracks to 1.00 for bud groove with an overall mean of 0.80, which was inconsistent with the current result. Altoveros et al. (2003) and Belay et al. (2022) also reported that the diversity index ranged from 0.18 to 1.00 and 0.08 to 0.94, with mean diversity index of 0.79 and 0.65, respectively, showing a wider range than the present study. In disagreement with the

present study, Balakrishnan et al. (2000) obtained a low index of 0.25 and 0.35 for the presence or absence of stalk corky patches and cracks, respectively. High diversity index values generally indicate that the trait is more polymorphic, diverse among the genotypes tested and present with relatively even distribution. In contrast, low values indicate that the trait is less polymorphic, genotypes in the population share traits. According to Blazakis et al. (2024) qualitative morphological traits are distinct for each variety and utilized for easy discrimination of varieties under cultivation.

The H' averaged across countries for different traits varied from 0.39 for the stalk growth crack to 0.76 for the canopy structure, with an overall average of 0.62 (Tables 6 & 7). The diversity of each trait within countries varied from 0.59 for stalk corky patches and the color of the leaves to 1.00 for the relative shape of dewlap. Low genetic diversity for stalk corky patches indicates that this trait is highly conserved within the countries. On the other hand, the proportion of diversity of traits between countries in terms of total variation ranged from 0.01 for the degree of bud extension to 0.43 for the relative shape of dewlap (Table 6). More than fifty percent of the traits exhibited high index values and had non-significant differences in index values among them.

Inconsistent with the current result, Esaya et al. (2018) found H' pooled over regions for various traits varied from 0.44 for stalk corky cracks to 0.94 for the relative degree of internode alignment and bud groove with an overall mean of 0.81. The high index values of the traits show high qualitative morphological trait diversity among the sugarcane genotypes considered for these traits. On the contrary, the low diversity index values of the other traits show the existence of limited diversity among the considered genetic materials for these traits.

Table 6. Estimates of Shannon-weaver diversity index (H′) for 16 qualitative characters partitioned within and between countries

Character	Code	H′	H_c	H_c/H'	$(H'-H_c)/H'$
Presence or absence of bud cushion	BUDCUSHION	0.94	0.64	0.68	0.33
Relative degree of bud extension	BUD EXTEND	0.68	0.67	0.99	0.01
Relative bud shape	BUDSHAPE	0.81	0.74	0.91	0.09
Canopy structure	CANOPY	0.91	0.76	0.84	0.16
Relative shape of dewlap	DEWLAPSHAPE	0.67	0.71	1.00	0.43
Relative plant erectness	ERECT	0.79	0.71	0.89	0.11
Internode alignment	INALIGN	0.56	0.55	0.98	0.02
Relative internode shape	INSHAPE	0.81	0.63	0.78	0.22
Color of the leaves	LEAFCOLOR	0.85	0.50	0.59	0.41
Type of outer auricle	AURICLEOUT	0.69	0.65	0.94	0.06
Shape of ligule	LIGSHAPE	0.60	0.54	0.90	0.10
Stalk corky cracks	STALKCORKC	0.71	0.47	0.66	0.34
Stalk corky patchs	STALKCORKP	0.99	0.58	0.59	0.41
Stalk growth cracks	STALKCRACK	0.50	0.39	0.78	0.23
Color of the exposed rind	RINDCOLE	0.88	0.71	0.81	0.19
Type of bud groove	BUDGROOVE	0.76	0.61	0.80	0.20
Mean		**0.76**	**0.62**	**0.82**	**0.20**

H′= Diversity index for each character calculated from entire data set; Hc = Average diversity index of each character for the 16 countries; Hc/H′= Proportion of diversity within countries; and (H′-Hc)/H′=Proportion of diversity between countries in relation to the total variation.

The H' pooled across the traits by countries ranged from 0.44±0.13 to 0.83±0.04, with an overall average of 0.62 (Table 7). The highest (0.83±0.04) diversity index (H') was recorded for India, followed by France and South Africa (0.82±0.03), and the lowest (0.44±0.13) value of H' was for Guyana and Thailand. However, no diversity was observed among genotypes from Mauritius (0.00) and Brazil (0.00), as only one genotype was considered for each country. Most countries (10 countries) showed a high Shannon-weaver diversity index in the present study (Table 7). Esayas et al. (2018) revealed an H' pooled across traits by region, ranging from 0.79 in Oromia to 0.85 in Tigray, with an overall average of 0.81. Their report also indicated that most of the regions recorded a high Shannon-Weaver diversity index. Belay et al. (2022) revealed high index (H') values (0.84) for genotypes from the Philippines, followed by France (H' = 0.71), while the lowest values were found among the sugarcane genotypes from Puerto Rico, Cuba, Guyana, South Africa, and Brazil. Esayas et al. (2016a) also revealed high index values for the genotypes from Ethiopia and the USA, which disagrees with the current study results. The high index value for different countries indicates the presence of high qualitative morphological trait variability among the sugarcane genotypes of these locations.

Table 7. Estimates of standardized Shannon-Weaver diversity index (H') of sugarcane germplasm for the qualitative traits by country of origin across the traits

Country	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Mean ± SE
Ethiopia	0.47	0.88	0.85	0.94	0.84	1.00	0.97	0.86	0.47	0.86	0.47	0.72	0.72	0.47	0.92	0.72	0.76±0.05
Sudan	0.54	0.81	0.77	0.97	0.67	0.95	0.00	0.99	0.95	0.95	0.81	0.00	0.95	0.54	0.97	0.81	0.73±0.08
USA	1.00	0.87	0.96	0.86	0.82	1.00	0.32	0.91	0.79	0.77	0.76	0.79	0.74	0.67	0.94	0.52	0.80±0.04
Cuba	0.90	0.96	0.88	0.87	0.71	0.83	0.76	0.89	0.70	0.74	0.80	0.34	0.90	0.34	0.90	0.90	0.78±0.05
India	0.76	0.88	0.92	0.83	0.88	0.91	0.51	0.94	0.99	0.61	0.85	0.77	0.99	0.50	0.95	0.96	0.83±0.04
France	1.00	1.00	0.91	0.91	0.73	0.81	0.64	0.81	0.90	0.84	0.77	0.74	0.90	0.74	0.92	0.49	0.82±0.03
Philippines	0.95	0.78	0.93	0.89	0.83	1.00	0.44	0.85	0.95	0.83	0.72	0.44	0.08	0.00	0.95	0.85	0.72±0.08
Barbados	0.96	0.71	0.80	0.87	0.96	0.91	0.59	0.92	0.71	0.83	0.75	0.84	0.98	0.39	0.95	0.71	0.81±0.04
Guyana	0.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	0.44±0.13
Australia	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.50±0.13
Thailand	1.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.44±0.13
South Africa	0.59	0.86	0.83	0.98	0.87	0.91	0.59	0.98	0.59	0.91	0.73	0.86	0.99	0.59	0.92	0.86	0.82±0.04
Brazil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00
Puerto Rico	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.69±0.12
Mauritius	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00
Mexico	0.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.75±0.11
Mean	**0.64**	**0.67**	**0.74**	**0.76**	**0.71**	**0.71**	**0.55**	**0.63**	**0.50**	**0.65**	**0.54**	**0.47**	**0.58**	**0.39**	**0.71**	**0.61**	**0.62**

1= BUDCUSHION, 2 = BUD EXTEND, 3 = BUDSHAPE, 4 = CANOPY, 5 = DEWLAPSHAPE, 6 = ERECT, 7 =INALIGN, 8 = INSHAPE, 9 = LEAFCOLOR, 10 = AURICLEOUT, 11=LIGSHAPE, 12 = STALKCORKC, 13 = STALKCORKP, 14 = STALKCRACK, 15 = RINDCOLE, 16 = BUDGROOVE

This suggests that the genotypes from these locations are the primary sources of genetic variation that could be utilized. Conversely, the lowest diversity index values for other countries elucidate limited qualitative morphological traits and, hence, genetic diversity among the sugarcane genotypes of these countries.

4.1.3. Cluster analysis

A total of 144 sugarcane genotypes were clustered into four different groups by cluster analysis using Euclidean distance. Hence, genotypes in the same cluster were hypothesized to have stronger relationships among them compared with those belonging to two separate clusters. This is similar to the results of Ekpélikpézé et al. (2016), who grouped 89 cultivars into four morphological groups with different characteristics. Similarly, Khalid et al. (2016) also clustered 16 sugarcane genotypes into four different clusters using qualitative traits. However, Esayas et al. (2016) divided 400 sugarcane genotypes into 20 clusters. Table 8 and Figure 2 show the distribution of genotypes in various diversity classes. Genotypes with the least genetic distance were grouped together and referred to as a cluster; each cluster contained a different number of genotypes. Cluster I contained the highest number of genotypes, 83 (57.6%) from fifteen different countries: Barbados (15), France (13), Cuba (11), India (10), USA (8), Philippines (5), Ethiopia (5), Sudan (5), South Africa (2), Puerto Rico (2), Australia (2), Mexico (2), Thailand (1), Brazil (1), and Guyana (1). Cluster II had five genotypes (3.5%) introduced from four countries viz India (Co-421), Guyana (D42/58), Barbados (B54/90), and France (FG06-114 and FG05-155). Cluster III had 51 genotypes (35.4 %) originating from thirteen countries that included Barbados (8), USA (7), India (6), France (6), Cuba (5), Philippines (5), Ethiopia (4), Sudan (4), Puerto Rico (2), South Africa (2), Guyana (1), Mauritius (1), Thailand (1). Similarly, cluster IV contained five genotypes (3.5%) originating from four countries, namely Barbados (DB386/60), the USA (CP001527 & CP52/86), Ethiopia (V422), and the Philippines (VMC9655) (Table 8, Fig. 2).

The genotype clustering pattern appeared independent of their geographical origin, which may be evidence suggesting that diversity in the distribution area is not a vital criterion for grouping accessions into different clusters rather based on the similarity index. This could be attributed to gene flow, exchange of genetic material, may developed from the same genetic material through breeding, and selection for similar traits. The observed phenomenon may also disclose the possibility of close similarity between their

evolutionary courses. Besides, genotypes from different origins may acquire similar genetic traits due to adaptation to similar environmental conditions, leading to clustering together despite different ancestry. This finding agreed with the results of Esayas et al. (2016), Iqbal et al. (2018), Mekonnen and Wakjira (2014), and Naznin et al. (2015). They found an absence of association among different clusters established with the origin of genotypes. Hence, geographical segregation is not the only agent causing genetic variability in sugarcane. Instead, evolution-driving forces such as genetic drift, mutation, natural and artificial selection, and germplasm exchange might have played an essential role in assigning the genotypes to different clusters (Kandamoorthy and Govindarasu, 2005; Senapati and Sarkar, 2005).

On the other hand, the clustering pattern of the genotypes revealed that genotypes originating from the same places did not form a single cluster because of direct selection pressure (based on biotic, abiotic, and agronomic factors), historical gene flow, and mixing with neighboring population. In addition, genetic recombination and random mutations can introduce new variations within a population, causing genotypes from the same origin to diverge and become more similar to genotypes from other origins. For instance, with an exception, the genotypes originating from Barbados were clustered in all (four) clusters. Similarly, the local landraces of Ethiopia were grouped into three clusters with other exotic genotypes at different frequencies; clusters I, III, and IV possessed three, five, and two local landraces, respectively (Table 8, Fig. 2). These results indicate that the Barbadose genotypes and the local landraces had diverse morphological traits although all originated from the same locations. The same results were reported by the previous studies (Esayas et al., 2016a, 2018; Belay et al., 2022).

Cluster I comprised sugarcane genotypes with bud cushion, bud extending above growth ring, ovate and squarish pentagonal bud shape, open semi-droopy canopy structure, squarish deltoid dewlap shape, semi-erect plant, slightly zigzag internode alignment, concave-convex internode shape, green leaves, transitional auricle shape, crescent with lozenge legule shape, devoid of stalk corky crack, devoid of stalk corky patch, devoid stalk growth crack, brownish green exposed rind color, and bud groove traits.

Sugarcane genotypes grouped in cluster III were mainly characterized by the presence of bud cushion, bud extending above growth ring, ovate bud shape, open semi-droopy canopy structure, squarish deltoid dewlap shape, moderately erect plant, slightly zig zag

internode alignment, concave and convex internode shape, green leaves, transitional auricle shape, crescent with lozenge legule shape, no stalk corky crack, no stalk corky patch, no stalk growth crack, brown exposed rind color, and presence of bud groove. Similarly, presence of bud cushion, bud extending above growth ring, squarish pentagonal bud shape, open droopy canopy structure, squarish deltoid dewlap shape, slightly prostate to nearly erect plant, slightly zigzag internode alignment, cylindrical internode shape, green leaves color, transitional and short lanceolate auricle shape, crescent with lozenge legule shape, absence of stalk corky crack, presence of stalk corky patch, absence of stalk growth crack, purple exposed rind color, and presence of bud groove were the main characteristics for the majority of genotypes grouped in cluster IV.

Table 8. Cluster analysis of 144 sugarcane genotypes with 16 qualitative traits

Cluster group	Number of genotypes	Genotypes in cluster										Origin country
I	83	44	80	71	88	28	38	37	47	54	67	Ethiopia, Sudan , Cuba, India, France, Philippines Barbados, Guyana Australia, S. Africa PortoRico, Mauritius Thailand, Mexico and USA
		70	62	18	30	21	41	56	100	86	19	
		81	102	112	66	133	123	98	105	64	127	
		12	108	94	32	122	10	65	33	114	103	
		143	39	7	25	128	138	31	1	106	110	
		5	135	142	83	129	6	72	73	140	120	
		69	4	96	126	77	58	45	99	113	60	
		43	42	101	78	35	36	116	136	26	107	
		121	34	63								
II	5	79	134	8	14	22						Barbados, France, India, and Guyana
III	51	144	74	104	141	48	124	85	111	109	9	Cuba, France, USA, Barbados, India, Brazil, Ethiopia Philippines, Mexico, Australia South Africa, Puerto Rico, Thailand
		16	90	89	137	132	27	131	17	15	68	
		40	55	49	130	91	76	11	24	97	46	
		84	139	29	59	118	51	117	50	125	82	
		93	75	87	20	61	53	3	115	57	92	
		2										
IV	5	23	52	119	13	95						Barbados, Philippines, USA, Ethiopia

Table 9 represents the distance (D) values among different clusters. Distance between the clusters can help distinguish different genotypes and determine the possible promising crosses for improving targeted traits. The analysis revealed that the inter-cluster distance among clusters varied from 5.252 to 9.695. The maximum inter-cluster distance (9.695) was recorded between clusters II and IV, followed by the distance between clusters III and IV (9.354). A more considerable inter-cluster distance implies that the two clusters are well-separated and more distinct from each other as they comprise divergent genotypes. The minimum inter-cluster distance was observed between clusters I and III (5.252), followed by the distance between clusters I and IV (5.734) (Table 9), indicating the clusters are close together or less distinct. Karpagam and Alarmelu (2017) also reported the maximum (14.08) inter-cluster distance between clusters IV and VI and the least distance between clusters II and IV, relatively more divergence than the present finding. Belay et al. (2021) reported intercluster distance values ranged from 9.77 to 12.74. The observed discrepancy with the present result may be due to difference in size and type of genotypes considered.

Table 9. Estimates of inter-cluster distances among the four clusters of 144 sugarcane Genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	0.000	8.623	5.252	5.734
Cluster II	8.623	0.000	9.005	9.695
Cluster III	5.252	9.005	0.000	9.354
Cluster IV	5.734	9.695	9.354	0.000

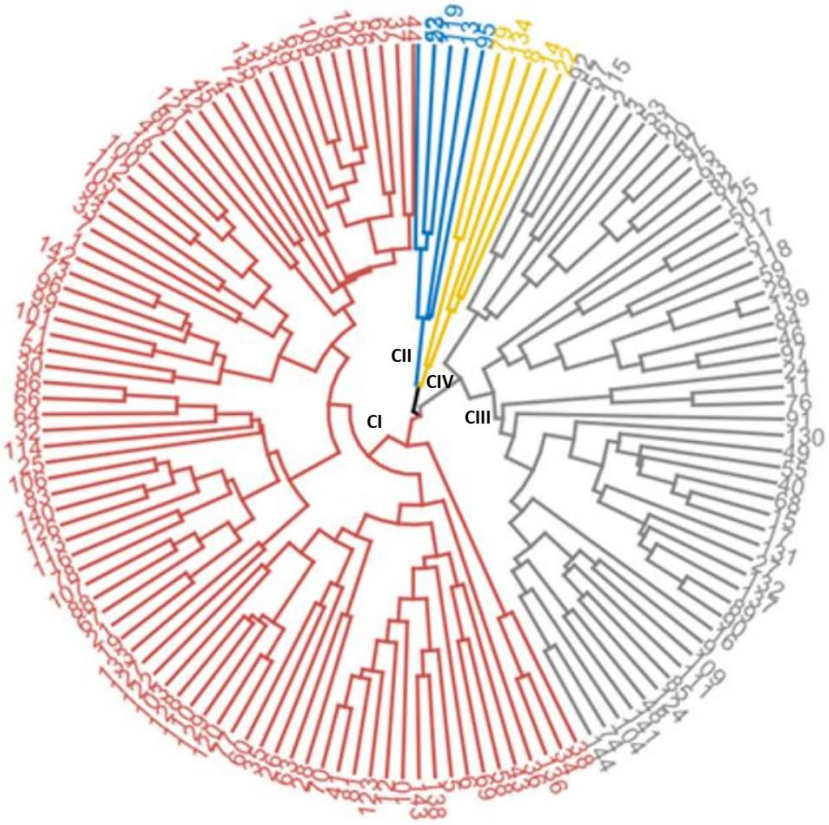

Figure 2. A dendrogram showing the clustering pattern of sugarcane genotypes based on morphological qualitative traits

In our study, qualitative traits revealed high variability among sugarcane genotypes; this could be utilized and conserved as diverse reference varieties for sugarcane crops. Furthermore, since morphological descriptors were distinct for each variety, our findings may help plant breeders and researchers identify, document, and group varieties along with their use in registration, ease discrimination in the cultivated fields, and help in selecting desirable genotypes easily through visual observation at the morphological level.

4.2. Quantitative Morphological Diversity

4.2.1. Variability based on analysis of variance

In any crop improvement program, the availability of genetically divergent germplasm material is a prerequisite for hybridization to develop new better hybrid varieties. A pooled analysis of variance (ANOVA) was performed for the quantitative traits; results were obtained (Table 10). The ANOVA result showed that the mean squares due to the genotypes were highly significant ($P < 0.01$) for all quantitative traits, showing a substantial level of genotypic variation. The present finding is in agreement with the results of Mancini et al. (2012), Smiullah et al. (2020), Alam et al. (2017), Singh et al. (2018b), Filho et al. (2021), and Tawadare et al. (2019), which demonstrated significant variability among the genotypes studied for all quantitative traits. In Table 10, the relatively large mean square of genotypes over GxL and the error mean square indicate that the genotypes varied in their actual inherent potential for the traits. This may provide the best opportunity to select widely adapted genotypes for the locations under study. The same result was reported by Esayas et al. (2016b), Sing et al. (2019), Esayas et al. (2022), and Singh et al. (2023).

Similarly, the mean square of the interaction between genotype and location (GL) demonstrated significant differences ($P < 0.05$), except for two traits (Table 10). This significant GL interaction for most traits explained that the mean performance of the genotypes was affected by the locations. This interaction was mainly due to the alteration in the relative ranking of the genotypes between the locations; genotypes that perform great in one location may perform lower in another and vice versa (Esayas et al., 2016b). Genotype-by-location (GL) interaction is the primary focus of plant breeding programs, as high interactions can reduce selection gains and obscure the detection of the best-performing varieties.

Table 10: Analysis of variance for 16 quantitative traits of sugarcane genotypes evaluated at two test locations (Metahara and Kessem) during 2021-2022

Traits	Location (df=1)	Replication (df=1)	Rep (Block) (df=22)	Genotype (df=143)	Genotype * Location (df=143)	Error (df=265)	CV %	R^2
SP (%)	15290.04**	51.40^{NS}	340.85**	321.54**	208.23**	65.66	20.8	0.85
TCHA	90642441150**	2354745785*	3143327219**	3392400586**	111198562**	555661502	20.1	0.85
MCHA	376743668^{NS}	1823839946**	2994973525**	1774710364**	497373139**	165988442	16.7	0.90
SH (cm)	1321.20**	69.41^{NS}	3719.13**	3113.23**	805.02**	537.46	11.2	0.81
SD (cm)	1007.35**	8.78**	15.31**	2.23**	1.47*	0.006	14.5	0.84
SCW(kg)	0.41**	0.01^{NS}	0.34**	0.28**	0.09**	0.06	18.9	0.77
NOI	116.46**	16.34**	29.55**	20.75**	2.75^{NS}	2.75	7.2	0.87
LW(cm)	82.89 **	0.59^{NS}	2.06**	0.90**	0.47**	0.35	14.1	0.78
LL(cm)	10703.63**	42.79^{NS}	478.41**	388.73**	142.47^{NS}	162.62	9.2	0.69
LA(cm^2)	2841106.90**	17768.89^{NS}	51973.97**	31188.39**	15501.58**	11519.32	18.3	0.78
Pol%	553.86**	19.69**	2.67**	4.10**	1.93*	1.48	6.8	0.79
Brix%	1073.33**	8.99**	4.84**	3.46**	2.35**	1.28	5.7	0.85
Purity%	589.84**	17.20*	11.94**	9.63**	5.02*	3.67	2.1	0.74
Sucrose%	178.08**	13.80**	2.31*	2.69**	1.50**	1.05	8.1	0.74
CYHA	73677.12**	8940.42^{NS}	39669.86**	30052.30**	10796.22**	6898.63	26.5	0.79
SYHA	20.67^{NS}	712.96**	712.04**	494.78**	206.57**	111.69	26.7	0.80

SP = Sprouting percentage, TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = single cane weight, NOI = number of internodes, CYHA = cane yield ton per hectare, Pol = Polarity, SYHA = sugar yield ton per hectare, LW = leaf width, LL = leaf length, LA = leaf area, CV = coefficient of variation, R2= coefficient of determination, NS = non-significant difference, *, and ** = difference at the significance level 5, and 1%.

The interaction of GL may indicate the significance of multilocation trials in obtaining accurate data. The present result was consistent with the results of Mebrahtom et al. (2017), in which the GL interaction was high for cane yield and yield attributes. Zhou (2019) revealed a significant difference in GL interaction for cane yield and sucrose content.

Guilly et al. (2017) also reported a significant GL interaction in each sugarcane trait considered and suggested that GL interactions were the most critical source of genotype-environment interactions. Previous studies that included different locations in Ethiopia also revealed considerable genetic variation in yield and yield attributes among evaluated sugarcane genotypes (Feyissa et al., 2014; Esayas et al., 2016b; Mebrahtom et al., 2016; Shitahun et al., 2022; Belay et al., 2023a).

The mean performance of the genotypes for all traits is displayed in Appendix Table 1. The genotypes exhibited variability in their mean performance in all considered traits. Genotype FG06-695 showed the highest percentage of sprouting (58.8%), while the lowest (14.46%) was produced by 2-111, with a mean value of 39.03%. Maximum mean values for TCHA (193965 tillers/ha), MCHA (142931 stalks/ha), CYHA (184.5 t/ha), and SYHA (23.63 t/ha) were observed for genotype CP70/321. In contrast, the minimum TCHA (37759 tillers/ha), MCHA (27931 stalks/ha), CYHA (3.11 t/ha), and SYHA (4.1 t/ha) were observed in genotypes DB377/60, FG05-155, FG06-747, and FG06-747, respectively. Similarly, stalk height (SH) ranged from the lowest, 138.3 cm in genotype C85-102, to the highest, 276.43 cm in genotype VMC9655, with a mean value of 208 cm. The maximum SD (3.1 cm) was recorded for the Co810 genotype, while the lowest (1.49 cm) was recorded for the B59/250 genotype. Regarding single cane weight (SCW), all genotypes ranged between 0.75 and 2.2 kg, with the highest (2.2 kg) being exhibited by the local landrace V141, while the lowest (0.75 kg) was shown by the genotype CP961252. Data presented in Appendix Table 1 revealed that genotypes NCo-334 and C8751 exhibited the maximum (27.25) and minimum (15.25) number of internodes, respectively (Appendix Table 1). The current results showed variation among the genotypes for the quantitative traits considered. Therefore, genotypes that exhibit superior performance in a particular trait can be considered in the selection strategy to improve the traits of the corresponding genotypes, given the traits in consideration have good heritability values and high genetic advance.

The coefficients of variation (CV %) for sixteen measured quantitative traits are shown in Table 10, ranging from 2.11% in Brix% to 26.68% in CYHA, with an average of 14.40, showing good experimental precision, environmental condition control, and strong treatment effects. Earlier studies have also revealed higher CV values for cane and sugar yields (Esayas et al., 2016; Belay et al., 2023a). Additionally, all traits had a high coefficient of determination (R^2, ≥ 0.69), implying a high level of experimental precision.

4.2.2. Comparison of the best 5% genotypes against commercial cultivars and check varieties

A list of the eight top genotypes (best 5%) for each of the quantitative traits and their comparative advantage over commercial cultivars and standard check varieties (B52/298 and NCo-334) is presented in Appendix Table 2. Appendix Table 3 also shows the mean performance of 11 commercial varieties cultivated in the study areas. The top 8 genotypes outperformed standard check varieties and the mean commercial cultivar (MCC) in cane yield and yield components, including TCHA (1.6–61.2% and 27.1–51.7%), MCHA (1.2–47.5 and 45.3–73.6%), SH (8.8–37.3 and 19.7–27.1%), SD (6.1–62.3 and 22.1–34.1%), SCW (19.7–87.2 and 30.5–55.4%) and CYHA (6.7–33.4 and 32.1–66.2%), respectively (Appendix Table 2). This suggests that these genotypes have potential for use in sugarcane breeding programme to improve cane yield and can also be considered as potential commercial cultivars after testing for ratooning ability. In line with the current result, Belay et al. (2023a) reported a mean performance of the top 10 genotypes for cane yield and yield components with comparative advantage over the standard check varieties in cane yield and yield components except for single cane weight.

Concerning sugar yield and sugar quality traits, the top 5% of genotypes showed advantage in Brix% (0.5 to 12.2% and 5.9 to 10.5%), Pol% (0.3% to 15.6% and 7.3 to12.0%), purity% (0.56% to 5.68% and 2.6% to 3.8%), sucrose% (0.3 to 20.0% and 14.5 to 20.16%), and SYHA (1.4% to 38.77% and 29.7 to 65.11%) over standard check varieties and commercial cultivar means, respectively (Appendix Table 2 and 3). This makes them highly essential for breeding program, which could be harnessed for improving the respective traits. Genotypes CP70/321, Mex57/197, D41/46, B657/150, CP6023, CO-967, and V141 showed superior sugar yield over standard check varieties and commercial cultivars in 1.4% to 38.77% and 29.7 to 65.11%, respectively. Belay et al. (2023a) also identified genotypes with superior sugar quality traits than the standard

checks. The magnitude of the mean advantages was more significant than the present findings for all sugar yield and yield components. The observed difference may be due to genotype differences and environmental conditions.

In the present study, the top 5% genotypes expressed a high genetic potential compared to commercial cultivars and standard check varieties in all traits studied. Genotypes vary in their inherent genetic makeup for the performance of various traits (Khalid et al., 2014). The generated information helps to understand the extent of genetic variation among the genetic materials and to identify potential individuals for further study. Generally, selected genotypes with top 5% for all quantitative traits can be considered a potential source of genetic materials for the future improvement of the respective quantitative traits through selection and hybridization.

4.2.3. Estimates of means and ranges

The mean performance and range value of sixteen quantitative traits are presented in Table 11. The mean value of TCHA was 117237.6, with a range of 14482.8 to 265344.8 tillers, with a difference of 250862 tillers per hectare. This showed substantial variation between the range value and the population mean, implying a substantial variation among the genotypes for this trait and the potential for improvement of this trait. The observed substantial variation coupled with high GCV, moderate heritability, and high genetic advance values recorded for TCHA and strong correlation with cane yield (Table 12) could aid in the improvement of yield through selection. High variability in the number of tillers (TCHA) in sugarcane reported by Esayas et al. (2018), Pandey et al. (2018), Belay et al. (2023b), which agrees with the current result.

Similarly, the average number of millable canes per hectare (MCHA) was 77309.5, with a range of 11724.1 to 162758.6, representing a difference of 151,034.5 stalks per hectare. This indicates a significant variation between the population mean and range values, implying substantial variation among the sugarcane genotypes considered for the MCHA, and the possibility of improving this trait. The observed significant variation in this trait, combined with its high GCV, moderate H^2, and high GAM value (Table 12), and strong correlation with cane yield (Table 13 & 14) may enable the development of a sugarcane variety with higher yield by selecting the best genotypes using this trait. Earlier studies reported similar findings (Esayas et al., 2018; Kumar et al., 2018; Belay et al., 2023b).

The mean value for LA was 587 cm^2, with a range of 168 cm^2 to 1169 cm^2 that extended to a range unit of 1001. This indicates a considerable variation between the range value and the mean in this trait, implying substantial variation among the tested genotypes, which is a potential source for improving this trait. This trait is associated with the canopy light interception capacity, which influences the photosynthetic rate of a crop. The larger the leaf area a genotype has, the better its capability to intercept and use available light. Hence, improvement in this trait could improve photosynthetic efficiency, thereby increasing yield in sugarcane.

The mean performance value for CYHA was found to be 99.32 t/ha, while the range for this trait was 8.08 to 232.19 t/ha, indicating a presence of significant variation between the mean and the range value of the trait. Similarly, the observed SYHA ranged from 0.96 to 26.88 t/ha, with a mean value of 12.52, showing a substantial variation between the population mean and the range value of SYHA. These results imply great variability between the tested genotypes for these attributes, paving the way for sugar and cane yield improvement through strategic selection and cross-breeding. The high GCV, moderate heritability and high genetic advance values for cane and sugar yield (Table 12) also confirmed the best opportunity to improve these traits through selection. High variation in CYHA and SYHA was also observed in studies by Esayas et al. (2018), Kumar et al. (2018), and Belay et al. (2023b).

The mean for SH was 208cm with a range of 118 cm to 305 cm with a variation of 187. The range of variation for SD ranged from 1.05 cm to 3.39 cm with a population mean value of 2.3 cm. Similarly, single cane weight varied from 0.36 kg to 2.43 kg, with a mean of 1.30 kg (Table 11). The observed huge variations between the ranges and the mean values of these traits showed the presence of considerable variations among the tested genotypes for these traits. Hence, this variation is basic for effective selections and sustainable improvement of these traits in sugarcane. Sucrose percent scored a mean value of 12.59% and a range value from 8.14 to 15.95%. The result showed a great variation between the mean and range values, revealing a significant variation among the genotypes for this trait and the potential for improvement.

It was further supported by the high heritability and genetic advance values for sucrose percent (Table 12) that genotypes could be selected based on how well they performed phenotypically for these traits.

Table 11. Basic statistics data of all sugarcane genotypes used in the study

Traits	Mean	Maximum value	Minimum value	Range unit
TCHA	117237.6 ± 21911	265344.80	14482.76	250862
MCHA	77309.54 ± 11883	162758.60	11724.14	151034.5
SH(cm)	208 ± 0.15	305	118	187
SD(cm)	2.3 ± 0.24	3.4	1.0	2.3
SCW(kg)	1.30 ± 0.19	2.43	0.36	2.07
NOI	21.26 ± 1.21	29	13	16
LW(cm)	4.19 ± 0.54	7.04	1.70	5.34
LL(cm)	138.79 ± 9.56	190	80	110
LA(cm^2)	587.03 ± 98.58	1169	168	1001
Pol%	17.97 ± 1.20	21.96	12.20	9.76
Brix%	19.83 ± 0.79	23.76	14.00	9.76
Purity%	90.66 ± 1.66	97.60	80.99	16.61
Sucrose%	12.59 ± 0.85	15.95	8.14	7.81
CYHA (t/ha)	99.32 ± 20.51	232.19	8.08	224.11
SYHA (t/ha)	12.53 ± 2.67	26.88	0.96	25.93

TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = Single cane weight, NOI = Number of internode, CYHA = Cane yield per hectare, Pol = Polarity, SYHA = Sugar yield per hectare, LW = Leaf width, LL = Leaf length, LA = Leaf area

The result indicated significant variation for this trait between the population mean and the range values. Similar results were reported by previous studies for stalk height (Gowda et al., 2016), SCW (Gowda et al., 2016; Esayas et al., 2018), sucrose percent (Belay et al., 2023b) and SD (Esayas et al., 2018).

A number of internodes revealed a range of 13 to 29, with a population mean value of 21.26. Pol percent ranged from 12.2 to 21.96%, with a mean value of 17.97%. Brix percent had a mean value of 19.83% and ranged from 14 to 23.76%, with a range unit of 9.71. The purity percentage values varied from 80.99 to 97.60, with a mean value of 90.66%. Similarly, LL ranged from 80 to 190 cm and LW from 1.70 to 7.04 cm, with a mean value of 138.79 cm and 5.34 cm, respectively (Table 11). The variability between

the sugarcane quantitative (quality) traits was also revealed by several previous authors (Ekpélikpézé et al., 2016; Jayabose et al., 2022).

4.2.4. Estimates of variance components, heritability, and genetic advance

The efficiency of selecting superior genotypes relies on the amount of inherent variation, heritability, and genetic advance for a particular trait. The estimates of variance components, coefficient of variation, heritability, and genetic advance the mean are displayed in Table 12.

4.2.4.1. Estimate of variance components

Genetic variance (σ^2g) is vital, as it refers to the extent of genetic variability in the traits. After dissecting the phenotypic variance (σ^2p), it was revealed that the genotypic variance was higher than the environmental variance, but lower than phenotypic variance for traits such as TCHA, MCHA, SH, SD, and NOI (Table 12). This finding implies that non-additive genetic effect such as dominance and epistasis may played a substantial role in the variability of these traits, hence the trait's heritability expected to be moderate to high. Similar to the present study results, higher σ^2g over environmental variance (σ^2e) was reported for single cane weight, cane yield, number of millable canes, and stalk height (Chaudhary, 2001); for the number of internodes, millable canes, and stalk weight (Jamoza et al., 2014); and for all traits considered except number of millable cane (Diribu et al., 2020). Phenotypic variance (σ^2p) was higher than genotypic variance (σ^2g), which in turn less than environmental variance (σ^2e) for traits such as SCW, LW, LL, LA, pol%, sucrose%, CYHA and SYHA (Table 12), indicating that the expression of these trait influenced by environmental factors, hence making direct selection of genotypes based on these traits challenging.

According to Sivasubramanian and Menon (1973), GCV and PCV values are described as high (> 20%), moderate (10-20%), and low (< 10%). In this study, the high values of GCV and PCV were observed in TCHA (24.4 and 26.4), MCHA (23.1 and 27.2), CYHA (69.9 and 87.3), and SYHA (67.7 and 88.8) (Table 12). High GCV and PCV together imply the presence of considerable genetic variability for such a trait, suggesting that the trait has good breeding potential and can be improved through effective selective breeding programs. The current results are in accordance with the previous reports (Esayas et al., 2016b; Diribu et al., 2020; Belay et al., 2023b) for the number of tillers (Bhatnagar, 2003)

for the number of millable cane; (Diribu et al., 2020; Belay et al., 2023b) for cane yield; and (Esaya et al., 2016b; Gowda et al., 2016) for sugar yield.

It is evident in Table 12 that, moderate GCV and high PCV were recorded in SP (13.64 and 23.0) and SCW (16.76 and 20.4). A similar result was reported by Belay et al. (2023b) and Esayas et al. (2016b) for SCW. The combination of moderate GCV and high PCV implies that the trait had moderate genetic variability and was strongly affected by environmental factors. Improving such traits via selective breeding may be more complex and require due attention to minimize environmental effects and enhance genetic variation. Moderate GCV and PCV were observed for SH (11.55 and 13.4) and LA (10.67 and 15.0). This indicates that the trait had modest genetic variability and was moderately affected by environmental and genetic factors, thus having a modest scope for genetic improvement via selective breeding.

The analysis result revealed that low GCV and moderate PCV were recorded for SD (5.92 and 10.1), NOI (9.98 and 10.7), and LW (7.83 and 11.3, respectively) (Table 12). Similar result was also reported by Belay et al. (2023b) for SD and NOI, Dereje (2018) for stalk diameter, and Esayas et al. (2016b) for the number of internodes per stalk. Low GCV and moderate PCV indicated that the trait had limited genetic variability and was more affected by environmental effects; hence, there is comparatively limited scope for improvement of these traits through selection, selection may not be effective. As a result, improving such traits may require expert attention to enhance genetic diversity, which includes introducing new germplasm or exploring genetic resources from related species or environmental modification.

Table 12 shows that low GCV and low PCV were exhibited in LL (5.65 and 7.3), Brix% (2.66 to 5.6), pol% (4.10 and 4.7), purity% (1.18 and 1.7), sucrose% (4.33 and 6.5), implying the presence of low variability in these quality traits (Table 12). Environment rather than genetic factors predominantly influenced the expression of the trait, thus having limited potential for improvement. Improving such traits may require strategies to enhance genetic diversity through exploring genetic resources from related populations or using genetic engineering techniques to create novel variations. Similar results were reported by Gowda et al. (2016) for pol%, purity% and Brix%; Diribu et al. (2020) for Brix% and purity%; and Dereje (2018) for purity percent.

This study also found that PCV values higher than their corresponding GCV for all traits we considered (Table 12), indicating that the apparent variation is not only due to genetics but also due to environmental effect. This finding is in agreement with the results reported by Dereje (2018), Kumar et al. (2018), Diribu et al. (2020), and Kumar (2023) but contradicts the results reported by Patil and Patel (2017). However, the closer difference between the PCV and GCV for TCHA, MCHA, SH, and NOI (Table 12), expressed the little influence of environmental factors for these traits. Earlier workers also reported similar results. GCV gives insight into the relative genetic variability present in quantitative traits of the base population. However, it cannot estimate the magnitude of the variation transferred to the next generation. Instead, GCV and heritability estimates would give the best understanding of the extent of advance to be expected from selection (Allard, 1960).

4.2.4.2. Heritability

Heritability is a valuable measure of how traits are passed from parents to their offspring. Heritability in a broad sense (H^2), is classified as low (0–30 percent), moderate (31–60%), and high (>60%) (Robinson et al., 1949). In this study, an estimate of heritability broadly varied from 32% for pol% to 87% for the NOI (Table 12). High heritability value was recorded for TCHA (86%), MCHA (72%), SH (74%), SCW (68%), NOI (87%), and CYHA (64%). This result conforms with the reports of Alam et al. (2017) for NOI; Belay et al. (2023) for TCHA, MCHA, and SCW; Gowda et al. (2016) and Esayas et al. (2016b) for the number of tillers, number of millable cane, single cane weight, number of internode, and cane yield; Diribu et al. (2020) for numbers of tiller, single stalk weight, cane height, and cane yield. The high heritability of a trait indicates that a substantial proportion of the observed variability in the trait was due to genetic makeup rather than environmental factors and confirms repeatability of the trait performance.

Hence, selection for improvements in such traits could be relatively easy and rewarding. The important stalk traits that were taken into consideration generally had heritability estimates that ranged from moderate to high. This means that simple selection could be used to enhance these traits. Based on the heritability estimates in the current study, the performance of the trait SCW, TCHA, NOI, CYHA, MCHA, and SH were repeatable and genotypes could be easily selected.

Table 12: Variance components, genotypic and phenotypic coefficients of variance, heritability, and GAM for 16 traits of sugarcane genotypes tested

Traits	σ^2p	σ^2g	σ^2e	σ^2gl	GCV	PCV	H^2	GA	GAM
SP (%)	80.4	28.3275	65.66	71.29	13.7	23.0	35	404.2	10.4
TCHA	959215882	820300506	555661502	0.00	24.4	26.4	86	3809114	32.5
MCHA	443677591	319334306	165988442	165692349	23.1	27.2	72	2442312	31.6
SH (cm)	778.3	577.1	537.46	133.78	11.6	13.4	74	2811.2	13.5
SD (cm)	5.6	1.9	0.06	7.32	5.9	10.1	34	165.4	7.1
SCW(kg)	0.1	0.05	0.06	0.02	16.8	20.4	68	22.5	17.3
NOI	5.2	4.5	2.75	0.00	9.98	10.7	87	291.6	13.7
LW(cm)	0.2	0.1	0.35	0.06	7.83	11.3	48	21.6	5.2
LL(cm)	102.2	61.6	162.62	0.00	5.7	7.3	60	572.8	4.1
LA(cm^2)	7797.1	3921.7	11519.32	1991.13	10.7	15.0	50	4346.4	7.4
Pol%	0.9	0.6	1.28	0.54	4.1	4.7	32	29.2	1.5
Brix%	1.0	0.3	1.48	0.23	2.7	5.6	53	53.1	3.0
Purity%	2.4	1.2	3.67	0.68	1.2	1.7	48	71.5	0.8
Sucrose%	0.7	0.3	1.05	0.23	4.3	6.5	44	34.5	2.7
CYHA(t/ha)	7513.1	4814.0	6898.63	1948.80	69.9	87.3	64	6785.1	68.3
SYHA(t/ha)	123.7	72.1	111.69	47.44	67.7	88.8	58	796.9	63.6

SP = Sprout percentage, TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = Single cane weight, NOI = Number of internode, CYHA = Cane yield per hectare, Pol = Polarity, SYHA = Sugar yield per hectare, LW = Leave width, LL = Leave length, LA = Leave area, ECV = Environmental coefficient, GCV=Genotypic coefficient of variance, PVC = Phenotypic coefficient of variance, H^2 = Heritability, GA = Genetic advance, GAM = genetic advance as percentage of mean

Analysis result in Table 12 elucidated that traits viz. SP (35%), SD (34%), LW (48%), LL (60%), LA (50%), Brix% (53), pol% (32), purity% (48), sucrose% (44%), and SYHA (58%) exhibited moderate heritability in this study. Moderate heritability indicates a balanced influence of genetic factors and environmental conditions on the phenotypic expression of a trait. It indicates a moderate breeding potential of the traits, highlighting the significance of dealing with both genetic and environmental factors in improving such traits. The same results were also reported for SD (Patra et al., 2022), Brix% (Esaya et al., 2016b), Brix%, Pol%, and sucrose% (Dereje, 2018). Heritability alone cannot predict the degree of genetic improvement that would arise via genotype selection. Instead, higher heritability should be coupled with higher estimates of GCV and GAM (Johnson et al., 1955).

4.2.4.3. Genetic advance

High heritability coupled with genetic advance as a percentage of the mean would guarantee more effective and efficient selection (Belay et al., 2023b). Genetic advance as population mean (GAM) is essential in predicting the expected genetic gain from one selection cycle (Hamdi et al., 2003). In this study, the expected genetic advance as a percentage mean varied from 0.8% for purity to 68.3% for CYHA (Table 12). Johnson et al. (1955) described the genetic advances as a percentage of the mean (GAM) as low (0–10%), moderate (11–20%), and high (above 20%). Accordingly, among the sixteen traits measured, the high genetic advance was found in TCHA (32.5%), MCHA (31.6%), CYHA (68.3%), and SYHA (63.6%) traits (Table 12). This result showed a significant opportunity to improve these traits by adopting an appropriate breeding approach, including direct selection. Earlier studies have also reported high genetic advances for sugar yield, cane yield, number of tillers, and number of millable canes (Esayas et al., 2016b; Gowda et al., 2016; Diribu et al., 2020; Belay et al. (2023b), which supported the present result. Contrary to the present finding, Kumar et al. (2017) reported intermediate genetic advances for the number of tillers, cane yield, and sugar yield.

As shown in Table 12, GAM values were found to be moderate in the SP (10.4%), SH (13.5%), SCW (17.33%), and NOI (13.7%) traits. This implies that improvement in these traits over successive generations is possible through selection, but the rate of progress would be slower than traits with high genetic advances. In line with the present results, moderate GAM for sprouting percentage and single stalk weight (Kumar et al., 2018),

stalk height (Gowda et al., 2016), and single cane weight and number of internodes (Kumar et al., 2017) were reported.

The analysis result also elucidated that, low GAM was observed for quantitative traits such as SD (7.1%), LW (5.2%), LL (4.1%), LA (7.4%), Brix% (3.0%), pol% (1.5%), purity% (0.8%), and sucrose% (2.7%) (Table 12). This implies that the trait's expected genetic progress or improvement was limited by selection. Hence, improvement of these traits is challenging through selective breeding. Similar results were also observed by earlier studies (Kumar et al., 2018; Belwal & Ahmad, 2020; Belay et al., 2023b) for Brix%, pol%, purity%, and sucrose%; (Gowda et al., 2016; Diribu et al., 2020) for purity percent. Inconsistent with the present results, Singh et al. (2019a) found a high genetic advance in the Brix and pol percent.

In general, high GCV, high heritability or high genetic advance alone does not always provide a high prediction of genetic gain to ensure effective selection for improvement. Hence, higher GCV should be coupled with higher estimates of broad sense heritability and GAM to provide accurate and valuable selection (Johnson et al., 1955). In this research, high GCV, H^2, and GAM were recorded for TCHA, MCHA, and CYHA traits (Table 12), indicating the involvement of additive gene action in these traits. This shows that selection of these traits based on simple phenotypic observation is possible. Many studies also reported strong positive correlation of these traits with cane yield (Belwal & Ahmad, 2020; Esayas et al., 2022; Sharma et al., 2023). Similar results for CYHA indicated that direct selection for yield seems plausible. Similar results were reported for number of millable canes (Chaudhary, 2001; Esayas et al., 2016b; Esayas et al., 2022), in conformity with current results. Furthermore, SYHA exhibited high GCV, moderate H^2, and high GAM (Table 12), indicating the possibility to improve this trait by direct selection. In conclusion, the results of the current study indicated the importance of TCHA, MCHA, CYHA, and SYHA as one of the selection criterion in sugarcane breeding program to improve sugarcane yield.

4.2.5. Association of traits

Analysis of traits association estimates the natural relationship between various traits and identifies the traits on which selection would be utilized for crop improvement. Path analysis, on the other hand, separates these correlations into the estimate of the direct and indirect contribution of a set of independent traits to the dependent traits. For this study the

genotypic and phenotypic correlation among the morphological quantitative traits of sugarcane genotypes are presented in Table 13 and 14.

4.2.5.1. Genotypic correlation

Cane yield had a positive and highly significant genetic correlation with SP (rg = 0.533**), TCHA (rg = 0.699**), MCHA (rg = 0.768**), SH (rg = 0.704**), SCW (rg = 0.368**), and NOI (rg = 0.407**) (Table 13), indicating that selection based on these multiple traits could be rewarding in improving CYHA in sugarcane genotypes. The genotypic association between yield components and cane yield indicates their inherent genetic relationship. The positive strong genetic association implies that genetic factors promoting high-yield components also tend to promote high cane yield, and the reverse is true for negative genetic correlation. The reports by Tabassum et al. (2023) and Kumar et al. (2022) were in conformity with the current findings, except for a number of internodes. Previous studies found similar results for stalk height, stalk weight, and number of millable canes (El-Taib, 2009; Masri et al., 2015); millable stalk number (Patel et al., 2006); single cane weight (Masri et al., 2008; Masri et al., 2022); and internode number (Jamoza et al., 2014; Esayas et al., 2016e).

In addition, there was a highly significant negative genetic association between CYHA and SD (rg = - 0.262**), which is consistent with the findings of Tahir et al. (2014). Table 13 shows that CYHA has a non-significant negative genetic association with pol percent (rg = - 0.047), Brix% (rg = - 0.019), and sucrose% (rg = - 0.026), but a non-significant positive correlation with purity% (rg = 0.078). This implies that difficulty in simultaneous improvement in SD, pol%, Brix%, and sucrose% and CYHA. In addition, improvement in purity% could have no influence on improvement of CYHA. Similar results were reported by Esayas et al. (2016e) for purity percent, and contrary results were also reported for Brix%, pol%, and sucrose%. In contrast to the current finding, Ali et al. (2018) and Tyagi et al. (2012) found a positive and highly significant correlation between cane yield and sucrose% at the genotypic level. Tahir et al. (2014) and Ali et al. (2018) found similar results with the present study for Brix%. Whereas Smiullah et al. (2013) revealed a positive relationship between cane yield and Brix% in contrast to the present result.

In this study, LW (rg = -0.266**) and LA (rg = -0.221**) had a negative and significant genetic correlation, while LL (rg = -0.057) demonstrated a non-significant and negative genetic association with cane yield.

Table 13: Genotypic correlation coefficients of 16 yield related traits and sugar yield of 144 sugarcane genotypes

	SP	TCHA	MCHA	SH	SD	SCW	NOI	CYHA	Pol	Brix	Purity	Sucrose	LW	LL	LA	SYHA
SP	—															
TCHA	0.775**	—														
MCHA	0.663**	0.870**	—													
SH	0.181*	0.259**	0.326**	—												
SD	-0.476**	-0.587**	-0.670**	-0.188*	—											
SCW	-0.205*	-0.260**	-0.338**	0.580**	0.610**	—										
NOI	0.091^{NS}	-0.010^{NS}	0.113^{NS}	0.558**	0.007^{NS}	0.421**	—									
CYHA	0.533**	0.699**	0.768**	0.704**	-0.262**	0.368**	0.407**	—								
Pol	0.131^{NS}	0.042^{NS}	-0.142^{NS}	-0.082^{NS}	0.144^{NS}	0.025^{NS}	0.286**	-0.047^{NS}	—							
Brix	0.161^{NS}	0.080^{NS}	-0.085^{NS}	-0.079^{NS}	0.089^{NS}	0.008^{NS}	0.264**	-0.019^{NS}	0.935**	—						
Purity	0.228**	0.186*	0.128^{NS}	-0.055^{NS}	-0.11^{NS}	-0.108^{NS}	0.191*	0.078^{NS}	0.700**	0.823**	—					
Sucrose	0.154^{NS}	0.077^{NS}	-0.098^{NS}	-0.081^{NS}	0.092^{NS}	-0.001^{NS}	0.282**	-0.026^{NS}	0.992**	0.945**	0.794**	—				
LW	-0.546**	-0.632**	-0.610**	-0.185*	0.702**	0.478**	-0.136^{NS}	-0.266**	0.001^{NS}	-0.043^{NS}	-0.261**	-0.046^{NS}	—			
LL	-0.029^{NS}	-0.042^{NS}	-0.134^{NS}	-0.066^{NS}	0.256**	0.130^{NS}	-0.384**	-0.057^{NS}	-0.263**	-0.270**	-0.275**	-0.286**	0.314**	—		
LA	-0.421**	-0.497**	-0.521**	-0.153^{NS}	0.655**	0.436**	-0.255**	-0.221**	-0.106^{NS}	-0.142^{NS}	-0.301**	-0.150^{NS}	0.900**	0.696**	—	
SYHA	0.549**	0.710**	0.750 **	0.681**	0.264**	0.356**	0.432**	0.994**	0.128^{NS}	0.159^{NS}	0.218**	0.150^{NS}	-0.270**	-0.088 ^{NS}	-0.237**	—

SPC = Sprout count, TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = Single cane weight, NOI = Number of internode, CYHA = Cane yield per hectare, Pol = Polarity, SYHA = Sugar yield per hectare, LW = Leave width, LL = Leave length, LA = Leave area, * and ** = significant at 5% and 1% probability level, respectively, NS = Non-significant difference

This kind of relationship with CYHA implies that direct selection based on these traits may not be practical and their indirect effect on CYHA via other traits should be investigated which is evident from path analysis. Contrary to our findings, Esayas et al. (2016e) identified a highly significant and strong positive genetic relationship between cane yield and leaf length, width, and area.

In Table 13, it was observed that the sprouting percentage (SP) exhibited a highly significant positive genetic association with TCHA (rg = 0.775**) and MCHA (rg = 0.663**). This indicates that an improvement in sprouting percentage would concurrently cause an enhancement in the number of tillers and millable cane numbers. These findings are consistent with the results of Tabassum et al. (2023). A significantly positive genetic association was found between SP and SD (rg = 0.181*). It also had a significantly negative (rg = -0.205*) association with SCW, showing the difficulty in simultaneously improving these two traits. It also had a non-significant and positive correlation with NOI (rg = 0.091), and with sugar quality traits such as pol% (rg = 0.131), Brix% (rg = 0.161), and sucrose% (rg = 0.154). Traits LW and LA had a negative and highly significant (rg = -0.546** and rg = -0.421**, respectively) genetic association with SP, while LL showed a negative and non-significant association (rg = -0.029). These results indicate improving the sprouting percentage significantly reduce these traits. This could be due to increased sprouting percentage increase tiller numbers and thus number of millable canes increasing the population leading to competition that can result in low leaf area.

A positive and highly significant genetic association was found between TCHA and MCHA (rg = 0.870**) implying that TCHA can be simultaneously improved with MCHA. Previous studies by Tabassum et al. (2023), Parihar (2020), and Tahir et al. (2014) also reported similar results for these traits. Similarly, TCHA and MCHA had positive and highly significant genetic association with SH (rg = 0.259**, rg = 0.326**) (Table 13) showing that increase in TCHA and MCHA could lead to increase in SH of the stalk. Hence, TCHA and MCHA can be used as selection criteria for this trait. TCHA and MCHA had negative and highly significant genetic association with SD (rg = -0.587**, rg = -0.670), SCW (rg = -0.260**, rg = -0.338**), LW (rg = -0.546**, rg = -0.610**), and LA (rg = -0.421**, rg = -0.521**), indicating that SD, SCW, LW, and LA decrease as the TCHA and MCHA increase, hence selection based on these multiple traits would not be rewarding in TCHA and MCHA improvement. This is consistent with the findings of El-Taib (2009), Tahir et al. (2014), and Masri et al. (2015) who showed a negative correlation

with SD. Parihar (2020) found similar results for SCW. In disagreement with the present finding, highly significant and positive genetic relationships of MCHA with SD and SCW were reported (Esayas et al., 2016e).

TCHA had a positive non-significant genotypic correlation with most sugar quality traits (Brix%, pol% and sucrose %), except purity percent, which showed positive and significant (rg = 0.186*) genetic association with TCHA. This implies that selection for sugar quality traits Brix%, pol%, and sucrose% based on TCHA would not be rewarding. The present result also indicated that all the quality traits had non-significant positive genetic correlations with MCHA, except purity percent, which had a negative association with MCHA. In conformity with the present result, Esayas et al. (2016e) reported positive and a non-significant correlation of MCHA with sugar quality traits.

Trait SH exhibited a positive and highly significant relationship with SCW (rg = 0.588**) and NOI (rg = 0.558**), indicating that improvement in SH leads to improvement in SCW and NOI, because the same genetic factor control the two traits. Hence, SCW and NOI could be considered as selection criteria for SH improvement. Earlier study by Tabassum et al. (2023) and Parihar (2020) also reported a similar result for the SCW. This study revealed that SH had a negative and non-significant association with all the sugar quality traits. Hence, SH could not be used as selection criteria for improvement of sugar quality traits. This is in conformity with Parihar's (2020) findings, which showed a negative and highly significant association between SH and purity% and sucrose%. Additionally, traits such as LL (rg = -0.066) and LA (rg = -0.153) were negatively and non-significantly correlated with SH, while SD (rg = -0.188**) and LW (rg = -0.185*) were negatively and significantly associated with SH. This relationship showed improvement in one of these traits would result in reduced performance of the other traits.

Result in Table 13 showed that SD had a highly significant positive genetic association with SCW (rg = 0.610**), LW (rg = 0.702**), LL (rg = 0.256**), and LA (rg = 0.655**). This indicates that improvement in leaf traits contribute to higher photosynthetic efficiency improving the stalk diameter which will ultimately improve SCW. Previous studies by Esayas et al. (2016e), Parihar (2020), and Tabassum et al. (2023) found similar results for SCW. Additionally, the present study found that SD had a non-significant positive genetic relationship with NOI, Brix%, pol%, and sucrose%, while purity% showed a non-significant negative relationship. This shows that during selection care

should be taken to optimize the simultaneous improvement of SD and sugar quality traits. The study result also found that SCW had highly significant positive genetic correlation with NOI, LW, and LA. A study by Esayas et al. (2016e) also found a similar association with the number of internodes per stalk. Regarding sugar quality, pol% and Brix% had a positive but non-significant genetic correlation with SCW, while purity% and sucrose% showed a non-significant and negative genetic relationship with SCW. This shows that the sugar quality traits have no impact on SCW improvement.

The genetic association between NOI and LW was negative and non-significant, but it exhibited a highly significant negative association with LL (rg = -0.384**) and LA (rg = -0.255**), indicating that LW, LL, and LA traits had negative effect on improvement of NOI. Thus, these traits could not be improved simultaneously with NOI. In contrast, previous findings reported a positive and highly significant genetic association between leaf length and leaf area (Esayas et al., 2016e). NOI showed positive and highly significant correlation with pol%, Brix%, and sucrose% and had a positive and significant genetic correlation with purity%. A previous study also revealed a highly significant positive genetic relationship between NOI with pol% and Brix% (Esayas et al., 2016e).

The estimated genotypic association coefficients shown in Table 13, reflecting that SYHA had a highly significant and positive genetic association with SP (rg = 0.549**), TCHA (rg = 0.710**), MCHA (rg = 0.750**), SH (rg = 0.681**), SD (rg = 0.264**), SCW (rg = 0.356**), NOI (rg = 0.432**), CYHA (rg = 0.994**), and purity percent (rg = 0.218**). The results indicate that genetic factor influencing these component traits also affect sugar yield in sugarcane genotypes. This implies that simultaneous improvement of these traits could result in improvement of SYHA. The current findings are in accordance with Sharma et al. (2023), except for NOI and purity percentage. Tabassum et al. (2023) also reported similar results except for the number of internodes. Comparable results were reported for cane yield, sprouting percentage, weight of single cane, tiller number, millable cane number and purity percentage (Patra et al., 2022). The maximum positive and significant genetic association of cane yield and sucrose percentage with sugar yield was also reported (Younis et al., 2020; Masri et al., 2022). In this research, the genotypic association of SYHA with pol% (rg = 0.128), Brix% (rg = 0.159), and sucrose% (rg = 0.150) was weakly positive and non-significant. These results agree with the report of Masri et al. (2022) and Sanghera et al. (2017). SYHA also demonstrated a highly significant negative genetic relationship with LW (rg = -0.270**) and LA (rg = -0.237**)

while exhibiting a non-significant and negative genetic correlation with LL. The leaf traits LL, LW, and LA have positive effects on cane yield compared to sugar quality traits and thus on sugar yield (Yavas et al., 2024).

The study found that all sugar quality traits had positive and a highly significant genetic correlation with each other. This implies that improving any one of these would exert a positive and highly significant improvement in the other quality traits; thus, improvement in any of these quality traits would improve the other traits positively and significantly. Similar results were reported in earlier studies (Tahir et al., 2014; Esayas et al., 2016a; Masri et al., 2022; Tabassum et al., 2023). Leaf area, length, and width exhibited positive and a highly significant correlation with each other, indicating that improving either of these leaf traits could lead to improvements in the others leaf traits. Similarly, previous studies also found a highly significant positive correlation of leaf areas with leaf length (Esayas et al., 2016e).

Overall result from correlation revealed that genotypic correlation coefficients found to be higher in magnitude than their corresponding phenotypic correlation coefficients, indicating a strong inherent relationship among the traits (Khan et al., 2013; Jamoza et al., 2014). The lower estimates of phenotypic correlation indicated that the actual genetic relationships between traits were affected or distorted by environmental factors at phenotypic level (Khan et al., 2012). Similar result was reported by Esayas et al. (2016e).

4.2.5.2. Phenotypic correlation

The pairwise phenotypic correlation coefficients were calculated to understand the association between various pair of traits. The results of the analysis are presented in Table 14. CYHA contained positive significant association with SYHA (rp = 0.957**), MCHA (rp = 0.728**), SH (rp = 0.609**), TCHA (rp = 0.542**), SCW (rp = 0.482**), SP (rp = 0.397**), and NOI (rp = 0.376**). This indicates that emphasis should be given to these traits to improve cane yield through selection. Thus, these traits can be selection criteria for improvement of CYHA. A similar result was reported for NOI (Ahmed et al., 2019). The present finding is in agreement with Ahmed et al. (2019), Sharma et al. (2023), Masri et al. (2022), and Kumar et al.(2022) for SCW, SH, and MCHA; Younis et al. (2020) and Tabassum et al. (2023) for SH and MCHA; and Esayas et al. (2016d) for MCHA, SCW, SH, TCHA, and SP. Furthermore, CYHA exhibited a highly significant negative phenotypic correlation with SD (rp = -0.112**). The negative relationship of SD with

CYHA indicates that improvement in SD could adversely contribute to improvement of CYHA. Similar results were reported by Esayas et al. (2016d) and Sharma et al. (2023).

TCHA had a highly significant positive association with the MCHA (rp = 0.704**) and SH (rp = 0.207**), and positive and non-significant correlation with NOI (rp = 0.037). This indicates that MCHA, SH, and NOI of sugarcane genotypes could be improved through improving its TCHA. Kumar et al. (2022) found similar results for SH, as did Belwal & Ahmad (2020) for the MCHA and Tahir et al. (2014) for MCHA and SD. It is evident in Table 14 that trait TCHA also had a highly significant negative association with SD (rp = -0.234**) and a negative and non-significant association with SCW (rp = -0.080), indicating selection based on these two traits adversely affect TCHA improvement, thus these traits could not be improved simultaneously.

The other cane yield component, MCHA, had a highly significant positive association with SH (rp = 0.286**), and NOI (rp = 0.113**), yet it had a highly significant negative association with SD (rp = - 0.397**) and SCW (rp = -0.133**). The observed positive association between MCHA and the two yield components indicates that improvement made on MCHA could simultaneously improve the SH and NOI. Conversely, the observed negative relationship between the traits indicates that an increase in MCHA may lead to a decrease in SD and SCW hence, selection based on these traits may not be effective for the improvement of MCHA. This result was consistent with the findings of Belwal & Ahmad (2020) and Tahir et al. (2014) for SH. The negative and highly significant correlation of MCHA with SCW was also reported by Parihar (2020).

The other yield component, stalk height (SH), showed highly significant negative relationships with SD (rp = - 0.134**) and positively and highly associated with SCW (rp = 0.560**), which were consistent with report by Kumar et al. (2022). This indicates that increase in SH could result in decrease in SD thus selection based on either of the traits affect the other adversely. The analysis result revealed that SD exhibited a highly significant positive association with SCW (rp = 0.318**) and NOI (rp = 0.412**) which is consistent with the results reported by Belwal & Ahmad (2020). This implies that selection based on SD could be effective in the improvement of SCW and NOI, thus SCW and NOI could be a selection criteria for improvement of SD.

In this study, SYHA showed positive and highly significant correlation with Pol% (rp = 0.328**), Brix% (rp = 0.308**), sucrose% (rp = 0.314**), and purity% (rp = 0.156**).

This indicates that selection based on these component traits ultimately improves SYHA. Thus, these traits can be selection criteria for SYHA. In agreement with the present findings, Esayas et al. (2016d) reported a closely phenotypic correlation ($P < 0.01$) of sugar yield with pol percent, purity percent and brix percent. The study estimated the correlation between different sugar yield components, and found positive and significant associations among them. Accordingly, Brix% contained a highly significant positive association with pol% (rp = 0.946**). Similarly, purity% had a highly significant positive interrelationship with pol% (rp = 0.562**) and Brix% (rp = 0.653**). This implies that selection based on these correlated yield components would be rewarding in improving either of the traits. Earlier study by Tahir et al. (2014) also reported a similar result for the association between Brix% and purity%. Contrary to the present results, Belwal & Ahmad (2020) reported a significant negative correlation of Brix% with purity%.

The present result also revealed that sucrose% showed highly significant positive association with Brix% (rp = 0.959**), pol% (rp = 0.958**), and purity% (rp = 0.681**). This suggested that selection based on these traits will improve the sucrose% of the cane. Previous studies by Tyagi & Lal (2007), Esayas et al. (2016d), Parihar (2020), and Masri et al. (2022) also pointed out results in line with the present finding. Disagreeing with the present result, Belwal & Ahmad (2020) revealed a negative association of sucrose% with Brix%.

4.2.6. Path coefficient analysis

4.2.6.1. Diagnosis of multicollinearity

Multicollinearity is detected with the help of variance inflation factor (VIF). If the VIF value for a given independent trait is greater than 10, then multicollinearity can be corrected by removing one of the correlated traits. In the present study, a multicollinearity diagnosis was performed for the eight traits considered. The diagnosis results revealed weak multicollinearity in each variable, which ensures the path coefficient is close to being unbiased. Traits TCHA (1.997), MCHA (2.086), SH (2.218), SD (1.473), SCW (2.158), NOI (1.522), Brix % (1.244), and LA (1.400) had small VIF values and were subjected to path coefficient analysis to determine direct and indirect effects of different traits on cane yield.

4.2.6.2. Phenotypic and genotypic path coefficient on CYHA

Lenka & Mishra (1973), categorized path coefficient values as follows: negligible (less than 0.1), weak (0.11–0.19), medium (0.2–0.29), strong (0.3–0.9), and very strong (≥ 1). The genotypic and phenotypic path coefficients were presented in Tables 15 and 16, respectively. According to the genotypic and phenotypic path coefficients, MCHA had a very strong positive direct effect on CYHA at the genotypic level (1.174) and the phenotypic level (0.759) (Tables 15 and 16). Besides, MCHA had a positive, highly significant correlation at genotypic (rg = 0.768**) and phenotypic (rp = 0.728**) levels with CYHA (Table 13 and 14). The highly significant and positive direct effect showed the true relationship between MCHA and CYHA, implying that MCHA can be the best selection criterion for CYHA improvement in a breeding program. This finding is consistent with that of Masri et al. (2015), Esayas et al. (2016b), and Masri (2022) at the genotypic level. Earlier studies by Masri et al. (2015), Esayas et al. (2016b), Gowda et al. (2016), Ahmed et al. (2019), and Tabassum et al. (2023) found that a number of millable canes had the strongest direct effect on CYHA at the phenotype level.

The indirect effect of TCHA on CYHA through MCHA was very strong (1.02183) at the genetic level and strong (0.53489) at the phenotypic level. This trait also had strong positive highly significant correlation with CYHA at genotypic and phenotypic level (Table 15 and 16). These results elucidate that improvement in TCHA indirectly enhances CYHA through improving MCHA, suggesting that TCHA can be used as best selection criterion in improving CYHA.

Similarly, SH (0.38207) had a strong positive indirect influence on CYHA through MCHA at the phenotypic level and had strong positive highly significant correlation with CYHA at the phenotypic level (Table 16). These results show that an increase in the height of the stalk leads to an increase in the MCHA, which enhances the CYHA. Thus, SH is can be used as best selection criterion in improving CYHA. On the contrary, the indirect effect of SD (- 0.78682, rg = -0.262**), SCW (- 0.3969, rg = 0.368**), and LA (- 0.61182, rg = -0.262**) on CYHA through MCHA was strong negative at genotypic level (Table 16). This observed relationship necessitates selection criteria that optimize MCHA with regard to SD, SCW and LA as these traits have positive correlation with cane yield.

The path analysis also revealed that SCW exerted a very strong positive direct effect on CYHA at the genetic level (1.06708) and a strong positive direct effect at the phenotypic

level (0.49977). Similarly, SCW showed a highly significant positive correlation with CYHA both at genetic (0.368**) and phenotypic levels (0.482**). The strong direct effect and significant positive correlation suggest positive relationships between dependent and independent traits. Hence, improvement in SCW could improve cane yield, which implies SCW could be considered as a selection criterion for cane yield.

Table 14: Phenotypic correlation coefficients of 16 yield and yield related traits of 144 sugarcane genotypes

	SP	TCHA	MCHA	SH	SD	SCW	NOI	CYHA	Pol	Brix	Purity	Sucrose	LW	LL	LA	SYHA
SP	—															
TCHA	0.529**	—														
MCHA	0.505**	0.704**	—													
SH	0.137**	0.207**	0.286**	—												
SD	-0.238**	-0.234**	-0.394**	-0.134**	—											
SCW	-0.058^{NS}	-0.080^{NS}	-0.133**	0.560**	0.318**	—										
NOI	0.096*	0.037^{NS}	0.113**	0.513**	-0.008^{NS}	0.412**	—									
CYHA	0.397**	0.542**	0.728**	0.609**	0.112**	0.482**	0.376**	—								
Pol	0.126**	0.119**	0.045^{NS}	0.001^{NS}	0.067^{NS}	0.099*	0.177**	0.129**	—							
Brix	0.106*	0.115**	0.034^{NS}	-0.015^{NS}	0.046^{NS}	0.064^{NS}	0.176**	0.107*	0.946**	—						
Purity	0.049 ^{NS}	0.101*	0.046 ^{NS}	-0.075 ^{NS}	-0.059^{NS}	-0.042^{NS}	0.040^{NS}	0.018^{NS}	0.562**	0.653**	—					
Sucrose	0.116**	0.119**	0.045^{NS}	-0.015^{NS}	0.045^{NS}	0.073^{NS}	0.159**	0.111**	0.985**	0.959**	0.681**	—				
LW	-0.115**	-0.198**	-0.257**	-0.030^{NS}	0.268**	0.273**	-0.047^{NS}	-0.041^{NS}	-0.089*	-0.113**	-0.147**	-0.108**	—			
LL	0.017^{NS}	-0.009^{NS}	-0.067^{NS}	0.007^{NS}	0.102*	0.086*	-0.182**	-0.020^{NS}	-0.132**	-0.133**	-0.055^{NS}	-0.123**	0.295**	—		
LA	-0.070 ^{NS}	-0.158**	-0.230**	-0.020^{NS}	0.259**	0.250**	-0.107*	-0.043^{NS}	-0.124**	-0.141**	-0.135**	-0.134**	0.897**	0.675**	—	
SYHA	0.409**	0.547**	0.712**	0.566**	0.104*	0.447**	0.387**	0.957**	0.328**	0.308**	0.156**	0.314**	-0.079^{NS}	-0.046^{NS}	-0.081^{NS}	—

SPC = Sprout count, TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = Single cane weight, NOI = Number of internode, CYHA = Cane yield per hectare, Pol = Polarity, SYHA = Sugar yield per hectare, LW = Leaf width, LL = Leaf length, LA = Leaf area, * and ** = significant at 5% and 1% probability level, respectively, NS = Non-significant difference

In agreement with the present result, previous studies were reported by Masri (2015), Esayas et al. (2016b), and Masri (2022) for genetic level while consistent with the earlier reports by Esayas et al. (2016b), Gowda et al. (2016), Ahmed et al. (2019), and Tabassum et al. (2023) for phenotypic level.

The results in Table 16 showed that SH (0.61846, rg = 0.704**), SD (0.65129, rg = -0.262**), NOI (0.449200, rg = 0.407**), and LA (0.46550, rg = -0.255**) exerted strong positive indirect influence on CYHA through SCW at the genetic level. Though these traits have negative direct effect on CYHA their positive strong indirect effect through SCW implies that the simultaneous improvement of those multi-traits enhances CYHA indirectly by improving SCW. MCHA exerted a strong negative (-0.36076) indirect influence on CYHA through SCW; conversely, it showed a positive, highly significant (rg = 0.768**) correlation with CYHA. This indicates that a higher number of millable cane leads to reduced single cane weight due to competition of canes for scarce resources, which resulting in lighter stalk, however, the total yield still benefits from having the higher MCHA, because the cumulative effect of many canes outweigh the reduction in single cane weight. Similarly increase in SCW can compensate the yield penalty due to reduction in MCHA. Therefore, during selection and breeding programs optimization of the traits' improvement should be considered to get optimum yield.

The path analysis also disclosed that the residual (unexplained) variation in the path analysis was 0.043 and 0.1195 on genotypic and phenotypic level, respectively. The observed low values of the residual effect at the genotypic and phenotypic level indicated that the independent traits (variables) included in the path analysis explained most of the variation in the dependent traits.

4.2.7. Development of Smith – Hazel selection index

Smith–Hazel selection index aid the plant breeders to distinguish desirable genotypes based on phenotypic performance and genetic path coefficient. Selection of genotypes based on improved cane yield is the most crucial aim of sugarcane breeding. However, cane yield is determined by several agronomic traits and environment factors. In this study, selection indices for cane yield were calculated using MCHA, SCW, and CYHA and the result is presented in Table 17. MCHA and SCW are cane yield components and were selected for the analysis due to their significant direct effects on CYHA and had strong positive correlation with CYHA.

Table 15: Estimates of genotypic direct effects (bold and diagonal) and indirect effects (off-diagonal) of traits via other independent traits on cane yield of 144 sugarcane Genotypes

	TCHA	MCHA	SH	SD	SCW	NOI	Brix%	LA	Corr. with CYHA
TCHA	**-0.14342**	1.02183	-0.07126	0.15212	-0.27779	0.00052	0.00777	0.00871	**0.699****
MCHA	-0.12483	**1.17398**	-0.08943	0.17375	-0.36076	-0.00576	-0.00823	0.00913	**0.768****
SH	-0.03719	0.38207	**-0.27478**	0.04873	0.61846	-0.02843	-0.00759	0.00268	**0.704****
SD	0.08416	-0.78682	0.05166	**-0.25924**	0.65129	-0.00036	0.00856	-0.01148	**-0.262****
SCW	0.03734	-0.39690	-0.15926	-0.15823	**1.06708**	-0.02147	0.00078	-0.00764	**0.368****
NOI	0.00145	0.13260	-0.15322	-0.00182	0.44920	**-0.05099**	0.02550	0.00446	**0.407****
Brix%	-0.01153	-0.10002	0.02158	-0.02295	0.00861	-0.01345	**0.09664**	0.00249	**-0.012^{ns}**
LA	0.07128	-0.61182	0.04210	-0.16984	0.46550	0.01299	-0.01376	**-0.01752**	**-0.255****

Residual value = 0.043

Table 16: Estimates of phenotypic direct effects (bold and diagonal) and indirect effects (off-diagonal) of traits via other independent traits on cane yield of 144 sugarcane genotypes

	TCHA	MCHA	SH	SD	SCW	NOI	Brix%	LA	Corr. With CYHA
TCHA	**0.03262**	0.53489	0.02031	-0.01051	-0.03995	0.00100	0.00479	-0.00167	**0.542****
MCHA	0.02298	**0.75936**	0.02802	-0.01774	-0.06658	0.00305	0.00143	-0.00243	**0.728****
SH	0.00676	0.21695	**0.09808**	-0.00605	0.28017	0.01381	-6.00E-04	-0.00021	**0.609****
SD	-0.00762	-0.29934	-0.01318	**0.04500**	0.15888	-2.00E-04	0.00193	0.00273	**-0.112****
SCW	-0.00260	-0.10099	0.05490	0.01428	**0.49977**	0.01109	0.00262	0.00264	**0.482****
NOI	0.00121	0.08604	0.05029	-0.00034	0.20609	**0.02693**	0.00732	-0.00113	**0.376****
Brix%	0.00375	0.02612	-0.00142	0.00208	0.03144	0.00473	**0.04169**	-0.00149	**0.107***
LA	-0.00516	-0.17473	-0.00195	0.01165	0.12529	-0.00288	-0.00588	**0.01055**	**-0.043^{ns}**

Residual value = 0.1195

TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = Single cane weight, NOI = Number of internode, LA = Leaf area, CYHA= cane yield per hectare, ** = highly significant (P < 0.01), ns = non-significant

CYHA was chosen as it is one of the primary traits that influence genotype performance. The analysis showed that the individual genetic advance value for cane yield was 25.16, for SCW was 0.16, and for MCHA were 53653.95. The individuals selected on the basis of these selection indices had higher mean values than that of the overall means for MCHA and CYHA and revealed higher genetic advances. Based on the selection indices, the sugarcane genotypes selected for multi-trait improvement were CP6023, CP70/321, Mex57/197, D41/46, CP971944, B657/150, B49-244, and the check variety NCo-334 (Table 17).

Table 17 : Genotypes selected on the basis of the Smith index for different combination of traits with selection intensity (I) = 5%

Row names	MCHA	SCW	CYHA
CP6023	117746.58	1.24	155.47
CP70/321	139722.13	1.29	178.40
Mex57/197	136315.39	1.22	167.14
D41/46	102902.68	1.58	163.12
CP971944	58969.30	1.03	77.72
B657/150	92768.38	1.62	152.62
B49-244	121793.00	1.19	144.70
NCo-334	116055.50	1.14	133.99
Mean of selected individuals	110784.12	1.29	146.64
Mean of all individuals	77309.54	1.30	99.32
Selection of individual	33474.58	-0.01	47.33
Expected genetic gain for 5%	53653.95	0.16	25.16

MCHA = Millable count per hectare, SCW = Single cane weight, CYHA = Cane yield per hectare

Similar result were reported by Singh and Khan (1998, 2003) that selection based on number of millable canes, cane yield, stalk height, and stalk weight, lead to improvement in cane yield. Tahir et al. (2014) also revealed that genotypes CPF-225, MS-2003-CR5-245, MS-2003-CR7-243, and MS-2003-CR8-407 were selected using either of the selection indices. Belay et al. (2024) also selected B552–11, FG04–466, B707–1, FG05 771, B491–18, DB386/60, B658–11, B528–30, CP70/321, and B517–40 using the same selection index. Among their genotypes CP70/321 was also selected in our present study.

4.2.8. Multivariate cluster and principal component analysis

4.2.8.1. Cluster analysis

Cluster analysis was performed based on the Euclidean distance matrix using the Unweighted Pair Group Method with the Arithmetic Mean technique (UPGMA) (Sneath and Sokal, 1973). The optimum number of clusters was identified based on the Euclidean distance using the K-means method and is shown in Figure 3. Consequently, the multivariate cluster analysis sequestered 144 genotypes into five main clusters using 16 quantitative traits (Table 18, Fig. 4). Four of the identified five main clusters were further divided into two subclusters (Fig. 4). The cluster pattern exhibited high homogeneity within a cluster and high heterogeneity between clusters. Clusters showed the genetic relationship among genotypes. The members of each group are also displayed in Table 18. Previous researchers have reported similar results in sugarcane. In a similar local study in Ethiopia, Esayas et al. (2016b) reported 400 genotypes grouped into 19 main groups and a singleton using 21 quantitative traits. Tawadare et al. (2019) revealed the separation of ten sugarcane genotypes into three clusters with ten agronomic traits. In another study, 135 F1 sugarcane hybrids were divided into three groups using a cluster analysis based on 11 attributes (Xu et al., 2023). Similarly, Siraree et al. (2018) demonstrated 92 sugarcane genotypes grouped into nine main groups based on seven traits. Furthermore, Mebrahtom et al. (2016) reported that 49 sugarcane genotypes were divided into ten groups.

The evaluated genotypes were divided into five different groups with a number of genotypes per cluster ranging from 1 to 124 (Table 18). Cluster I had the most genotypes (124), of which 18 genotypes were introduced from India, seven from South Africa (Natal), two from Guyana, 13 from Cuba, 17 from France, 24 from Barbados, 13 from the USA, one from Thailand, two from Puerto Rico, one from Brazil, 10 from Ethiopia, seven from the Philippines, seven from Sudan, one from Mexico, and one from Australia (Table 18, Fig. 2).

These genotypes may have similar genealogical histories, share similar parental sources, gene flow, and indicate the use of a common genetic pool for the development of varieties due to the exchange of germplasm between countries. All local landraces also belong to this group, highlighting the effect of sharing related genealogical history during the evolution of these genotypes.

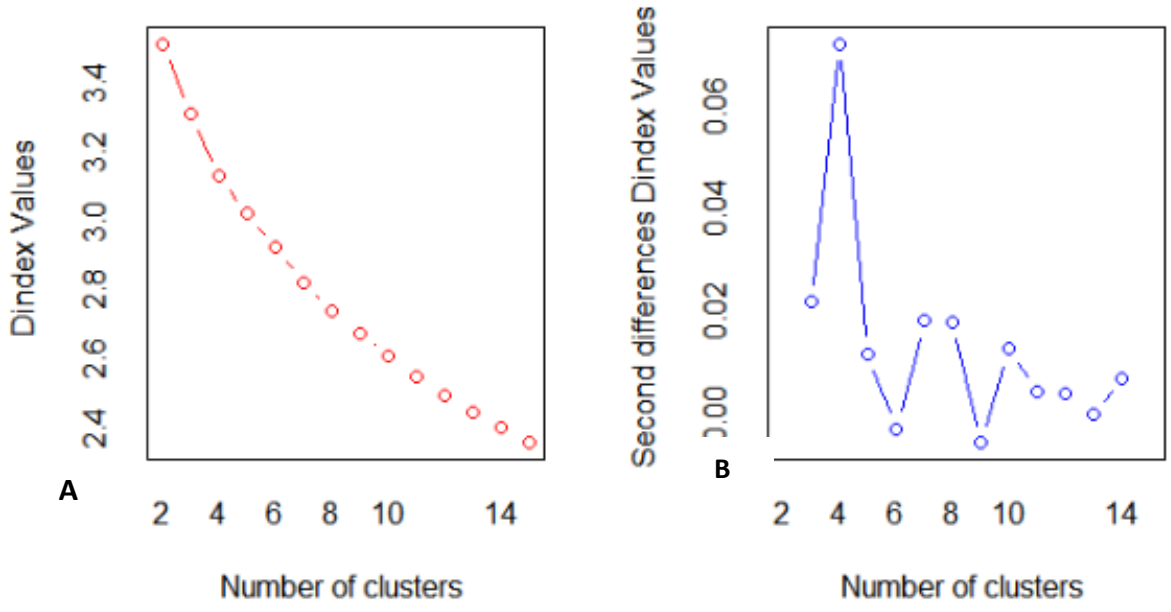

Figure 3. Graphics of Dindex showing the best number of clusters for 144 sugarcane genotypes a) value of Dindex b) Second difference Dindex value

Cluster II comprised nine genotypes: one from Mauritius (M202/46), two from Philippines (PSR97-092 & VMC9647), two from the USA (CP6023 & CP70/321), one from Australia (Q-200), one from Mexico (MEX57/197), one from Thailand (MPT96-273), and one from France (FGO5-155). Cluster III consisted of eight genotypes: two from Sudan (2-333 & 2-111), one from Philippines (VMC9655), one from Cuba (C85-102), two from the USA (CPOO1527 & CP96-1252), one from India (Co680) and one from France (FGO6-114). Similarly, Cluster IV had two genotypes: one from France (FGO4798) and one from the Philippines (VMC96-134).

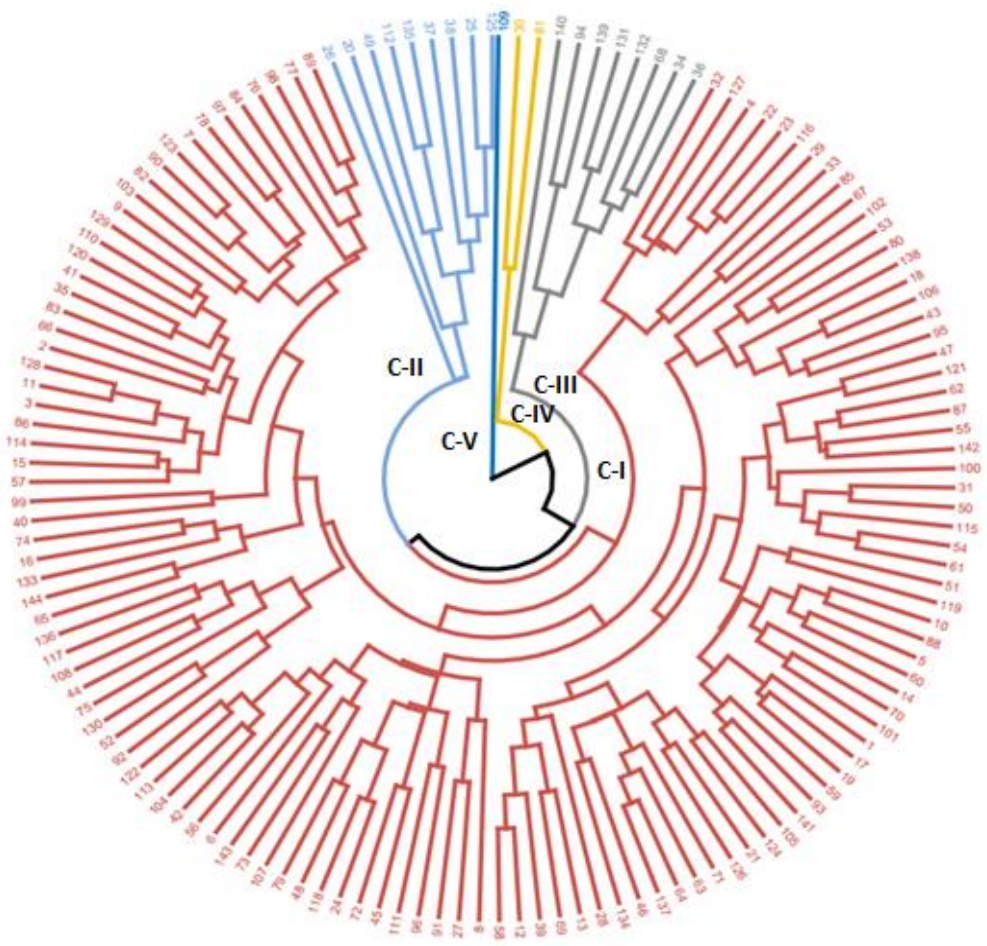

Figure 4. Dendrogram of 144 sugarcane genotypes depicting the genetic similarity obtained from the UPGMA cluster analysis (see the corresponding genotype name in Table 1)

Furthermore, cluster V consisted of one genotype (singleton) from Cuba (C88-356), suggesting that this genotype had significantly higher or lower values for the traits that make them phenotypically dissimilar from the other genotypes (Table 18, Fig.2). The present study also revealed that sugarcane genotypes belonging to the same regions were grouped into different clusters; conversely, genotypes from different sources were grouped into the same group, showing a lack of association between genotypic diversity and geographic origins (Table 18, Fig. 2). The exchange of germplasm and the environment are possibly the main factors influencing the distribution pattern of genotypes in the clusters.

Table 18: Group constellation of sugarcane genotyes based on cluster analysis

Cluster	Frequency	Genotype							origin
I	124	1	19	45	64	84	103	123	S. Africa,
		2	21	46	65	85	104	124	Mauritious,
		3	22	47	66	86	105	126	USA, Philipines
		4	23	48	67	87	106	127	
		5	24	50	69	88	107	128	Austeralia, Mexico,
		6	27	51	70	89	108	129	Thiland, France,
		7	28	52	71	90	110	130	Cuba, Sudan,
		8	29	53	72	91	111	133	India, Ethiopia,
		9	31	54	73	92	113	134	Barbados, Guyana,
		10	32	55	74	93	114	136	Puerto Rico, and
		11	33	56	75	95	115	137	Brazil
		12	35	57	76	96	116	138	
		13	39	58	77	97	117	141	
		14	40	59	78	98	118	142	
		15	41	60	79	99	119	143	
		16	42	61	80	100	120	144	
		17	43	62	82	101	121		
		18	44	63	83	102	122		
II	9	20	25	26	37	38	49	112	S.Africa, Mauritious, Philipiness, USA, Austeralia, Mexico, Thiland, and France
		125	135						
III	8	34	36	68	94	131	132	139	Sudan, Cuba, USA, Philipiness,India, France
		140							
IV	2	30	81						Cuba and Philipiness
V	1	109							Cuba

Therefore, the selection of parent genotypes for crossing solely through geographic diversity could not be effective (Sharma et al., 2014). The present result is in line with reports by Esayas et al. (2016b); Mebrhatom et al. (2016); and Belay et al. (2023a). The present findings are also supported by Chandra et al. (2007) and Ali et al. (2021), who revealed a lack of association between agronomic traits and place of origin in other crops.

The divergence analysis was computed using Euclidean distance, and the mean distance within a cluster (intra-cluster) and between the clusters (inter-cluster) is presented in Table 19. It is evident in Table 19 that the intercluster distance between the clusters ranged from 6.41 to 11.52. The highest distance was recorded between Clusters III and Clusters V (D = 11.52), followed by Clusters II and Cluster V (D = 11.46), and Cluster I and Group V (D =

8.18) (Table 19). The relatively high distance was attributed to substantially diverse quantitative morphological traits between the members of the two groups, which imply a significant genetic divergence between the two groups. As a result, members of these genetically divergent clusters could be used as parental materials in hybridization to produce desirable offspring with significant heterosis and variation in future generations. However, the shortest distance between clusters was discovered between clusters I and II (D = 6.41), followed by clusters I and IV (D = 6.55) and I and III, indicating genetically less diverse pairs of clusters; thus, crossing between members of these groups may not be effective in obtaining desirable recombinants (Singh et al., 2018b).

Furthermore, Cluster I had the highest intra distance within clusters (D = 4.97), followed by Cluster II (D = 4.76), indicating that genotypes of Cluster I were more divergent than the genotypes of the other groups, resulting in high genetic diversity. On the contrary, the lowest intra-cluster distance was observed in Cluster IV (D = 4.24), which implies that the genotypes assigned in this Cluster exhibited relatively less genetic divergence. However, the mean distance of all Clusters (D = 3.67) was less than that of the first Cluster (D = 4.97). This indicates that the genotypes in Cluster I were the farthest away from the genotypes in any other group. The genotypes grouped into Cluster I comprised genotypes with the highest sugar yield per hectare (SYHA; 14.86 t ha-1) and stalk height (SH; 208.95 cm) compared to the genotypes of all other clusters, based on a cluster mean analysis (Table 20). In agreement with the present finding Belay et al. (2023a) reported intercluster eculidian distances of 6.06 to 11.73 based on agromorphological traits. The largest genetic distance was recorded between clusters C3 and C5 (11.73) and the smallest was obtained between C1 and C2 (6.06). Gowda et al. (2017) reported the highest intercluster distance between cluster I and VII (23.79), which is higher than the present result.

In general, the intercluster distances were greater than the intra-cluster distances, showing greater genetic variation between genotypes of different clusters (Wu, 2006). Sanghera et al. (2015) also reported that intercluster distances were higher than intra-cluster ones.

The mean values of 16 quantitative morphological traits for each cluster are shown in Table 20. In this study, the mean values varied between the clusters for all traits. Cluster I genotypes had the highest mean value for the traits SH (208.95 cm) and SYHA (14.86 t ha-1), indicating that they can be used in future breeding programmes based on stalk height and sugar yield per hectare.

Table 19: Average intra- (**Bold**) and inter-cluster distance for five clusters of sugarcane genotypes

Cluster	I	II	III	IV	V
I	**4.967057**	6.410903	7.275202	6.550085	8.183625
II		**4.759223**	7.111491	8.129732	11.463318
III			**4.376526**	7.531173	11.524787
IV				**4.236928**	7.235733
V					**0.000000**

The genotypes in cluster II had the highest mean values for SP (41.3%), TCHA (124009 tillers), MCHA (83065.13 stalks), SCW (1.40 kg), NOI (21.70 internodes), and CYHA (112.6 t ha^{-1}) compared to the others. In this study, MCHA and SCW are also the traits identified for direct selection to improve cane yield (see 4.2.8 and 4.2.9). This shows that the genotypes assigned to this cluster have a significant genetic potential for these traits; therefore, these genotypes can also be used as parents in hybridization programs to develop high-yielding sugarcane varieties. (Table 20).

Compared to the other clusters, the highest mean purity percentage (91.26%) was observed in the genotypes grouped in cluster III (Table 20). Similarly, the genotypes of cluster IV showed the highest mean for pol% (18.53%), sucrose% (13.03%), and Brix% (20.32%) than the rest of the clusters (Table 20). This implies that genotypes in this cluster could be utilized in selection and hybridization programs to improve sugar quality. Therefore, these genotypes can be selected to improve the purity percentage in sugarcane genotypes. Furthermore, the genotype in cluster V (C88-356) had the highest mean values for SD (28.5 mm), LW (4.54 cm), and LA (642.5 cm^2) compared to the other clusters (Table 20). This demonstrated that genotype C88-356 had a high genetic potential for these traits and, as a result, could help improve the cane stalk thickness, leaf area, and leaf width in the sugarcane breeding program.

As previously stated, there was a significant difference in mean performance among the clusters for the majority of traits; however, each cluster performed better for specific traits, providing an excellent opportunity to identify diverse parent materials across the groups for future sugarcane yield and quality trait improvement. The phenotypic variability observed among the 144 sugarcane genotypes suggests that quantitative traits can unfold diversity among sugarcane genotypes. Esayas et al. (2016b), Mebrahtom et al. (2016), Shitahun et al. (2022), and Belay et al. (2023a) also reported similar results.

Table 20: Means of sixteen quantitative traits in 144 sugarcane genotypes in different clusters

	Cluster					
Traits	I	II	III	IV	V	Mean
SP (%)	39.14	41.03	35.39	32.35	35.30	36.64
TCHA	117563.34	124008.62	114698.28	76637.93	117413.79	110064.39
MCHA	78267.14	83065.13	67241.38	44568.97	52758.62	65180.25
SH (cm)	208.95	208.58	204.44	182.7	164.55	193.84
SD (cm)	2.32	2.38	2.37	2.26	2.85	2.44
SCW (kg)	1.29	1.40	1.28	1.13	1.17	1.25
NOI	21.3	21.70	20.9	17.80	18.8	20.10
LW (cm)	3.93	4.13	4.12	4.35	4.54	4.21
LL (cm)	135.91	135.19	140.73	142.69	140.65	139.03
LA (cm^2)	539.62	564.62	582.76	625.68	642.46	591.03
Pol%	17.88	18.37	18.22	18.53	17.63	18.13
Brix%	19.79	20.15	19.98	20.32	20.08	20.06
Purity%	90.59	91.18	91.26	91.20	87.87	90.42
Sucrose%	12.54	12.92	12.82	13.03	12.04	12.67
CYHA(t/ha)	100.34	112.64	87.95	51.70	62.45	83.02
SYHA(t/ha)	14.86	11.86	10.36	14.30	9.54	12.18
Mean	**12316.24**	**13021.34**	**11449.6**	**7652.831**	**10713.62**	**11030.73**

SP = Sprouting percent; TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = Single cane weight, NOI = Number of internodes, CYHA = Cane yield per hectare, Pol = Polarity, SYHA = Sugar yield per hectare, LW = Leaf width, LL = Leaf length, LA = Leaf area

4.2.8.2. Principal component analysis

Principal component analysis (PCA) provides information on the contributions of critical traits to total variability among sugarcane genotypes. It helps to understand the pattern of variation between genotypes (Sumbele et al., 2020). PCA has been extensively utilized in agricultural research to categorize genotypes and classify variables. In this study, the PCA analysis for 16 quantitative traits of 144 sugarcane genotypes was carried out and the result is presented in Table 21. The PCA analysis extracted a total of 16 PCs, which was equal to the number of original attributes. However, according to Kaiser's rule (Kaiser, 1958), PCs with eigenvalues < 1 provide less information than one of the original traits and, therefore, are not worth keeping. Accordingly, the first four main components, with eigen values greater than one were retained which, are responsible for 81.44% of the overall variation (Table 21). Similarly, traits with the most significant absolute values closer to unity had a greater impact on grouping than those with the smallest absolute

values closer to zero (Chahal & Gosal, (2002). Hair et al. (2019) also suggested that component loadings greater than ± 0.3 are assumed to be meaningful and significant.

The present result agrees with Tawadare et al. (2019), Esayas et al. (2022), and Sumbele et al. (2021), who reported four main components in sugarcane genotypes which cumulatively explained 76.72%, 92%, and 75.95% of the total variation, respectively. The current study's findings differed slightly from those of Esayas et al. (2016b), Belay et al. (2023a), and Ong'ala et al. (2016), who identified six, six, and three PCs, accounting for 79.26%, 84.13%, and 80.85% of the overall variation, respectively. The disagreement of these results may be attributed to the different numbers and types of traits and genotypes considered in their study. The current study, as well as the findings of the previous investigations, revealed that only a few primary variables account for the significant variation observed among the genotypes tested, rather than the small effect of many minor traits.

The first principal component (PC1), which had an eigenvalue of 2.19, represented 30% of the overall observed variation with high component loadings of the traits TCHA (0.35), MCHA (0.36), CYHA (0.35), and SYHA (0.38) (Table 21); suggesting that yield and yield component traits highly accounted for the variation in this PC. Therefore, the discrimination of the genotypes into diverse groups was due to the relatively more substantial contribution of a few traits than the small contribution of each characteristic. The present result is consistent with Esayas et al. (2016b), in which cane yield, stalk number, and sugar yield were crucial contributors to the large variability in sugarcane genotypes clustered in the first PC. The present result also aligns with Belay et al. (2023a) for the significant contribution of the number of millable stalks, sugar yield, stalk length, cane yields, and number of tillers to variation in PC1.

The second component (PC2) had an eigenvalue of 1.90, representing 23% of the total variation (Table 21). This result was agreed with Ahmed & Gardezi (2017b); Belay et al. (2023a); Sumbele et al. (2021), who found 21.9%, 20.69%, and 20.09% total variation in PC2, respectively. The result in Table 21 revealed that PC2 had distinctly high positive factor loads for sugar quality traits such as Brix% (0.47), pol% (0.47), purity% (0.36) and sucrose% (0.47), indicating that the second PC differentiated genotypes mainly on the basis of sugar quality traits. The findings of the present study disagree with earlier results that reported stalk length, sugar yield, SCW, cane yield, number of internodes, and stalk

diameter (Belay et al., 2023a), single cane weight and stalk diameter (Esayas et al., 2016b); and the height of the plant and the length of the stalk (Sumbele et al., 2021) in second principal component (PC2). It is evident in Table 21 and Figure 5 that the first two axes PCs of the main components explained more than half of the total variability (52%) observed, indicating the major contribution of yield related and sugar quality traits for the overall observed variation.

The third component (PC3) contained an eigenvalue of 1.72 and contributed 18% of the overall variation. This result slightly agrees with the report by Esayas et al. (2016b), where PC3 explained 13.36% of the total variation. Traits such as SH (0.33), SD (0.32), SCW (0.52), LW (0.35), and LA (0.34) had the maximum positive loading in this PC (Table 21**).** Belay et al. (2016b) found that SL, SCW, and SD were significant contributing factors in PC3, which supports the current result.

Furthermore, the fourth component (PC4) accounted for 11% of the variation and was characterized mainly by NOI (0.40), LL (-0.51), and LA (-0.41) (Table 21). Similarly to the present result, Belay et al. (2016b) and Sumbele et al. (2021) reported an 11.16% and 10.07 % contribution of PC4 to overall variation, respectively. The current study found a significant difference in the contribution of the traits investigated to the overall observed phenotypic variation among sugarcane genotypes. The observed variability in the traits' contribution help sugarcane breeders for sugarcane improvement program.

The PCA biplot that loaded variables and genotypes at a time on a single graph shows how strongly each trait affects a principal component and is correlated to each other and how distances the genotypes from each other. The principal component biplot based on 16 quantitative morphological traits is presented in Figure 5. It represented the interrelationships or correlations between the quantitative traits measured, allowing it to distinguish between cane yield and its components (high PC1 score) and quality traits (high PC2 score). The angle between a vector and an axis shows the correlation level of the corresponding variable to the principal component. If the angle is close to 0o the variable has strong correlation, implying that the variable align closely with the direction of principal components. On contrary, if the angle between them close to 90o, the variable contain little to no correlation to that component

Table 21. Eigenvalues, total variance, cumulative variance, and eigenvectors for 16 quantitative characters in 144 sugarcane genotypes.

Traits	PC1	PC2	PC3	PC4
SP (%)	0.29	-0.10	-0.06	-0.30
TCHA	0.35	-0.15	-0.08	-0.29
MCHA	0.36	-0.23	-0.07	-0.10
SH (cm)	0.22	-0.16	0.33	0.26
SD (cm)	-0.18	0.20	0.32	0.02
SCW (kg)	0.01	0.02	0.52	0.19
NOI	0.20	0.07	0.25	0.40
LW(cm)	-0.24	0.06	0.35	-0.23
LL(cm)	-0.16	-0.09	0.17	-0.51
LA(cm^2)	-0.26	0.01	0.34	-0.41
Pol%	0.17	0.47	0.03	-0.08
Brix%	0.18	0.47	0.01	-0.09
Purity%	0.18	0.36	-0.08	-0.10
Sucrose%	0.18	0.47	0.01	-0.10
CYHA(t ha^{-1})	0.35	-0.17	0.29	-0.06
SYHA(t ha^{-1})	0.38	-0.08	0.28	-0.09
Eigenvalues	2.19	1.90	1.72	1.30
Variance (%)	0.30	0.23	0.18	0.11
Cumulative (%)	0.30	0.52	0.71	0.81

SP = Sprouting percent; TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = Single cane weight, NOI = Number of internodes, CYHA = Cane yield per hectare, Pol% = Polarity, SYHA = Sugar yield per hectare, LW = Leaf width, LL = Leaf length, LA = Leaf area, PC = Principal component

Similarly, the angle between pairs of vectors indicates the correlation between the corresponding variables. A small angle (acute, < 90^o) shows that the traits are positively correlated, while an angle of 90° between the vectors shows that the traits are not correlated. An angle close to 180° indicates that the traits are negatively correlated (Khan et al., 2022). Furthermore, the length of the vector indicates the contribution (loading) of the variable to the respective PC. The longer the vector, the more that variable contributes to the PC. Accordingly, NOI, SP, SYHA, TCHA, LA, LW, MCHA, CYHA, and SH had small angles with the first principal component (PC1), indicating a high correlation with this component. Conversely, Brix%, pol%, purity%, and sucrose% had small angles with the second principal component (PC2), suggesting that their correlation with this component is high. Based on the length of their vector, TCHA, MCHA, and SYHA had significant contribution (high loading) to PC1, while sucrose%, Brix%, and Purity% had strong contribution (high loading) to PC2.

In Figure 5, it was clear that the LL trait was located on the opposite axis of NOI, pol%, Brix%, purity%, and sucrose% in the biplot diagram, indicating a negative correlation with these traits. The trait SD, LW, and LL were on the opposite axis (180^{o}) to the SH, SP, and NOI, respectively, indicating negatively associated to each other. This implies that improving one trait may negatively affect the other and cannot be improved simultaneously. Belay et al. (2023a) also reported the same result. This result disagrees with the report of Mebrahtom et al. (2016), who revealed a positive correlation between stalk height and stalk diameter traits. Similarly, the diameter of the stalk had an obtuse angle with the number of internodes (NOI), indicating a negative correlation between them. The present result agrees with the report by Mebrahtom et al. (2016); however, it is inconsistent with the results reported by Kumar & kumar (2014).

Figure 5 also indicates that traits such as SP, SH, SYHA, CYHA, TCHA, and MCHA had an acute angle (< 90°) among them, which was highly correlated. This result implies that improving one of these traits enhances the other traits, thus increasing the efficiency of breeding programmes. Contrary to the present study, Mebrahtom et al. (2016) reported a negative correlation between MCHA and TCHA with CYHA and SYHA. The present finding agrees with Mebrahtom et al. (2016) for a positive correlation between CYHA, SH, and SYHA traits. A strong positive association was reported between SH and CYHA (Rewati & Joshi, 2005), consistent with the present finding. The previous similar study by Belay et al. (2023a) revealed a positive correlation between SH, TCHA, MCHA, and CYHA, supporting the present result.

In contrast, SP, SH, SYHA, CYHA, TCHA, and MCHA negatively correlated with SD, LA, and LW (Fig. 5). In Figure 5, it was also evident that quality traits such as pol%, Brix%, purity%, and sucrose% clustered closely together, indicating that these traits are positively correlated, suggesting that an improvement in one of these traits leads to an improvement in others and vice versa. This result is in line with the report of Mebrahtom et al. (2016). According to Falconer & Mackay (1996) the correlation between traits revealed by this PCA approach may result from a genetic link between controlled trait loci or a pleiotropic effect. Information on the correlation between quantitative traits is vital to improving breeding efficiency and effectiveness.

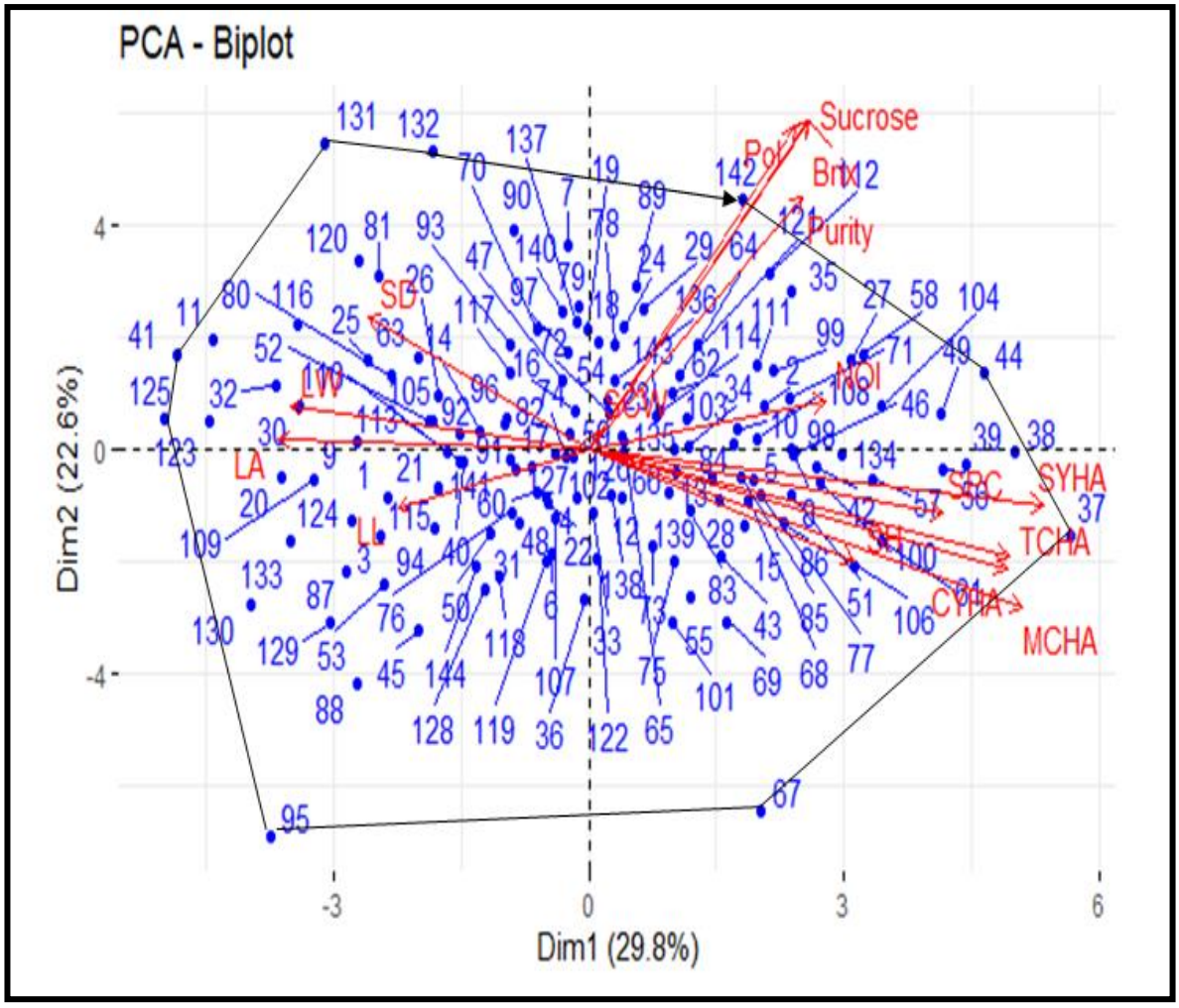

Figure 5. Biplot graph depicting trait loading, genotype distribution pattern, genotype, and trait relationships (Please see Table 1 for the genotype code number)

In addition Figure 5 illustrates the distribution of genotypes in the PC1 and PC2 plots. All sugarcane genotypes were evenly distributed along the biplot, elucidating the diversity among the genetic materials studied. The grouping pattern illustrated the clustering of sugarcane genotypes based on the similarity of the traits. The geometric distances between genotypes in the biplot indicate the genetic distances among them regarding the traits measured. Commonly, genotypes that are close together are assumed to be similar and found near the origin. In contrast, genotypes far apart from each other and far from the origin (x, y) are genetically diverse. Consequently, in this study, nine genotypes (37, 41, 44, 67, 95, 125, 131, 132, and 142) are located outside the center of the origin (x, y) in the direction of the vector relative to the other genotypes and far apart from each other; therefore, they are assumed to be more morphologically divergent (Fig. 5).

The biplot diagram showed that genotype 37 had a maximum mean value for TCHA (193966), MCHA (142931), CYHA (184.5 t/ha) and SYHA (23.59 t/ha). Genotypes 132 and 44 had a maximum mean value for SD (3.04cm) and NOI (27.25 internodes), respectively. Similarly, genotype 131 recorded the highest mean value for pol% (20.48%) and Brix% (22.30%), while genotypes 132 and 142 produced the highest mean for purity (94.09%) and sucrose% (14.66%), respectively. Furthermore, genotype 41 produced the highest mean value for LW (5.72cm) and LA (860.13 cm^2). In contrast, genotypes 125 and 67 had minimum values for MCHA (27931) and SD (1.49cm), respectively, and genotype 95 had minimum mean values for pol% (14.03), Brix% (16.23 %), purity% (83.68 %) and sucrose% (9.31%) (Appendix Table 1). This suggests that genotypes with higher mean values of these traits could be targeted for selection breeding. Since these genotypes are divergent from others due to the maximum and minimum mean values for some main traits, they could be potential genetic material in the sugarcane crop improvement program. The same results were reported (Ahmed & Gardezi, 2017b).

The principal component analysis (PCA) also showed the relationship between the different traits and genotypes concerning PC1 and PC2. The genotypes assigned in the first quadrant were similar in percentages of pol%, Brix%, purity%, sucrose%, and NOI. In the second quadrant, the genotypes were correlated with SD and LW, while the genotypes falling in the third quadrant were correlated with LL. Similarly, the genotypes plotted in the fourth quadrant were similar in SH, SP, TCHA, MCHA, CYHA, and SYHA. Closure and overlapping genotypes on the two PC axes comprised similar phenotypic expressions of the trait. Furthermore, the biplot showed that the genotypes were distributed along all quadrants. Overall, the PCA analysis showed high discriminatory power in identifying key traits responsible for variability in a population, thus classifying genotypes based on their similarity that can be used to select divergent parent materials for hybridization for future improvement of varieties.

4.3. Molecular Diversity, Population Structure, and Marker-Trait Association

4.3.1. Molecular diversity study

4.3.1.1. Level of polymorphism of the SSR loci

The genotypic assessment of genetic resources provides decisive information for sugarcane breeders to use more genetically divergent parents in crop improvement programs. One way to assess genetic variability in germplasm is to detect diverse alleles at

different gene loci using molecular marker techniques (Solomon et al., 2010). In the present study, 482 alleles were revealed within the genotype data set. Of which, 434 (90%) polymorphic alleles were detected. The total number of alleles produced by a single locus ranged from 14 (SCM32) to 40 (SOGL41), with a mean value of 24.1 alleles per locus (Table 22).

Polymorphism in SSR may result from changes to the SSR region caused by expansion, contraction or interruption (Ahmad et al., 2018), which in turn resulted from polymerase slippage during DNA replication, point mutation, unequal crossing over during meiosis, transposable element, and genetic drift (Zhao et al., 2023). The present results were higher than what had previously been reported, which found fewer allele numbers per locus (7.4) (Singh et al., 2019b), 8.2 per locus (Singh et al., 2018b), and 7.0 alleles per locus (Singh et al., 2020). Likewise, using similar SSR markers used in this study, Esayas et al. (2014; 2016c) reported somewhat fewer alleles per locus for the markers SOMS167 (10), SOMS168 (10), SOMS 173 (21), SOMS147 (22), and SOGL41 (15) than the present study. On the contrary, the current finding was somewhat lower than other previous reports of studies done using SSR markers, which found a higher number of alleles per locus, 46 (Xiong et al., 2022) and average 38 (Fickett et al., 2020). The discrepancies in the results of these studies could be due to differences in genotype source, type of genotypes, the number of genotypes, the number of markers used and their level of polymorphism, and the detection method.

Furthermore, sugarcane allelic patterns are complex due to their heterozygosity and level of polyploidy in the genome (Nawaz et al., 2010). The wide range of alleles per locus recorded in the present study indicated significant genetic variation among all genotypes. The allelic diversity of SSR markers in each of these 144 genotypes suggests their robustness in estimating the genetic relationships among these genotypes. Nevertheless, the number of alleles amplified by any SSR marker was not directly associated with its capability to discriminate the sugarcane genotypes.

The length of PCR fragment amplified from SSR locus can differ due to natural variation in number of tandem repeat unit at that locus, it can also vary between genotypes. In this study the size of the PCR fragment produced from 20 SSR-loci ranged from 50 bp to 1400 bp (Table 22).

Table 22. Details of 20 SSR primer pairs including; primer name, primer sequences, annealing temperatures (Ta), number of alleles, size range of amplified alleles

SSR Name	Forward Primers (5'-3')	Reverse primers (3'-5')	Annealing Temp.(^{o}C)	Size of amplified fragments	No. of bands
SCM4	CATTGTTCTGTGCCTGCT	CCGTTTCCCTTCCTTCCC	54	55 - 250	15(13)
SCM32	GATGAAGCCGACACCGAC	AGTTGCCTGTTCCCATTT	52	110 - 460	14(12)
UGSM542	ACCTCCACCTCCACCTCAGTTC	CGTTCAGCTTCAGGGTGTCGAT	55	50 - 380	20(16)
UGSM585	GAAGAGGAGGAGAGGAGAAG	TGGGATGGTTGTTGACTG	53	80 - 1350	25(23)
UGSM665	GTTACCATCCCATCCCAC	TGTCCCTCGTTCACAGAC	55	60 - 450	17(15)
UGSM667	CTATCCTCTTGTTGGGTCCT	TCCGCACCTCCGTTCACC	54	50 - 1100	19(18)
UGSM681	ACACATCGCTTTCCCACA	GCATACCTGTCGTCGTCT	55	100 - 1000	30(30)
SMC1825LA	CACGTCCTTCCGCCTTGA	TCATCGTTCGTCGCACTG	56	75 - 960	27(25)
SMC286CS	TCAAATGGGACCTTATTGGAG	TCCCTCGATCTCCGTTGTT	54	70 - 300	26(26)
SMC477CG	CCAACAACGAATTGTGCATGT	CCTGGTTGGCTACCTGTCTTCA	58	52 - 400	20(20)
SMC36BUQ	GGGTTTCATCTCTAGCCTACC	TCAGTAGCAGAGTCAGACGCTT	58	65 - 520	21(18)
SOMS167	AGCAGAGACACACGCACA	ACAAGAGGAGGTTCAGGG	54	100 - 1200	29(25)
SOMS168	ATGGCGTCTCGTCTCGTT	ACCTCAGTCTTGTCTTCCTTC	50	69 - 1100	26(23)
SOMS147	AGCGAACCCTAATGGAGA	GGGAGACATCGTAGACCTG	55	64 - 470	29(27)
SOGL41	TGAGGACGGGATGAAGAC	CGGTTACTGTTTGAGGGAG	52	150 - 1400	40(35)
SOMS173	GTGGACGAGAAGTGGAAGT	ATAGGAGGGCAGGACAAG	52	50 - 500	21(17)
P-89	AGAGAGAAAGAGAGGCGG	CTTCACGGAGCGAGAGAC	55	80 - 520	28(24)
P-90	CTTCCACAACCAGAGCAG	GGAGACAGAGGCGAACAG	55	54 - 1000	31(28)
P-100	AACGCCTCCGACAGTGAG	GACGAACCAACCAGAGCC	53	100 - 1300	28(23)
P-142	TAAGAATCGTTCGCTCCAGC	CTTGTGGATTGGATTGGAT	56	50 - 250	16(12)

The difference in size of PCR fragments in a particular locus is also reported by previous studies (Liu et al., 2011; You et al., 2013; Esayas et al., 2016c). Example of the illustration of the banding pattern of PCR amplified DNA fragments in 8% polyacrylamides gel in the current study is presented in Figure 6. The present study revealed a considerable SSR polymorphism ranging from 75 to 100% with a mean of 88.59% polymorphic loci (Table 23), indicating the robustness and discriminating power of the SSR markers. The highest level of polymorphism was detected in UGSM681, SMC477CG, and SMC286CS (100%) followed by UGSM667 (94.7%). These findings are further supported by similar studies, where 94%, 93.5% and 97.3% mean polymorphism were reported by Singh et al. (2010); Bisht et al. (2017b); Wu et al. (2019), respectively.

The polymorphic information content (PIC), which defines the magnitude of information retrieved from each locus, ranged from 0.42 to 0.92, with an average value of 0.62 per locus (Table 23). According to Botstein et al. (1980), the informative level or polymorphic information content of the primer is classified as high for PIC > 0.5, moderate for 0.25< PIC <0.5), and low for PIC < 0.25. Accordingly, the locus SMC286CS had the highest PIC value (0.92), followed by UGSM681 (0.82) and UGSM667 (0.81). In contrast, the lowest (0.42) was discovered at locus SCM4, followed by 0.47 at locus P90 (Table 23). Large alleles number and high PIC values (19-40, >0.63) were found with 10 SSR markers: UGSM667, UGSM681, SMC1852LA, SMC286CS, SMC477CG, SMC36BUQ, SOMS168, SOMS147, SOGL41, and P89, which showed significant potential of discriminating the 144 sugarcane genotypes into different genetic clusters. The moderate to high PIC values infer that most SSR markers had high discriminatory power.

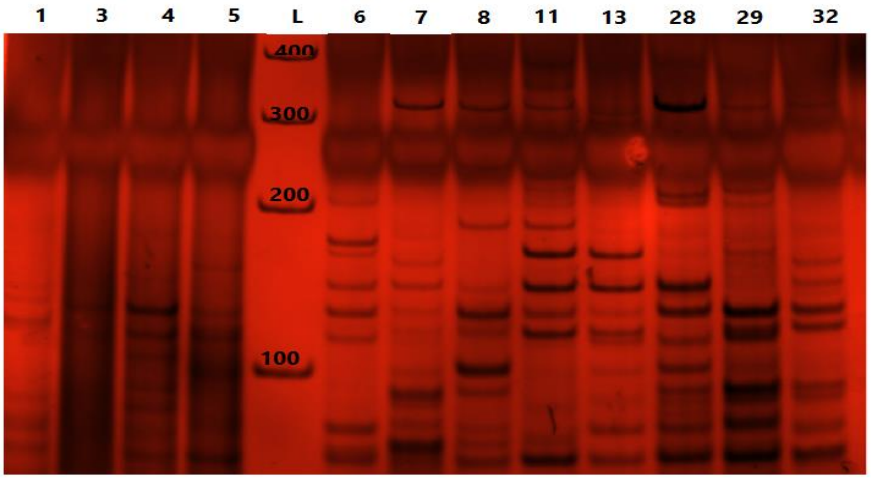

Figure 6. Banding pattern of PCR amplified DNA fragments on 8% polyacrylamide gel using primer UGSM542.

The findings indicate that microsatellite loci have a significant deal of potential for examining genetic divergence in the germplasm of sugarcane. The observed results indicate high genetic diversity in the sugarcane population considered. Singh et al. (2020) and Pinto et al. (2006) reported similar results with a PIC range of 0.35 to 0.90 and 0.28 to 0.90, with a mean value of 0.66 per locus each, respectively. The number of alleles generated by a marker and its PIC value depend on the microsatellite sequences' repeat number and sequence (Rahman et al., 2010).

4.3.1.2. Genetic diversity based on SSR markers

In this study, the number of different alleles (Na) ranged from 1.75 in locus P142 to 2.0 in loci UGSM681 and SMC286CS, with an average of 1.90 (Table 23). In another similar study, Esayas et al. (2014) found number of different alleles ranged from 1.09 to 2, with a mean of 1.75. In this study, the locus UGSM585 had the highest number of effective alleles (Ne = 1.82), followed by P-90 (Ne = 1.81), indicating that the genotypes in these loci have a high genetic diversity, which is generally desirable in breeding programs. In contrast, the lowest number of effective alleles (Ne = 1.36) was found in P-142 (Table 23). The average effective number of alleles for 20 SSR loci was 1.59, which is consistent with the average reported in studies by Esayas et al. (2014) and Esayas et al. (2016c), which were 1.52 and 1.55, respectively, for 22 SSR markers.

The Shannon information index (I), an indicator of genetic variability in a population, ranged from 0.23 (P-142) to 0.48 (SCM4), with a mean of 0.35 in this study. However, the average Shannon information index of the locus in this study (0.35) was considerably lower than the values reported by Esayas et al. (2016c) and Esayas et al. (2014), who described an average of 0.47 and 0.45 per locus, respectively.

Moreover, the Nei's gene diversity (h) in the polymorphic alleles ranged from 0.31 (SCM32) to 0.61 (USGSM585), with a mean of 0.48. The variability in Nei's gene diversity values among SSR markers shows differences in their ability to detect polymorphism. The low value recorded for the SCM32 marker indicates reasonably low genetic diversity, suggesting that this marker identifies fewer alleles or that the majority of the genotypes had the same allele(s) at this locus. Thus, marker SCM32 was found to be less informative and unsuited for distinguishing between genotypes. The mean value (h = 0.48) in this study across SSR loci were significantly higher than those reported by Bisht et al. (2017b) and Qian et al. (2013), who reported mean gene diversity h = 0.260 and h =

0.178 between markers, respectively. Consistent with the present result, Esaya et al. (2014; 2016) also reported Nei's gene diversity index ranging from 0.167 to 0.440 and 0.129 to 0.473, respectively.

The analysis result also showed that total gene diversity (Ht) values ranged from 0.22 (UGSM667) to 0.44 (UGSM585), with an average of 0.34. This result indicates that there was low overall genetic diversity at the UGSM667 locus while high total genetic diversity at the UGSM585 locus; this USGSM585 locus had high discriminatory power to differentiate divergent parents, making this marker more helpful in capturing broader allelic differences across populations. Similarly, within-population gene diversity (Hs) varied from 0.11 in UGSM667 to 0.28 in UGSM585, with a mean value of 0.21. The low intra-population diversity at the UGSM667 locus is likely due to inbreeding, founder effects, or a narrow genetic base. In contrast, a higher level of diversity within populations at the UGSM585 locus suggests more gene flow or a larger effective population size.

On the other hand, more than half of the loci (56.3%) were recorded for greater than the average (0.37) population differentiation (Gst). The microsatellite loci SMC286CS (Gst = 0.48), SOMS173 (Gst = 0.46), and USGM665 (Gst = 0.45) have resulted in significant genetic differentiation across the population (Table 23). Thus, these populations are genetically distinct from each other. This result implies low gene flow and strong population structure due to reduced genetic exchange at this locus. The observed result suggests that markers SMC286CS, SOMS173, and USGM665 are powerful enough to discriminate genotypes within and among the population, making the selection of divergent parents easier for hybridization. The lowest genetic differentiation was observed at the SOMS167 locus (Gst = 0.28), which resulted from high gene flow (1.29) between the populations, which may facilitate genetic similarity between populations and minimize the risk of inbreeding. Furthermore, the analysis revealed that the evaluated microsatellite loci had a gene flow ranging from 0.54 (SMC286CS) to 1.29 (SOMS167), with a mean of 0.89. Marker SOMS167 (1.29), UGSM681 (1.17), SMC36BUQ (1.11), SCM32 (1.02), and P-89 (1.02) exhibited high gene flow, bigger than 1, suggesting frequent gene exchange between populations which cause less differentiation of sugarcane genotypes. The tendency of low differentiation at these markers is likely due to shared alleles among populations or genotypes.

Table 23. Estimated indices of genetic diversity per locus evaluated across 144 sugarcane cultivars

Primer	Na	Ne	I	h	Ht	Hs	Gst	Nm	PPL (%)	PIC
SCM4	1.93	1.54	0.48	0.32	0.34	0.18	0.43	0.66	86.7	0.42
SCM32	1.86	1.51	0.46	0.31	0.31	0.20	0.33	1.02	85.7	0.62
UGSM542	1.80	1.58	0.33	0.48	0.32	0.20	0.37	0.85	80.0	0.55
UGSM585	1.92	1.82	0.43	0.61	0.44	0.28	0.37	0.85	92.0	0.52
UGSM665	1.88	1.59	0.34	0.50	0.34	0.18	0.45	0.61	88.2	0.74
UGSM667	1.95	1.46	0.27	0.41	0.22	0.11	0.35	0.93	94.7	0.81
UGSM681	2.00	1.61	0.36	0.54	0.38	0.25	0.30	1.17	100.0	0.82
SMC1825LA	1.96	1.69	0.39	0.57	0.40	0.26	0.35	0.93	92.6	0.68
SMC286CS	2.00	1.37	0.24	0.39	0.32	0.17	0.48	0.54	100.0	0.92
SMC477CG	2.00	1.56	0.34	0.52	0.34	0.21	0.34	0.97	100.0	0.74
SMC36BUQ	1.86	1.61	0.34	0.50	0.31	0.20	0.31	1.11	85.7	0.64
SOMS167	1.86	1.70	0.38	0.55	0.36	0.25	0.28	1.29	86.2	0.56
SOMS168	1.88	1.61	0.34	0.50	0.34	0.20	0.40	0.75	88.5	0.66
SOMS147	1.93	1.70	0.38	0.55	0.37	0.24	0.34	0.97	93.1	0.63
SOGL41	1.90	1.68	0.38	0.55	0.40	0.24	0.39	0.78	87.5	0.66
SOMS173	1.81	1.46	0.27	0.41	0.29	0.15	0.46	0.59	80.9	0.60
P-89	1.89	1.50	0.30	0.45	0.29	0.19	0.33	1.02	85.7	0.68
P-90	1.87	1.81	0.42	0.59	0.40	0.26	0.34	0.97	87.1	0.47
P-100	1.86	1.67	0.37	0.52	0.37	0.22	0.38	0.82	82.1	0.50
P-142	1.75	1.36	0.23	0.36	0.24	0.15	0.35	0.93	75.0	0.63
Mean	**1.90**	**1.59**	**0.35**	**0.48**	**0.34**	**0.21**	**0.37**	**0.89**	**88.59**	**0.64**

Na = Number of different alleles; Ne = Effective number of alleles; I = Shannon's Information index; H = Nei's gene diversity; HT = Total genetic diversity, HS = within population heterozygosity; Gst = Genetic differentiation statistics by locus; Nm = estimate of the number of migrants (gene flow) = 0.5(1 - Gst)/Gst, PPL = Percentage of polymorphic Loci

4.3.1.3. Genetic variability within and among populations

Table 24 summarizes the different estimates of genetic diversity for all population loci in this study. For population sizes ranging from 5 to 26, the mean estimates of genetic diversity within the population were: band frequency (BF) 0.59, observed different alleles (Na) 1.75, effective alleles (Ne) 1.53, Shannon's information index (I) 0.45, Nei's gene diversity (h) 0.30, unexpected heterozygosity (uHe) 0.32, and percentage of polymorphic loci (PPL%) 79.23% (Table 24). In the present study, different levels of polymorphic loci were recorded in various sugarcane populations. The percentage of polymorphic loci per population is the proportion of genetic markers in the DNA genome where individuals within a population display different forms or alleles. The study result revealed that Barbados had the highest percentage of polymorphic loci (PPL = 87.34%) of the ten sugarcane populations studied, followed by India and the United States (86.31%). This

result could be attributed to large number of genotypes (Barbados = 26, India = 24, and the USA = 19) in the three populations (Table 24). The percentage of polymorphic loci exhibited within a population can be attributed to population size, migration patterns, genetic drift, and natural selection (Song et al., 2006). This finding is consistent with the results of another study conducted by Esayas et al. (2016c), who found a high (86.54%) percent of polymorphic loci in the Indian population, followed by 78.08% and 77.31% in the Barbados and Hawaii populations, respectively. In a separate study, the percentage of polymorphic loci in introgression groups ranged from 63.44% in the EIH population to 89.77% in the SSH population (Elumalai & Srinivasan, 2023).

The result in Table 24 showed that the number of different alleles (Na) that refers to the number of distinct forms of a particular gene present in a population, ranging from 1.47 to 1.86. The maximum number of observed different alleles (Na = 1.86) was found in the Barbados and India populations. In contrast, the minimum number of observed alleles (Na = 1.47) was recorded in the sugarcane population of Guyana. This result implies that the two populations have higher genetic diversity than Guyana, as exhibited by their high percentage of polymorphic loci. The results reported by Esayas et al. (2016c) are similar to the results of the present study, which found several different alleles ranging from 0.523 in Cuba to 1.796 in the Indian population with an average of 1.369. The actual number of different alleles in a population can vary depending on the locus and the population itself. Some loci may have only a few alleles, while others may have many variants.

Effective allele, which has significant effect on a trait, can be used to determine genetic diversity and variation within a population at a specific gene locus. This helps to explain the observed genetic variations. In this study, the sugarcane population in India had the most effective alleles (Ne = 1.60), followed by Barbados (Ne = 1.58). In contrast, the Guyana population had the fewest effective alleles (Ne = 1.41), followed by the South African population (1.46). This indicates the highest genetic diversity in Barbados and Indian populations while the lowest recorded in Guyana. Another study by Elumalai and Srinivasan (2023) found that the SSH (PIO x PIS) mating group had the highest number of effective alleles (Ne = 2.534), which is slightly higher than the current result. However, the present study showed slightly higher numbers than the report by Esayas et al. (2016c), who found an average range of effective alleles per locus in Cuba (Ne = 1.144) to (Ne = 1.489) in Canal Point populations with an overall mean of 1.365. Moreover, Ali et al. (2019) reported that *Saccharum officinarum* exhibited the highest number of effective

alleles (Ne = 1.70), followed closely by *Saccharum spontaneum* (Ne = 1.64). In contrast, *Erianthus arundinaceous* had the lowest number of effective alleles (Ne = 1.30). These findings are important because higher numbers of effective alleles indicate more significant genetic variation. This peculiarity can significantly enhance the variability observed within the population and thus its ability to adapt to different environments, boosting its overall fitness.

The Shannon Information Index (I) is another parameter used to measure genetic diversity. In this study, India and Barbados had the highest Shannon information indices (I = 0.49), followed by the Cuban sugarcane population (I = 0.48) with an average of 0.32. In contrast, the lowest Shannon information index (0.34) was recorded for the Guyana population (Table 4). This implies that India and Barbados population had the highest genetic diversity while the lowest recorded for Guyana. Similar findings were reported by Esayas et al. (2016c), who found the highest Shannon's information index (0.438) in Indian accessions, followed by 0.411 in those at Canal Point, with an overall mean of 0.321. Elumalai & Srinivasan (2023) found slightly higher values, with hybrids of mating groups having the highest index, PIO x PIS (I = 0.815), followed by PIS x Co (I = 0.759) and PIR x PIO (I = 0.728).

The Nei gene diversity (h), also called expected heterozygosity, measures genetic diversity within the population. The analysis result revealed that the values of Nei diversity ranged from 0.23 to 0.34, with a mean value of 0.30. The sugarcane population from India demonstrated the highest Nei gene diversity (h = 0.34). However, the lowest Nei gene diversity was observed in the Guyana populations (h = 0.23). A high Nei diversity value indicates a high level of genetic variability within a population. Sixty percent of the populations considered exhibited gene diversity (h = 0.23–0.34) greater than the mean value of 0.30 (Table 24). Similarly, the result in Table 24 elucidated that the percentage of polymorphic loci in different populations ranged from 60.17% (Guyana) to 87.34% (Barbados), with an average of 79.23% (Table 24). The high percentage of polymorphic loci indicates high genetic diversity in population, which enable the breeder to select divergent genotypes for the improvement of desirable traits through hybridization. Furthermore, the highest unexpected heterozygosity values (uHe) were detected in the population of India, Barbados, and Cuba (uHe = 0.34), while the lowest was recorded for Guyana (uHe = 0.26) (Table 24). High unexpected heterozygosity indicates that the observed level of genetic diversity at a locus is greater than expected. This could be

attributed to factors like gene flow, outbreeding, non-random mating, hybrid origin, and low recombination rate.

Table 24. Diversity indices and allelic pattern in 10 populations averaged over the 20 SSR Loci

Population	BF	N	Na	Ne	I	h	uHe	PPL (%)
France	0.58	19	1.84	1.57	0.47	0.32	0.33	85.48
Barbados	0.58	26	1.86	1.58	0.49	0.33	0.34	87.34
USA	0.58	19	1.85	1.56	0.48	0.32	0.33	86.31
India	0.60	24	1.86	1.60	0.49	0.34	0.34	86.31
Cuba	0.59	15	1.85	1.57	0.48	0.33	0.34	85.68
Ethiopia	0.57	10	1.76	1.54	0.45	0.31	0.32	80.08
Philippines	0.60	13	1.72	1.53	0.44	0.30	0.31	77.59
Sudan	0.58	8	1.73	1.52	0.43	0.30	0.31	77.59
Guyana	0.58	5	1.47	1.41	0.34	0.23	0.26	60.17
South Africa	0.60	5	1.57	1.46	0.38	0.26	0.29	65.77
Mean	0.59	14	1.75	1.53	0.45	0.30	0.32	79.23
SE	0.00	0.10	0.01	0.01	0.004	0.003	0.003	2.98

BF = band frequency, N = sample size, Na = number of different alleles, Ne = no.of effective alleles, I = Shannon information index, h= Nei's gene diversity, uHe = unexpected Heterozygosity= (2N / (2N-1)) * He, PPL = percentage of polymorphic loci, SE = standard error

In general, the Indian population had the highest observed number of alleles (Na = 1.86), effective number of alleles (Ne = 1.60), Nei gene diversity (h = 0.34), Shannon's information index (I = 0.49), and unexpected heterozygosity (uHe = 0.34). Similarly, the Barbados population had the same observed number of alleles (Na = 1.86), Shannon's information index (I = 0.49), and unexpected heterozygosity (uHe = 0.34) as the Indian population, as well as the second highest effective number of alleles (Ne = 1.58) and Nei's gene diversity (h = 0.33). These results suggest that the India and Barbadose populations are genetically more diverse than others and could be used as a source of genetic materials for breeding program. Historically, India and Barbados contributed more sugarcane breeding populations and are sources of many of the current modern commercial cultivars. Furthermore, the present result also indicated that the local landraces, the Ethiopian population, scored the average value for all diversity parameters (Na = 1.76, Ne = 1.54, I = 0.45, h = 0.31, uHe = 0.32, and PPL = 79.23%) (Table 24).

4.3.1.4. Banding patterns across populations

Figure 7 illustrates the banding pattern of 144 sugarcane genotypes classified into ten populations. The number of total bands per population ranged from 420 to 480. The majority of the bands were found in India's sugarcane population (480), followed by Cuba and the United States (479 and 477 bands, respectively) and the lowest is recorded for Guyana (Demerara) (420). The Indian population had the highest expected mean heterozygosity (0.337), followed by Barbados and Cuba, with mean values of 0.332 and 0.326, respectively (Fig. 7), indicating high genetic diversity. High mean heterozygosity usually observed in large populations that have not undergone genetic drift (stable population). High band number does not always lead to high heterozygosity. The private bands utilized to differentiate populations from each other were not identified; all populations did not have a private band (Fig. 7) indicating that the populations do not have unique allele loci that could be utilized to differentiate the populations.

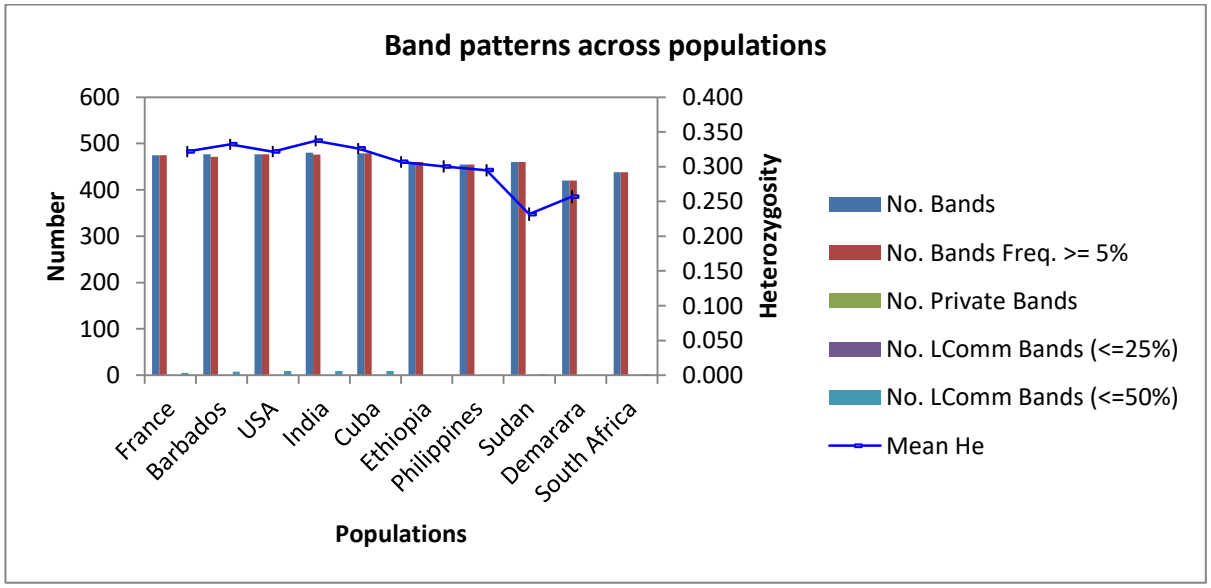

Figure 7. Graphic representation of band patterns across populations.

4.3.1.5. Genetic relatedness and population differentiation in sugarcane genotypes

The present study showed the highest genetic distance of 0.075 between the Sudan and Guyana populations, followed by the distance between the USA and Guyana (0.070). Sugarcane populations of the United States and Barbados had the least genetic distance (0.006), followed by Cuba and India (0.009) (Table 25). This result implies higher genetic divergence between the genotypes of the Sudan and Guyana populations, possibly due to

low gene flow or less exchange of genetic materials between the two countries. Gene flow was the highest between Cuba and India (18.98), followed by the USA and Barbados (13.64), in contrast, the lowest obtained between the USA and Guyana (2.47) followed by Sudan and Guyana (3.08) (Table 25). Therefore, genotype selection for hybridization from the diverse Sudan and Guyana populations would be effective in the breeding program, as genotypes from these populations could have unique features for improving sugar yield, fibre, ratooning ability, and resistance to biotic and abiotic stress-related traits. The narrow genetic distance observed between the United States and Barbados populations could indicate an adjoining geographical area with high gene flow through genetic material exchange between these countries and thus use of similar parental genotypes leading to narrow genetic base.

Table 25. Unbiased genetic distance (lower triangle) and gene flow (upper triangle) among the 10 sugarcane populations in Ethiopia.

Pop.	France	Barb.	USA	India	Cuba	Eth.	Philip.	Sudan	Guyana	S. Africa
France	—	6.51	4.30	8.68	7.10	8.08	4.65	5.56	3.00	4.96
Barbados	0.016	—	13.64	9.75	12.91	9.37	3.92	11.65	3.17	3.22
USA	0.022	0.006	—	6.89	9.75	6.89	3.08	5.85	2.47	3.48
India	0.011	0.013	0.016	—	18.98	12.25	4.96	9.01	3.72	6.69
Cuba	0.015	0.012	0.013	0.009	—	11.65	5.70	9.75	4.38	4.47
Ethiopia	0.014	0.02	0.019	0.016	0.017	—	5.43	12.25	3.04	6.16
Philippines	0.024	0.037	0.038	0.028	0.024	0.026	—	3.92	2.47	4.96
Sudan	0.028	0.025	0.028	0.022	0.023	0.029	0.042	—	3.08	3.78
Guyana	0.051	0.066	0.07	0.056	0.058	0.066	0.047	0.075	—	2.23
South Africa	0.041	0.066	0.055	0.048	0.054	0.047	0.044	0.062	0.062	—

Genetic distance (GD) was also estimated between geneotypes of different populations and the distances were ranged from 80 between two France population genotypes FGo4-275 and FG04-798 to 227 between genotypes FGo5-336 (France population) and CP36/11 (USA population)(data not shown). This result aligns with the dendrogram result of cluster analysis, in which genotypes FGo5-336 and CP 36/11 were assigned in different clusters (Fig. 5). This relationship indicates that these genotypes were geographically diverse having different genealogical history and evolved independently. These genotypes can possess unique characteristics for improving cane and sugar yield and resistance to biotic and abiotic stress-related traits. The second highest genetic distance was also found between genotypes FGo5-155 and B46/81 (222) and between FGo5-155 and CP47/193

(221) (data not shown). Esayas et al. (2016c) also reported genetic variations between genotypes based on genetic similarity distances.

In this study, estimates of genetic differentiation ranged from 0.013 to 0.101. The highest and statistically significant genetic differentiation was observed between South African population and the Guyana population (PhiPT = 0.101, p = 0.012), followed by the Guyana and Philippines, and the USA and Guyana populations (PhiPT = 0.092, p = 0.001) (Table 26). According to Wright et al. (1951), the values of genetic differentiation between populations or their progeny ranging from 0.0 to 0.05, 0.05 to 0.15, 0.15 to 0.25, and more than 0.25 indicate low, moderate, high, and very high genetic divergences, respectively.

Table 26. Genetic differentiation of population measured by PhiPT (lower triangle) between the ten sugarcane populations with p-values (upper triangle)

Pop	Fra	Bar	USA	India	Cuba	Eth	Philip	Sudan	Dema	S. Afr.
France	—	0.001	0.001	0.001	0.005	0.012	0.001	0.004	0.003	0.018
Barbados	0.037	—	0.010	0.001	0.016	0.009	0.001	0.047	0.001	0.001
USA	0.055	0.018	—	0.001	0.008	0.006	0.001	0.002	0.001	0.003
India	0.028	0.025	0.035	—	0.042	0.028	0.001	0.015	0.001	0.023
Cuba	0.034	0.019	0.025	0.013	—	0.039	0.004	0.049	0.017	0.008
Ethiopia	0.030	0.026	0.035	0.020	0.021	—	0.003	0.094	0.005	0.050
Philippines	0.051	0.060	0.075	0.048	0.042	0.044	—	0.002	0.003	0.027
Sudan	0.043	0.021	0.041	0.027	0.025	0.020	0.060	—	0.002	0.025
Guyana	0.077	0.073	0.092	0.063	0.054	0.076	0.092	0.074	—	0.012
S. Africa	0.048	0.072	0.067	0.036	0.053	0.039	0.048	0.065	0.101	—

It is presumed that genetic differentiation between populations increases with geographical distance as gene flow between populations decreases with increased distance between the populations. Accordingly, gene flow was lowest between the South Africa and Guyana populations (2.23), followed by between the Guyana and Philippines populations (2.47) and the USA and Guyana populations (2.47) (Table 26). No significant ($P > 0.05$) differentiation was observed between the populations of Sudan and Ethiopia, suggesting that these population share a high degree of genetic similarity possibly due to the adjoining geographical region, which promote gene flow through germplasm exchange, environmental similarity (analogues selective pressure), historical connection etc. In contrast, Table 26 revealed that low differentiation was recorded between Cuba and India populations (PhiPT = 0.013, $p < 0.042$), suggesting that low genetic differentiation underlines the importance of human-mediated gene flow between these regions. Genotypes from populations with considerable differentiation can be exploited for crop

improvement in the future sugarcane breeding programme of the country. In supporting the present result, You et al. (2013) also elucidated that high level of gene flow primarily caused low genetic differentiation (Gst) among populations. Compared to wild sugarcane (Gst = 0.209) (Chang et al., 2002) and weedy rice genetic differentiation (Gst = 0.387) (Zhang et al., 2012), the Gst (0.047) of the present 144 sugarcane genotypes was relatively at low level. Esayas et al. (2016c) revealed PhiPT values among pairs of sugarcane populations ranging from 0.0018 (between Tigray and Gambella) to 0.4003 (between Benshangul and Amhara).

4.3.1.6. Genetic relationship within and among populations

A distance-based molecular variance assessment (AMOVA) was performed to determine variability between and within populations. The study used bands produced by twenty primers in ten sugarcane populations. The result showed that a significant percentage (96%) of total allelic diversity was due to individuals within a population, while only 4% was distributed between populations (Table 27). The observed lower proportion of genetic variation among populations than within populations could be attributed to the high gene flow between populations leading to narrow genetic base, which might have arisen due to the exchange of sugarcane germplasm between breeding programs worldwide.

Table 27. Analysis of molecular variance (AMOVA) within and among the sugarcane populations

Source	df	SS	MS	Est.Var	PV%	FST	Pvalue	Nm
Among population	9	1232.042	136.89	3.528	4	0.04	0.001	12.32
Within Population	134	11721.327	87.47	87.780	96		0.001	
Total	143	12953.368		91.001	100			

df = degree of freedom, SS = sum of squares, MS = mean square, Est. Var. = estimated variation, PV = percent of variation, F*st* = fixation indices, Nm = gene flow

The AMOVA results also showed a highly significant genetic variation ($p < 0.001$) within and between sugarcane populations (Table 27). Earlier studies by Wu et al. (2019), Glynn et al. (2009), Esayas et al. (2014), and Huang et al. (2022) also reported similarly lower proportions among populations with genetic variation of 5%, 3.4%, 3%, and 1%, respectively. Another study by Glynn et al. (2009) also revealed that 96.6% of within

population variations. Furthermore, Tazeb et al. (2017) pointed out more variability within the groups than between them. The present analysis also found high gene flow (Nm = 12.32) and statistically significantly low genetic differentiation (Fst value = 0.039; P < 0.001) (Table 27). In the present study, the observed low genetic variation among populations indicates less involvement of geographic location in population differentiation and more frequent gene flow in these regions. The present findings were in agreement with Wu et al. (2019), who reported high gene flow (Nm = 4.98) and a low fixation index value (Fst = 0.048).

4.3.2. Cluster analysis

Plant breeders must identify various genotypes from the gene pool to develop superior cultivars in breeding programs. One of the practical approaches for detecting genetic variation between genotypes is the UPGMA-based clustering method. In this study, genotypic data on 482 alleles from the 144 sugarcane genotypes was used to estimate the genetic distance of Nei (1978) between genotypes for cluster analysis.

The dendrogram constructed based on Nei's (1978) genetic distance using UPGMA showed the relationships among the 144 sugarcane genotypes studied (Fig. 8). The UPGMA cluster tree analysis discriminates 144 sugarcane genotypes according to genetic distance into four main clusters that have 65, 26, 40, and 13 genotypes in C1, C2, C3, and C4, respectively (Fig. 8). Each main cluster was subdivided into two sub-clusters; accordingly, C1 could be further subdivided into C1A with 51 genotypes and C1B with 14 genotypes. C2 could also be subgrouped into C2A with 14 genotypes and C2B with 12 genotypes. C3 could be further subdivided into C3A with ten genotypes and C3B with 30 genotypes. Similarly, C4 could be further subgrouped as C4A with four genotypes and C4B with nine genotypes (Fig.8). Genotypes originating in Barbados and the USA comprised 30% and 25% of the genotypes in C3. Sugarcane populations from Barbados and India represented 26.9% and 23.1% of the C2 genotypes, respectively. Ten local landraces were assigned to C1 (8), C2 (1), and C4 (1).

In Figure 8, it was remarkably observed that no single group in the tree only comprised genotypes with the same geographic affinities. The genotypes in a single cluster exhibited a uniform allelic pattern, indicating they were more genetically similar than others due to shared alleles through gene flow among genotypes. These findings are consistent with the results of Singh et al. (2011) and Khalid et al. (2019), who evaluated sugarcane genotypes

using SSR markers and reported dividing the genotype population into four main clusters. Another similar study by Elumalai & Srinivasan (2023) also revealed that the UPGMA method divided sugarcane hybrids into three major clusters and further classified them into 11 subclusters. Contrary to the present finding, Padmanabhan & Hemaprabha (2018) reported a trend of clustering 133 sugarcane genotypes into two major groups based on time and region of origin.

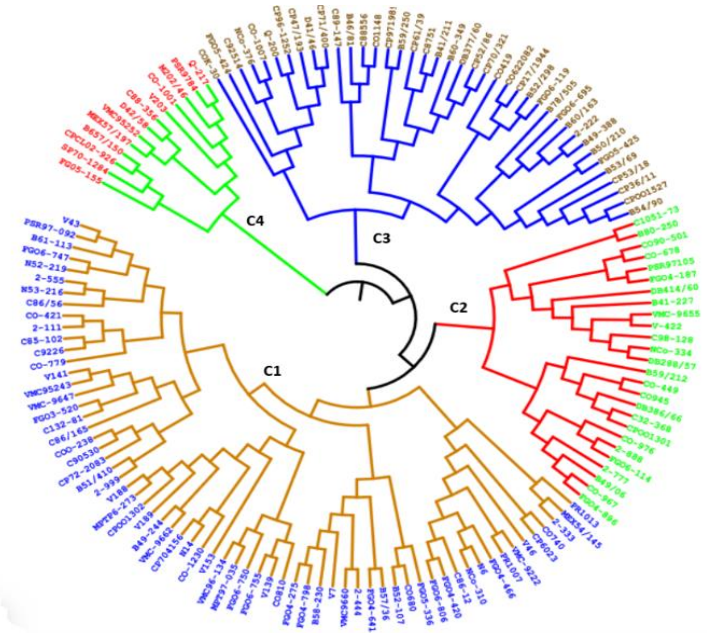

Figure 8. Circular dendrogram constructed from 144 sugarcane genotypes through the unweighted pair group method with arithmetic mean (UPGMA) in MEGA11 using SSR markers.

4.3.3. Principal coordinate analysis

Principal coordinate analysis (PCoA) can also be used to compute the genetic structure of sugarcane genotypes using a genetic distance matrix generated from SSR marker data. PCoA was carried out in this study based on 144 sugarcane genotypes using 20 polymorphic loci of the SSR. The PCoA grouped the genotypes into four major clusters regardless of geographical origin, validating the result of UPGMA cluster analysis (Fig. 9). Previous research has also reported PCoA that divided the population into two major groups (Padmanabhan & Hemaprabha, 2018), three distinct clusters (Elumalai & Srinivasan, 2023), three gene pool groups (Esayas et al., 2014), and four groups (Ahmed & Gardezi, 2017b). The analysis has also showed that, in all geographical populations, genotypes from the same collection site were often found in different clusters. In contrast, genotypes from different countries of collection are often clustered together (Figs. 8 and

9), indicating the possibility of exchanging genetic materials between countries globally. Previous studies by Esayas et al. (2014) and Ahmed & Gardezi (2017b) also found that the UPGMA and the neighbour-joining-based cluster analysis results confirmed the PCoA results.

A two-dimensional scatter plot generated by PCoA showed an almost uniform distribution of genotypes across all populations around the center of the two-dimensional coordinate plane, with distinct population groupings (Fig.9). The first three PCoA coordinates exhibited a cumulative variation of 19.85%. The PCoA plot's first and second dimensions contributed 7.83% and 6.30% of the total population variability, respectively. In similar study, Padmanabhan & Hemaprabha (2018) reported 8.34% and 3.22%, respectively, and Wu et al. (2019) found 8.41% and 6.71% variation in the first and second coordinate axes, which is consistent with the current findings. Similarly, 10.59% of the total variance by the first two coordinates was reported by Fickett et al. (2020). Other studies, on the other hand, reported higher variation in the first two coordinates than the current findings: 16.42% and 8.29% (Elumalai & Srinivasan, 2023), 49.8% and 13.69% (Esayas et al., 2014), and 45.71% and 15.02% (Esayas et al., 2016c) respectively. Furthermore, Ahmed & Gardezi (2017b) reported that PCoA-1 and PCoA-2 accounted for 31% of the total variability.

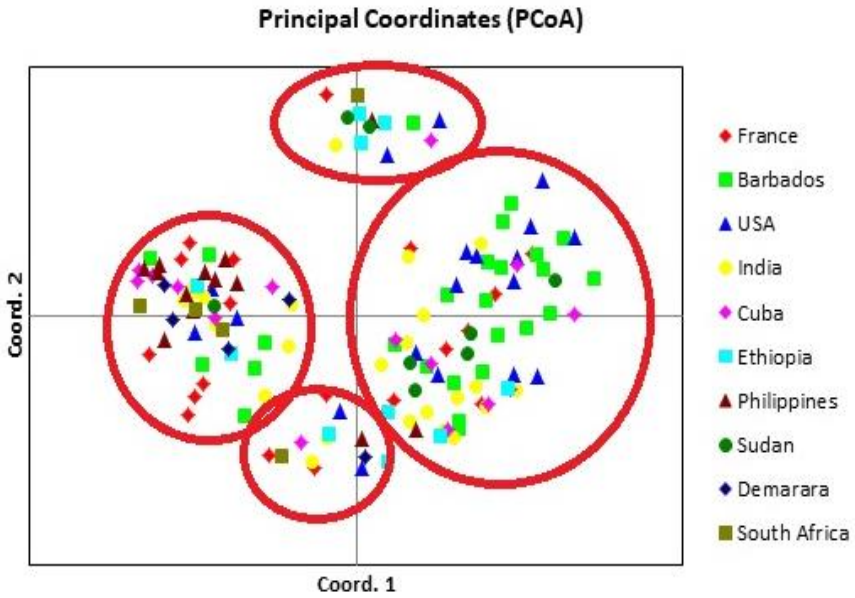

Figure 9. Scatter diagram of the principal coordinate analysis showing the distribution of the ten sugarcane population on the two coordinates based on Nei's genetic distance.

4.3.4. Genetic analysis of population structure

The genetic architecture of a set of genotypes can be precisely determined by analyzing population structure with SSR molecular markers (Varshney et al., 2007). The optimal K value of 6 was obtained by calculating the log-likelihood using the STRUCTURE software. Similarly, the maximum value of the ad hoc measure (ΔK) analysis (Evanno et al., 2005) indicated a sharp peak at K = 6 (Fig. 10A & B), which revealed that 144 sugarcane genotypes were categorized into six subpopulations (Pop1, Pop2, Pop3, Pop4, Pop5, and Pop6) (Fig. 10A, B & 11). Earlier research by Singh et al. (2020) studied the structure of 139 sugarcane genotypes and revealed the optimal or best value of K at 3 (K = 3) using the structure harvester. Padmanabhan & Hemaprabha (2018) also estimated the best value of K at 5 (maximum ΔK of 5) and divided 133 genotypes into five subpopulations. Elumalai & Srinivasan (2023) used a model-based Bayesian approach to classify 172 introgressed hybrids at various nobilized stages (F1, BC1, and BC2) into three genetic subgroups (K = 3). Fickett et al. (2020) also elucidated the grouping of 190 sugarcane clones into five major subpopulations through structure analysis.

The Bayesian approach assesses the percentage of the genome of individuals originating from different populations. This research used 60% of the membership coefficient as a threshold value for individual genotypes to be categorized into their genetic groups. The structure analysis result (bar plot, Fig. 11) depicts the genetic admixture between the six subpopulations, with each subpopulation having a small to high proportion of shared membership with other subgroups.

Among the 144 sugarcane genotypes studied, 133 (92.4%) were assigned to six subpopulations with a membership probability greater than 0.6. The remaining eleven genotypes (7.6 %) were an admixture in subpopulations, with membership probability less than 0.6 (Fig. 11). Accordingly, 1, 2, 1, 6 and 1 admixed genotypes were grouped into subpopulation 1 (CP001527), 3 (Co1148, C86-165), 4 (FG05-424), 5 (C86/12, B52-107, DB414/60, Co740, DB288/57, C132-81), and 6 (CP52/86), respectively. As a result, subpopulations 1, 2, 3, 4, 5, and 6 had 95.8% (23), 100% (13), 94.7% (36), 92.3% (12), 85.7% (36), and 92.9% (13) pure genotypes, respectively with more than 0.6 memberships, indicating a low level of admixture or genetic mixing in each subpopulation (Fig. 11), implying significant genetic differentiation among subpopulations due to limited gene flow among them. Pocovi & Mariotti (2015) revealed that among the 63 accessions,

34 (54%) have more than 0.60 memberships in any given of the five clusters, while 46% (29 accessions) share similar membership coefficients for at least two groups.

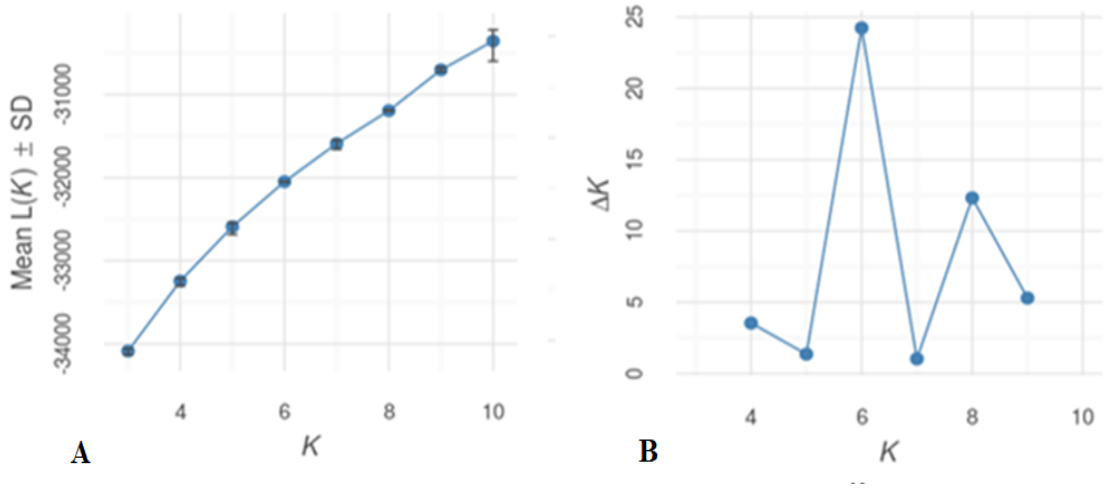

Figure 10. Delta K showing the number of subpopulations A) Average log-likelihood K-value (LnP (K)) against the number of K. B) The optimal number of K values (K = 6) determined by web based pophelper algorism

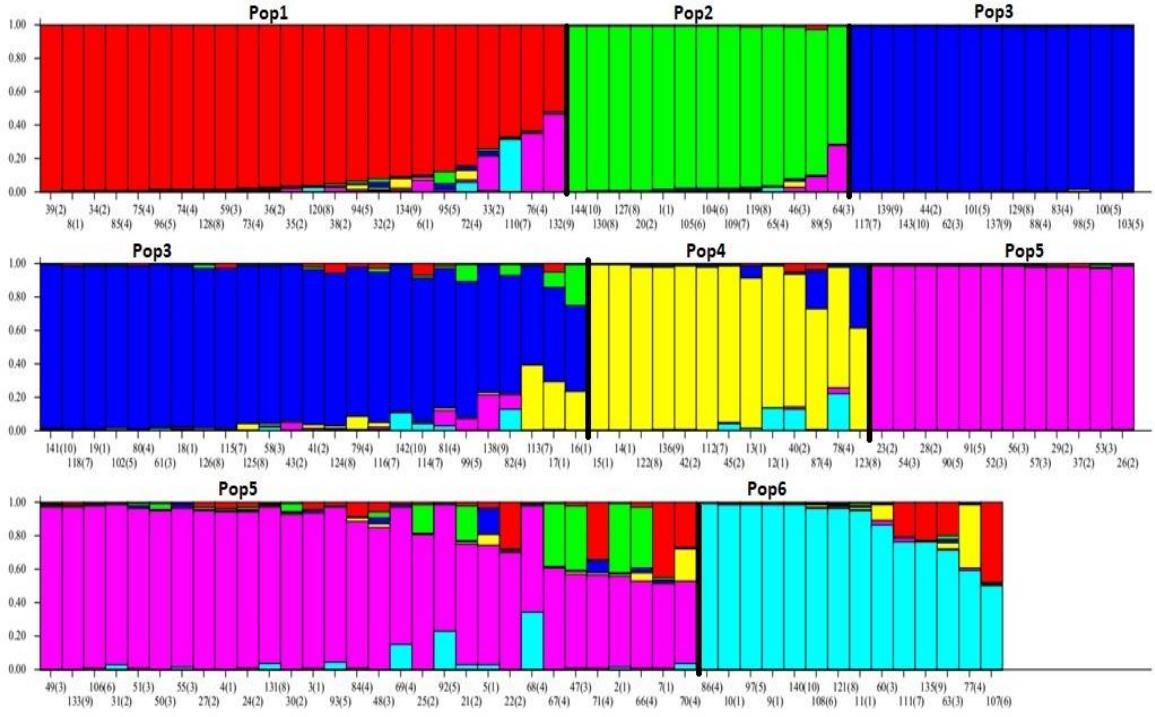

Figure 11. Population structure Bar plots developed based on 20 SSR markers for 144 genotypes of sugarcane. The genotypes grouped into six subgroups (K = 6) and each genotypes is represented by a single vertical bar that is broken into six colored segments. Pop1 genotypes are coded red, Pop2 (green), Pop3 (dark blue), Pop4 (yellow), Pop5 (pink), and Pop6 (light blue). Populations with one solid color that is not shared by another group are genetically distinct. Populations that share colors are more similar, share certain proportion of ancestral alleles in their genome.

Like distance-based clustering methods cluster analysis and principal coordinate analysis, the Bayesian-based clustering (structure analysis) grouped the sugarcane genotypes based on genetic similarity rather than geographic affinity. Esayas et al. (2016c) found a similar result. Among the six subpopulations, subpopulation 5 (in pink) had the most sugarcane genotypes (42) with a higher proportion of Barbados genotypes (12 genotypes), indicating high genetic divergence compared to the other subpopulations. Subpopulation 1 (in red) comprised 24 genotypes, with most of the genotypes originating in Barbados (7 genotypes). Subpopulation 2 (in green) included 13 genotypes, among which the larger proportions (3 genotypes) were of Philippine origin. Subpopulation 3 (in blue) consisted of 38 genotypes, with a higher proportion of India (6) and Cuba (6) originating genotypes. Thirteen genotypes were identified in subpopulation 4 (in yellow), with a more significant proportion of genotypes originating from France (4). Furthermore, subpopulation 6 (light blue) had 14 genotypes, of which French-origin genotypes were observed in a large proportion (3 genotypes). Subpopulations 2, 3, and 6 each comprised genotypes from nine original populations, indicating high genetic divergence compared to the other subpopulations (Fig. 11). On the other hand, ten Ethiopian local landraces considered in this study were grouped into different subpopulations: 2 landrace in subpopulation 1, 4 in subpopulation 2, 3 in subpopulation 3, and 1 in subpopulation 6, indicating a lack of connection between geographical origins and clustering.

In this study, Figure 12 illustrates the different parameters of genetic diversity computed using structure analysis. The mean FST (fixation index) values confirmed the existence of differences within subpopulations. Subpopulation 6 had the greatest FST value (0.33), followed by subpopulations 4 (0.31), 2 (0.25), 1 (0.20), 3 (0.17), and 5 (0.14%). The analysis also revealed that subpopulation 5 had the most genotypes per population, expected heterozygosity (He), and the lowest value of population differentiation (Fst), indicating the presence of less genetically distinct genotypes or high admixture. Subpopulation 6 had high FST (population differentiation) and low expected heterozygosity, indicating the presence of genetically distinct genotypes or fewer admixtures. Therefore, genotypes clustered in subpopulation six can be used as parent material for hybridisation in a sugarcane variety improvement program, as it comprises relatively divergent genotypes. According to Wright et al. (1978), Fst values below 0.05 indicate negligible genetic differentiation, while those above 0.15 to 0.25 indicate significant genetic differentiation. Similar to the current study, Pocovi and Mariotti (2015)

found that cluster 3 had the highest FST value (0.63), followed by cluster 5 (0.38) and cluster 1 (0.29), indicating that cluster genotypes varied. In this study, the highest proportion of membership (Q = 0.286) was observed in subpopulation 3. The minimum membership (Q = 0.087) was recorded for subpopulation 4. This implies that subpopulation 3 contribute the considerable proportion of genetic ancestry.

Furthermore, this study revealed a mean alpha value of 0.0428. In agreement with the current results, Wright et al. (1978) revealed a low mean alpha value, 0.1772. According to Elumalai & Srinivasan (2023), when the mean value of alpha is close to zero, most individuals are basically from one population or another, while alpha > 1 means most individuals are mixed. Consequently, the low alpha value of this study indicates a low admixture between the genotypes generated in the present study. The observed results enable breeders to select more divergent parents for hybridization. It is known that evolutionary agents such as natural selection, gene mutation, and gene flow are the main reasons for altering the population's genetic structure.

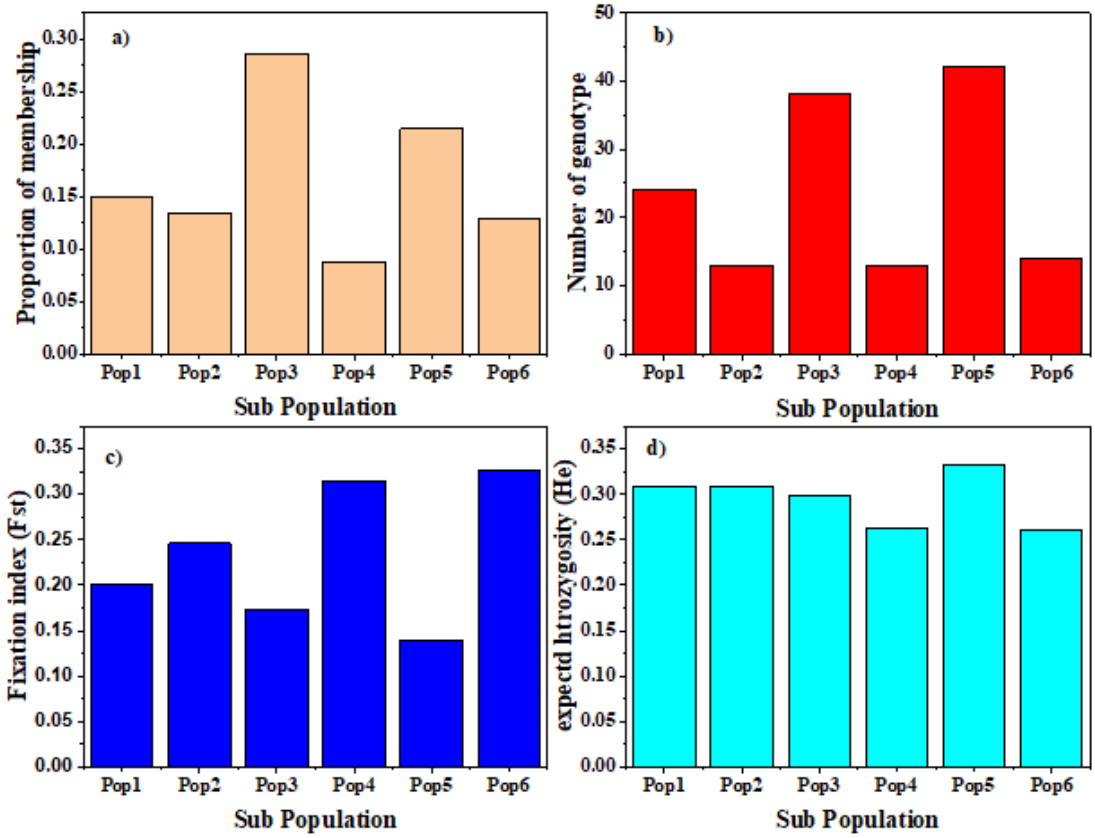

Figure 12. Graphic illustration of population structure parameters. a) Proportion of membership, b) Number of genotypes, c) Fixation index, and d) expected heterozygosity

It has been revealed that gene flow values greater than 1.00 would resist the effect of genetic drift and prevent the differentiation of populations (Johnson et al., 2018). Previous researchers reported the same result (Padmanabhan & Hemaprabha, 2018; Elumalai & Srinivasan, 2023). Limited admixture is typical in sugarcane, where breeding programs conserve distinct genetic lineages.

4.3.5. Linkage disequilibrium and marker trait association study

4.3.5.1. Linkage disequilibrium

The linkage disequilibrium (LD) of the studied genotypes was determined using 20 pair of SSR markers. In total 8.58% ($r^2 \geq 0.05$), 7.4% ($r^2 \geq 0.1$), and 0.29% ($0.3 < r^2 < 0.70$) of the marker pairs based on r^2 estimates showed significant LD. High r^2 estimate indicate strong LD, suggesting that the two markers at different loci on a chromosome are inherited together. Low r^2 estimate suggest weak LD, indicating that the two SSR markers (alleles) independently inherited. The darker block or large square indicates that SSR in that region are inherited together with higher level of correlation (Fig. 13).

4.3.5.2. Marker trait association

This study used TASSEL 5.0 (Bradbury et al., 2007) to determine marker-trait association (MTA) in sugarcane using 20 SSR markers and sixteen quantitative traits with the general linear model (GLM) and mixed linear model (MLM). Markers with a low frequency of alleles ($p < 0.05$) were removed before MAT analysis with TASSEL ver. 5.0 (Bradbury et al., 2007) as it can bias LD estimates between loci (Mohlke et al., 2001). The association analysis results using GLM and MLM are presented in Table 28. A significant association between SSR markers and quantitative traits was identified on the R2 value > 0.1 and p-value < 0.05.

The study identified 67 significant marker-trait associations for 16 traits in the GLM (64) and MLM (3) models, with p-values < 0.01 (Table 28). Using similar study, Siraree et al. (2017) identified 60 MTAs with 22 qualitative traits and 21 MTAs for quantitative traits using the MLM model, which was more significant than our present result. In comparison to GLM, the MLM model, which combines the population structure (Q) and kinship (K) matrix, reduces type I and II errors while eliminating false positives (Bradbury et al., 2007). Thus, MLM method reduced the number of significant associations.

Yu et al. (2005) also suggested that MLM is a more efficient model for marker-trait association in structured populations.

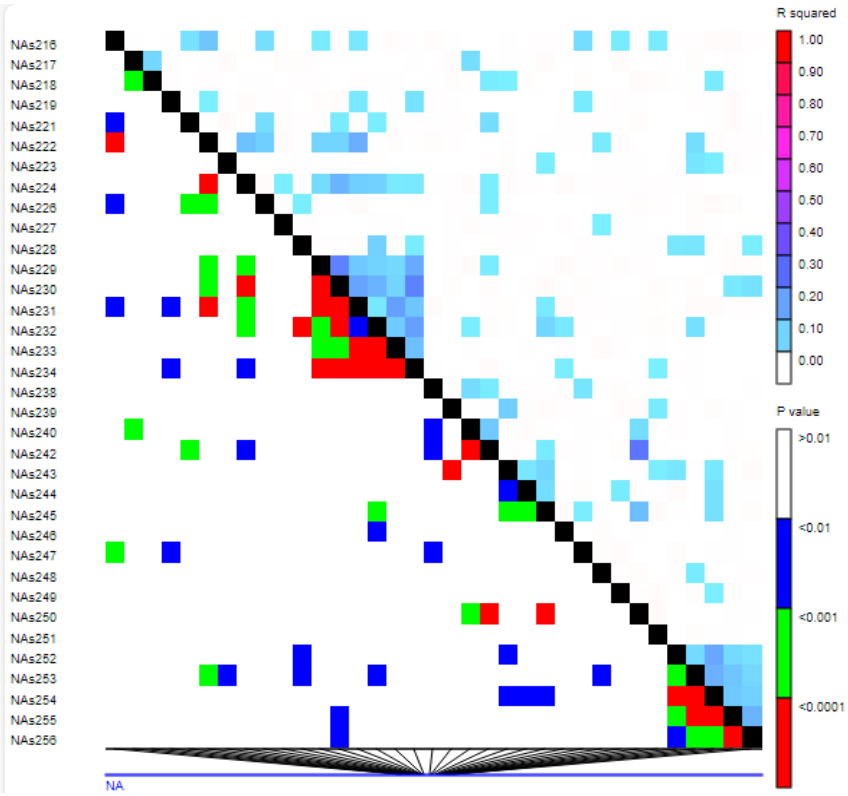

Figure 13. The heat map of linkage disequilibrium (LD) value (R^2) based on 20 SSR marker. Markers were arranged along the x and y axes according to their genomic locations, with each cell in the heat map representing a specific marker pair. The R^2 values for each marker pair are displayed in the bottom half of the heat map, using a color gradient that ranges from white (0.0) to red (1.0) in increments of 0.1. In the upper half of the heat map, the p-values corresponding to each R^2 estimate are shown, with shades of color indicating significance: non-significant values ($p > 0.01$) are represented in white, while highly significant values ($p < 0.0001$) appear in red.

Coefficient of determination (R^2) shows the percentage of the phenotypic variance explained by each marker (Table 28). In this study, R^2 values varied from 0.05167 to 0.12312 with an average of 0.06671 using GLM, while it ranged from 0.09526 to 0.10574 with an average of 0.0997 using MLM analysis. In the GLM model, marker locus UGSM665-8 associated with MCHA had the highest phenotypic variance (R^2) values (12.3%), followed by locus P-142-7 associated with LW (10.7%), and P-90-30 linked with purity% (9.0%). Their relatively high phenotypic variance (R^2) values indicate a significant association between markers and traits and strong genetic control over the trait with a few environmental effects. While, low R^2 value may be attributed to insufficient

marker density, rare alleles, and complex allelic interactions (Yang et al., 2010; Debibakas et al., 2014). Two and three detected MTAs exhibited phenotypic variance (R^2) ≥ 10% in GLM and MLM at P < 0.01, respectively, suggests that the marker is in strong LD with a causal gene. The QQ plot showed significant association of markers for the quantitative traits (Fig.14).

In this research, both GLM and MLM analysis identified marker alleles UGSM665-8, SMC36BUQ-21, and P-142-7 as significantly associated (p < 0.001) with the MCHA, SCW, and LW traits, respectively. Therefore, marker locus UGSM665-8, SMC36BUQ-21, and P-142-7 are a useful and informative marker to identify the genes encoding respective traits. These markers locus can be used as reliable markers to improve traits in question using marker-assisted selection methods. However, the correlation between quantitative traits and SSR marker alleles in this study was not strong (moderate), as evidenced by the moderate value of phenotypic variation (R2) (Table 28).

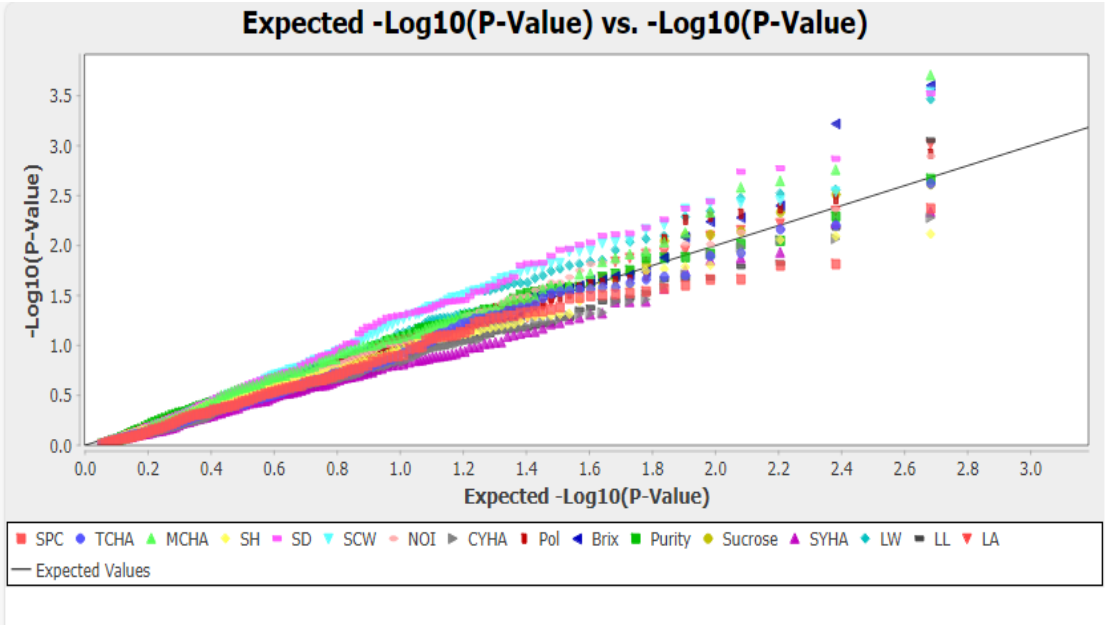

Figure 14. Quantile-Quantile (QQ) plot and distribution of marker trait association in sugarcane genotypes based on GLM analysis using TASSEL software

Therefore, these associated trait markers should be validated with another breeding sugarcane population before being utilized for marker-assisted selection to ensure consistency. In contrast to the current study, Siraree et al. (2017) reported a high frequency of phenotypic variation explained by EST-SSR markers; for example, the marker IISR_236 explained 37% phenotypic variation in CCS percent and 38% in corrected Brix, while the marker IISR_279 explained 37% phenotypic variation in plant height.

Based on the results of GLM model, some marker loci were associated with more than one trait at the same time. For example, marker locus P-89-12 showed simultaneously significant association with MCHA, CYHA, SYHA, and SP. Marker loci UGSM665-11 associated with SP, TCHA, MCHA traits concurrently. Marker locus UGSM542-14 had significant association with LA, LW, MCHA, and TCHA concurrently. In addition, molecular loci SOMS167-9, SOMS167-21, and UGSM681-2 each contained significant association with Brix%, Pol%, and sucrose% at the same time. Similarly, marker locus UGSM665-11 had significant correlation with traits such as SP, TCHA, and MCHA at once with phenotypic variation (R^2) of 0.063, 0.057, and 0.066, respectively. Traits such as MCHA, SYHA and SD were concurrently linked significantly with SOMS173-21 marker locus and collectively explained 19.4% of the phenotypic variation, while the individual r^2 value ranged from 0.060 to 0.070. In addition, marker loci P-142-7, P-89-27, P-90-31, P-90-9, SCM4-12, SCM4-9, SOMS167-29, UGSM665-8, and UGSM681-3 each had simultaneously significant association with two traits (Table 28). It is suggested that one locus may be involved in conferring multiple traits, which is the result of gene–gene interactions or pleiotropism (Lehner, 2011). Similar result was reported by Saeidnia et al. (2021).

The present study also revealed that certain marker loci associated only with single trait. Marker loci P-100-16 and SMC477CG-14 had a single association with LA. This implies the two specific loci are linked to variation in LA trait. There was also a significant single association between stalk diameter and loci P-90-27 (R^2 = 6.2%), SMC36BUQ-16 (R^2 = 5.2%), SOMS173-18 (R^2 = 5.9%), and UGSM665-9 (8.4%). The marker loci P-100-7 and SMC36BUQ-21 had a significant single association with SD, accounting for 5.7% and 7.5% of total phenotypic variation, respectively. Similarly, the marker loci P-142-15, SOMS167-6, UGSM681-28, and SOMS167-17 were significantly associated with LW, with explained phenotypic variability ranging from 5.6 to 7.3%. Furthermore, a single trait association was found for loci P-89-17 with CYHA, P-89-22 and SOMS168-6 with TCHA, P-90-26 with SYHA, SMC1825LA-24 with SH, SMC286CS-10 and UGSM667-13 with NOI, SMC286CS-24 and SOGL41-8 with Brix%, and UGSM585-6 with MCHA. The observed single trait association of the locus may be due to functional specificity of the locus, monogenic traits, lack of epistatic interaction or absence of pleiotropic loci. Understanding this genetic phenomenon help the researchers or breeders use marker-assisted selection more effectively, targeting specific traits without unintended effect on

other characters. Siraree et al. (2017) used the MLM model to identify a single MTA for a few quantitative traits, such as corrected Brix value and fibre content, as well as qualitative traits, such as bud cushion, bud size, growth crack, pithiness, and bud shape. Similar result was reported by Bilal et al. (2015).

As presented in Table 28, two and more marker loci had a significant association with one trait concurrently, this phenomena is often referred to as polygenic inheritance, where multiple genes with minor individual effects collectively determine the phenotype. For instance, marker P-89-12 and UGSM665-11 were significantly associated with SP, while markers P-89-22, SOMS168-6, UGSM542-14, UGSM665-8, UGSM665-11, and UGSM681-3 were significantly associated with TCHA. In addition, statistically significant associations were found between five polymorphic markers (P-89-12, SOMS173-21, UGSM542-14, UGSM665-11, and UGSM665-8) and MCHA, with explained phenotypic variation of 8.5%, 7.0%, 8.1%, 6.3%, and 12.3%. As shown in Table 28, marker alleles P-89-27 and SMC1825LA-24 were significantly associated with SH in the GLM model, explaining 12.1% of the collective phenotypic variability. Four markers were found to be significantly associated with SD (P-90-27, SMC36BUQ-16, SOMS173-21, SOMS173-18, and UGSM665-9), accounting for 5.2%, 5.2%, 6%, 5.4%, and 8.4% of phenotypic variation, respectively. Four marker alleles, namely P-89-27, P-90-31, SMC286CS-10, and UGSM667-13, showed a significant relationship with NOI.

These results indicate that more than one marker could influence a quantitative trait (additive gene action), hence breeders can select individuals carrying multiple favorable alleles rather than relying on a single marker for marker assisted selection. Previous research by Siraree et al. (2017) identified two markers, each associated with CCS percent, leaf blade width, and plant height; three each with internode diameter, NMCs, and sucrose content; and four markers linked with purity percent. Understanding these multiple loci association enables the breeders to improve the complex traits in sugarcane such as cane and sugar yield through marker assisted selection. Banerjee et al. (2015) also found that four markers were significantly associated with cane diameter, seven markers with cane length and NMCs, eleven markers with number of nodes, six with sucrose percent, and five markers with average cane weight using the MLM.

In general, significant marker-trait association results revealed the potential application of trait-linked alleles in developing improved sugarcane genotypes through marker-assisted

breeding. In the future, it would be valuable to validate the present MTA results using diverse sugarcane populations and identify high agronomic trait-linked alleles to advance marker-assisted breeding in sugarcane.

Pervious study by Chun-Yan et al. (2024) analyzed marker-trait associations using three breeding traits: plant height, stem diameter, and Brix% and 37 SSR markers using 62 sugarcane genotypes based on the MLM model. They detected 20 markers associated with plant height, stem diameter, and Brix traits, which explained 5.04%–27.98% of phenotypic variation. Debibakas et al. (2014) detected DArT and AFLP makers associated with the sugarcane yellow leaf virus. Similarly, Gouy et al. (2015) used DArT, AFLP, and SSR markers in 184 sugarcane genotypes to identify a significant association between three agromorphological traits. Furthermore, Bilal et al. (2015) discovered a link ($P < 0.01$) between SSR markers CEMB-14A and CEMB-14B and tillers per plant, while CEMB-5A, CEMB-5C, and CEMB-7B were associated with cane weight ($P < 0.05$).

Table 28: Association between SSR markers and quantitative traits (MTA) in 144 sugarcane genotypes showing the marker, F value, significance level (p), and phenotypic variation (R^2) value

Sir. /No.	Trait	GLM (P+G+Q)				MLM (P+G+Q+K)		
		Marker Allele	F value	P value	R^2 value	F value	P value	R^2 value
1	SP	UGSM665-11	6.94	0.010	0.06			
2	SP	P-89-12	6.89	0.010	0.06			
3	TCHA	UGSM665-8	9.13	0.003	0.07			
4	TCHA	P-89-22	8.79	0.004	0.07			
5	TCHA	UGSM665-11	8.26	0.005	0.07			
6	TCHA	UGSM681-3	7.69	0.007	0.06			
7	TCHA	SOMS168-6	7.18	0.008	0.06			
8	TCHA	UGSM542-14	7.15	0.009	0.06			
9	MCHA	UGSM665-8	16.83	0.000	0.12	12.34	0.001	0.11
10	MCHA	P-89-12	11.06	0.001	0.08			
11	MCHA	UGSM542-14	10.54	0.002	0.08			
12	MCHA	SOMS173-21	8.97	0.003	0.07			
13	MCHA	UGSM665-11	8.02	0.005	0.06			
14	SH	SMC1825LA-24	8.68	0.004	0.07			
15	SH	P-89-27	7.32	0.008	0.06			
16	SD	UGSM665-9	11.64	0.001	0.08			
17	SD	SOMS173-21	8.14	0.005	0.06			
18	SD	SOMS173-18	7.20	0.008	0.05			
19	SD	P-90-27	6.91	0.010	0.05			
20	SD	SMC36BUQ-16	6.89	0.010	0.05			
21	SCW	SMC36BUQ-21	10.35	0.002	0.07	11.69	0.001	0.10
22	SCW	P-90-31	9.10	0.003	0.07			
23	SCW	P-100-7	7.80	0.006	0.06			
24	NOI	P-89-27	10.01	0.002	0.08			

S/N	Trait	GLM (P +G+ Q)			MLM (P+G+Q+K)		
		Marker Allele	F value	P value	R2 value	F value	P value	R2 value
25	NOI	SMC286CS-10	9.09	0.003	0.07			
26	NOI	P-90-31	8.15	0.005	0.06			
27	NOI	UGSM667-13	7.87	0.006	0.06			
28	LL	UGSM585-6	9.10	0.003	0.07			
29	LW	P-142-7	14.67	0.000	0.11	11.75	0.001	0.10
30	LW	UGSM542-14	10.08	0.002	0.08			
31	LW	UGSM681-3	9.80	0.002	0.07			
32	LW	SOMS167-17	9.56	0.003	0.07			
33	LW	P-142-15	8.44	0.004	0.06			
34	LW	UGSM681-28	7.36	0.008	0.06			
35	LW	SOMS167-6	7.23	0.008	0.06			
36	LA	P-142-7	10.87	0.001	0.08			
37	LA	SMC477CG-14	7.59	0.007	0.06			
38	LA	P-100-16	7.19	0.008	0.06			
39	LA	UGSM542-14	6.87	0.010	0.05			
40	Brix%	SOMS167-9	10.31	0.002	0.08			
41	Brix%	SOGL41-8	9.94	0.002	0.08			
42	Brix%	SOMS167-21	8.47	0.004	0.06			
43	Brix%	UGSM681-2	8.11	0.005	0.06			
44	Brix%	SMC286CS-24	6.92	0.010	0.05			
45	Pol%	SOMS167-9	9.48	0.003	0.07			
46	Pol%	UGSM681-2	8.19	0.005	0.06			
47	Pol%	SCM4-12	7.96	0.006	0.06			
48	Pol%	SOMS167-21	7.74	0.006	0.06			
49	Pol%	SCM4-9	7.38	0.008	0.06			
50	Purity%	P-90-30	11.74	0.001	0.09			
51	Sucrose%	SOMS167-9	9.44	0.003	0.07			
52	Sucrose%	UGSM681-2	8.09	0.005	0.06			
53	Sucrose%	SCM4-12	8.03	0.005	0.06			
54	Sucrose%	SOMS167-21	7.58	0.007	0.06			
55	Sucrose%	SCM4-9	7.44	0.007	0.06			
56	CYHA	SOMS167-29	8.92	0.003	0.07			
57	CYHA	P-90-9	8.24	0.005	0.07			
58	CYHA	P-89-12	7.44	0.007	0.06			
59	CYHA	P-89-17	6.93	0.010	0.06			
60	SYHA	P-89-12	8.16	0.005	0.07			
61	SYHA	SOMS173-21	7.98	0.006	0.06			
62	SYHA	P-90-26	7.95	0.006	0.06			
63	SYHA	P-90-9	7.13	0.009	0.06			
64	SYHA	SOMS167-29	6.93	0.010	0.06			

SP = Sprouting percent; TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height, SD = Stalk diameter, SCW = Single cane weight, NOI = Number of internodes, CYHA = Cane yield per hectare, Pol = Polarity, SYHA = Sugar yield per hectare, LW = Leaf width, LL = Leaf length, LA = Leaf area, Q= population structure, K= kinship, GLM= general linear model, MLM = Mixed linear model

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Knowledge of the magnitude and distribution genetic variability within the genotype population is useful in designing crop improvement programs and maintaining the diversity. The information obtained from diversity analysis is useful in making the crosses and selection of divergent parents to maximize heterosis. To better understand phenotypic relationships among sugarcane genotypes, this study quantified qualitative morphological traits in 144 genotypes using descriptive analysis. Among the traits studied, exposed rind color had a relatively even frequency distribution across its phenotypic classes, indicating that this trait varies significantly in the sugarcane population. The study also discovered that stalk corky patches had the highest Shannon diversity index (H'), and stalk growth cracks had the lowest, implying that stalk corky patches characters had high discriminatory power and useful for selection or conservation of genotypes. Similarly, India's population had the highest Shannon-Weaver diversity index (H') pooled across characters by country, while the Mauritius population had the lowest diversity index. The multivariate cluster analysis, on the other hand, divided 144 sugarcane genotypes into four major clusters, regardless of geographical origin, implying that geographical diversity may not always be an indicator of genetic variability when grouping genotypes into different clusters. This information could be used to identify specific genotypes based on observable phenotypes, conserve these germplasm resources, and guide future improvement work of the sugarcane crop.

The analysis of variance (ANOVA) showed significant genetic variability among the genotypes for all sixteen quantitative traits considered. This indicates inherent genetic variation among the genetic materials. The study also discovered that traits like TCHA, MCHA, and CYHA had high GCV, heritability, and genetic advance as a percentage of the mean. Similarly, SYHA had high GCV, moderate heritability, and high genetic advance, indicating that the additive gene action controls these traits. As a result, a selection strategy based on these traits may produce superior genotypes with higher cane and sugar yields.

Comparing a variety's phenotypic performance against standard checks and commercial cultivars is valuable for selecting outstanding and well-adapted varieties to the country's

diverse agroecology. Accordingly, the present comparison of the top 5% genotypes with standard check varieties and commercial cultivars revealed that genotypes CP70/321, Mex57/197, D41/46, B657/150, CP6023, CO-967, and V141 outperformed standard check varieties and commercial cultivars in terms of sugar yield. Thus, these high-performing genotypes in sugar yield could replace existing commercial cultivars if they maintain higher performance across multiple locations and seasons. Again, these genotypes could be used for future sugar yield trait improvement programs.

The principal component analysis is used to identify major traits that contribute to total genetic variability. The present study revealed that the first four principal components contributed 81.44% of the overall variation. This result implies that breeders can emphasize these four components when selecting genotypes. PC1 represented the yield potential of each genotype. At the same time, PC2 reflected the quality potential of each genotype, implying that yield and quality are the principal attributes driving genetic diversity among the genotypes studied. In addition, the multivariate cluster analysis separated the 144 genotypes into five different clusters regardless of their geographical origin, indicating an international germplasm exchange. This approach allows breeders to select divergent parents from different clusters to improve traits of interest and maximize hybrid vigor.

The correlation and path coefficient analysis are vital statistical approaches to understanding complex traits like sugarcane yield, which is affected by multiple, often interrelated yield components. The study also found that SP, TCHA, MCHA, TCHA, SCW, NOI, pol%, brix%, sucrose%, and sugar yield had a significant positive phenotypic correlation with cane yield. Similarly, SP, TCHA, MCHA, SH, SCW, and NOI had a significant positive genotypic association with cane yield. This result implies that selecting genotypes with greater values for these components can result in higher cane yield. Correspondingly, the path analysis revealed that MCHA and SCW strongly and positively affected cane yield at phenotypic and genotypic levels. This implies that direct selection for significant MCHA and SCW will result in significant genetic improvement in cane yield.

Furthermore, the Smith-Hazel selection index analysis identified eight well-performing sugarcane genotypes such as CP6023, CP70/321, Mex57/197, D41/46, CP971944, B657/150, B49-244, and NCo-334. This indicates the presence of significant

morphological traits diversity among the genotypes considered, that could be used to improve yield and quality of sugarcane either through direct selection or hybridization.

The genetic analysis based on 20 SSR markers revealed that the considered markers were polymorphic, effective, and suitable to estimate genetic diversity within a population. The cluster analysis classified the genotypes into four distinct clusters based on their genetic background rather than their geographical origin, as confirmed by principal coordinate analysis and Bayesian structure analysis. The sugarcane populations studied showed moderate to high genetic diversity, with the Indian population having the most diversity, followed by the Barbados population, indicating that the two populations will be a good source of divergent parents for improvement of traits of interest though hybridization.

In addition, the result in the present study demonstrated that among the SSR markers studied, ten markers, such as UGSM665, UGSM667, UGSM681, SMC1852LA, SMC286CS, SMC477CG, SMC36BUQ, SOMS168, SOGL41, and P89 had high discriminatory power with PIC values above the average and high diversity value. Furthermore, SSR markers UGSM665, SMC36BUQ, and P-142 were found to be associated with MCHA, SCW, and LW traits, respectively, through the GLM and MLM model. In general, all the diversity and population structure analyses revealed the existence of genetic variability in the sugarcane germplasm. Similarly, the marker-trait association study identified markers associated with the agronomic traits. Information obtained from the result of the present study would enable sugarcane breeders to select the most divergent parents for crossing to improve the desired traits in the breeding program. Furthermore, the breeders can also use markers that are linked to certain desirable traits in marker-assisted selection.

5.2. Recommendations

- Breeders and researchers can use the genetic information generated by this study in future breeding programmes and genetic resource conservation and management.

- The top 5% genotypes such as CP70/321, Mex57/197, D41/46, B657/150, CP6023, CO-967, and V141 that showed superiority in sugar yield over standard check varieties and commercial cultivar should be further reevaluated in the two locations to check their performance for future use in breeding program as well as in commercial production.

- Genotypes identified based on multiple traits selection analysis (Smith-Hazel selection index), such as CP6023, CP70/321, Mex57/197, D41/46, CP971944, B657/150, and B49-244 should be reevaluated over multiple locations for further use in sugarcane production and breeding.

- The identified polymorphic SSR markers namely, UGSM665, UGSM667, UGSM681, P89, SMC1852LA, SMC286CS, SMC477CG, SMC36BUQ, SOMS168, and SOGL41 could be used for diversity and population structure analysis of sugarcane in the future improvement programs.

- SSR markers UGSM665, SMC36BUQ, and P-142 that found associated with MCHA, SCW, and LW traits, respectively, could be used for marker assisted selection in the breeding program after their verification and validation.

- Although the present research findings provide valuable insight into the genetic diversity and population structure of sugarcane germplasm in the Ethiopian sugar industry, it is essential to conduct more robust and advanced research in the future, considering the limitations of the present research. Therefore, we recommend the following as future work lines:

    - ❖ The sugarcane population shall be reevaluated using more informative molecular markers like SNP markers to discriminate the genotypes and assess the diversity precisely.

    - ❖ These genotypes have to be reconsidered for major traits like drought resistance, salinity tolerance, and disease resistance using SNP maker and other advanced molecular techniques

    - ❖ A large number of exotic genotypes and landraces shall be considered over multiple locations in the future study.

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7. APPENDICES

Appendix Table 1: Mean performance of 144 sugarcane genotypes for sixteen qualitative traits

Gen.	SP	TCHA	MCHA	SH (cm)	SD (cm)	SCW(kg)	NOI	CYHA (t/ha)
1	35±10.9a-t	77759±15930.6j-t	67414±10296.8o-L	174.3±37.8k-z	2.18±0.4b-k	0.9±0.2h-n	19.3±2.1e-s	63.2±19.9g-q
2	45.6±13.7a-o	141379±23121.5a-p	92069±14493.7d-A	218.5±11.1a-y	2.31±0.3a-k	1.3±0.2b-n	25.3±1.7a-e	113.8±3.7a-p
3	32.8±10.3b-t	125690±8069.8a-q	74655±16726.8m-K	159.8±11.4t-z	2.55±0.8a-k	0.9±0.1k-n	16.5±0.6 o-s	63.5±17.0g-q
4	57.8±13a-b	140345±21986.6a-p	78448±5528i-I	197.8±26.2d-z	2.55±0.4a-k	1.3±0.5a-n	20.0±1.2c-s	104.6±34a-q
5	37.5±5.5a-t	125345±26197.1a-q	101724±6383.2b-u	223.3±11.5a-w	2.18±0.5b-k	1.2±0.2d-n	24.3±1.5a-i	118.8±24.5a-p
6	49.8±11a-m	136034±37750.4a-p	63793±15333.5q-L	215.3±49.8a-y	2.29±0.3a-k	1.3±0.6a-n	21.3±2.5b-r	91.6±47.4b-q
7	30.6±11.6c-t	72586 ±44193.1l-t	49138±41449.7C-L	196.0±26.3d-z	2.30±0.4a-k	1.2±0.3 b-n	22.5±1a-m	81.1±46.7c-q
8	42.4±7.2a-r	128621±20562.8a-q	94138±14689.3d-y	211.5±42.5a-y	2.03±0.3d-l	1.2±0.2 b-n	23.3±1.5a-l	116.4±38.3a-p
9	24.8±9.8m-t	74483±28250.6k-t	58793±24874.6v-L	171.3±33.7l-z	2.48±0.3a-k	1.5±0.8a-n	19.3±1.7f-s	87.1±52.0b-q
10	40.9±12.1a-s	138966±28799.5a-p	94828±12639.2d-y	200.8±8.3d-z	2.47±0.4a-k	1.4±0.3 a-n	21.8±1.0a-o	135.7±21.9a-j
11	27.4±15h-t	58103±13029.1q-t	41897±12225.6F-L	158.0±14.7u-z	2.64±0.6a-j	1.3±0.2a-n	20.0±2.2c-s	55.8±17.7g-q
12	48.8±8a-m	127414±25524.6a-q	81207±11082.9h-H	188.0±16.4h-z	2.35±0.5a-k	1.4±0.1a-n	22.8±2.1a-m	111.8±24.7a-q
13	47.6±18.9a-n	117759±27124.7b-r	86552±11710.6f-C	234.3±12.2a-q	2.43±0.4a-k	1.5±0.4a-n	24.3±2.8a-i	126.5±16.4a-o
14	26.7±6.3j-t	89483±27823e-t	60172±15186.8v-L	234.3±46.8a-q	2.31±0.3a-k	1.6±0.4a-k	21.0±2.0b-r	102.7±33.2a-q
15	37.5±6.5a-t	124483±22695.8a-r	110690±11053.3a-n	202.0±23.1c-z	1.75±0.3k-l	1.0±0.4g-n	18.5±1.7 i-s	106.8±42.4a-q
16	29.9±4.4e-t	99138±19436.2d-t	63966±10250.5q-L	220.8±13.3a-x	2.57±0.3a-k	1.6±0.2a-m	25.8±1.5a-c	102.0±16.8a-q
17	38.5±3a-t	128793±30586.2a-q	61207±38292.4u-L	182.0±35.4i-z	2.43±0.3a-k	1.3±0.5 b-n	23.3±1.3a-l	89.5±85.8b-q
18	49.8±11a-m	104655±25566.1c-t	61035±20087u-L	209.8±16.9a-y	2.32±0.3a-k	1.3±0.1a-n	22.8±2.1a-m	81.6±27.9c-q
19	38.2±8.5a-t	126724±14503.3a-q	69138±23632.6o-K	223.3±12.5a-w	2.17±0.4b-k	1.3±0.2a-n	22.3±1.5 a-o	94.2±42.9 b-q
20	42.6±4.5a-r	110345±14212c-t	51897±21229.5z-L	168.8±14.7m-z	2.51±0.3a-k	1.2±0.2b-n	18.8±2.1 h-s	62.2±21.5h-q
21	37±10a-t	116034±22145.6b-s	78793±23208.3h-I	201.0±19.1c-z	2.58±0.6a-k	1.1±0.2e-n	17.5±1.7k-s	87.8±38.0b-q
22	30.1±5.1d-t	88966±11905.3e-t	75172±27626.4m-J	238.0±24.6a-m	2.41±0.3a-k	1.4±0.2a-n	22.8±1.3a-m	108.2±44.2a-q
23	40.9±24.8a-r	136379±28331.2a-p	74828±13355.1m-K	204.0±48.4b-z	2.19±0.3b-k	1.2±0.4b-n	22.5±3.3a-m	90.3±39.9b-q
24	49±8.9a-m	110000±40168.1c-t	62586±19107.1s-L	179.3±26.6i-z	2.70±0.3a-i	1.4±0.4a-n	25.0±1.8a-f	88.3±44.7b-q

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Gen	SP	TCHA	MCHA	SH	SD	SCW	NOI	CYHA
25	36±15.1a-t	153319±13134.7a-j	119655±16412.2a-h	190.8±14.6h-z	2.65±0.4a-j	1.5±0.3a-n	22.5±2.1a-m	77.0±31.6c-q
26	35.3±4.9a-t	120345±25597.9a-r	97241±19740.7c-x	177.0±15.4i-z	2.47±0.3a-k	1.4±0.1a-n	20.0±1.6 c-s	93.7±22.0b-q
27	52.4±20.2a-j	141164±61890.1a-p	92759±17424.3d-z	194.8±2.9e-z	1.90±0.3g-l	0.9±0.4h-n	22.8±0.5a-m	89.9±23.8b-q
28	44.4±20.8a-o	112457±25664.5c-t	60517±11011.1v-L	221.8±14.9a-w	2.14±0.3c-l	1.3±0.2a-n	21.5±1.7 a-q	109.6±29.6a-q
29	40.3±4.5a-t	97586 ±16188.5d-t	56552±18565.3w-L	211.5±21.9a-y	2.73±0.2a-i	1.6±0.4a-n	22.0±0.8 a-o	88.3±23.9b-q
30	29.2±8.9f-t	118621±25439.5a-r	75172±23916.9m-J	192.5±14.8g-z	2.36±0.2a-k	1.2±0.2b-n	19.0±1.4 g-s	61.9±24.5h-q
31	42.9±10.9a-r	104569±15985c-t	103793±9647a-r	222.3±21.3a-w	2.49±0.5a-k	1.2±0.5b-n	21.0±2.4b-r	90.8±27.7b-q
32	33.2±7.1a-t	148621±30235.2a-l	118276±4080.1a-i	165.3±18.3p-z	2.83±0.4a-e	1.4±0.4a-n	19.5±2.1e-s	74.5±33.4d-q
33	58±14.2a	148103±26878.1a-l	131379±12394.6a-d	264.8±20.2a-e	2.28±0.3a-k	1.7±0.2a-h	22.0±2.2 a-o	107.4±21.4a-q
34	51.5±12.2a-j	159138±24489.2a-g	91207±7080.9d-B	207.8±16.9a-z	2.31±0.3a-k	1.4±0.2a-n	22.5±1.3 a-m	138.9±15.6a-i
35	50±6.8a-l	113621±28665 c-t	73966±6178.1m-K	213.5±10.3a-y	2.14±0.3c-l	1.3±0.2a-n	17.8±1.9k-s	90.6±14.8b-q
36	44.4±10.3a-o	115690±34424.7b-s	71220 ±7903n-K	185.5±22.8h-z	2.02±0.5d-l	0.8±0.1n	17.3±1.5 m-s	77.5±0.3c-q
37	56.4±12.2a-c	177586±39301.5a-c	130000±15292.1a-e	225.8±21.9a-u	1.92±0.5f-l	1.3±0.3b-n	23.3±1.0a-l	184.5±13.1a
38	52±21.8a-i	131034±18937.3a-q	79655±12597.6h-I	232.8±18.0a-q	2.08±0.4c-l	1.2±0.1b-n	23.3±1.5a-l	167.9±7.6ab
39	44.5±9.8a-o	78103±24631.3j-t	56724±8740.5w-L	263.8±18.5a-f	1.86±0.3h-l	1.3±0.3a-n	23.8±0.5a-j	146.3±18.3a-g
40	17.4±8.5q-t	102931±13318c-t	76552±9201.1k-J	213.5±24.5a-y	2.37±0.3a-k	1.5±0.2a-n	25.5±1.7a-d	96.3±24.4b-q
41	27.7±5.5g-t	120690±25458.1a-r	89310±15365.2e-C	216.0±23.6a-y	2.58±0.4a-k	1.6±0.4a-n	24.8±1.0a-g	55.8±31.1g-q
42	52±12.9a-j	161379±26483.7a-f	104310±16000.1a-r	214.0±21.6a-y	2.10±0.4c-l	1.0±0.2e-n	23.8±0.5a-j	112.2±13.1a-q
43	33.1±19.1a-t	155172±30558.3a-h	141897±9993ab	223.3±26.4a-w	2.34±0.3a-k	1.4±0.2a-n	23.3±1.7a-l	143.9±28.5a-h
44	45.1±9.5a-o	140690±33621.4a-p	75690±11025.5l-J	239.3±16.7a-l	1.91±0.3f-l	1.1±0.2e-n	27.3±1.7a	137.6±17.3a-j
45	27.7±23.8g-t	162586±52677.4a-f	113448±15620.3a-m	271.0±9.0a-c	2.26±0.4a-k	1.5±0.4a-n	20.8±1.7 c-s	101.5±42.4a-q
46	58.9±20.6a-b	83966±15547.9g-t	67931±9158o-L	237.8±25.4a-m	2.04±0.3d-l	1.3±0.2b-n	20.0±1.8 c-s	117.5±38.3a-p
47	47.3±5a-n	122241±27594.1a-r	81035±11574.4h-H	160.0±19.6t-z	2.50±0.4a-k	1.4±0.3a-n	22.0±1.8 a-o	90.5±16.5b-q
48	38±9.9a-t	112931±29212.8c-t	70000±10390.7n-K	221.8±22.6a-w	2.65±0.4a-j	1.2±0.3b-n	22.0±1.2a-o	90.3±16.8b-q

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Gen	SP	TCHA	MCHA	SH	SD	SCW	NOI	CYHA
49	55.8±17.3a-d	153319±13134.7a-j	119655±16412.2a-h	210.8±19.2a-y	1.94±0.2f-l	1.3±0.4 b-n	23.0±1.4a-m	155.2±59.4a-e
50	45.6±5.5a-o	120345±25597.9a-r	97241±19740.7c-x	200.5±13d-z	2.37±0.2a-k	1.5±0.3a-n	19.0±2.0 g-s	137.4±33.9a-j
51	53.4±15.4a-g	141164±61890.1a-p	92759±17424.3d-z	203.0±14.3c-z	2.70±0.4a-i	0.9±0.2j-n	22.8±1.5a-m	79.7±14.4c-q
52	39.5±7.4a-t	112457±25664.5c-t	60517±11011.1v-L	168.5±14.6m-z	1.79±0.2j-l	1.3±0.2 b-n	18.0±1.4 i-s	70.0±21.5f-q
53	32.4±5.3b-t	97586.2±16188.5d-t	56552±18565.3w-L	209.3±13.8a-y	2.63±0.3a-j	1.3±0.1b-n	24.5±1.3a-h	70.8±21.3f-q
54	22.7±4.6n-t	118621±25439.5a-r	75172±23916.9m-J	224.3±18.6a-w	2.08±0.3c-l	1.4±0.2a-n	23.8±2.2a-j	107.2±42.1a-q
55	39.3±7.5a-t	104569±15985c-t	103793±9647a-r	265.3±13.2a-d	2.19±0.2b-k	1.4±0.2a-n	23.0±1.4a-m	138.9±40.5a-i
56	51.2±11.1a-k	148621±30235.2a-l	118276±4080.1a-i	224.5±19.7a-w	1.93±0.3f-l	1.2±0.1b-n	23.5±1.7a-j	140.8±20.9a-h
57	53.2±7.4a-h	148103±26878.1a-l	131379±12394.6a-d	202.3±20.8c-z	2.18±0.3b-k	1.1±0.2e-n	20.3±1.3 c-s	143.4±14.1a-h
58	43.4±9.8a-p	159138±24489.2a-g	91207±7080.9d-B	217.8±12.8 a-y	2.22±0.3b-k	1.6±0.2a-l	22.0±1.8 a-o	148.8±34.1a-f
59	32.8±8.1b-t	113621±28665 c-t	73966±6178.1m-K	199.3±16.9d-z	2.39±0.3a-k	1.3±0.2a-n	17.8±0.5k-s	96.1±18.3b-q
60	41.2±10a-r	115690±34424.7b-s	71220 ±7903n-K	189.8±29.2h-z	2.28±0.4a-k	1.5±0.1a-n	24.5±1.3a-h	102.8±20.9a-q
61	46.1±3.6a-o	177586±39301.5a-c	130000±15292.1a-e	224.8±21.2a-v	2.68±0.3a-i	1.1±0.1e-n	20.5±1.0 c-s	141.4±21.2a-h
62	45±28.1a-o	131034±18937.3a-q	79655±12597.6h-I	216.3±15.2a-y	2.18±0.3b-k	1.6±0.3a-m	21.3±2.2 b-r	121.5±28.0a-p
63	26.7±11.6j-t	78103.4±24631.3j-t	56724±8740.5w-L	187.5±14.8h-z	2.33±0.4a-k	1.3±0.2b-n	22.3±1.7 a-o	71.9±28.7d-q
64	29.7±10.8e-t	102931±13318c-t	76552±9201.1k-J	235.0±14.4a-p	2.59±0.3a-k	1.6±0.1a-m	23.0±1.6a-m	122.2±12.8a-p
65	32.6±4b-t	120690±25458.1a-r	89310±15365.2e-C	237.0±17.3a-n	2.17±0.3b-k	1.4±0.2a-n	21.5±2.1 a-q	124.2±35.8 a-p
66	55.9±15.9a-d	161379±26483.7a-f	104310±16000.1a-r	180.5±26.1i-z	2.07±0.4c-l	0.9±0.2h-n	18.0±2.2 i-s	97.6±22.7b-q
67	46.3±11.8a-o	155172±30558.3a-h	141897±9993ab	226.8±31.3a-u	2.07±0.3c-l	1.0±0.2e-n	20.0±1.4 c-s	146.6±17.7a-g
68	42.9±12.6a-r	140690±33621.4a-p	75690±11025.5l-J	264.0±30.7a-f	1.27±0.3d-l	1.5±0.3a-n	22.5±1.0a-m	111.9±17.3a-q
69	52.7±22.4a-j	162586±52677.4a-f	113448±15620.3a-m	234.0±17.9a-q	1.98±0.3d-l	1.1±0.2e-n	21.3±2.9 b-r	128.2±15.9a-m
70	40.9±16.3a-r	83965.5±15547.9g-t	67931±9158o-L	213.3±12.8a-y	2.04±0.3d-l	1.4±0.2a-n	21.5±1.9 a-q	93.8±19.7b-q
71	48.3±16.1a-n	122241±27594.1a-r	81035±11574.4h-H	226.3±39.6a-u	2.40±0.4a-k	1.5±0.4a-n	23.8±3.3a-j	116.5±28.1a-p
72	40.2±13.4a-t	112931±29212.8c-t	70000±10390.7n-K	187.8±22.6h-z	1.86±0.6i-l	1.3±0.3a-n	23.8±0.5a-j	91.0±25.0b-q

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Gen	SP	TCHA	MCHA	SH	SD	SCW	NOI	CYHA
73	39.2±4.9a-t	106379±17969.7c-t	73793±6494m-K	254.5±17.5a-h	2.85±0.4a-d	2.1±0.2a	23.3±1.0a-l	155.7±5.3a-d
74	33.6±15.5a-t	169483±55308.4a-d	69655±2815.5n-K	178.3±11.2i-z	2.24±0.3a-k	1.0±0.2g-n	17.8±1.9k-s	66.3±10.8f-q
75	55.1±4a-e	141207±52596.9a-p	107586±31588.9a-o	199.8±16.2d-z	2.44±0.3a-k	1.1±0.2e-n	21.5±1.0a-q	119.5±40.6a-p
76	30.9±20.3c-t	108966±62590.4c-t	82759±31471.1g-F	201.3±29.1c-z	1.96±0.3e-l	0.9±0.2i-n	22.0±1.4a-o	73.3±36.0d-q
77	43.9±14.7a-p	130517±21555.6a-q	84828±8120.4g-D	236.0±19.4a-o	2.48±0.2a-k	1.5±0.3a-n	25.0±0.8a-f	123.2±21.4a-p
78	37±17.8a-t	131724±23087.1a-q	56724±18957.2w-L	208.3±23.7a-z	2.36±0.3a-k	1.5±0.3a-n	18.5±1.3h-s	85.0±25.9b-q
79	43.1±5.3a-q	117241±34303b-r	56552±6253w-L	206.8±38.5a-z	2.62±0.3a-k	1.7±0.1a-j	23.0±1.4a-m	94.7±26.3 b-q
80	25.2±8.4l-t	78793±23124i-t	40345±15579.7H-L	194.0±19.9f-z	2.36±0.4a-k	1.3±0.2b-n	21.3±2.1b-r	51.7±22.7k-q
81	35.5±8a-t	74828±13199.9k-t	38793 ±14057.1I-L	165.5±14.8p-z	2.30±0.5a-k	1.1±0.2e-n	16.5±1.9o-s	41.5±9.4pq
82	30.2±10.4d-t	103793±21409c-t	76724±24130.5k-J	206.8±28.8a-z	2.27±0.2a-k	1.1±0.2e-n	21.0±2.4b-r	83.0±30.9c-q
83	43.4±6a-p	130172±14360.5a-q	79655±10897.1h-I	228.3±20.5a-t	2.38±0.4a-k	1.8±0.4a-f	21.5±2.4a-q	143.9±26.0a-h
84	47.3±12.7a-n	125862±14839.6a-q	102931±7263.2a-t	204.3±9.3b-z	2.16±0.3c-k	1.2±0.1b-n	22.0±2.4a-o	121.6±22.4a-p
85	39.5±7.8a-t	140690±66353.4a-p	82241±29782.5h-F	244.3±42.3a-k	2.26±0.4a-k	1.4±0.4a-n	21.5±2.6a-q	116.0±48.6a-p
86	43.6±9.2a-p	161379±45549.4a-f	123621±19135.5a-g	184.5±39.1h-z	1.96±0.3e-l	0.8±0.1l-n	19.3±0.5f-s	100.3±29.8b-q
87	32.4±4.2b-t	125862±62180a-q	72414±19964.3m-K	178.5±16.1i-z	2.06±0.3c-l	0.9±0.2h-n	15.3±1.5s	70.0±25.9f-q
88	37.3±9.3a-t	129310±41319.9a-q	87414±18178.6f-C	185.0±25.1h-z	2.12±0.3c-l	1.0±0.2g-n	15.8±1.5q-s	87.1±32.8b-q
89	37±12.6a-t	154483±49369.4a-i	78621±5220.8h-I	154.5±11w-z	2.18±0.3b-k	1.1±0.2e-n	17.3±1.0m-s	82.7±26.0c-q
90	28.2±6.9g-t	71035±20842.3m-t	50172±16856B-L	191.8±17.5g-z	2.42±0.3a-k	1.6±0.3a-n	22.0±1.8a-o	81.4±37.2c-q
91	43.4±12.5a-p	144310±58003.2a-o	81207±17225.6h-H	155.0±19.6v-z	1.91±0.3f-l	0.8±0.2n	15.5±2.1rs	63.7±34.2g-q
92	39.7±5.9a-t	88621±19083.2e-t	63448±8219.4r-L	202.3±15.4c-z	2.43±0.4a-k	1.3±0.1a-n	20.3±1.3c-s	85.0±12.2b-q
93	14.5±4t	78448±17027.4i-t	53966±12108.3y-L	246.5±22.4a-i	2.78±0.1a-f	1.7±0.5a-i	23.5±2.4a-j	93.5±41.4b-q
94	27.9±9.4g-t	79655±21942.9h-t	60345±15480.8v-L	205.3±30.2b-z	2.18±0.3b-k	1.3±0.2a-n	22.3±1.5a-o	79.2±35.4 c-q
95	35.5±6.8a-t	115172±25810.7c-s	81552±13017h-G	197.0±26.0d-z	1.94±0.3f-l	0.8±0.2mn	19.0±2.2g-s	64.2±21.0g-q
96	38.2±21.7a-t	118793±2999.5a-r	60027±19151.3v-L	199.5±7.7d-z	2.6±0.2a-k	1.3±0.2b-n	21.0±1.4 b-r	74.8±24.7d-q

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Gen	SP	TCHA	MCHA	SH	SD	SCW	NOI	CYHA
97	51.7±11.2a-j	100345±10926.2d-t	73966±16962.1m-K	167.0±22.0n-z	2.56±0.2a-k	1.2±0.1b-n	18.8±1.0 h-s	89.0±19.0b-q
98	43.9±13a-p	136034±35054.5a-p	102931±14774a-t	226.3±9.9a-u	2.35±0.3a-k	1.5±0.2a-n	21.5±1.7 a-q	159.2±24.2a-c
99	51.2±12.4a-k	135172±31978a-p	84483±15549.1g-D	203.3±15.8c-z	1.90±0.3g-l	1.0±0.2e-n	19.0±0.8 g-s	88.4±31.7b-q
100	55.1±14.6a-e	171379±30741.9a-d	102931±7258a-t	223.8±12.2a-w	2.01±0.2d-l	1.3±0.2a-n	22.0±2.6a-o	131.0±33.1a-l
101	35.3±6.8a-t	141034±16479.7 a-p	81379±14759.9h-H	273.8±24.1ab	2.54±0.3a-k	1.8±0.2a-e	23.3±1.3a-l	148.8±13.0a-f
102	37.5±8.5a-t	110517±5556.6c-t	71897±18215n-K	240.5±15.8a-l	2.22±0.3b-k	1.4±0.2a-n	19.8±1.0 d-s	102.1±33.6a-q
103	40.2±10a-t	123793±39096a-r	79483±17667.2h-I	253.0±18.5a-h	2.31±0.3a-k	1.5±0.2a-n	24.0±1.2a-i	118.8±39.3a-p
104	38.5±4.3a-t	157069±13923.2a-g	118397±14006.1a-i	215.5±15.4a-y	2.42±0.6a-k	1.1±0.1e-n	24.3±1.3a-i	127.5±31.3a-n
105	31.1±20.7c-t	110517±28764.4c-t	74310±10525.2m-K	198.8±42.2d-z	3.10±0.1a	1.4±0.2a-n	17.5±0.6 l-s	102.1±11.5a-q
106	53.2±12.5a-h	144655±26884a-o	127586±32494.4a-f	204.8±11.5b-z	2.26±0.3a-k	1.1±0.2e-n	23.3±1.0a-l	137.1±29.3a-j
107	34.8±5.6a-t	110862±11749.5c-t	87414±14590.5f-C	228.3±9.9a-t	2.20±0.4b-k	1.2±0.1b-n	19.3±1.0 e-s	104.7±17.5a-q
108	41.4±18.4a-r	137586±35513.3a-p	107931±13775.9a-o	211.5±17.4a-y	2.11±0.3c-l	1.1±0.1e-n	20.5±0.6 c-s	119.3±16.2a-p
109	35.5±6a-t	117414±30544.7b-r	52759±8696.2z-L	164.5±21.8q-z	2.85±0.0a-d	1.2±0.2c-n	18.8±0.5h-s	62.5±17.6h-q
110	41.4±13.6a-r	78448.3±30957.1i-t	55000±15249.3y-L	215.8±20.2a-y	2.93±0.1a-c	1.3±0.2a-n	19.5±1.0e-s	73.4±24.6d-q
111	42.9±9.2a-r	142931±51907.3a-o	72241±42234n-K	222.5±20.2a-w	2.43±0.3a-k	1.2±0.2b-n	24.5±0.6a-h	83.6±26.0c-q
112	40.9±27.9a-r	149138±45298.6a-k	76724±9190.4k-J	201.8±36.5c-z	2.74±0.3a-h	1.4±0.2a-n	20.0±2.2 c-s	106.2±24.7a-q
113	29.5±8.3e-t	105517±36139.5c-t	76897±23606.6j-I	175.5±13.5j-z	2.38±0.4a-k	0.9±0.3i-n	16.8±0.5 n-s	73.5±37.2d-q
114	38.2±14.7a-t	97413.8±20723.2d-t	71724±10787.5n-K	238.3±30.2a-m	2.78±0.3a-f	1.7±0.7a-i	22.5±0.6a-m	126.6±20.6a-o
115	38.5±10a-t	107069±47355.3c-t	65345±16440p-L	187.3±16.1h-z	2.27±0.4a-k	1.1±0.3e-n	17.5±2.4k-s	73.5±28.8 d-q
116	17.2±12.8r-t	40517.2±21999.7s-t	35690±15249.3J-L	161.8±29.2s-z	2.55±0.1a-k	0.8±0.4k-n	22.0±3.9a-o	31.1±16.9q
117	36±10a-t	71206.9±27270.5m-t	56207±35122.8x-L	193.3±22.0g-z	2.32±0.8a-k	1.0±0.2f-n	22.5±2.9a-o	71.7±33.9e-q
118	25.5±5.5k-t	117586±57822.8b-r	58966±9329.5v-L	243.0±13.6a-k	2.68±0.4a-i	2.0±0.3a-c	21.5±1.3a-q	126.9±30.9a-o
119	37.5±8.4a-t	96034.5±28426.1d-t	71552±11125.7n-K	245.0±14.1a-j	2.76±0.4a-g	2.0±0.2a-d	22.3±1.0 a-o	140.8±23.3a-h
120	32.6±5.3b-t	66551.7±15852.1p-t	44483±13706.6D-L	166.0±12.3o-z	2.40±0.3a-k	1.0±0.2f-n	19.5±1.3e-s	44.8±16.3m-q

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Gen	SP	TCHA	MCHA	SH	SD	SCW	NOI	CYHA
121	36±5.6a-t	78793±21621.7i-t	57069±12431.3w-L	231.3±19.1a-s	2.35±0.1a-k	1.2±0.1b-n	26.8±1.5ab	67.4±13.0f-q
122	37±13.6a-t	164483±30389.2a-e	92241±10110.4d-z	227.5±6.0a-u	2.78±0.3a-f	1.6±0.4a-n	20.3±1.9 c-s	140.9±24.3 a-h
123	27.2±7.4i-t	48793±12658.8r-t	39138±11830.1I-L	162.3±5.3r-z	2.46±0.2a-k	1.1±0.2e-n	16.0±1.2p-s	43.2±20.0o-q
124	37±8.1a-t	86724±27697.3f-t	56552±15676.1w-L	239.5±28.1a-l	2.63±0.5a-j	1.5±0.2a-n	24.8±2.2a-g	86.8±21.8b-q
125	14.9±9.5s-t	48448±49113.8r-t	27931±13821.8L	209.5±31.6a-y	2.73±0.3a-i	1.8±0.3a-f	20.5±2.6 c-s	53.9±35.0j-q
126	50.7±23.4a-m	105862±32894.5c-t	83621±20016.8g-E	181.8±14.6i-z	2.16±0.4c-k	1.0±0.2g-n	22.5±1.3a-m	83.0±39.9c-q
127	21.3±13.3o-t	70690±30623.1n-t	55862±20597.5y-L	234.3±11.1a-q	2.82±0.4a-e	2.0±0.3ab	23.3±1.7a-l	123.5±54.7a-p
128	35.8±12.6a-t	102414±26564.4c-t	62164±12335.5t-L	234.3±17.7a-q	2.69±0.3a-i	1.7±0.2a-j	22.3±1.0 a-o	105.2±27.8a-q
129	33.1±8.3a-t	115345±20746.1b-s	70000±22702.8n-K	165.0±21.5p-z	2.62±0.3a-k	1.0±0.3f-n	17.5±1.3 k-s	75.9±41.9c-q
130	25.2±9l-t	78448±19362.6i-t	62241±17504.9s-L	151.5±25.5x-z	2.33±0.5a-k	0.9±0.1i-n	17.5±0.6k-s	57.0±23.6g-q
131	18.4±10.2p-t	96035±35063.5d-t	44138±19676.8D-L	138.3±7.4z	2.78±0.3a-f	1.1±0.1e-n	19.8±1.3d-s	44.0±15.1n-q
132	27.2±4.8 i-t	75000±26378k-t	40690±9201.1G-L	148.8±21.5yz	3.04±0.1ab	1.2±0.2 d-n	19.8±1.3 d-s	48.7±18.5l-q
133	29.7±8.1e-t	101897±63613.9c-t	64310±27120.7q-L	175.3±14.6j-z	2.49±0.3a-k	1.3±0.3 b-n	19.3±2.2f-s	76.7±30.7c-q
134	41.2±16.3a-r	191379±68037ab	117241±37168.9a-k	232.3±10.6a-r	2.34±0.2a-k	1.2±0.2c-n	18.0±2.7g-s	135.0±35.6a-k
135	34.8±5.3a-t	118103±20235.4a-r	76035±16440l-J	261.3±15.6a-g	2.43±0.4a-k	1.5±0.2a-n	24.5±2.1a-h	113.2±28.9a-q
136	39±6.9a-t	103966±29627.8c-t	65517±8101.6p-L	234.3±24.9a-q	2.66±0.2a-j	1.7±0.2a-k	22.8±0.5a-m	109.3±22.2a-q
137	22.5±10.9n-t	109622±25046.2c-t	68266±8451.8o-L	202.5±16.9c-z	2.57±0.3a-k	1.4±0.5a-n	21.5±1.3a-q	99.7±23.4b-q
138	45.8±9a-o	131379±23220.7a-q	83966±5071.8g-E	205.5±10.8b-z	2.19±0.3b-k	1.1±0.2e-n	18.5±2.1 i-s	92.8±8.9b-q
139	46.1±9.6a-o	122241±33114.8a-r	71035±7822.8n-K	276.5±8.5a	2.37±0.3a-k	1.8±0.2a-g	23.8±1.7a-j	126.5±19.1a-o
140	40.7±8.4 a-s	105690±20196.2c-t	42931±28263.9E-L	200.3±12.3d-z	2.37±0.3a-k	1.3±0.3 b-n	19.5±2.1e-s	76.8±31.1c-q
141	54.4±17.9a-f	115345±25472.9b-s	74655±11281.4m-K	188.3±14.9h-z	2.49±0.4a-k	1.4±0.2a-n	21.8±2.2a-o	105.4±32.6a-q
142	33.8±7.2a-t	112759±15135.8c-t	72931±11295.4m-K	200.3±5.7d-z	2.33±0.3a-k	1.0±0.2e-n	24.0±1.8a-i	76.8±23.0c-q
143	40±13.5a-r	120690±14866.3a-r	85172±11325.2g-D	192.5±8.6g-z	2.11±0.3c-l	1.0±0.1e-n	21.8±2.2 a-p	86.5±13.7 b-q
144	40.9±14.4a-r	78793±21621.7i-t	86121±22755.8g-C	231.3±19.1a-s	2.25±0.4a-k	1.2±0.2 b-n	20.0±1.4 c-s	88.6±16.7b-q

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Gen	Pol%	Brix%	Purity %	Sucrose%	SYHA	LW	LA	LA
1	17.2±2.0 a-h	19.6±1.7a-k	87.8±2.8c-f	11.8±1.56a-h	7.3±1.9 j-r	4.0±0.5a-j	560.6±135a-j	73.0±19.1a-e
2	19.0±1.5a-g	21.1±1.3a-g	90.0±2.1 a-e	13.2±1.39 a-h	15.0±1.5a-q	4.1±0.6a-j	545.5±112.4a-j	150.4±15a-e
3	16.4±1.3 c-h	18.1±1.2g-l	90.4±2.2a-e	11.5±1.10a-h	7.3±2.3j-r	3.7±0.8 b-j	557.5±174.6a-j	73.3±22.7a-d
4	17.9±2.3a-h	20.1±1.9 a-k	88.9±3.2a-e	12.3±2.05a-h	12.5±2.8a-r	4.8±1.0a-i	694.4±208.2a-h	124.9±28.1a-e
5	17.8±2.5 a-h	19.6±1.6a-k	91.2±1.6a-e	12.5±1.92a-h	14.6±2.9a-r	3.7±1.0b-j	473.9±156.4c-j	146.2±28.9b-e
6	17.5±2.0 a-h	20.1±1.5 a-k	87.1±1.8 d-f	11.9±1.76a-h	10.9±2.6c-r	3.8±0.6a-j	555.2±71.9a-j	108.6±25.7a-e
7	19.7±1.2a-f	21.5±1.1a-e	91.7±1.9 a-e	13.9±0.94 a-e	11.0±4.6 c-r	4.0±0.7a-j	527.1±120.8a-j	110.4±45.7a-e
8	18.2±1.8 a-g	20.0±1.9a-k	90.8±1.3a-e	12.7±1.25 a-h	14.5±3.6a-r	3.5±1.0 d-j	431.9±140.7 e-j	145.3±36.4c-e
9	17.0±1.7 a-h	18.5±2.0 e-l	91.6±1.4 a-e	12.0±1.12a-h	10.4±4.3d-r	4.3±1.5a-j	606.5±236.0a-j	104.4±42.8a-e
10	18.5±2.0 a-g	20.5±2.1 a-i	90.4±0.7a-e	13.0±1.45a-h	17.5±2.6a-j	3.7±0.5b-j	552.7±105.8a-j	174.7±25.7a-d
11	17.9±1.3a-g	19.7±1.2 a-k	92.1±2.5a-e	12.6±1.07 a-h	7.0±2.4j-r	4.8±1.5a-i	749.5±268.9a-g	70.4±23.7a-d
12	17.7±2.7a-h	19.9±2.2a-k	88.6±3.1a-f	12.2±2.26a-h	13.6±3.8a-r	4.5±1.0a-i	607.4±218.0a-j	136.2±38.3a-e
13	18.1±1.2 a-g	20.0±1.0a-k	90.4±3.0a-e	12.7±1.09a-h	16.0±2.3 a-p	4.1±1.4a-j	635.0±308.6a-j	159.9±22.7a-d
14	17.5±1.3a-h	18.9±1.1b-l	92.3±1.9a-e	12.4±1.09a-h	13.0±5.1a-r	4.7±0.8a-i	674.3±122.3a-i	129.7±51.1a-e
15	17.7±1.7a-h	19.5±2.2a-k	90.8±2.0a-e	12.4±1.01a-h	13.2±5.2a-r	3.1±0.4 h-j	391.5±47.7 h-j	132.3±51.8b-e
16	18.4±0.6 a-g	20.6±0.6 a-i	89.2±2.9a-e	12.7±0.71a-h	13.0±2.6 a-r	4.1±0.5a-j	540.0±95.4a-j	129.8±25.8a-e
17	17.9±1.2a-h	20.0±1.4 a-k	89.3±0.8a-e	12.4±0.73 a-h	10.8±10.2c-r	4.3±0.5a-j	562.3±87.9a-j	108.1±102a-e
18	18.9±0.8a-g	20.7±1.0a-i	91.4±3.1a-e	13.3±0.72a-h	10.8±3.8 c-r	4.2±0.1a-j	588.8±49.0a-j	108.3±38.1a-e
19	19.6±0.7a-f	21.5±1.1a-e	91.3±2.1a-e	13.8±0.30a-h	12.9±5.6a-r	5.0±0.8 a-g	740.5±167.9a-g	128.7±56.2a-d
20	17.2±0.6 a-h	19.4±0.7a-l	88.9±2.1a-e	11.9±0.48a-g	7.4±2.6j-r	5.2±0.7a-e	784.7±154.6a-d	74.0±26.5 a-d
21	17.6±1.5 a-h	19.7±1.8a-k	89.3±2.0a-e	12.2±1.05a-h	10.8±4.8c-r	4.3±0.3a-j	644.5±70.2a-j	108.0±48.4a-d
22	17.3±1.0 a-h	19.4±1.2a-l	89.2±1.6a-e	12.0±0.73a-h	12.8±5.0 a-r	4.1±0.7a-j	588.9±134.1a-j	128.4±50.5a-e
23	18.3±1.3a-g	20.2±1.5a-k	90.4±2.4a-e	12.8±0.93a-h	11.3±4.4c-r	4.2±0.5a-j	569.8±103.8a-j	113.1±43.7 a-e
24	19.0±2.0a-g	20.9±2.1 a-g	90.8±1.4a-e	13.3±1.37 a-h	11.4±5.0c-r	3.9±0.7a-j	522.9±163.3a-j	113.9±50.4a-e

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Gen	Pol%	Brix%	Purity %	Sucrose%	SYHA	LW	LA	LA
25	18.2±0.5 a-g	20.1±0.0a-k	90.5±2.4 a-e	12.7±0.66a-h	9.9±4.5 e-r	5.5±0.4 a-c	715.1±65.9a-h	99.3±45.3a-e
26	18.1±1.2a-g	20.2±1.3 a-k	90.0±2.1a-e	12.6±0.91a-h	11.7±2.4 c-r	5.1±0.7a-f	635.0±79.4a-j	117.3±23.6 b-e
27	19.0±3.2a-g	20.4±2.6 a-i	93.3±2.0 a-c	13.5±2.06a-h	12.3±3.9 a-r	3.4±0.2d-j	425.1±22.8g-j	122.7±39.4 b-e
28	18.4±2.5 a-g	20.7±2.3 a-h	88.8±2.9a-f	12.7±1.99 a-h	14.3±5.9a-r	3.2±0.8g-j	450.6±124.3 d-j	143.4±58.9a-e
29	19.4±1.0 a-f	21.5±1.2a-e	90.2±1.3 a-e	13.5±0.62a-h	11.9±3.1c-r	3.9±1.3a-j	434.1±196.4e-j	118.9±31.3a-d
30	18.0±1.5 a-g	20.1±1.2 a-k	89.7±3.0a-e	12.5±1.27a-h	7.8±3.3 i-r	4.8±0.3a-i	728.1±103.3a-h	77.6±33.0a-e
31	17.4±2.4a-h	19.6±2.7a-k	88.8±2.6 a-f	12.0±1.7a-h	10.7±2.9c-r	3.9±0.5a-j	555.6±96.1a-j	106.8±28.5 a-e
32	18.0±1.4a-g	20.0±1.5a-k	89.9±1.2a-e	12.5±0.98a-h	9.3±4.1f-r	5.5±0.4ab	771.1±57.6a-f	93.1±40.9a-d
33	17.8±0.9 a-h	20.1±1.1a-k	88.6±2.2a-f	12.2±0.6 a-h	13.2±2.9a-r	4.3±0.8a-j	651.1±165.6a-j	131.7±29.3ab
34	18.5±2.4a-g	20.3±2.0a-j	91.4±1.1a-e	13.0±1.57a-h	18.1±2.6a-i	4.8±0.8a-i	777.9±185.7a-e	180.8±26.3b-e
35	19.8±1.7a-e	21.4±2.0 a-f	92.8±1.9 a-e	14.1±1.12a-h	12.9±2.9 a-r	3.5±0.8d-j	443.1±105.0e-j	128.9±28.8a-e
36	15.9±2.9f-h	17.5±2.6 i-l	91.3±1.5a-e	11.2±1.94a-d	8.7±1.5g-r	3.3±0.8 e-j	478.4±160.5b-j	86.8±14.9b-e
37	18.2±1.7 a-g	19.9±2.1 a-k	91.6±1.1 a-e	12.8±1.06 c-i	23.6±1.8a	3.8±0.6 b-j	463.8±72.4c-j	235.9±18.3a-e
38	19.0±0.7 a-g	20.6±1.0 a-i	92.4±1.8a-e	13.5±0.37a-h	22.8±1.2ab	3.9±0.7a-j	531.2±154.1a-j	227.7±12.2b-e
39	18.7±1.1a-g	20.2±1.2a-k	92.6±2.7a-e	13.3±0.87 a-h	19.5±3.2a-f	3.3±1.1 f-j	425.7±170.3g-j	194.9±32.0a-e
40	17.4±1.2a-h	19.3±1.3a-l	90.1±1.2 a-e	12.2±0.84a-h	11.8±3.4 c-r	4.1±0.9a-j	552.2±138.6a-j	117.6±34.5a-d
41	17.8±2.0a-h	19.4±2.1a-l	91.7±1.5a-e	12.6±1.47a-h	6.8±3.7k-r	5.7±1.2a	860.1±206.4 a	68.1±37.4b-e
42	18.0±1.9 a-g	19.4±2.3a-k	92.8±1.5a-e	12.8±1.21a-h	14.4±2.5a-r	3.3±0.7f-j	422.7±108 g-j	144.1±24.6a-e
43	17.1±1.9a-h	18.9±2.2 b-l	90.4±1.9 a-e	11.9±1.32 a-h	17.1±3.8a-k	3.7±0.8b-j	536.0±151a-j	171.2±38.3a-e
44	19.7±1.6a-f	21.3±2.0 a-g	92.5±1.6a-e	14.0±1.0a-h	19.2±2.7a-g	3.6±1.2 b-j	488.4±179.7b-j	192.1±27.4a-d
45	16.8±2.4a-h	19.3±2.1a-l	86.9±3.0 ef	11.3±1.64 a-e	11.6±3.1 c-r	3.9±1.2a-j	590.5±209.6a-j	116.2±30.6a-e
46	18.5±0.8a-g	20.2±1.7 a-k	92.1±4.0a-e	13.1±0.2a-h	15.4±5.3a-q	3.6±0.6c-j	506.9±126.3b-j	154.3±52.6a-e
47	18.6±0.2a-g	20.5±0.6 a-i	91.2±2.6 a-e	13.0±0.21a-h	11.8±2.1c-r	3.9±0.6a-j	549.0±61.0a-j	117.9±20.8 b-e
48	17.4±0.3a-h	19.3±0.6a-l	90.1±1.3a-e	12.2±0.22 a-h	11.0±1.9 c-r	4.1±1.6a-j	512.0±200.6b-j	109.9±18.6a-e

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Gen	Pol%	Brix%	Purity %	Sucrose%	SYHA	LW	LA	LA
49	19.0±1.2 a-g	20.3±1.4a-j	93.2±3.2a-c	13.5±0.98a-h	20.0±6.5a-e	3.7±0.9 b-j	497.8±121.0b-j	199.7±64.8ab
50	17.7±1.0a-h	19.9±0.8a-k	89.0±3.3a-e	12.2±0.98a-h	17.0±5.6a-l	4.9±0.5 a-h	800.2±78.5 a-c	170.0±56.1b-e
51	17.4±1.3a-h	18.7±1.5 c-l	93.0±2.0a-d	12.4±0.92a-h	9.9±2.1e-r	2.6±0.4 j	326.9±70.1 j	99.0±21.1a-e
52	18.0±0.8a-g	19.9±0.7 a-k	90.6±2.9a-e	12.6±0.83a-h	8.8±2.7g-r	4.0±0.9a-j	585.7±160.9a-j	88.0±27.2a-e
53	16.1±2.2d-h	18.2±2.4 g-l	88.6±0.9a-f	11.1±1.44a-h	7.8±2.3i-r	4.1±0.1a-j	544.1±52.5a-j	77.7±23.2 a-e
54	18.4±0.2 a-g	20.2±0.7a-k	91.8±3.0 a-e	12.9±0.22 d-i	13.9±5.5a-r	4.3±0.9a-j	578.0±190.4a-j	138.6±54.5 a-e
55	17.3±1.4a-h	19.4±1.8a-l	88.7±1.5a-e	12.0±0.84a-h	16.6±5.0 a-n	4.2±1.3a-j	581.8±213.1a-j	166.1±50.0 b-e
56	18.5±1.3 a-g	20.1±1.8a-k	92.2±2.7a-e	13.1±0.76a-h	18.3±2.0a-i	3.6±0.6b-j	472.6±138.9 c-j	183.1±19.9a-e
57	18.2±1.9a-g	19.8±1.5 a-k	91.8±1.5a-e	12.8±1.17a-h	18.5±3.0a-h	4.0±0.8a-j	542.4±136.7a-j	184.5±29.6a-e
58	19.9±0.8a-d	21.9±1.0 abc	91.3±2.9a-e	14.0±0.72 a-h	20.9±4.9 a-d	4.1±1.0a-j	617.5±198a-j	208.9±49b-e
59	18.2±2.5 a-g	20.1±2.9a-k	90.6±1.9a-e	12.8±1.74a-e	12.4±3.7 a-r	4.1±0.9a-j	508.4±162.1 b-j	124±36.8a-e
60	17.4±1.1a-h	19.4±1.0a-l	89.4±2.2a-e	12.0±0.89 a-h	12.4±1.7a-r	3.8±1.0a-j	537.0±205.4a-j	123.6±17.4b-e
61	17.7±0.8 a-h	19.6±0.2a-k	90.1±4.2a-e	12.3±1.04a-h	17.5±3.8a-j	3.8±0.7a-j	487.9±92.4b-j	174.8±37.7a-d
62	18.8±1.0a-g	20.5±1.1 a-i	91.7±1.5 a-e	13.3±0.75a-h	16.1±3.7a-p	4.4±0.5a-j	668.9±88.7a-i	161±37.2 b-e
63	18.1±2.0a-g	19.9±1.1 a-k	90.8±2.0a-e	12.7±1.41a-h	9.2±4.2 f-r	4.6±0.5 a-i	591.9±86.1a-j	92.2±41.8b-e
64	18.8±0.9a-g	20.6±1.2a-i	91.2±1.1 a-e	13.2±0.58 a-h	16.2±2.2 a-p	4.4±0.8a-j	556.2±98.2a-j	161.8±22.1 a
65	18.2±1.6 a-g	20.0±1.9a-k	91.2±1.0 a-e	12.8±1.04 a-h	15.8±4.6a-q	4.0±0.9a-j	680.6±143.8a-i	158.5±45.5a-e
66	17.7±1.9 a-h	19.0±1.8 b-l	93.2±3.1 a-c	12.6±1.05 a-h	12.4±3.8a-r	4.0±0.4a-j	583.7±130.6a-j	124.3±37.7a-e
67	15.2±3.7gh	19.1±2.3 b-l	88.2±3.6a-f	10.5±2.89a-h	16.3±5.7a-o	3.9±0.6a-j	553.5±93.4a-j	162.7±56.5a-e
68	17.8±0.6a-h	19.7±0.9a-k	90.6±3.3 a-e	12.4±0.58 hi	14.0±2.6a-r	3.5±0.6d-j	476.1±160.3b-j	139.5±25.7a-e
69	16.9±2.0 a-h	18.9±2.2b-l	89.4±1.9a-e	11.7±1.41a-h	15.1±2.9 a-q	4.4±0.3a-j	628.3±75.3a-j	150.9±29.2a-e
70	18.9±2.0a-g	20.6±2.1 a-i	91.9±3.3a-e	13.4±1.53a-h	12.5±3.0a-r	4.6±0.5 a-i	657.4±66.5 a-j	125.5±30.2a-e
71	19.0±0.4 a-g	20.8±0.8 a-h	91.4±2.5a-e	13.4±0.37a-h	15.6±3.7 a-q	3.9±0.5a-j	516.8±87.6 b-j	155.5±37.1 b-e
72	18.6±1.2a-g	21.0±1.4 a-g	88.7±2.1a-f	12.8±0.83a-h	11.5±2.5 c-r	3.6±0.4b-j	459.7±71.2c-j	115.1±24.7a-e

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Gen	Pol%	Brix%	Purity %	Sucrose%	SYHA	LW	LA	LA
73	17.2±0.4 a-h	19.2±0.5a-l	89.6±2.2a-e	11.9±0.46 a-h	18.5±1.2 a-h	4.6±0.8a-i	642.0±171.3a-j	185.3±11.5a-e
74	17.9±2.3 a-h	19.5±1.8a-k	91.7±1.7a-e	12.6±1.46a-h	8.4±2.1h-r	3.8±0.2a-j	562.2±13.1a-j	84.1±21.1 a-e
75	17.6±1.8a-h	20.0±2.4 a-k	89.3±5.6a-e	12.0±1.62 a-h	14.2±4.1a-r	4.4±0.5a-i	592.4±82.5a-j	141.7±40.8a-d
76	17.3±2.6a-h	19.1±1.6 b-l	90.9±2.4a-e	12.1±1.64a-h	9.1±5.2 f-r	4.0±0.8a-j	608.6±119.7a-j	91.5±51.6 a-e
77	17.3±1.6a-h	19.0±1.9 b-l	91.2±3.3 a-e	12.2±1.11a-h	14.9±1.6a-q	3.9±0.3a-j	525.6±83.7a-j	148.8±15.9 a-e
78	18.5±1.6a-g	19.8±0.9 a-k	93.2±4.9a-c	13.2±1.62a-h	11.2±3.7c-r	4.1±0.5a-j	547.6±139a-j	112.1±37.3a-e
79	19.1±1.5 a-g	20.7±1.9a-i	92.6±1.8a-e	13.6±0.93 a-h	13.0±1.5 a-r	4.9±0.8a-i	712.5±144.4a-h	129.7±14.9a-e
80	18.2±1.0a-g	20.1±1.1a-k	90.5±1.7 a-e	12.7±0.69a-h	6.5±2.7 l-r	4.3±0.4a-j	591.8±71.3a-j	65.0±27.1a-e
81	19.0±1.3 a-g	20.5±1.6 a-i	92.7±1.7 a-e	13.5±0.75a-h	5.7±1.5 p-r	4.3±0.5a-j	608.2±112.7a-j	56.6±14.6a-e
82	17.8±2.3 a-h	19.4±2.0a-l	92.1±1.2 a-e	12.6±1.45a-h	10.6±4.4c-r	4.7±0.5a-i	642.7±86.6a-j	106.0±44.5 b-e
83	17.4±0.8a-h	19.1±0.8a-l	90.9±4.3a-e	12.2±0.93a-h	17.4±2.3a-j	4.9±0.6 a-i	606.3±122.6a-j	173.7±23.4 a-e
84	18.6±0.9a-g	20.5±1.0a-i	91.1±4.1a-e	13.1±0.95a-h	15.8±2.8 a-q	4.5±0.3a-i	621.6±51.8a-j	158.4±28.4a-e
85	17.7±2.8 a-h	19.1±1.6b-l	92.6±5.3a-e	12.6±2.34a-h	15.0±6.5a-q	3.7±0.9b-j	543.7±196.7a-j	149.7±64.6 b-e
86	17.9±1.2a-h	19.7±1.6 a-k	90.7±1.3a-e	12.5±0.7 a-h	12.6±4.3 a-r	3.1±0.8g-j	392.1±82.9 h-j	126.4±43.2a-e
87	16.7±3.0a-h	18.6±1.9 d-l	88.2±2.5a-f	11.7±2.37a-h	8.2±4.5 h-r	4.5±0.6 a-i	652.3±92.1 a-j	82.5±45.2a-e
88	16.0±3.6e-h	18.2±2.1f-l	86.9±4.9 ef	10.8±2.64a-h	9.5±5.2 e-r	4.3±0.6a-j	633.6±113a-j	95.4±52.3 a-e
89	19.6±1.6a-f	21.2±1.7a-g	92.7±1.4a-e	14.0±1.17 e-i	11.5±3.8c-r	4.3±0.4a-j	577.2±105.9a-j	115.4±38.4b-e
90	19.5±1.5a-f	21.1±1.7a-g	92.5±1.6 a-e	13.9±0.99a-e	11.4±5.5 c-r	4.6±1.5 a-i	599.5±224.2a-j	113.7±54.6a-e
91	17.2±2.8 a-h	18.5±2.2 e-l	93.1±0.8a-c	12.3±1.92 a-f	8.2±5.4 h-r	4.2±0.3a-j	560.6±88.9a-j	82.1±54 a-e
92	17.9±1.8 a-h	19.8±1.9a-k	90.3±2.2a-e	12.5±1.28a-h	10.6±2.1c-r	5.1±0.3 a-f	664.6±90.3a-j	106.1±21.1b-e
93	18.3±1.5 a-g	19.9±1.6 a-k	91.7±2.1a-e	12.9±1.15a-h	12.2±3.5c-r	4.1±1.0a-j	529.9±190a-j	121.8±34.8c-e
94	16.7±0.4a-h	18.8±0.8b-l	88.8±2.7a-f	11.5±0.42a-h	9.2±0.9 f-r	4.5±0.3a-i	531.1±82.7a-j	91.9±8.7 a-e
95	14.0±2.1h	16.2±2.3 l	83.7±1.9f	9.3.0±1.23a-h	5.9±2.9o-r	4.1±0.7a-j	577.2±135.2a-j	59.4±28.9a-e
96	18.1±1.0a-g	20.0±1.5a-k	90.3±1.9a-e	12.6±0.64 i	9.5±3.5 e-r	3.7±0.5 b-j	528.3±44.4a-j	95.4±35.5a-e

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Gen	Pol%	Brix%	Purity %	Sucrose%	SYHA	LW	LA	LA
97	18.9±1.6a-g	20.7±2.5a-h	91.9±3.8a-e	13.3±0.76a-h	11.9±2.9 c-r	3.9±0.7a-j	572.6±170a-j	119.1±28.8a-c
98	18.7±1.2a-g	20.4±1.6a-j	91.7±1.2a-e	13.2±0.75 a-h	21.0±3.5 a-c	4.3±0.4a-j	682.2±28.5a-i	210.0±34.9a-e
99	19.0±1.3 a-g	20.4±1.6a-i	93.0±1.8 a-d	13.5±0.78a-h	12.1±2.3 c-r	3.4±0.8e-j	462.8±86.3 c-j	120.6±22.8a-e
100	18.3±1.7a-g	20.0±1.5a-k	91.1±3.2 a-e	12.8±1.48a-h	17.1±5.5 a-k	4.3±0.6a-j	623.9±161.2a-j	170.9±54.9a-e
101	16.0±1.7 e-h	17.6±1.9h-l	90.7±1.1a-e	11.2±1.22 a-h	16.6±1.8a-n	4.1±0.6a-j	539.1±99.4a-j	166.1±17.5a-d
102	18.1±2.0a-g	19.8±1.3a-k	91.1±2.2 a-e	12.7±1.26 c-i	13.1±4.7a-r	4.1±0.7a-j	618.9±129.8a-j	130.8±46.6a-e
103	18.4±2.3 a-g	20.1±2.2a-k	91.9±1.7a-e	13.0±1.43a-h	15.7±6.5 a-q	5.2±1.2a-e	717.5±273.3a-h	157.5±65.1b-e
104	18.7±2.1a-g	20.3±2.5a-j	92.2±2.7a-e	13.3±1.39a-h	16.6±2.8 a-n	4.0±1.0a-j	507.4±174 b-j	166.1±27.8a-c
105	17.9±1.0a-h	19.6±1.2 a-k	91.3±1.1a-e	12.6±0.56a-h	12.9±1.8 a-r	4.5±0.7a-i	720.3±142.7a-h	128.8±17.7b-e
106	17.1±2.5a-h	18.8±2.2b-l	91.0±1.6a-e	12.0±1.59a-h	16.7±5.5 a-m	3.5±0.8 d-j	438.8±119.9e-j	167.1±55.3a-e
107	17.3±3.6a-h	19.2±2.5a-l	90.1±1.4a-e	12.1±2.55a-h	12.7±3.5a-r	4.4±1.0a-j	589.0±154.8a-j	127.1±35.2a-e
108	18.5±1.2a-g	20.3±1.3a-j	91.0±2.0 a-e	13.0±0.92a-h	15.4±1.9 a-q	4.2±0.4a-j	593.4±80.4a-j	154.3±18.6a-d
109	17.6±1.9 a-h	20.1±2.3a-k	87.9±2.1b-f	12.0±1.27a-h	7.5±1.9 j-r	4.8±0.9 a-i	725.2±178.2a-h	74.6±19.1a-e
110	18.0±2.4 a-g	20.3±2.1a-j	88.8±3.9a-f	12.3±1.45 a-h	9.2±3.6 f-r	3.9±0.7a-j	534.3±102.2a-j	92.5±36.2b-e
111	18.8±1.5 a-g	20.3±1.7a-j	92.5±2.2a-e	13.3±0.98a-h	10.9±2.4c-r	3.9±0.8a-j	498.2±163.1 b-j	109±24.4 a-e
112	20.2±1.6a-c	22.0±1.6ab	92.1±2.4 a-e	14.3±1.26 a-c	15.4±4.8a-q	4.0±0.8a-j	560.4±158a-j	154.2±48.1 a-e
113	17.9±2.2 a-h	20.1±1.4a-k	89.3±1.1a-e	12.4±1.43a-h	9.5±5.2 e-r	4.1±0.5a-j	573.4±82.9a-j	94.9±51.9b-e
114	18.2±1.3 a-g	20.0±1.4a-k	90.7±1.3a-e	12.7±0.95a-h	16.0±2.8 a-p	4.6±0.3 a-i	582.2±50.9a-j	159.6±28.2a-e
115	17.5±2.0a-h	19.6±1.5a-k	89.4±1.5a-e	12.1±1.22a-h	9.1±4.3f-r	4.6±0.7a-i	620.7±89.4a-j	91.4±42.7c-e
116	18.7±1.4a-g	21.1±1.8a-g	88.9±2.2a-e	12.9±0.85a-h	4.1±2.3r	4.7±0.7a-i	548.6±58.3a-j	41.0±23.2a-e
117	18.3±1.8a-g	20.0±1.4a-k	91.5±2.2a-e	12.9±1.03a-h	9.1±4.1 f-r	4.3±0.5a-j	557.3±52.3a-j	90.6±41.1 a-e
118	16.4±1.0 c-h	18.5±1.4 e-l	89.0±1.9a-e	11.3±0.59a-h	14.4±4a-r	4.9±0.7a-h	676.2±130.3a-i	144.4±39.8a-e
119	16.6±0.8a-h	18.7±1.3c-l	89.0±2.8a-e	11.5±0.37a-h	16.1±2.6a-p	5.0±0.2a-g	718.1±19.6a-h	161.3±25.7 a-d
120	19.1±2.1a-g	20.6±2.6 a-i	92.7±1.7a-e	13.6±1.33a-h	6.1±2.4n-r	4.8±0.5a-i	706.4±89.5a-h	61.4±24.1a-d

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Gen	Pol%	Brix%	Purity %	Sucrose%	SYHA	LW	LA	LA
121	18.8±0.6a-g	20.5±1.2a-i	91.8±2.3a-e	13.3±0.21a-h	8.9±1.7 g-r	3.0±0.4 ij	350.1±88.7ij	89.3±17.4a-e
122	17.6±2.0 a-h	19.8±2.2a-k	88.5±1.8a-f	12.1±1.46a-h	17±3.5 a-l	5.0±1.0 a-g	759.8±123.2a-g	169.5±34.6a-c
123	17.4±1.4a-h	19.4±1.9a-l	89.8±1.9a-e	12.1±0.89a-h	5.3±2.7qr	4.6±1.3a-i	622.5±185.1a-j	53.0±26.9ab
124	16.9±1.7a-h	19.0±1.8b-l	88.8±1.5a-f	11.6±1.16a-h	10.2±3.1 e-r	5.2±0.7a-f	816.7±149.4 ab	101.9±30.6 a-e
125	17.3±1.1a-h	19.1±1.3a-l	90.5±1.9a-e	12.1±0.79a-h	6.6±4.4 k-r	4.6±0.8 a-i	745.6±147.9 a-g	65.7±43.8 b-e
126	17.7±1.5a-h	19.3±1.9a-l	92.1±2.6a-e	12.6±0.86 a-h	10.5±5.2c-r	4.0±0.8a-j	556.4±123.7a-j	105.0±52.1a-e
127	17.5±2.0a-h	19.5±2.4a-k	89.8±1.7a-e	12.1±1.24a-h	16.2±9 a-p	4.7±0.5a-i	607.6±103.7a-j	162±89.9a-e
128	16.2±1.6d-h	18.2±1.9f-l	89.1±1.5a-e	11.2±1.01c-i	11.8±3.3 c-r	4.2±0.5a-j	613.5±114.5a-j	117.6±32.7a-e
129	15.3±0.4gh	17.1±0.6kl	90.0±1.3 a-e	10.7±0.19 f-i	8.1±4.4h-r	4.4±0.9a-j	631.4±154.3a-j	80.8±44.1 a-e
130	15.3±1.1gh	17.2±1.4 j-l	89.4±1.2a-e	10.6±0.65g-i	6.0±2.3o-r	3.9±0.7a-j	543.5±114a-j	59.8±23.3a-e
131	20.5±0.9a	22.3±1.3 a	91.9±1.5a-e	14.5±0.42ab	6.3±2.1 m-r	5.3±0.5 a-d	743±108.1a-g	63.4±20.9a-d
132	19.6±1.6 a-f	20.8±1.8a-h	94.1±0.7a	14.1±1.06a-d	6.7±2.1 k-r	4.1±0.8a-j	586.3±131.2a-j	67.2±21.0a-e
133	16.5±1.7 c-h	18.5±1.8 e-l	89.2±1.6a-e	11.4±1.19a-h	9.0±4.4 f-r	4.7±1.6a-i	739.9±305.1a-g	89.7±43.8a-e
134	19.1±1.6 a-g	21.1±1.3a-g	90.7±2.5 a-e	13.4±0.84a-h	18.3±5.7a-i	4.1±0.5a-j	586±122.3a-j	182.6±56.6a-e
135	18.1±1.2 a-g	19.8±1.5a-k	91.4±2.7 a-e	12.7±0.9a-h	14.4±4.2a-r	4.4±0.5a-i	648.8±125.3a-j	144.3±42.1b-e
136	18.5±1.6a-g	20.1±1.8a-k	91.8±2.2 a-e	13.1±1.01 a-h	14.3±3.2a-r	4.2±1.1a-j	623.8±222.6a-j	143±32.4a-e
137	18.9±1.4a-g	20.6±1.5a-i	91.5±1.5a-e	13.3±1.03 a-h	13.1±3.6a-r	4.5±0.3 a-i	572.2±72.7a-j	131.3±36.5a-e
138	17.6±1.8a-h	19.4±2.3a-k	90.7±2.2 a-e	12.3±1.08a-h	11.4±1.3 c-r	4.0±0.7a-j	597.1±164.2a-j	114.1±13.0a-e
139	18.1±0.6a-g	20.3±1.1a-j	89.4±2.5a-e	12.5±0.4a-h	15.8±2.2a-q	4.7±0.5a-i	665.3±163.2a-j	158.4±22.1a-d
140	18.7±1.3a-g	20.2±1.9 a-k	92.6±2.7a-e	13.3±0.73 a-h	10.0±3.9e-r	4.1±1.1a-j	594±232.8a-j	100.5±38.6a-e
141	17.6±1.6a-h	19.4±1.9a-l	90.5±2.0a-e	12.3±1.1a-h	13.1±2.9a-r	4.9±0.8a-h	743.4±89.4a-g	131.4±28.9a-e
142	20.4±1.4 ab	21.8±1.9 a-d	93.8±1.8 ab	14.7±0.79 a	11.3±3.8 c-r	3.9±0.8a-j	539.8±135.3a-j	113.2±37.5a-d
143	18.4±1.7a-g	20.3±2.1a-k	91.0±1.6a-e	12.9±1.12a-h	11.2±2.0 c-r	3.8±0.3a-j	517.4±48.4b-j	111.8±19.8de
144	16.5±2.7 b-h	18.4±2.2e-l	90.2±2.0 a-e	11.5±1.7a-h	10.5±3.2c-r	4.2±1.1a-j	649.2±195.5a-j	104.7±31.7e

Appendix Table 2. Comparison of mean performance of best 5% sugarcane genotypes against the mean of the standard check varieties and commercial cultivar

			Comparative advantage (% over)						Comparative advantage (% over)						Comparative advantage (% over)		
Code	Genotype	Mean	B52-298	NCo-334	MCC	Code	Genotype	mean	B52-298	NCo-334	MCC	Code	Genotype	Mean	B52-298	NCO-334	MCC
		SP (%)						**TCHA**						**MCHA**			
33	FGO6-695	58.87	29.20	30.79	45.29	37	CP70/321	193965.5	61.17	21.20	51.65	37	CP70/321	142931.0	46.99	21.20	73.60
46	CO-421	57.99	27.00	28.84	43.12	134	CO-0238	191379.3	59.03	19.58	49.63	67	B59/250	141896.6	45.92	20.32	72.34
4	C86/56	57.84	26.95	28.50	42.75	61	CO1148	177586.2	47.56	10.96	38.84	38	Mex57/197	137586.2	41.49	16.67	67.10
37	CP70/321	56.37	23.73	25.24	39.12	38	Mex57/197	176551.7	46.70	10.32	38.03	57	CO-1230	131379.3	35.11	11.40	59.57
66	DB414/60	55.88	22.65	24.15	37.91	100	CO-449	171379.3	42.41	7.08	33.99	61	CO1148	130000.0	33.69	10.23	57.89
49	CP6023	55.80	22.48	23.97	37.72	74	FGO4641	169482.8	40.83	5.90	32.51	106	NCO-376	127586.2	31.21	8.19	54.96
75	V153	55.15	21.05	22.53	36.11	122	FGO4420	164482.8	36.68	2.77	28.60	86	V203	123620.7	27.13	4.82	50.14
100	CO-449	55.15	21.05	22.53	36.11	69	CO419	162586.2	35.10	1.59	27.12	49	CP6023	119655.2	23.05	1.46	45.33

SP = Sprouting percentage MCC = mean commercial cane TCHA= Tiller count per hectare MCHA = Millable count per hectare

			Comparative advantage (% over)						Comparative advantage (% over)						Comparative advantage (% over)		
Code	Genotype	Mean	B52-298	NCo-334	MCC	Code	Genotype	mean	B52-298	NCo-334	MCC	Code	Genotype	Mean	B52-298	NCO-334	MCC
		SH(cm)						**SD(cm)**						**SCW(kg)**			
139	VMC9655	276.4	37.92	15.58	27.10	105	CO-810	3.10	18.32	62.30	36.14	73	V141	2.12	42.52	87.20	55.36
101	CO-779	273.8	36.63	14.49	25.90	132	CPOO1527	3.04	16.15	59.32	33.64	127	V46	2.00	34.45	76.60	46.57
45	B57/36	266.1	32.78	11.27	22.36	73	V141	2.58	8.78	49.21	25.16	118	V7	2.00	34.45	76.60	46.57
33	FGO6-695	264.8	32.14	10.73	21.77	109	C88-356	2.85	8.78	49.21	25.16	119	V188	1.97	32.44	73.95	44.37
68	CO680	264.0	31.74	10.39	21.40	32	FGO4-466	2.83	8.02	48.17	24.28	101	CO-779	1.84	23.70	62.47	34.84
39	CO-967	263.7	31.59	10.27	21.26	127	V46	2.82	7.56	47.54	23.76	125	FGO5-155	1.82	22.35	60.71	33.38
135	Q-200	260.9	30.19	9.09	19.97	122	FGO4420	2.78	6.11	45.55	22.09	83	V189	1.82	22.35	60.71	33.38
55	PR1007	260.3	29.89	8.84	19.70	93	2-111	2.78	6.11	45.55	22.09	139	VMC9655	1.78	19.66	57.17	30.45

SH = stalk height MCC = mean commercial cane SD = stalk diameter SCW = single cane wieght

			Comparative advantage (% over)						Comparative advantage (% over)						Comparative advantage (% over)		
Code	Genotype	Mean	B52-298	NCo-334	MCC	Code	Genotype	mean	B52-298	NCo-334	MCC	Code	Genotype	Mean	B52-298	NCO-334	MCC
		NOI						**CYHA(t/ha)**						**Pol%**			
44	NCO-334	27.25	43.42	0.00	18.22	37	CP70/321	184.52	34.31	34.10	66.23	131	C85-102	20.48	15.64	4.12	11.97
121	2-555	26.75	40.79	-1.83	16.05	38	Mex57/197	167.88	22.20	22.00	51.24	142	2-888	20.43	15.36	3.86	11.70
16	CO-976	25.75	35.53	-5.50	11.71	98	D41/46	159.17	15.86	15.67	43.39	112	VMC9647	20.23	14.23	2.85	10.60
40	B41/227	25.50	34.21	-6.42	10.63	73	V141	155.65	13.30	13.11	40.22	58	B657/150	19.94	12.59	1.37	9.02
2	CO740	25.25	32.89	-7.34	9.54	49	CP6023	155.19	12.96	12.78	39.81	35	FGO5-336	19.83	11.97	0.81	8.42
77	VMC95243	25.00	31.58	-8.26	8.46	101	CO-779	148.84	8.34	8.17	34.09	7	B52-107	19.73	11.41	0.31	7.87
24	B78/505	25.00	31.58	-8.26	8.46	58	B657/150	148.78	8.30	8.12	34.03	44	NCO-334	19.67	11.07	0.00	7.54
124	FGO6-806	24.75	30.26	-9.17	7.38	67	B59/250	146.62	6.73	6.55	32.09	89	PSR97105	19.62	10.78	-0.25	7.27

NOI = Number of internode MCC = mean commercial cultivar CYHA = Cane yield per hectare Pol% = polarity

			Comparative advantage (% over)						Comparative advantage (% over)						Comparative advantage (% over)		
Code	Genotype	Mean	B52-298	NCo-334	MCC	Code	Genotype	mean	B52-298	NCo-334	MCC	Code	Genotype	Mean	B52-298	NCO-334	MCC
		Brix%						**Purity%**						**Sucrose%**			
131	C85-102	22.30	12.12	4.78	10.49	132	CPOO1527	94.09	5.68	1.71	3.81	142	2-888	14.66	19.95	4.91	20.16
112	VMC9647	21.96	10.41	3.18	8.81	142	2-888	93.83	5.39	1.43	3.52	131	C85-102	14.47	18.40	3.55	18.61
58	B657/150	21.86	9.90	2.71	8.31	27	CPCL02-926	93.34	4.84	0.90	2.98	112	VMC9647	14.33	17.25	2.55	17.46
142	2-888	21.80	9.60	2.43	8.02	49	CP6023	93.25	4.74	0.80	2.88	35	FGO5-336	14.13	15.62	1.12	15.82
7	B52-107	21.51	8.14	1.07	6.58	66	DB414/60	93.22	4.71	0.77	2.85	132	CPOO1527	14.07	15.13	0.69	15.33
29	MPT97-035	21.49	8.04	0.97	6.48	78	C92-514	93.19	4.67	0.74	2.81	58	B657/150	14.02	14.72	0.33	14.92
19	DB386/60	21.46	7.89	0.83	6.33	91	CPO4156	93.10	4.57	0.64	2.71	44	NCO-334	13.97	14.31	0.00	14.51
35	FGO5-336	21.38	7.49	0.46	5.94	99	NCO-310	93.03	4.49	0.56	2.64	89	PSR97105	13.97	14.31	0.00	14.51

Appendix Table 3. Mean performance of 16 quantitative morphological traits for 11 commercial sugarcane cultivars grown in Metahara and Kessem Sugar Estates

Code	Genotype	SP	TCHA	MCHA	SH	SD	SCW	NOI	CYHA	Pol%	Brix%	Purity %	Sucrose%	SYHA	LW	LL	LA
44	NCO-334	45.01	160043.1	117931.0	239.15	1.91	1.13	27.25	137.60	19.67	21.28	92.51	13.97	19.21	3.61	134.25	488.35
100	CO-449	55.15	171379.3	102931.0	223.83	1.93	1.30	22.00	130.99	18.25	20.01	91.14	12.84	17.09	4.29	143.75	623.86
50	B52/298	45.56	120344.8	97241.38	200.40	2.62	1.49	19.00	137.38	17.71	19.89	89.03	12.22	17.00	4.93	162.50	800.20
2	CO740	45.10	141379.3	92068.97	219.13	2.31	1.26	25.25	113.78	18.98	21.08	90.02	13.23	15.04	4.05	134.00	545.52
68	CO680	42.89	140689.7	75689.66	264.00	1.98	1.49	22.50	111.94	17.80	19.66	90.60	12.44	13.96	3.46	134.75	476.05
54	CO-678	22.74	118620.7	75172.41	231.68	2.19	1.40	23.75	107.16	18.39	20.17	91.26	12.93	13.86	4.27	133.25	578.01
4	C86/56	57.84	140344.8	78448.28	197.58	2.55	1.35	20.00	104.63	17.86	20.09	88.86	12.34	12.49	4.79	143.25	694.35
47	C132-81	47.33	133448.3	64655.17	162.63	2.52	1.40	22.00	90.50	18.62	20.53	90.75	13.04	11.79	3.87	143.00	549.00
40	B41/227	17.40	78793.1	65344.59	213.43	2.37	1.48	25.50	96.32	17.43	19.34	90.12	12.16	11.76	4.06	136.25	552.18
63	SP70-1284	26.72	78103.5	56724.14	187.48	2.37	1.25	22.25	71.90	18.08	19.90	90.83	12.68	9.22	4.59	128.75	591.91
103	Mex-54/245	39.95	123793.1	79482.76	252.85	2.31	1.48	24.00	118.83	18.40	20.05	91.93	12.99	15.75	5.22	133.75	717.48
	Mean	**40.52**	**127903.6**	**82335.40**	**217.47**	**22.77**	**1.36**	**23.05**	**111.00**	**18.29**	**20.18**	**90.64**	**12.80**	**14.29**	**4.28**	**138.86**	**601.54**

SP = Sprouting percent, TCHA = Tiller count per hectare, MCHA = Millable count per hectare, SH = Stalk height (cm), SD = Stalk diameter (cm), SCW = Single cane weight (kg), NOI = Number of internodes, CYHA = Cane yield ton per hectare, Pol = polarity (%), SYHA = Sugar yield ton per hectare, LW = Leaf width (cm), LL = Leaf length (cm), LA = Leaf area (cm^2)