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RESEARCH ARTICLE

Integrating genetic diversity and biochemical profiling for biofuel-efficient maize genotypes

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Abstract

This study explores the genetic diversity, biochemical composition and trait associations in maize (*Zea mays* L.) to assess its potential as a dual-purpose crop for food and biofuel production. Significant variations in lignocellulosic traits among the evaluated genotypes indicate opportunities for enhancing bioethanol yields. A comparative biochemical study reveals the cellulose content in the kernel is 2.15 times higher than in stover, whereas the hemicellulose and lignin content in stover are 4.6 times and 5.3 times higher, respectively, compared to the kernel. The inbred lines DQL 2159, DQL 222-1-1 and DQL 2272 exhibited significantly higher cellulose contents of 37.05 %, 35.98 % and 35.53 %, respectively, along with significantly lower lignin contents of 20.65 %, 22.25 % and 22.53 % in maize stover. Correlation analysis shows that shoot dry weight (r_p = 0.29), stalk diameter (r_p = 0.33) and plant height (r_p = 0.48) are positively associated with biomass yield. Biochemical studies reveal a strong negative correlation (r_p = -0.59) between kernel lignin and kernel cellulose content, indicating that higher cellulose leads to lower lignin. This finding is valuable for selecting high-cellulose, low-lignin genotypes. Path coefficient analysis further identifies plant height, number of leaves per plant, stalk diameter and kernel cellulose content as key contributors to grain yield, suggesting that selection for these traits could enhance biofuel production. Identifying desirable traits that enhance biofuel efficiency, such as high cellulose and low lignin content, enables targeted breeding for improved biomass conversion. Cluster analysis revealed that Cluster III exhibited superior performance across the majority of traits evaluated, making it the most promising group for utilization in biofuel breeding programs. Notably, genotypes such as DQL 2037, DQL 2272, DQL 2159 and DQL 222-1-1 emerge as promising candidates for biofuel applications due to their high grain and stover yields, alongside elevated cellulose and hemicellulose content. Collectively, these findings provide a comprehensive framework for targeted breeding strategies aimed at developing high-yielding, biofuel-efficient maize cultivars for climate smart agriculture systems.

Keywords: biochemical composition; biofuel; correlation; D^2 analysis; path analysis

Introduction

The global shift toward sustainable energy systems has intensified the demand for renewable biofuels that reduce dependency on fossil fuels and mitigate climate change. It emphasises the urgent need to transition to renewable energy sources, with a particular focus on lignocellulosic biomass especially maize stover as a sustainable feedstock for second-generation biofuel production. Efficient biofuel production depends on the biochemical composition of the biomass specifically, high cellulose and low lignin content. Maize, as a widely cultivated crop, generates over 1.5 billion tonnes of stover annually (1), presenting a vast yet underutilized resource. Second-generation biofuels from such residues can reduce lifecycle greenhouse gas emissions by up to 86 % compared to fossil fuels, aligning with global climate mitigation goals (1). Maize, with its high biomass yield, wide adaptability and established agronomic base, holds significant promise as a feedstock for second-generation bioethanol. However, optimizing both grain yield and biomass quality, particularly traits like cellulose content and lignin composition, remains a key challenge. Therefore, assessing genetic diversity and identifying genotypes with favourable biochemical traits is essential to advance breeding efforts aimed at developing climate-smart maize varieties that contribute to carbon neutrality and sustainable energy systems.

Maize, often referred to as the “Queen of Cereals,” has the highest genetic yield potential among cereal crops (1). India ranks as the sixth-largest maize producer, cultivating 10.74 million hectares and yielding 38.09 million tonnes of grain in year 2022-23 (1). This production generates approximately 24.34 million tonnes of agricultural residue, including stalks, leaves, husks and cobs (2). Improper disposal, particularly open -field burning, releases significant greenhouse gases such as CO_2, CH_4 and N_2O (3). These residues also represent a promising renewable resource for biofuel production, offering a sustainable alternative to conventional fuel options. It can reduce the waste accumulation and environmental pollution caused due to indiscriminate residue burning (3). The statistical data for area, production and productivity of the world, India & Odisha have been presented in Fig. 1-3, respectively (4, 5).

This substantial maize production generates vast quantities of agricultural residues, particularly, with only 22.2 % comprising grain and the remaining 77.8 % consisting of plant biomass. The stover biomass comprises 45 % stalk, 18 % leaf, 11.1 % cob and 3.7 % husk on a dry weight basis (6). Traditionally, these residues have been underutilized or left to decompose in fields, leading to potential environmental concerns such as

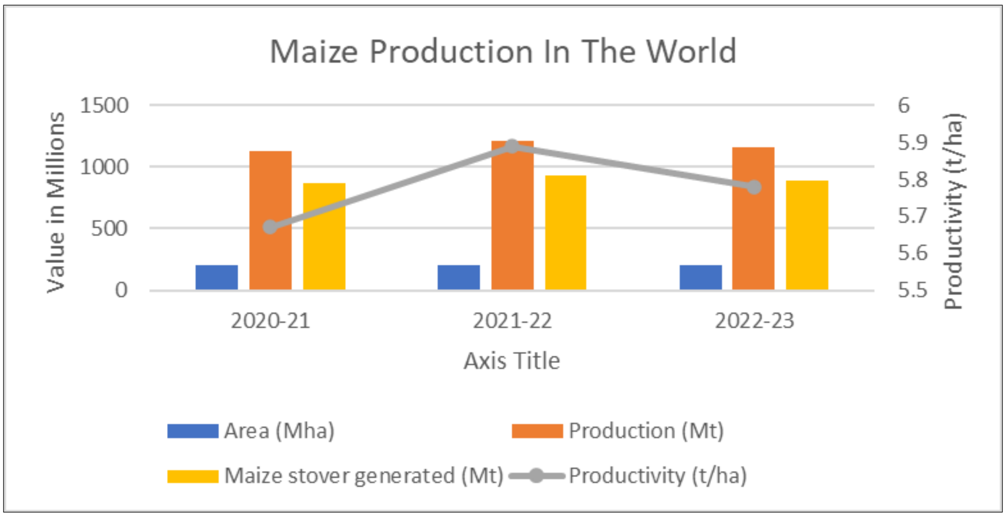

Fig. 1. Maize production statistics of World.

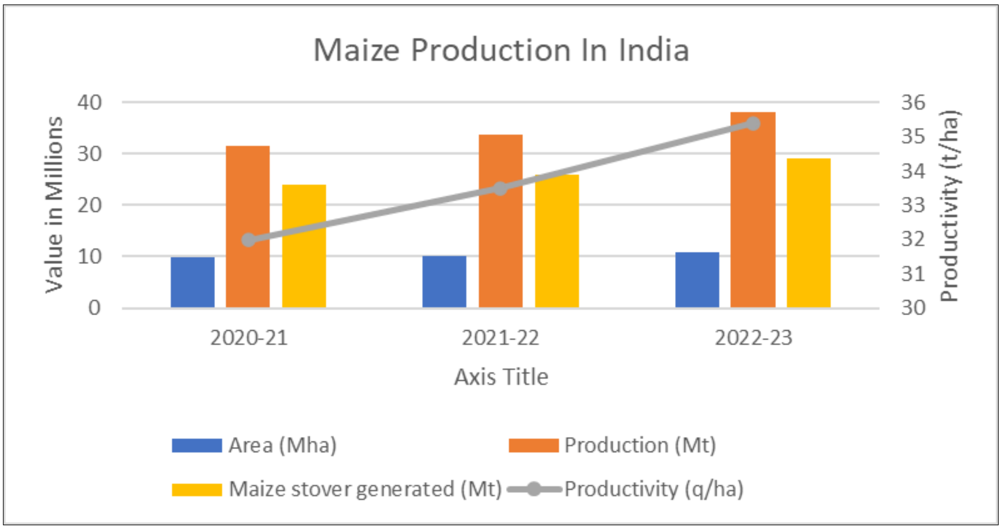

Fig. 2. Maize production statistics of India.

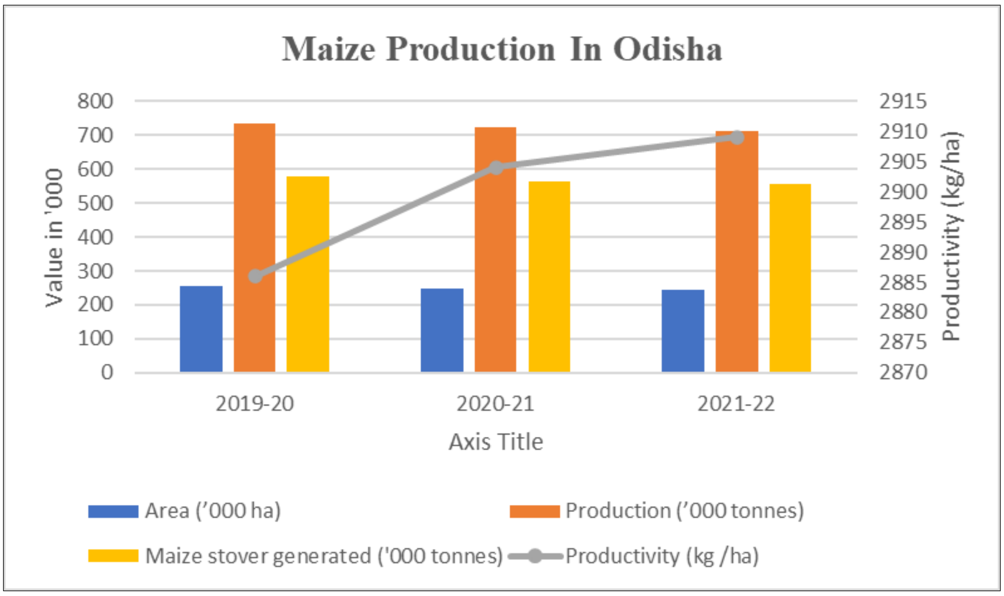

Fig. 3. Maize production statistics of Odisha.

increased greenhouse gas emissions. Furthermore, maize stover presents a valuable lignocellulosic biomass resource for biofuel production, offering a sustainable pathway to convert agricultural waste into renewable energy (7).

Harnessing maize stover for biofuel not only provides an alternative energy source but also addresses critical issues in stover management. Utilizing these residues for bioethanol production can mitigate environmental impacts associated with traditional disposal methods and enhance the economic viability of maize cultivation by adding value to what was once considered waste. This approach aligns with the United Nations Sustainable Development Goals (SDGs) 7 and 13, which aim to ensure access to affordable, reliable, sustainable and modern energy and to take urgent action to combat climate change and its impacts, respectively (8).

Maize possesses a robust genetic foundation that enables the simultaneous enhancement of grain yield and biomass quality. Exploring the genetic diversity and biochemical traits of maize genotypes is essential for improving both productivity and biofuel potential. Identifying key traits linked to high biofuel efficiency can significantly advance breeding programs focused on developing dual-purpose varieties optimized for food and renewable energy use. Key traits include high stem cellulose and low lignin content, increased plant height, greater leaf area and high shoot dry weight, all of which contribute to improved biomass quantity and quality for biofuel production (9). Such advancements could lead to more efficient biofuel production processes, contributing to a circular economy and reinforcing the role of maize as a versatile crop in the pursuit of sustainable energy solutions (10).

Materials and Methods

Experimental site

In the Kharif season of 2023, a study was conducted at EB-II, Odisha University of Agriculture & Technology (OUAT), Bhubaneswar (20°52′ N, 82°52′ E), utilizing a randomized block design (RBD) with three replications to ensure statistical reliability. The research focused on key biofuel-related morphological traits, including plant height, leaf area, number of leaves per plant, stalk diameter, shoot dry weight, 100-grain weight and grain yield per plant. These traits were selected based on their established relevance to biomass accumulation and biofuel conversion efficiency, as supported by previous studies highlighting their contribution to biofuel yield and feedstock quality (7-10). A total of 30 maize genotypes were evaluated as listed in Table 1.

In maize cultivation, proper agronomic practices are essential for optimal crop growth and yield. Land is prepared through deep ploughing followed by 2-3 harrowing, incorporating 10-12 t/ha of well-decomposed FYM. Seeds are treated with Captan or Thiram @ 2.5 g/kg to prevent seed-borne diseases. A seed rate of 25 kg/ha was employed, with planting arranged at a spacing of 60 × 20 cm. Nutrient management involved a balanced application of nitrogen, phosphorus and potassium (N:P:K) at a ratio of 120:60:40 kg/ha, with nitrogen administered in three split doses to align with the crop's growth stages. Irrigation was provided at three critical growth stages i.e., at the knee-high, tasselling and silking and grain-filling stages, to ensure proper crop development. Weed control was effectively managed through the application of pre-emergence herbicide Atrazine @ 1 kg a.i./ha, complemented by two hand weeding at 20 and 40 days after sowing. Pest management includes Carbofuran 3G @ 10 kg/ha at sowing for stem borer and fungal diseases like downy mildew and rust are controlled using resistant varieties and fungicides such as Mancozeb. Harvesting is done when husks turn brown and grains harden (at ~20 % moisture), followed by drying to 12-13 % for safe storage.

Morphological data were systematically recorded at key growth stages, emphasizing traits critical to biomass accumulation and biofuel production potential. At maturity, both stover and cob samples were collected from each replicate for biochemical analysis. The stover samples were sun-dried for seven days and stored in moisture-free conditions until further analysis. Biochemical parameters analysed included cellulose, hemicellulose and lignin content in both stover and kernels, as these components are directly associated with biofuel production potential. This comprehensive experimental approach facilitated robust screening, evaluation and biochemical characterization of maize genotypes for traits pertinent to biofuel production.

Biochemical analysis

Using an Aminex HPX-87H BioRad column using High-Performance Liquid Chromatography (HPLC), the amounts of cellulose and hemicellulose were measured. In High-Performance Liquid Chromatography (HPLC), the mobile phase typically consists of a mixture of water and organic solvents like methanol, acetonitrile, or ethanol, often with a small amount of acid (e.g., phosphoric or acetic acid) to adjust pH. The flow rate usually ranges from 0.5 to 1.5 mL/min, with 1.0 mL/min being standard for most analyses. Detection

Table 1. List of maize genotypes

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	DQL 614-4	11	DQL 2313	21	QIL-4-2800
2	VQL 26	12	DQL 158	22	QIL-4-3080
3	DQL 2261-1	13	DQL 2187	23	QIL-4-2825
4	DQL 182	14	DQL 2311	24	QIL-4-2830
5	DQL 222-1-1	15	DQL 2344	25	QIL-4-2047
6	DQL 2229	16	DQL 257	26	QIL-4-2124
7	DQL 2159	17	DQL 2099	27	QIL-4-2234
8	DQL 2272	18	DQL 72154	28	QIL-4-2295
9	DQL 2037	19	DQL 72242	29	QIL-4-2297
10	VQL 1	20	QIL-4-2784	30	QIL-4-2300

methods vary based on the analytes, with UV-Visible (UV-Vis) detection commonly used for compounds that absorb UV light (wavelengths between 190 and 400 nm). Other detection methods include fluorescence detection for compounds that fluoresce, refractive index (RI) detection for non-UV-absorbing compounds and mass spectrometry (MS) for high-sensitivity, specific analyte identification when coupled with HPLC (LC-MS) (11). Hexane (3 hr, 60-70 °C), ethanol (6 hr, 60-70 °C) and water (12 hr, 60-70 °C) were used in a three-step Soxhlet extraction process to extract extractives from ground stover biomass and kernel samples, separately. After drying and treating the extractive-free samples with 72 % sulphuric acid, they were diluted and autoclaved. Following filtration, the filtrates' sugar contents were examined using HPLC and the lignin concentration was evaluated using the residues (12-14). Three repeated samples were used for the biochemical examination of biofuel properties for each genotype and the results showed negligible to no variations. Nevertheless, the mean was calculated using the mean of the three replicates.

Statistical analysis

Observations for seven biofuel-related morphological traits were recorded and mean performance was analysed using ANOVA. The analysis of variance (ANOVA) method was used to evaluate the obtained data set in order to test for statistically significant differences between treatments. Using the least significant difference or critical difference test at a 5 % significance level, the mean effects of the treatments were calculated using the WINDOSTAT version 9.30 program. The mean effect genotypes are compared using critical difference (CD) at 5 % level of significance ($p = 0.05$).

Multivariate analysis

Correlation studies are instrumental in biofuel research as they identify relationships between various plant traits and biomass composition, which are crucial for biofuel production. Correlation studies among various morphological and biochemical traits can guide the selection of genotypes with optimal biofuel production capacities (15). Such insights enable breeders to focus on traits that directly enhance biofuel efficiency.

Path analysis, on the other hand, further refines this understanding by distinguishing between the direct and indirect effects of traits on biofuel-related outcomes. The path analysis was carried out using the software WINDOSTAT version 9.30. This method allows to construct models that elucidate causal relationships among traits, thereby identifying key factors that directly influence biomass quality and quantity (16). By applying path analysis, traits with the most significant direct impact on biofuel production can be prioritized, leading to more targeted and effective breeding strategies.

Genetic diversity analysis

The genetic divergence was studied using D^2 Mahalanobis analysis, which is a robust approach to understand the genetic variability and relationships among different populations or species (17). By using Tocher's method (18) all the genotypes were clustered into different groups on the basis of biofuel related morpho-biochemical characters under study. The intra and inter-distance were computed. Higher inter-cluster distances (i.e., Clusters III and IV) indicate greater genetic divergence among genotypes, suggesting higher potential for heterosis and transgressive segregants when used in hybridization. Thus, selecting parents from distant clusters enhances breeding utility for trait improvement. The D^2 analysis was carried out using the software WINDOSTAT version 9.30.

Results

The analysis of variance (ANOVA) indicated the presence of significant genetic variation among the genotypes with respect to the traits studied in the present investigation. Also, it was observed that the variances for all the traits under observation were statistically significant. ANOVA results for thirteen morpho-biochemical traits in maize inbred lines are presented in Table 2.

Mean performances

The mean performances of the thirty maize genotypes for biofuel-related traits were analysed to assess their potential for efficient bioethanol production. The mean serves as a crucial statistical measure, providing an overall estimate of trait performance across genotypes and enabling the identification of superior lines. In this study, significant variations were observed among genotypes for key morphological traits such as plant height, leaf area, number of leaves per plant, stalk diameter, shoot dry weight, 100-grain weight and grain yield per plant (Fig. 4a, b). Several genotypes, including DQL 2037, DQL 2272, DQL 2159, DQL 222-1-1 and DQL 182, demonstrated notable performance in key biofuel-related morphological

Table 2. ANOVA for thirteen morpho-biochemical traits in maize inbred lines

Sl. No.	Source	Mean Sum of Squares (MSS)		
		Replication	Treatment	Error
	Degrees of freedom	2	29	58
1	Plant height (cm)	35.211	2180.596**	14.062
2	Leaf area (cm^2)	48.344	64735.774**	75.319
3	Number of leaves per plant	0.433	5.636**	0.203
4	Stalk diameter (cm)	0.025	3.604**	0.120
5	Shoot dry weight (g)	40.833	7647.205**	13.672
6	100 grain weight (g)	0.010	82.790**	0.196
7	Grain yield per plant (g)	10.741	1164.351**	3.999
8	Stem cellulose (%)	0.039	11.626**	0.014
9	Stem hemicellulose (%)	0.001	11.790**	0.010
10	Stem lignin (%)	0.001	11.737**	0.010
11	Kernel cellulose (%)	0.001	20.273**	0.003
12	Kernel hemicellulose (%)	0.002	2.110**	0.001
13	Kernel lignin (%)	0.001	0.687**	0.001

**$p < 0.01$.

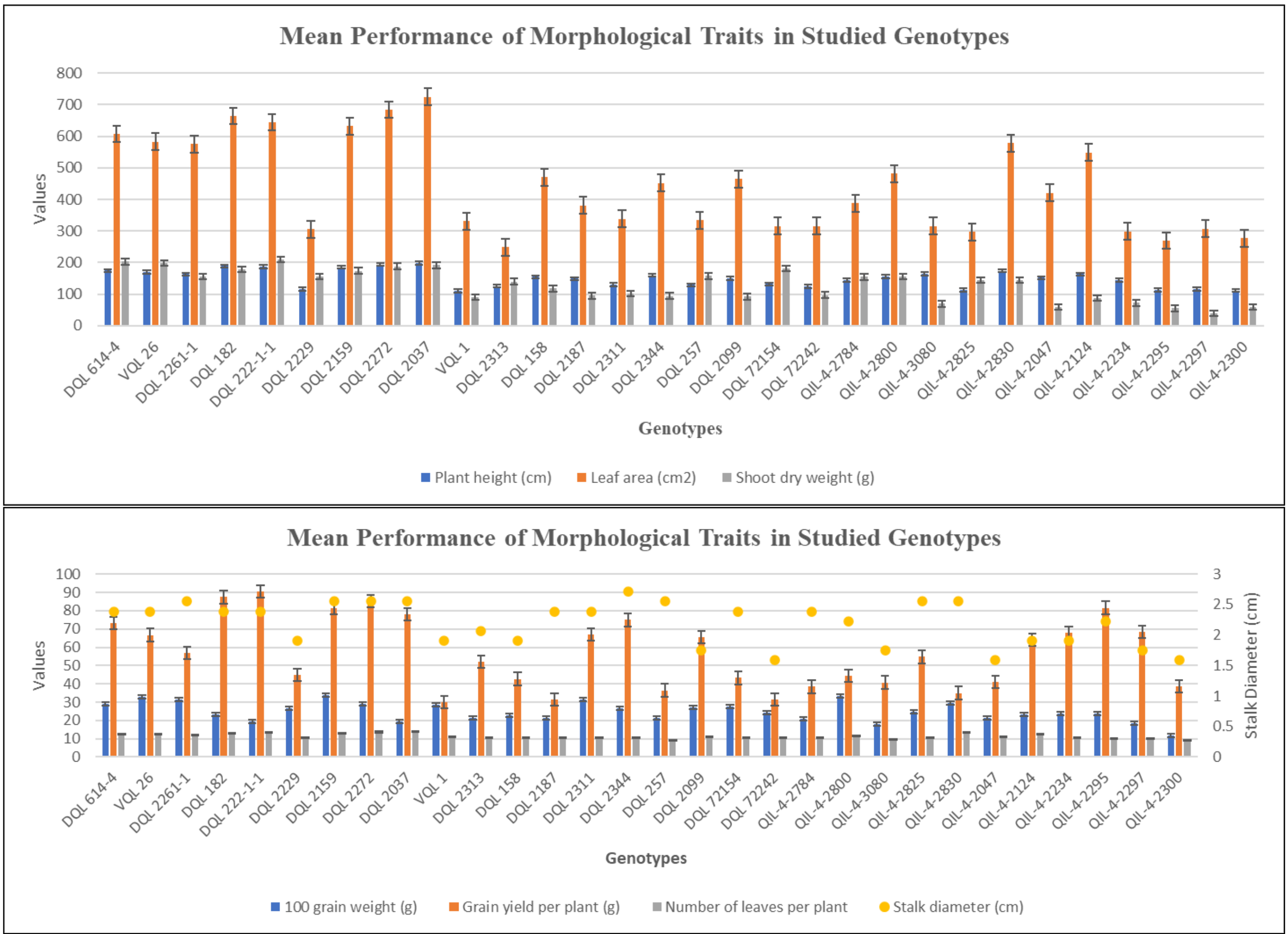

Fig. 4. (a) Characterization of biofuel related morphological traits in maize genotypes (plant height, leaf area & shoot dry weight); (b) Characterization of biofuel related morphological traits in maize genotypes (100 grain weight, grain yield, number of leaves per plant & stalk diameter).

traits such as plant height, grain yield per plant and shoot dry weight per plant. Among these, DQL 2037 recorded the highest mean values for plant height (198 cm), leaf area (725 cm²) and number of leaves per plant (15), making it a strong candidate for biomass production. In contrast, DQL 222-1-1 excelled in yield-related traits, achieving the highest stover yield (209.67 g) and grain yield (90.50 g).

Compositional biochemical characterization

The biochemical characterization of thirty maize genotypes was conducted to assess their cell wall polymer composition, specifically cellulose, hemicellulose and lignin accumulation. These polymers are crucial for biofuel production, as cellulose and hemicellulose serve as primary carbon sources for fermentation into biofuels. In contrast, lignin, which is more resistant to degradation, can negatively impact the efficiency of enzymatic hydrolysis and fermentation processes. Understanding the balance of these polymers is essential for optimizing maize genotypes for higher biofuel yield and more efficient processing. For efficient bioethanol production, an ideal biomass profile includes high cellulose and hemicellulose content with minimal lignin accumulation (9). The lignocellulosic stover biomass contained cellulose ranging from 28.05 % to 37.05 %, hemicellulose between 7.06 % and 15.81 % and lignin from 20.65 % to 28.35 %. The kernel exhibited markedly higher cellulose levels (65.36 % to 75.02 %), notably lower hemicellulose levels (1.09 % to 4.45 %) and lignin content varying between 3.56 % and 6.07 %. The average value of cellulose, hemicellulose and lignin found in stover (32.5 %, 11.5 % and 24 %, respectively) and kernel (70 %, 2.5 % and 4.5 %, respectively) highlights the significant differences in composition, with the kernel being more cellulose-rich and the stover containing higher amounts of hemicellulose and lignin. Cellulose content in kernel was approximately 2.15-fold higher than in stover are 4.6 times and 5.3 times higher, respectively, compared to the kernel.

Among the evaluated genotypes, DQL 2037, DQL 2272, DQL 222-1-1, DQL 2159 and DQL 182 exhibited significantly higher levels of cellulose and hemicellulose while maintaining relatively lower lignin content. Notably, DQL 2159 recorded the highest stem cellulose content (37.05 %) and the lowest stem lignin content (20.65 %), making it an excellent candidate for stover-based biofuel production. Similarly, DQL 2272 had the highest kernel cellulose content (75.02 %) and the lowest kernel lignin content (3.50 %), indicating its suitability for biofuel extraction from maize kernels. These promising genotypes can be effectively utilized in breeding programs aimed at developing maize varieties with enhanced biochemical efficiency for biofuel applications. Fig. 5 illustrates the biochemical composition of maize stover across the genotypes, while Fig. 6 present graphical representations of cellulose, hemicellulose and lignin content of kernel in the studied genotypes. To improve the robustness of the observed differences, the mean ± SE and CV % for all the morpho-biochemical traits under study have been presented in Table 3.

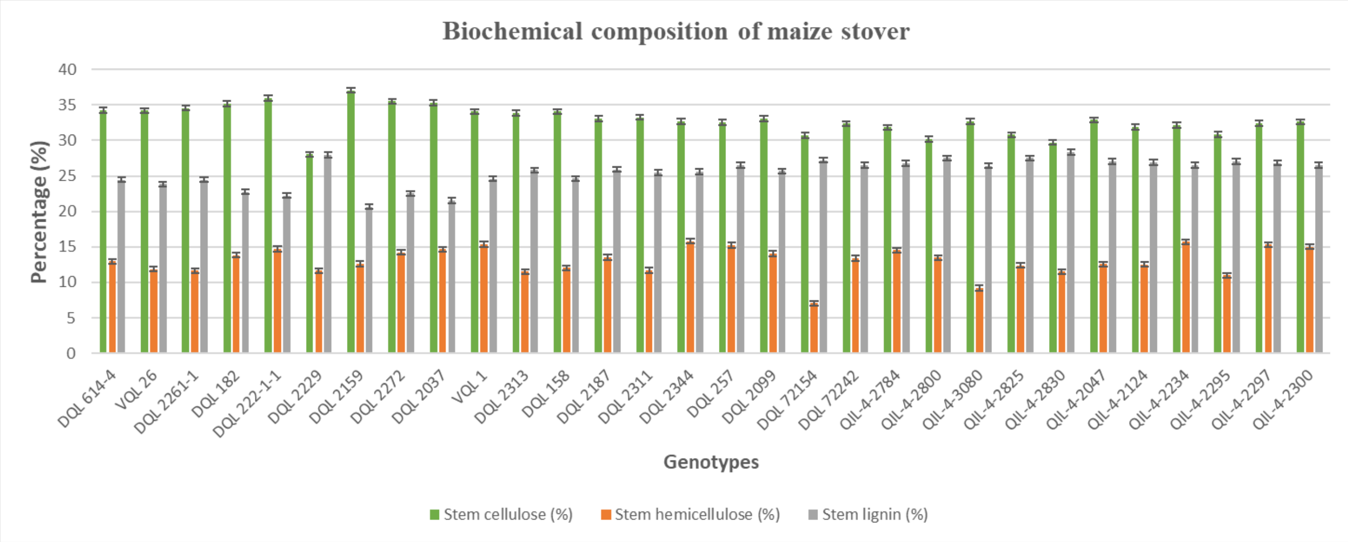

Fig. 5. Characterization of biochemical composition of maize stover.

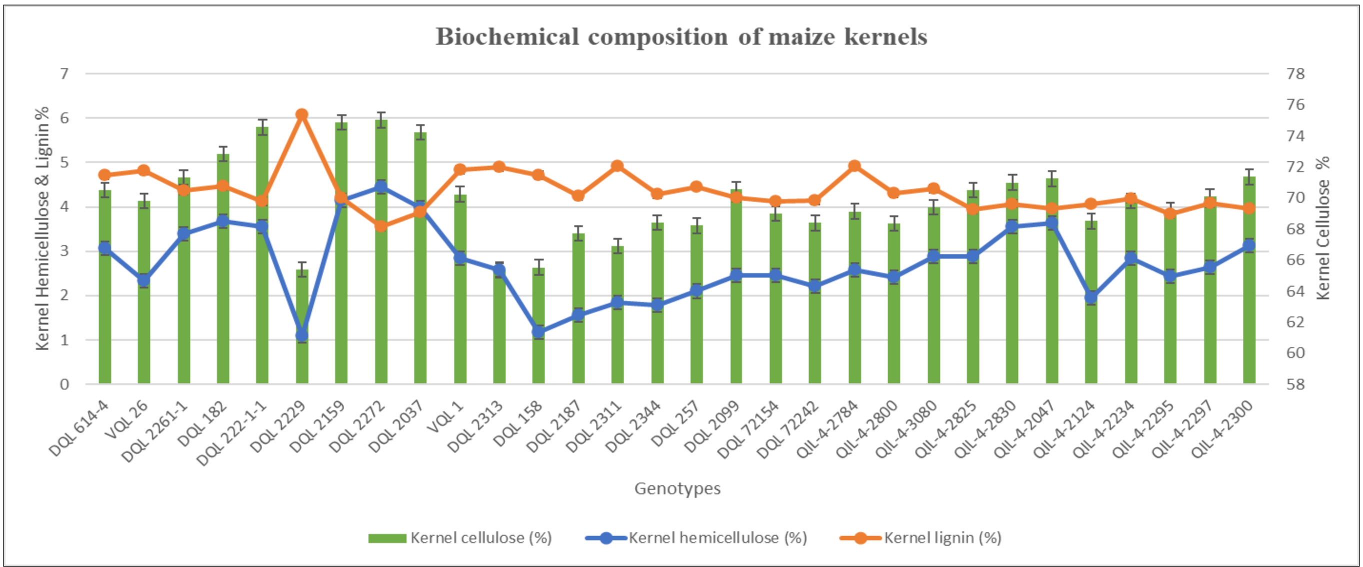

Fig. 6. Characterization of biochemical composition of maize kernels.

Table 3. Descriptive statistics for thirteen morpho-biochemical traits

Traits	Mean	Range	CV (%)	SE(±m)	CD (0.05)
Plant height (cm)	149.91	110-198	2.5	2.17	6.13
Leaf area (cm^2)	441.76	248.47-725	1.96	5.01	14.18
Number of leaves per plant	11.23	9-14	4.02	0.26	0.74
Stalk diameter (cm)	2.19	1.60-2.70	5.03	0.2	0.57
Shoot dry weight (g)	129.07	40-209.67	2.86	2.13	6.04
100 grain weight (g)	24.9	11.55-33.95	1.78	0.26	0.72
Grain yield per plant (g)	56.71	22.03-90.5	3.53	1.15	3.27
Stem cellulose (%)	32.92	28.05-37.05	0.36	0.07	0.19
Stem hemicellulose (%)	13.04	7.06-15.81	0.13	0.01	0.03
Stem lignin (%)	25.53	20.65-28.35	0.04	0.01	0.02
Kernel cellulose (%)	69.92	65.36-75.02	0.08	0.03	0.09
Kernel hemicellulose (%)	2.72	1.09-4.45	1.13	0.02	0.05
Kernel lignin (%)	4.36	3.56-6.07	0.54	0.01	0.04

A few genotypes such as DQL 2037, DQL 2272, DQL 2159 and DQL 222-1-1, exhibited mean values that were consistently higher for desirable traits, such as increased biomass accumulation and elevated cellulose and hemicellulose levels, while maintaining lower lignin content, which is essential for efficient enzymatic digestion and ethanol conversion. These genotypes outperformed others and demonstrated enhanced potential for biofuel production, highlighting their suitability for further genetic improvement and large-scale bioenergy applications.

Estimates of heritability and genetic advance for morphological and biochemical traits

The coefficient of variation (CV), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were assessed for 30 elite maize genotypes across morphological and biochemical traits. Higher CVs were noted in morphological traits, especially stalk diameter and shoot dry weight, while biochemical traits exhibited lower CVs. GCV and PCV values showed close agreement, with PCV slightly higher than GCV across all traits, indicating limited environmental influence. Traits like shoot dry weight, grain yield and kernel hemicellulose content exhibited high GCV and PCV, suggesting greater scope for genetic improvement. Heritability estimates for all traits were very high, ranging from 82.98 % in grain yield to 99.65 % in leaf area, with biochemical traits showing heritability above 99 %. Genetic advance as a percentage of mean was high (>20 %) for all morphological traits, indicating the predominance of additive gene action and potential for improvement through selection. Among biochemical traits, kernel hemicellulose content showed the highest genetic advance (63.42 %), while kernel cellulose content had the lowest (7.66 %). The above findings have been presented in Table 4.

Correlation coefficient analysis

Correlation coefficient analysis reveals the relationship among traits, with genotypic correlations consistently higher than phenotypic ones due to environmental influence. This aids in refining selection strategies for enhanced crop improvement. Genotypic and phenotypic correlations were analysed to determine the degree and direction of association among various biofuel related morpho-biochemical traits. The results for 30 maize genotypes are presented in Table 5.

Grain yield per plant showed the highest positive and significant correlation with stem cellulose content (0.527, 0.524), leaf area (0.516, 0.512), kernel cellulose content (0.513, 0.510), number of leaves per plant (0.515, 0.487) and plant height (0.486, 0.478) at both genotypic and phenotypic levels. Other positively correlated traits included stalk diameter (0.348, 0.333), kernel hemicellulose content (0.376, 0.373) and shoot dry weight (0.289, 0.287). In contrast, a strong negative correlation was observed with stem lignin content (-0.640, -0.637) and kernel lignin content (-0.239, -0.230). Meanwhile, a positive but non-significant correlation was noted with 100-grain weight (0.1452, 0.1437) and stem hemicellulose content (0.1954, 0.1947).

Plant height exhibited significant positive correlations with several traits, notably leaf area (correlation coefficients of 0.928 and 0.919), number of leaves per plant (0.851 and 0.807), stalk diameter (0.458 and 0.436), shoot dry weight (0.571 and 0.563) and stem cellulose content (0.577 and 0.569). Similarly, shoot dry weight showed significant positive associations with 100-grain weight (0.415 and 0.411) and stem cellulose content (0.319 and 0.318). Conversely, a non-significant negative correlation was observed between shoot dry weight and stem hemicellulose content (-0.1174 and -0.117).

Stem cellulose content exhibited significant positive correlations with stem hemicellulose content (0.281), kernel cellulose content (0.582) and kernel hemicellulose content (0.530). Conversely, it showed significant negative correlations with stem lignin content (-0.921) and kernel lignin content (-0.228). Additionally, kernel cellulose content was significantly positively correlated with kernel hemicellulose content (0.904) and significantly negatively correlated with kernel lignin content (-0.593). To visually represent the correlation matrix, effectively highlighting the strength and direction of associations among key agronomic and biochemical traits, a heat map has been presented in Fig. 7.

Path coefficient analysis

Path coefficient analysis is a vital tool in biofuel-oriented maize breeding as it dissects the correlations among traits into direct and indirect effects, helping breeders identify traits that most significantly influence biomass and grain yield. In the present study, both phenotypic and genotypic path analyses revealed that certain traits exert notable direct effects on grain yield per plant, thus, guiding the selection of key traits for dual-purpose maize improvement.

Among the traits analysed, kernel cellulose content exhibited the highest positive direct effect on grain yield at both phenotypic (0.2257) and genotypic (0.2856) levels. This strong influence may be attributed to the integral role of cellulose in kernel structure and grain filling, where enhanced cellulose deposition could be associated with improved kernel integrity and mass, thereby boosting yield. Additionally, cellulose contributes to dry matter accumulation, which is positively correlated with grain development and final kernel weight.

Other traits with considerable positive direct effects included number of leaves per plant (0.1533, 0.3255), stalk diameter (0.1409, 0.1643) and plant height (0.106, 0.1675), indicating that vegetative vigor contributes significantly to assimilate production and partitioning toward reproductive structures. A robust stalk and taller plant stature are generally associated with greater photosynthetic capacity and improved translocation efficiency of assimilates to the developing grains. Conversely, traits such as leaf area (-0.1941, -0.3969), shoot dry weight (-0.2141, -0.2387), kernel hemicellulose (-0.2088, -0.2763) and notably stem lignin content (-1.0548, -1.0094) exerted strong negative direct effects on grain yield. The pronounced negative impact of stem lignin is likely due to its structural rigidity and resistance to degradation, which, while

Table 4. Estimates genetic parameters for the thirteen morpho-biochemical traits in maize inbred lines

Sl. No.	Genetic parameters	CV	GCV	PCV	$h^2_{(bs)}$	GA	GAM
1	Plant height (cm)	2.5	17.93	18.10	86.09	54.83	36.57
2	Leaf Area (cm^2)	1.96	33.23	33.29	87.65	301.90	68.34
3	Number of leaves per plant	4.02	11.98	12.63	89.90	2.63	23.40
4	Stalk diameter (cm)	5.03	15.66	16.45	90.65	2.11	30.71
5	Shoot dry weight (g)	2.86	39.08	39.19	89.47	103.64	80.30
6	100 seed weight (g)	1.78	21.07	21.14	95.29	10.77	43.25
7	Grain yield per plant (g)	3.53	34.68	34.86	82.98	40.31	71.07
8	Stem cellulose (%)	0.36	5.98	5.99	99.65	4.05	12.29
9	Stem Hemicellulose (%)	0.13	15.20	15.20	99.90	4.08	31.32
10	Stem Lignin (%)	0.04	7.75	7.75	99.00	4.07	15.96
11	Kernel Cellulose (%)	0.08	3.72	3.72	99.90	5.35	7.66
12	Kernel Hemicellulose (%)	1.13	30.81	30.83	99.87	1.73	63.42
13	Kernel lignin (%)	0.54	10.97	10.99	99.76	0.98	22.58

Table 5. Estimates of phenotypic and genotypic correlation coefficients among thirteen morpho-biochemical traits in maize genotypes

	Plant height (cm)	Leaf Area (cm^2)	Number of leaves per plant	Stalk diameter (cm)	Shoot dry weight (g)	100 seed weight (g)	Stem cellulose (%)	Stem Hemicellulose (%)	Stem lignin (%)	Kernel cellulose (%)	Kernel hemicellulose (%)	Kernel lignin (%)	Grain yield per plant
Plant height (cm)^p	1	0.919**	0.807**	0.436**	0.563**	0.244*	0.569**	0.0548	-0.666**	0.598**	0.535**	-0.254*	0.478**
Plant height (cm)^g	1	0.928**	0.851**	0.458**	0.571**	0.250*	0.577**	0.0553	-0.672**	0.604**	0.541**	-0.256*	0.486**
Leaf Area (cm^2)^p		1	0.890**	0.474**	0.639**	0.347**	0.581**	0.1516	-0.715**	0.661**	0.550**	-0.228*	0.512**
Leaf Area (cm^2)^g		1	0.938**	0.494**	0.642**	0.349**	0.584**	0.1518	-0.716**	0.662**	0.551**	-0.229*	0.516**
Number of leaves per plant^p			1	0.442**	0.608**	0.390**	0.490**	0.0565	-0.639**	0.627**	0.589**	-0.213*	0.487**
Number of leaves per plant^g			1	0.498**	0.646**	0.416**	0.518**	0.0595	-0.675**	0.663**	0.618**	-0.227*	0.515**
Stalk diameter (cm)^p				1	0.653**	0.432**	0.209*	-0.0163	-0.360**	0.276*	0.225*	-0.0788	0.333*
Stalk diameter (cm)^g				1	0.683**	0.458**	0.226*	-0.0169	-0.379**	0.289*	0.236*	-0.0827	0.348**
Shoot dry weight (g)^p					1	0.411**	0.318*	-0.117	-0.516**	0.337*	0.318*	0.1735	0.287*
Shoot dry weight (g)^g					1	0.415**	0.319*	-0.1174	-0.517**	0.338*	0.320*	0.174	0.289*
100 seed weight (g)^p						1	0.0401	-0.239*	-0.1764	0.0301	0.0294	0.1891	0.1437
100 seed weight (g)^g						1	0.0401	-0.239*	-0.1769	0.0304	0.0296	0.1887	0.1452
Stem cellulose (%)^p							1	0.281*	-0.919**	0.581**	0.529**	-0.227*	0.524**
Stem cellulose (%)^g							1	0.281*	-0.921**	0.582**	0.530**	-0.228*	0.527**
Stem Hemicellulose (%)^p								1	-0.243*	0.276*	0.1326	-0.1345	0.1947
Stem Hemicellulose (%)^g								1	-0.243*	0.276*	0.1328	-0.1347	0.1954
Stem Lignin (%)^p									1	-0.642**	-0.530**	0.1305	-0.637**
Stem Lignin (%)^g									1	-0.642**	-0.530**	0.1307	-0.640**
Kernel Cellulose (%)^p										1	0.903**	-0.592**	0.510**
Kernel Cellulose (%)^g										1	0.904**	-0.593**	0.513**
Kernel Hemicellulose (%)^p											1	-0.542**	0.373**
Kernel Hemicellulose (%)^g											1	-0.543**	0.376**
Kernel lignin (%)^p												1	-0.238*
Kernel lignin (%)^g												1	-0.239*
Grain yield per plant^p													1
Grain yield per plant^g													1

*$p < 0.05$, **$p < 0.01$.

^{p}Phenotypic correlation; ^{g}Genotypic correlation.

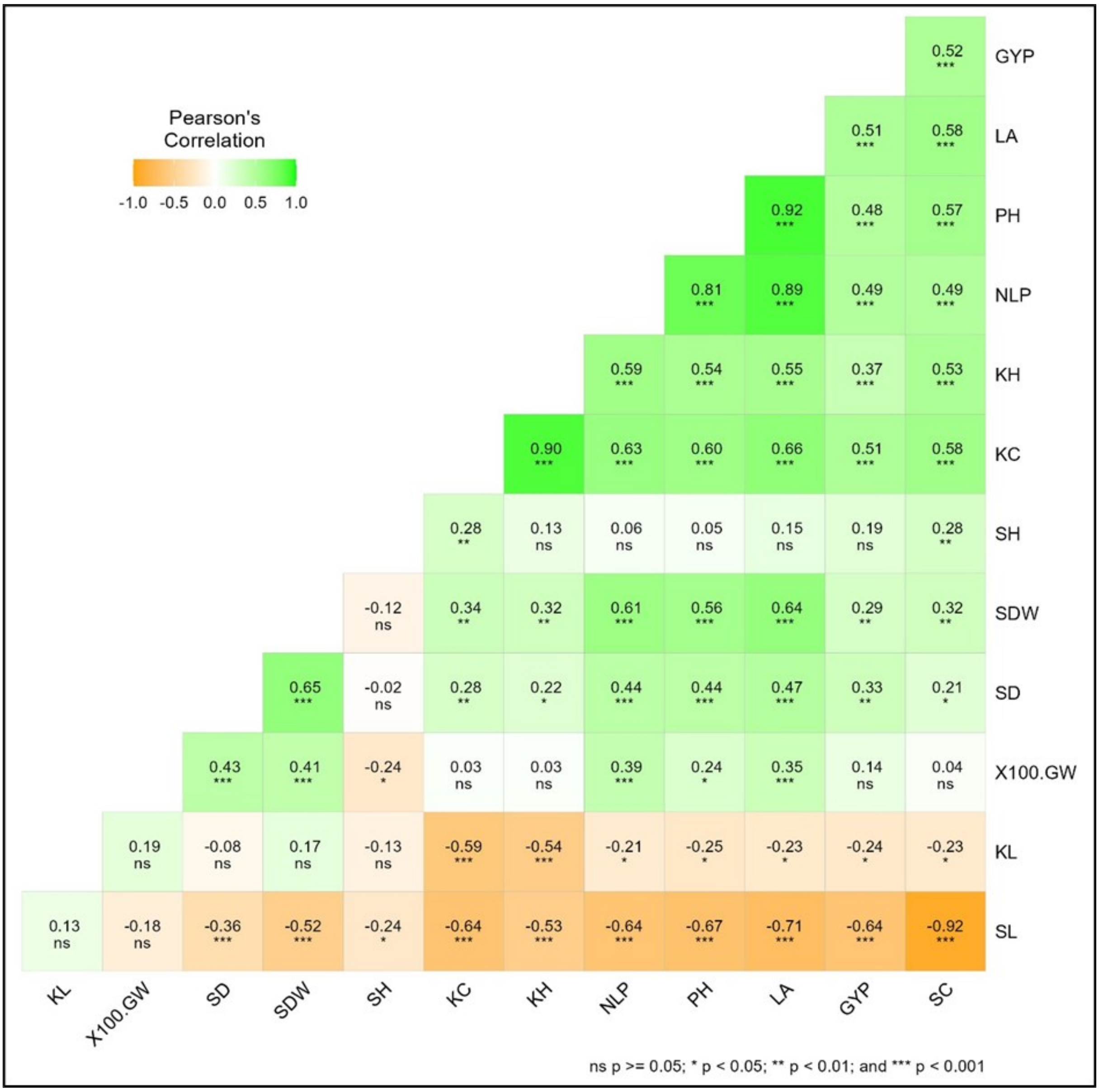

Fig. 7. Heatmap representing the strength and direction of associations among key biofuel related agronomic and biochemical traits of maize (PH: plant height; LA: leaf area; NLP: no. of leaves per plant; GYP: grain yield per plant; SDW: shoot dry weight , SD: Stem diameter; X100.GW: 100 grain weight; SC: stover cellulose; SH: stover hemicellulose; SL: stover lignin; KC: kernel cellulose; KH: kernel hemicellulose; KL: kernel lignin).

beneficial for biomass stability and biofuel feedstock quality, may compete with grain yield by diverting metabolic resources away from grain filling. The negative direct effect of shoot dry weight specifically on biofuel yield, despite its positive correlation with biomass, may be due to variations in biomass composition. High shoot dry weight can include greater amounts of non-fermentable materials, thus, reducing enzymatic efficiency. Therefore, biomass composition i.e., cellulose, hemicellulose and lignin content are more critical than quantity alone for optimizing biofuel production. This highlights the importance of focusing on both biomass quantity and its biochemical composition when selecting genotypes for biofuel optimization.

Notably, kernel cellulose (0.2257, 0.2856) content exerts the most substantial positive direct effect, while stem lignin (-1.0548, -1.0094) content has the most pronounced negative direct effect on grain yield. It also suggests a possible trade-off between biofuel-relevant cell wall components and reproductive efficiency. Excessive allocation of carbon towards complex polymers like lignin may limit carbohydrate availability for grain development. Therefore, selecting higher kernel cellulose content, along with favorable morphological traits like plant height and stalk diameter, can enhance grain yield without compromising biomass quality, making them ideal targets for dual-purpose maize breeding aimed at food and biofuel applications.

Additionally, traits such as stem lignin content, number of leaves per plant, stem cellulose content, kernel cellulose content and leaf area have significant indirect effects on grain yield. These effects are mediated through their interactions with other agronomic and biochemical traits. Therefore, these characteristics should be considered critical selection factors in breeding programs aiming to achieve high and reliable maize yields. The comparable direct influences of plant height, number of leaves per plant and stalk diameter further indicate that direct selection based on these attributes would be effective due to their inherent associations with grain yield. The genotypic and phenotypic path diagram indicating cause and effect relation between yield affecting attributes and grain yield per plant has been demonstrated through Fig. 8 and 9 respectively.

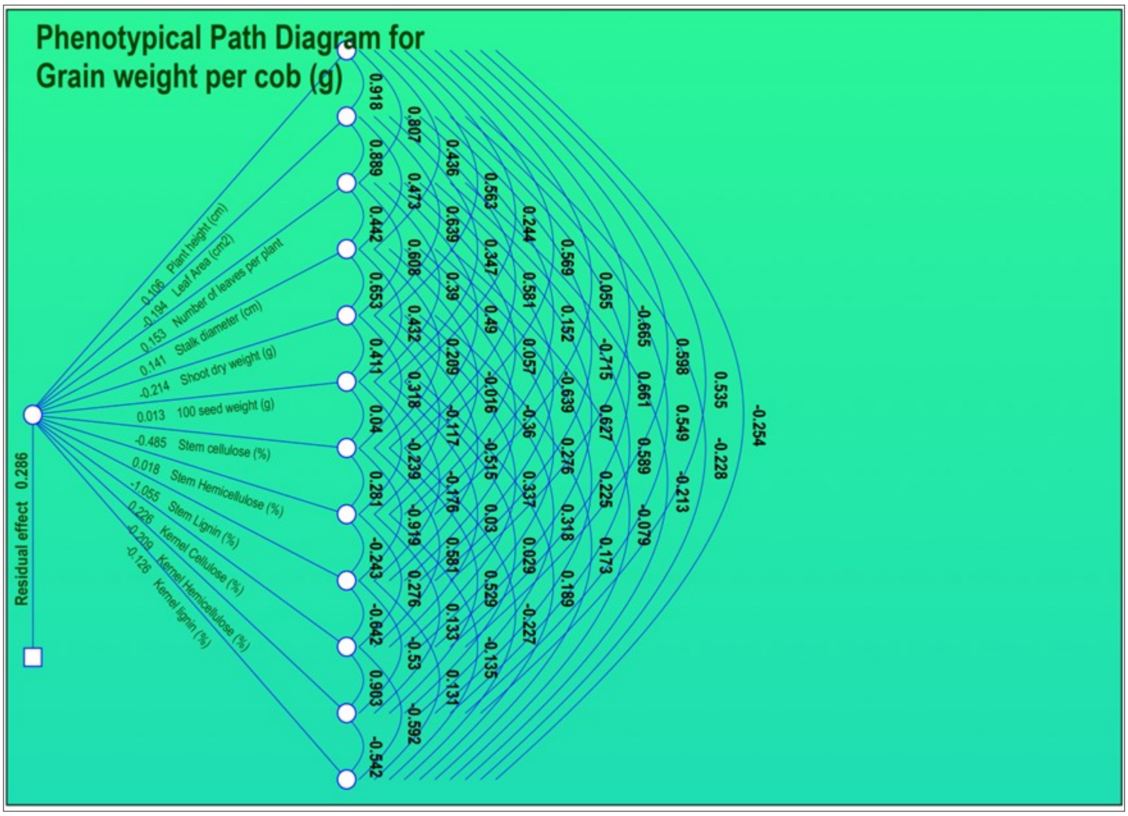

Fig. 8. Phenotypic path diagram indicating cause and effect relation between various traits and yield in maize.

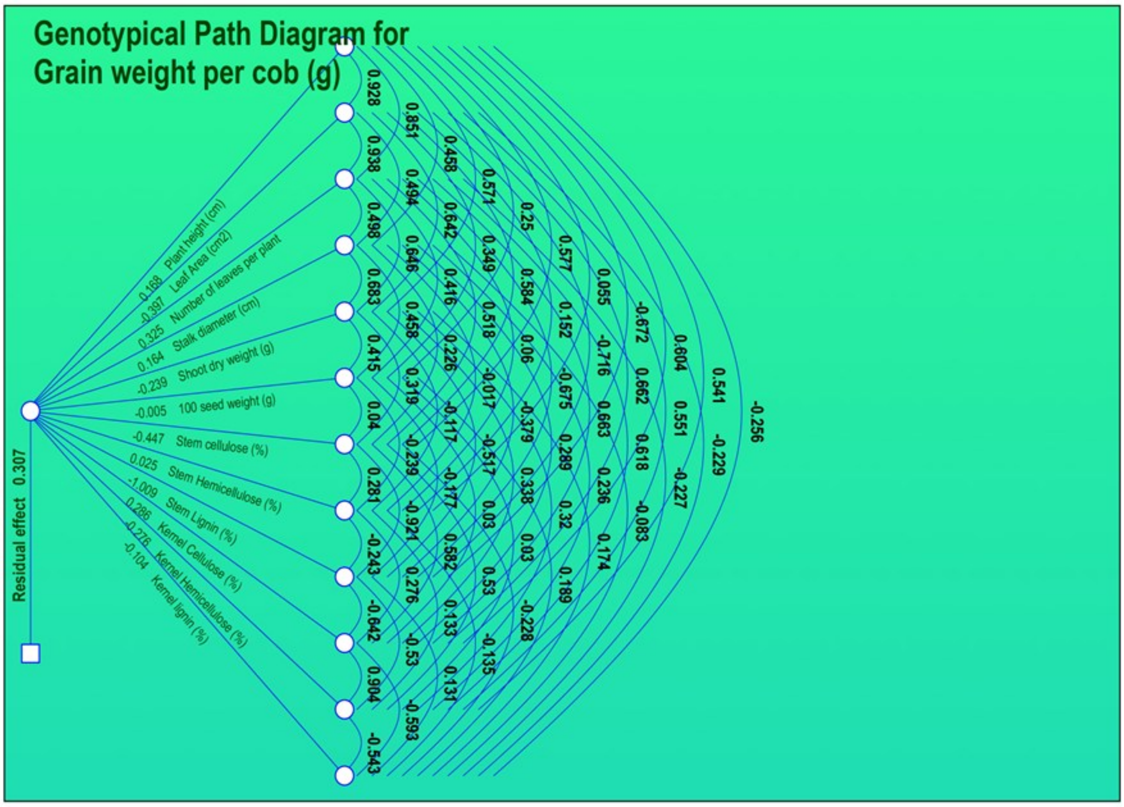

Fig. 9. Genotypic path diagram indicating cause and effect relation between various traits and yield in maize.

Genetic diversity analysis

Employing Tocher's method, the thirty maize genotypes were categorized into six distinct, non-overlapping clusters. Cluster I encompassed the largest group with 15 genotypes, followed by Cluster II with 7 genotypes and Cluster III with 5 genotypes; notably, Clusters IV, V and VI were solitary, each containing a single genotype (Fig. 10). The intra-cluster distances varied from 0 to 16, 971.12, with Cluster III exhibiting the highest intra-cluster distance (16, 971.12), succeeded by Cluster II (16, 168.75). With respect to inter-cluster distances, the highest inter-cluster distance was observed in between Clusters III and IV (297, 277.7), followed by Clusters I and III (214, 413.9). Intra and inter-cluster distance of thirty maize inbred lines based on D^2 values have been presented in Fig. 11. The mean performance of different clusters for various characters has been presented in Table 6. A detailed cluster mean analysis revealed that Cluster III possessed genotypes with superior means for traits such as plant height (190.6 cm), leaf area

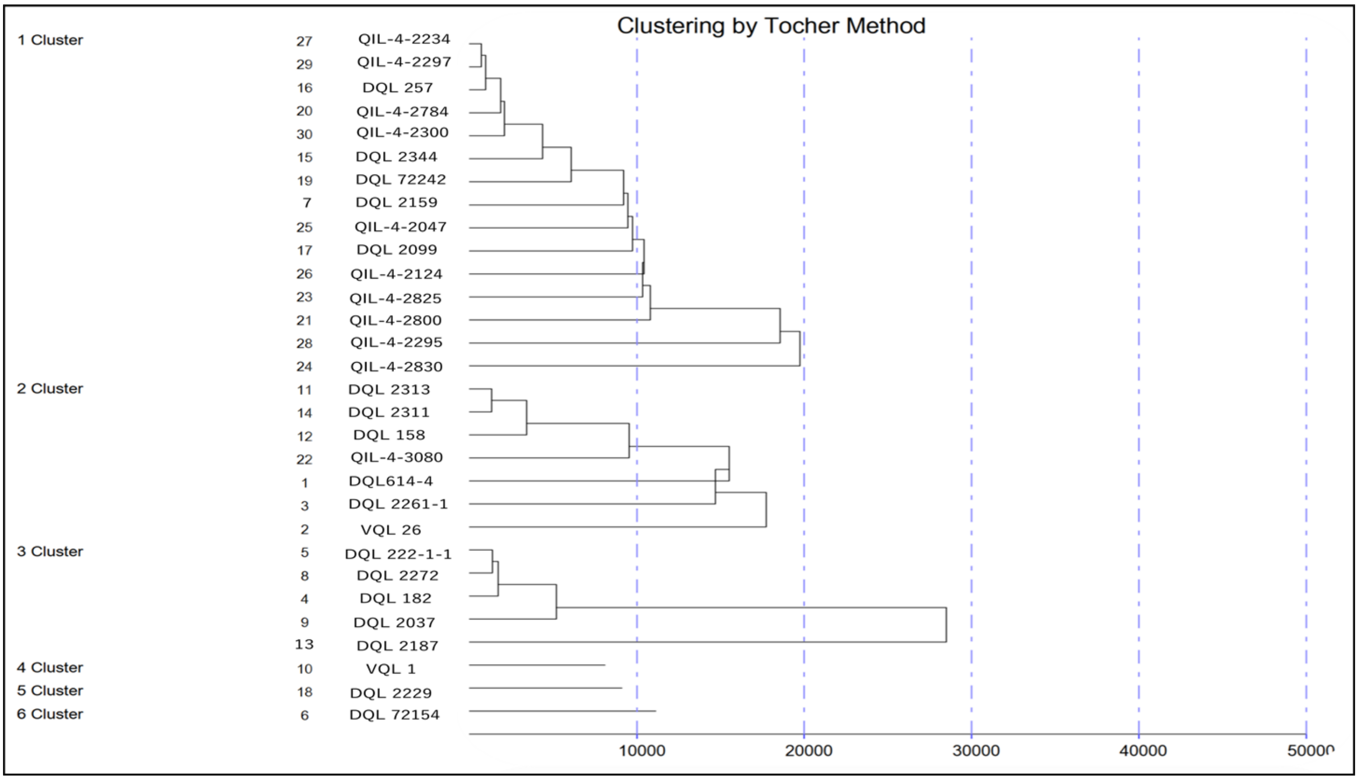

Fig. 10. Dendrogram of 30 maize inbred lines using Tocher's method.

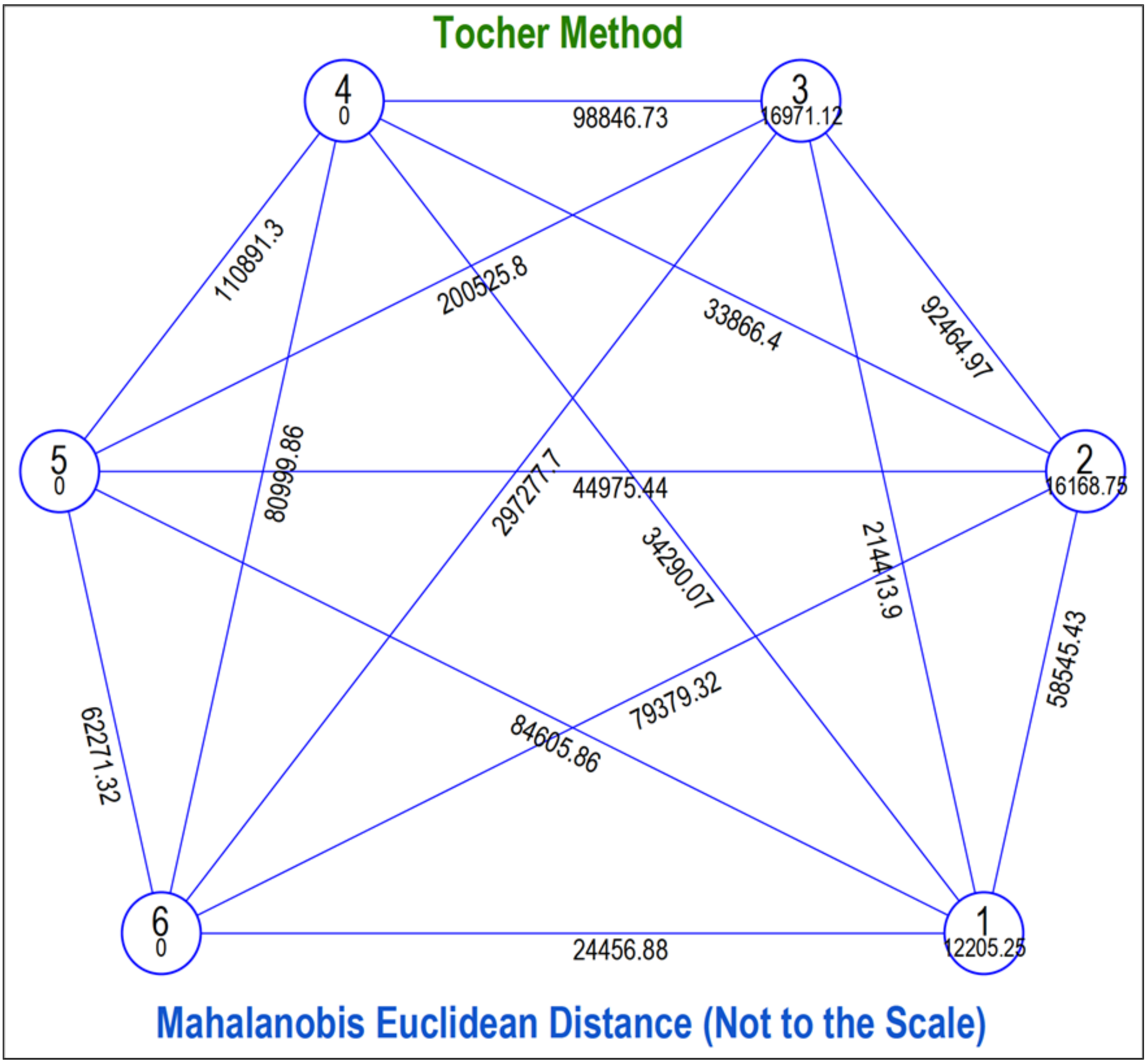

Fig. 11. Intra and inter-cluster distance of thirty maize inbred lines based on Mahalanobis Euclidean distance.

Table 6. Mean performance of different clusters for various traits was evaluated based on three replicates

	Plant height (cm)	Leaf Area (cm²)	Number of leaves per plant	Stalk diameter (cm)	Shoot dry weight (g)	100 seed weight (g)	Stem cellulose (%)	Stem Hemicellulose (%)	Stem lignin (%)	Kernel cellulose (%)	Kernel hemicellulose (%)	Kernel lignin (%)	Grain yield per plant (g)
Cluster 1	140.18	387.50	10.67	6.63	100.93	23.42	50.75	31.94	15.34	26.75	69.51	2.54	4.18
Cluster 2	154.67	448.30	11.19	6.93	141.19	28.50	57.03	33.84	11.56	25.01	68.41	2.47	4.69
Cluster 3	190.60	670.05	13.40	7.80	188.40	25.17	84.53	35.80	14.03	21.94	74.30	3.96	4.05
Cluster 4	110.00	331.38	11.00	6.00	91.00	26.50	30.00	34.08	13.74	24.61	70.25	2.84	4.84
Cluster 5	132.33	315.31	10.33	7.50	181.33	27.55	43.31	30.72	7.06	27.21	69.00	2.46	4.12
Cluster 6	116.67	305.08	10.33	6.00	155.33	26.58	45.00	28.05	11.65	27.98	65.38	1.09	6.07

(670.05 cm²), number of leaves per plant (13.4), stalk diameter (7.8 cm), shoot dry weight per plant (188.4 g), grain yield per plant (84.53 g), stem cellulose content (35.8 %), kernel cellulose content (74.3 %) and kernel hemicellulose content (3.96 %). Conversely, this cluster exhibited the lowest means for stem lignin content (21.94) and kernel lignin content (4.05 %), traits that are highly desirable for biomass production aimed at biofuel applications. Thus, Cluster III demonstrated the most advantageous trait profile for biofuel biomass production, whereas Cluster VI presented the least favourable characteristics among the studied clusters.

Discussion

Assessing the mean performance of maize genotypes for biofuel-relevant traits is essential, as it helps identify superior lines with high biomass production and optimal cell wall composition. This evaluation makes it easier to choose the genotypes best suited for producing ethanol, increasing the effectiveness and sustainability of bioenergy projects (19-21). Seven biofuel related morphological traits were analyzed. Among these stover and grain yields most significantly influence the biomass output and thus, the biofuel recovery. Hence, increased yield is one of the most important traits for efficient biofuel recovery at commercial levels. Many genotypes, such as DQL 2037, DQL 2272, DQL 2159, DQL 222-1-1 and DQL 182, performed remarkably well in terms of important morphological parameters relevant to biofuel, such as plant height, grain yield per plant and shoot dry weight per plant. For breeding dual-purpose maize genotypes, these genotypes that demonstrate high grain and stover yields play a crucial role in selection (9).

The biochemical composition of biomass significantly influences its suitability for biofuel production. High concentrations of cellulose in crops like maize facilitate enzymatic hydrolysis and fermentation, leading to increased bioethanol yields. Conversely, lignocellulosic biomass, rich in cellulose and hemicellulose but also containing recalcitrant lignin, requires pretreatment to break down structural barriers and enhance enzymatic accessibility (7). Optimizing these biochemical traits through genetic improvement and process engineering can enhance biofuel production while maintaining sustainability and economic viability (9).

While traits like high cellulose content and low lignin levels are desirable for enhancing lignocellulosic biomass quality and biofuel conversion efficiency, they can sometimes present trade-offs with grain yield (3). For instance, lignin provides essential structural integrity to plant tissues, contributing to resistance against lodging and biotic stresses. However, as observed in this study, high stem lignin content exerted a strong negative direct effect on grain yield, likely due to competition for photosynthates that would otherwise support reproductive development (7). Conversely, higher cellulose levels in kernels positively influenced yield, possibly due to their role in enhancing kernel mass and dry matter content (6). These contrasting effects highlight a critical breeding challenge like improving biomass traits for biofuel production without compromising grain productivity. Achieving this balance requires integrative breeding strategies, such as selecting genotypes with optimized lignin composition (e.g., reduced recalcitrant lignin without weakening structural function) and enhanced cellulose deposition in reproductive organs, ensuring both yield stability and biofuel suitability (9). Multi-trait selection indices and genomic tools can aid in identifying and advancing genotypes that meet both objectives (10).

The biochemical characterization of maize genotypes reveals significant potential for improving biofuel production efficiency. The observed variation in lignocellulosic composition, specifically in cellulose, hemicellulose and lignin content, indicates that certain maize genotypes possess favorable characteristics, such as higher cellulose and lower lignin content, for enhanced biofuel production (7-9). These traits are crucial for optimizing biomass conversion to biofuels, as high cellulose and hemicellulose content, combined with lower lignin levels, are desirable for improved fermentation efficiency and reduced biomass recalcitrance. While genotypes like DQL 2037, DQL 2272 and DQL 222-1-1 showed considerably higher levels of cellulose and hemicellulose with relatively lower level of lignin accumulation. These genotypes can be further used in the breeding programme to develop biofuel efficient maize varieties (22, 23). These genotypes, which exhibit superior grain and stover yield per plant, hold significant potential for developing dual-purpose maize varieties suitable for both grain production and biofuel applications.

High heritability and substantial genetic advance across most traits indicate the predominance of additive gene action, suggesting good potential for selection-based improvement. Morphological traits showed higher variability, while biochemical traits exhibited high heritability but lower variation.

Correlation studies in maize biofuel research identify key traits influencing biomass conversion efficiency, such as the positive correlation between stover yield and grain yield, enabling simultaneous improvement of both traits (24). These insights guide targeted breeding strategies to enhance biofuel production efficiency (9, 24). In maize, certain trait pairs exhibit significant correlations influencing biofuel efficiency. For instance, shoot dry weight and stalk diameter (r_p = 0.653, r_g = 0.683), as well as kernel cellulose and hemicellulose contents (r_p = 0.903, r_g = 0.904), show strong positive correlations, suggesting that as one trait increases, so does the other. Conversely, traits like stem lignin content and kernel hemicellulose content are negatively correlated, indicating that higher lignin levels may be associated with lower hemicellulose content (7, 11). Additionally, traits such as 100-seed weight versus kernel cellulose and kernel hemicellulose show no significant correlation. Notably, lignin content in both the stem and kernel displays negative correlations with most of the traits studied. Identifying the negative correlation between cellulose and lignin content is particularly beneficial for selecting maize genotypes optimized for biofuel production, as lower lignin content often facilitates more efficient biomass conversion (11, 25).

Path analysis is essential in biofuel-related maize studies as it quantifies the direct and indirect effects of key traits on biomass yield, aiding in targeted breeding strategies (e.g., see Fig. 8 and 9). Traits like plant height, number of leaves per plant, stalk diameter, kernel cellulose and stem hemicellulose show strong positive direct effects on grain yield, making them valuable selection criteria for improving biomass production. Conversely, traits such as stem and kernel lignin content exhibit negative direct effects, highlighting their role in reducing biofuel efficiency. Kernel cellulose content contributes the highest positive impact, while stem lignin content exerts the most negative effect on grain yield. Additionally, traits like stem lignin, stem cellulose, kernel cellulose and leaf area significantly influence grain yield indirectly through their interactions. Given the comparable influence of plant height, number of leaves and stalk diameter, direct selection based on these attributes could be highly effective (26, 27). Understanding these relationships enhances breeding efforts to develop high-yielding, biofuel-efficient maize varieties.

Cluster analysis using the Mahalanobis D^2 method grouped thirty maize genotypes into six clusters, with Cluster III showing the highest intra-cluster variability. The greatest inter-cluster distances were observed between Clusters III and IV, followed by Clusters I and III, indicating significant genetic divergence. Cluster mean analysis highlighted Cluster III as the most promising, exhibiting superior performance in key biofuel -related traits, including plant height, leaf area, shoot dry weight and cellulose content, while also having the lowest lignin content an essential trait for biomass conversion (28-31). These characteristics make it highly desirable for selection in biofuel breeding programs. In contrast, Cluster VI exhibited the least favourable trait combinations. With the highest cluster means for most traits under study, Cluster III stands out as the best choice for future selection and genetic improvement aimed at biofuel production.

The study highlights the potential of maize as a future “Energy crop” (7). Significant variation in lignocellulosic traits suggests their dual-purpose utility for food and energy. Based on mean performance and genetic diversity analysis, four superior genotypes viz., DQL 2037, DQL 2272, DQL 2159 and DQL 222-1-1 were identified with high grain and stover yield, along with increased cellulose and hemicellulose content. These lines hold promise for hybridization programs or direct biofuel production. While this study identifies promising genotypes, further validation across diverse environments is necessary. Integrating morphological, biochemical and genetic data will aid in developing high-performing maize cultivars for sustainable biofuel applications (22-33).

Despite providing valuable insights into the genetic diversity and biochemical composition of maize genotypes for biofuel potential, the present study has certain limitations. Firstly, the evaluation was conducted at a single location, which may not fully capture the genotype × environment interactions influencing biomass traits and grain yield. Multi-location trials would be necessary to assess the stability and adaptability of identified genotypes across diverse agro-climatic conditions. Secondly, while biochemical parameters such as cellulose, hemicellulose and lignin content were assessed, the study did not include direct measurements of enzymatic digestibility or saccharification efficiency, which are critical for determining actual bioethanol yield. Incorporating such functional assays in future research would provide a more comprehensive evaluation of feedstock quality and its practical utility in biofuel conversion processes.

Conclusion

This study highlights the potential of maize as a dual-purpose crop for both food and biofuel production. Significant variations in lignocellulosic traits among maize genotypes suggest opportunities for enhancing bioethanol yields. Notably, genotypes such as DQL 2037, DQL 2272, DQL 2159 and DQL 222-1-1 exhibit high grain and stover yields coupled with elevated cellulose and hemicellulose content, suggesting their suitability for biofuel production. Correlation analysis indicates that traits such as shoot dry weight and stalk diameter have a positive association with biomass yield. Additionally, plant height exhibits a strong positive correlation with leaf area, collectively contributing to higher biomass production. Biochemical studies further reveal a strong negative correlation between kernel lignin and kernel cellulose content. This is a highly desirable finding, as it suggests that an increase in cellulose content corresponds to a decrease in lignin, making it a valuable criterion for selection. Path coefficient analysis further identifies plant height, number of leaves per plant, stalk diameter and kernel cellulose content as key contributors to grain yield, suggesting that selection for these traits could enhance biofuel production. Cluster analysis identified Cluster III as the most promising group for biofuel breeding programs, demonstrating superior performance in eleven out of thirteen traits, with the exception of 100-grain weight and stem hemicellulose content. Hence, more genotypes should be selected from this cluster for enhanced bioethanol recovery. The findings of this study offer valuable guidance for both breeding programs and bioenergy industries. Genotypes such as DQL 2037 and DQL 2272, which exhibited favorable lignocellulosic profiles along with competitive agronomic performance, can serve as promising candidates for pre-breeding efforts aimed at improving dual-purpose maize cultivars. Moreover, their biochemical characteristics make them suitable for further evaluation in pilot-scale bioethanol conversion trials. Strategic utilization of these genotypes can accelerate the development of maize lines tailored for sustainable biofuel production without compromising yield potential. Additionally, selecting genotypes with high heritability and genetic advance ensures that improvements can be consistently passed on to future generations, facilitating breeding for dual-purpose crops that provide both food and biofuel benefits. Building upon these findings, breeders can also incorporate advanced breeding techniques to accelerate the development of hybrids with improved biofuel traits. Collectively, these findings provide a comprehensive framework for targeted breeding strategies aimed at developing high-yielding, biofuel-efficient maize cultivars. Future breeding programs should evaluate general and specific combining abilities to develop hybrids with enhanced heterosis for biofuel traits, improving biomass yield and biofuel efficiency. This will lead to the development of a Climate Smart Agriculture system that pursues three key objectives of enhanced yield, sustainable biofuel production with reduced greenhouse gas emissions and effective agri-waste management. Additionally, it will provide farmers with an extra source of income. As the demand for green fuels intensifies, research on maize-based biofuel production needs to evolve, focusing more on breeding for improved biofuel related traits in maize. Advancements in genetic improvement, multi-environment testing, process optimization, life cycle analysis and sustainable farming will maximize the role of maize in a circular bioeconomy, balancing food, feed and fuel production.

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Authors' contributions

Conceptualization was done by DS and DL. Material preparation, conduct of experiment, data collection and analysis were performed by PM, MKM and MD. The first draft of the manuscript was written by PM and checked by DS. Statistical analysis was done by AM, MRM and KCP. The manuscript was reviewed, read and edited with significant contributions by DM and KCS. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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