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RESEARCH ARTICLE

Biochemical profiling of quality protein maize inbred lines for optimized biofuel production

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Abstract

The rising demand for sustainable energy sources has necessitated the exploration of crops with enhanced biofuel production potential. Quality Protein Maize (QPM) offers a promising alternative for biofuel research due to its unique biochemical composition and high biomass yield. This study evaluated QPM inbred lines for biofuel potential through morpho-biochemical analysis. Key morphological traits, including plant height, leaf area, stalk diameter and grain yield, were assessed. Biochemical profiling measured cellulose, hemicellulose and lignin content in stover and kernels. Initial screening identified 30 high-performing lines for further biochemical evaluation. The lignocellulosic stover biomass exhibited cellulose content ranging from 28.05 % to 37.05 %, hemicellulose from 7.06 % to 15.81 % and lignin from 20.65 % to 28.35 %. In contrast, biochemical profiling of the kernel revealed significantly higher cellulose content (65.36 % to 75.02 %), lower hemicellulose levels (1.09 % to 4.45 %) and lignin content ranging from 3.56 % to 6.07 %. Inbred lines DQL 2037 and DQL 222-1-1 exhibited significantly ($p=0.05$) superior mean performance for biofuel-related morphological traits. Inbred lines DQL 2159, DQL 222-1-1 and DQL 2272 exhibited significantly higher cellulose of 37.05, 35.98 and 35.53 %, respectively along with a significantly lower lignin content of 20.65, 22.25 and 22.53 % respectively in maize stover. Principal Component Analysis (PCA) and correlation matrix techniques were employed to identify key traits contributing to variability and to cluster genotypes with superior biofuel-related traits. The identification of high-performing inbred lines with favourable lignocellulosic profiles provides a strong foundation for breeding programs focused on developing maize genotypes tailored for biofuel production. This study highlights the potential of QPM inbred lines as a renewable resource for biofuel production and provides a framework for breeding strategies aimed at enhancing biofuel efficiency.

Keywords

biofuel; cellulose; hemicellulose; lignin; quality protein maize

Introduction

The growing global energy demand and environmental concerns associated with fossil fuels have driven the search for renewable and sustainable energy sources. Among these, biofuels derived from plant biomass have emerged as a promising alternative to reduce carbon emissions and dependence on non-renewable resources (1). During biofuel production, biomass quantity, digestibility and lignocellulosic composition significantly influence energy output. Lignocellulosic biomass, primarily composed of cellulose, hemicellulose and lignin, is a key substrate for biofuel production primarily found in the secondary cell wall of biomass. High-end cellulose and hemicellulose availability is preferred for effective biofuel production, according to earlier findings. However, efficient biofuel production depends on identifying and utilizing crop genotypes with optimal biomass composition and yield.

Maize (*Zea mays* L.) is one of the most widely cultivated crops globally, renowned for its high biomass productivity and adaptability to diverse agroecological conditions. With an average productivity of 5.96 t/ha, maize is currently the most widely produced crop in the world in terms of area coverage, output and utilisation, with over 170 countries generating approximately 1241.6 million metric tonnes of maize collectively from an area of 208.2 million ha (2). Maize is primarily utilised for feed (61 %), food (17 %) and industry (22 %) (3). Stover is the term for the leftovers of maize, which include cobs, husks, stalks and leaves. The resulting heterogeneous biomass accounts for around half of the dry weight of whole plants. However, the relative amounts of each stover component were calculated; for 1 kilogramme of dried maize grains, these amounts are around 0.50 kg for stalks, 0.22 kg for leaves, 0.15 kg for cobs and 0.14 kg for husks (4).

QPM differs from conventional maize primarily due to its enhanced amino acid profile, achieved through genetic modifications. It contains higher lysine and tryptophan content as compared conventional maize. This biochemical alteration not only improves its nutritional value but also affects its starch composition and kernel texture (5). Compared to conventional maize, QPM possesses a softer endosperm with altered lignocellulosic composition, making it easily fermentable for bioethanol production. The reduced lignin content in QPM enhances enzymatic hydrolysis, facilitating higher biofuel recovery. These traits make QPM a promising alternative feedstock for bioethanol production. QPM developed through genetic enhancement for improved lysine and tryptophan content, has primarily been utilized for nutritional purposes. However, its potential as a biofuel crop remains underexplored. QPM inbred lines possess diverse morphological and biochemical traits that can be harnessed to improve biofuel efficiency, particularly through targeted breeding programs (5).

Biochemical profiling methods like High-Performance Liquid Chromatography (HPLC) quantifies cellulose & hemicellulose contents of the biomass. It provides critical insights into their lignocellulosic composition, which directly influences their suitability for biofuel production (6). Key traits such as cellulose and hemicellulose content contribute to fermentable sugar yields, while lignin content affects biomass recalcitrance and downstream processing efficiency (7). Identifying inbred lines with favourable biochemical profiles can accelerate the development of maize genotypes optimized for biofuel applications (8).

This study aims to investigate the morpho-biochemical traits of QPM inbred lines to evaluate their potential for optimized biofuel production. QPM has not been extensively studied for biofuels primarily because its breeding focus has been on improving protein quality for human and animal nutrition rather than optimizing lignocellulosic composition for biofuel production (7). Additionally, conventional maize hybrids have been the primary choice for biofuel research due to their higher biomass yield and well-characterized lignocellulosic traits. However, the softer starch content in QPM enhances its digestibility and fermentability, ultimately improving biofuel production efficiency (6). By assessing a diverse panel of QPM inbreds for key morphological and biochemical traits, this research seeks to identify superior genotypes with enhanced lignocellulosic biomass and efficiency. The findings will contribute to a deeper understanding of the biofuel potential of QPM and provide a foundation for future breeding efforts targeting renewable energy applications.

Materials and Methods

Screening of QPM inbred lines

The initial screening of 60 QPM inbred lines was conducted at the All India Coordinated Research Project on Maize, O.U.A.T., Bhubaneswar (20°52′ N, 82°52′ E) during Kharif 2022, following a Randomized Block Design (RBD) with three replications under uniform agronomic practices. Based 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, 30 promising inbred lines were selected. Stover weight and grain yield were prioritized as they are positively correlated to biomass availability and ethanol yield. The selected lines, listed in Table 1, were further evaluated during Kharif 2023 and Kharif 2024 at the same location. Observations for seven biofuel-related morphological traits were recorded and mean performance was analysed using ANOVA. Pooled analysis for RBD was done to find the mean effect across the seasons. But no significant effect was recorded for the seasons. The mean effect genotypes are compared using critical difference (CD) at 5 % level of significance (p=0.05).

Standard agronomic practices were followed to ensure optimal growth. A seed rate of 20 kg/ha with a standard spacing of 60×20 cm was taken for growing the crop. Balanced N:P:K dose of 150:80:40 kg/ha, with nitrogen applied in three splits was applied to meet the crop nutrient demand. Three irrigation was provided at critical crop growth stages for proper crop stand. Efficient weed control through pre-emergence herbicides and timely hand weeding was followed to enhances crop competitiveness.

Morphological data were recorded at key growth stages, focusing on traits critical to biomass accumulation and biofuel production potential. At maturity, plant samples both stover and cobs were collected systematically from each replicate for biochemical analysis. These stover samples were sun dried for 7 days and stored under moisture free area until further analysis. The biochemical parameters analysed included cellulose, hemicellulose and lignin content, in both stover and kernels which are directly associated with biofuel production potential (8). This experimental approach ensured robust screening, evaluation and biochemical characterization of QPM inbred lines for biofuel-related traits.

Biochemical characterization

The concentrations of cellulose and hemicellulose were measured using High Performance Liquid Chromatography (HPLC) (Shimadzu, Japan) (9). The Aminex HPX-87H BioRad

Table 1. List of QPM inbred lines

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

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column was used in a biochemical investigation (9). Three-step extraction procedure using Soxhlet apparatus was undertaken. Ground samples of stover biomass and kernel were separately taken for all the lines under study. These samples were taken in thimble and placed in syphon tubes. The Soxhlet apparatus was run using 3 different solvents i.e., hexane, ethanol and water for each sample individually. Residual solvents were eliminated through a rotary evaporator The extractive free samples of stover biomass and kernel powder were dried in hot air oven for an hour at 100 °C. Then 300 mg of the extractive free samples of stover biomass and kernel powder were separately taken and put in the pressure tube. Carefully, 3 ml of 72 % H_2SO_4 was added. The tube was then submerged in the water bath at 30 °C for an entire hour. After that 84 millilitres of deionised water were added and it was autoclaved for an hour. Next, 0.22 µm nylon filter paper was used to filter the sample. The filtrate is then added to the HPLC apparatus (SHIMADZU, UFLC) using a syringe. The chromatograms that were generated for each sample were then examined to determine the percentage of sugar composition, specifically cellulose and hemicellulose content. The residue left in the filter paper after filtration was used to calculate the amount of acid-insoluble lignin and a UV-visible spectrophotometer was used to quantify the amount of soluble lignin in the filtrate (liquid). The HPLC-performed analysis is accurate and repeatable. For each inbred line, biochemical analysis for biofuel traits was conducted using three replicated samples, which showed little to no significant differences. However, the mean of the three replicates was used for calculations.

Statistical Analysis

To test the statistical significance difference among treatment, the generated data set were analysed using the Analysis of Variance (ANOVA) method. The WINDOSTAT version 9.30 software was used to determine the mean effects of the treatments by applying least significant difference or critical difference test at a 5 % significance level.

Principle component analysis (PCA)

PCA is a statistical method that transforms correlated variables into a smaller number of uncorrelated variables called principal components, effectively reducing dataset dimensionality while preserving variance. While studying biofuel-related traits, it helps identify key traits, such as biomass yield, cellulose and lignin content that contribute significantly to variation. Key statistics in PCA include the mean vector, covariance matrix, eigen values and eigen vectors, which together help identify directions capturing the most variance in the data (10). The process involves standardizing data, computing the covariance matrix, performing eigen decomposition, selecting principal components with the largest eigen values and transforming the data accordingly (10). PCA clusters genotypes based on their performance, enabling the selection of superior lines with desirable biofuel traits. By reducing redundancy among correlated traits, PCA ensures a focus on comprehensive, non-redundant information. Additionally, it provides visual insights into genotype-trait relationships, aiding in the identification of outliers and high-performing genotypes. This systematic approach optimizes breeding efforts and streamlines decision-making in biofuel crop improvement programs. The multivariate analysis was carried out using software WINDOSTAT version 9.30. The correlation matrix was generated using the ggplot2 package of R software (version 4.4.1).

Results

Mean performance of maize varieties for morphological traits

The 60 QPM inbred lines were screened for biofuel-related morphological traits. Based on their performances for the seven characteristics mentioned above, 30 inbred lines were determined to be superior. These selected lines were subsequently cultivated in two seasons. Morpho-biochemical observations were recorded for the thirteen biofuel related traits for two seasons. The analysed genotypes showed minimal to no significant variation in traits across seasons, indicating the stability of biofuel-related traits. Therefore, the mean values from both seasons are presented in Table 2 for clarity.

Some inbred lines including DQL 2037, DQL 2272, DQL 2159, DQL 222-1-1 and DQL 182 showed considerably good performance with respect to key biofuel related morphological traits viz., plant height, grain yield per plant and shoot dry weight per plant. Inbred line DQL 2037 exhibited the highest mean performances for plant height (198 cm), leaf area (725 cm^2) and number of leaves per plant. Whereas, inbred line

Table 2. Combined mean performance of maize inbred lines for biofuel related morphological traits (2023-2024)

Sl. No.	Genotypes	Plant height (cm)	Leaf area (cm^2)	Number of leaves per plant	Stalk diameter (cm)	Shoot dry weight (g)	100 grain weight (g)	Grain yield per plant (g)
1	DQL 614-4	175.00	607.62	12.33	2.39	203.33	28.89	73.30
2	VQL 26	169.67	582.64	12.33	2.39	198.33	32.98	66.71
3	DQL 2261-1	163.33	574.85	12.00	2.55	156.00	31.62	56.79
4	DQL 182	189.33	664.34	13.00	2.39	178.00	23.48	87.44
5	DQL 222-1-1	186.67	644.39	13.33	2.39	209.67	19.60	90.50
6	DQL 2229	116.67	305.08	10.33	1.91	155.33	26.58	45.00
7	DQL 2159	185.00	632.46	13.00	2.55	174.33	33.95	81.59
8	DQL 2272	194.00	684.08	13.67	2.55	188.00	29.26	85.10
9	DQL 2037	198.00	725.00	14.00	2.55	192.00	19.58	78.00
10	VQL 1	110.00	331.38	11.00	1.91	91.00	28.50	30.00
11	DQL 2313	125.67	248.47	10.67	2.07	140.00	21.23	52.00
12	DQL 158	154.67	470.85	10.67	1.91	118.00	22.99	42.67
13	DQL 2187	149.00	380.58	10.67	2.39	95.00	21.40	31.36
14	DQL 2311	130.33	338.29	10.67	2.39	102.67	31.47	67.00
15	DQL 2344	160.00	451.80	10.67	2.71	94.33	26.81	75.00
16	DQL 257	129.67	334.18	9.00	2.55	158.00	21.35	36.47
17	DQL 2099	150.00	464.72	11.00	1.75	92.33	27.15	65.54
18	DQL 72154	132.33	315.31	10.33	2.39	181.33	27.55	43.31
19	DQL 72242	125.00	315.16	10.33	1.59	97.67	24.26	31.37
20	QIL-4-2784	144.33	387.78	10.33	2.39	155.00	20.66	38.41
21	QIL-4-2800	156.00	481.57	11.33	2.23	156.00	33.51	44.50
22	QIL-4-3080	164.00	315.41	9.67	1.75	70.00	18.14	40.73
23	QIL-4-2825	113.00	296.75	10.67	2.55	144.67	24.75	54.74
24	QIL-4-2830	174.33	578.04	13.33	2.55	145.00	29.55	35.03
25	QIL-4-2047	151.67	420.36	11.00	1.59	60.00	21.34	41.14
26	QIL-4-2124	163.67	548.36	12.33	1.91	88.00	23.24	64.04
27	QIL-4-2234	144.33	299.16	10.33	1.91	72.67	23.59	68.00
28	QIL-4-2295	113.67	269.44	10.00	2.23	55.33	23.59	81.59
29	QIL-4-2297	116.33	307.52	10.00	1.75	40.00	18.50	68.44
30	QIL-4-2300	111.67	277.06	9.00	1.59	60.00	11.55	38.63
	Mean	149.91	441.76	11.23	2.19	129.07	24.90	56.71
	CV (%)	2.50	1.96	4.02	5.03	2.86	1.78	3.53
	SE(±m)	2.17	5.01	0.26	0.20	2.13	0.26	1.15
	CD (0.05)	6.13	14.18	0.74	0.57	6.04	0.72	3.27
	CD (0.01)	8.15	18.87	0.98	0.75	8.04	0.96	4.35
	Range	110.00 -198.00	248.47 - 725.00	9.00 -14.00	1.60 - 2.70	40.00 - 209.67	11.55-33.95	30.00 - 90.50

DQL 222-1-1 performed the best with respect to yield related traits like stover yield (209.67g) and grain yield (90.50g). These genotypes exhibiting both high grain yield and stover yield per plant will be key in breeding dual purpose genotypes of maize.

Mean performance of maize varieties for biochemical traits

The thirty maize genotypes were biochemically characterized for cell wall polymers i.e., cellulose, hemicellulose and lignin accumulation. Biomass with high cellulose and hemicellulose content and low lignin levels is optimal for bioethanol production. Inbred lines like DQL 2037, DQL 2272, DQL 222-1-1, DQL 2159 and DQL 182 are having considerably higher level of cellulose and hemicellulose with relatively lower level of lignin accumulation. Inbred line DQL 2159 exhibited highest stem cellulose (37.05 %) and lowest stem lignin content (20.65 %), thus, suitable for biofuel production from stover. The inbred line DQL 2272 demonstrated the highest kernel cellulose content (75.02 %) and the lowest kernel lignin content (3.50 %), making it well-suited for biofuel production from the kernel. These inbred lines can be further be used in the breeding programme to develop biochemically biofuel efficient maize varieties. Fig.1 represents the biochemical performance of stover of maize inbred lines. Fig. 2-4 graphically represents the cellulose, hemicellulose and lignin content present in the maize inbred lines.

Genetic Diversity analysis using PCA

PCA is a dimensionality reduction technique used to transform a large set of correlated variables into a smaller set of uncorrelated variables called principal components. Table 2 displays the component loading of various features, percent variability, cumulative percent variability, principal components (eigen value larger than one) and eigen values (latent root). A total of 13 principal components were generated, of which only four had an eigen value greater than one. The contribution of these fours PCs was 84.50 % in the overall variability among the genotypes. The first principal component accounted for the highest variability (55.93 %). The major contributing traits included plant height, leaf area, shoot dry weight per plant, stem cellulose content, stem lignin content, kernel cellulose content, kernel hemicellulose content and kernel lignin content. The contribution of the rest of the principal component were PC 2 (13.41 %), PC 3 (7.85 %) and PC 4 (7.31 %) of the total variability among the genotypes. Cluster analysis based on PCA scores formed the 2 -D scattered diagram (Fig. 5) and the genotypes falling in same cluster were positioned closer to each other in the scattered diagram. The 2-D scatter diagram showed the presence of six different groupings. The interrelationships between maize traits are efficiently revealed by the genotype by trait biplot (Fig. 6).

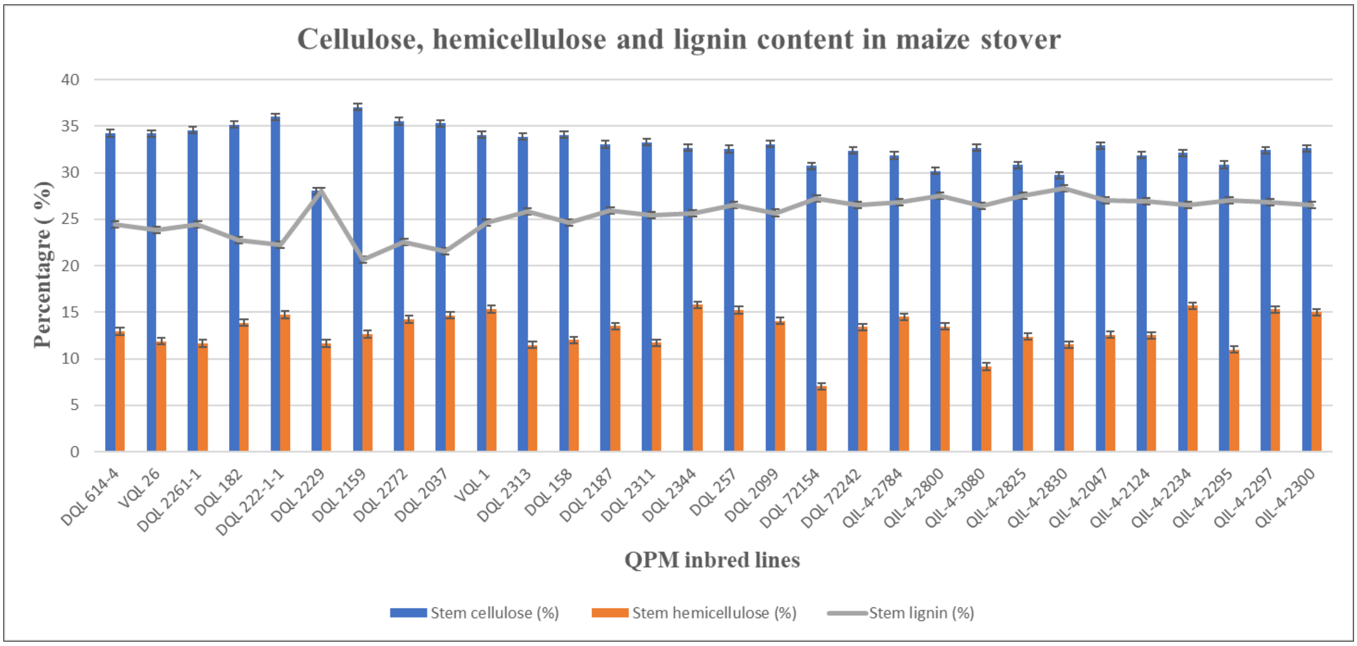

Fig. 1. Estimation of cellulose, hemicellulose and lignin content in maize stover.

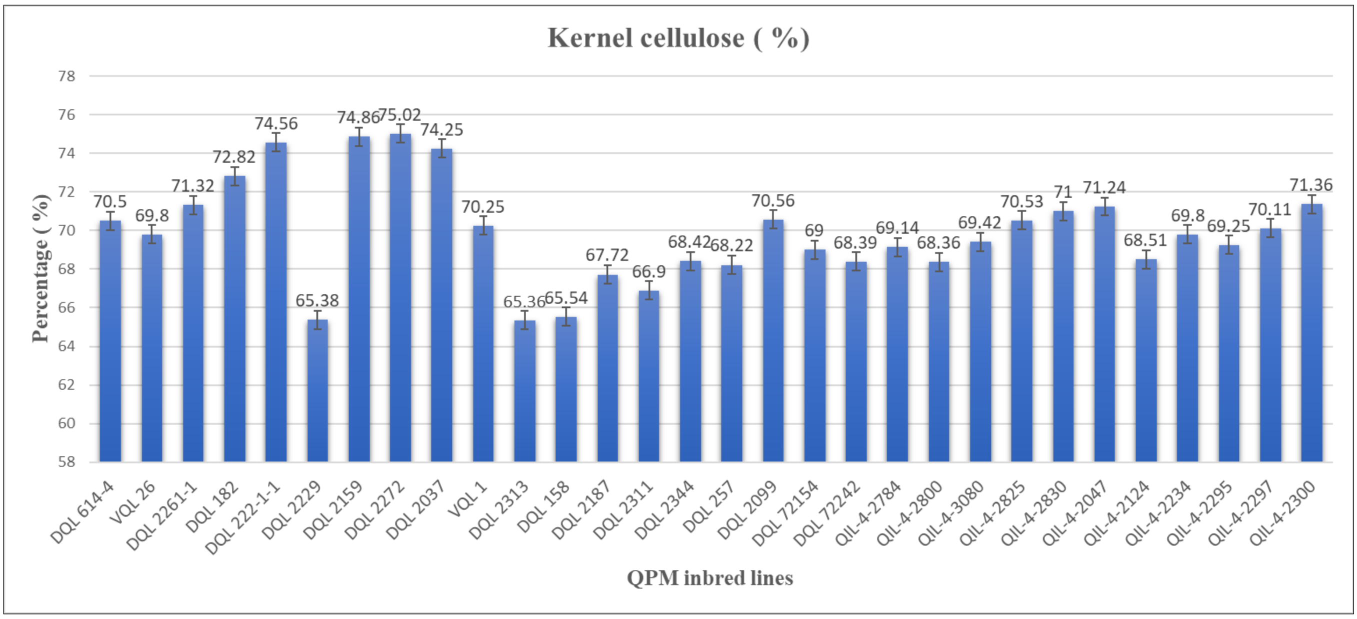

Fig. 2. Estimation of kernel cellulose content in maize genotypes.

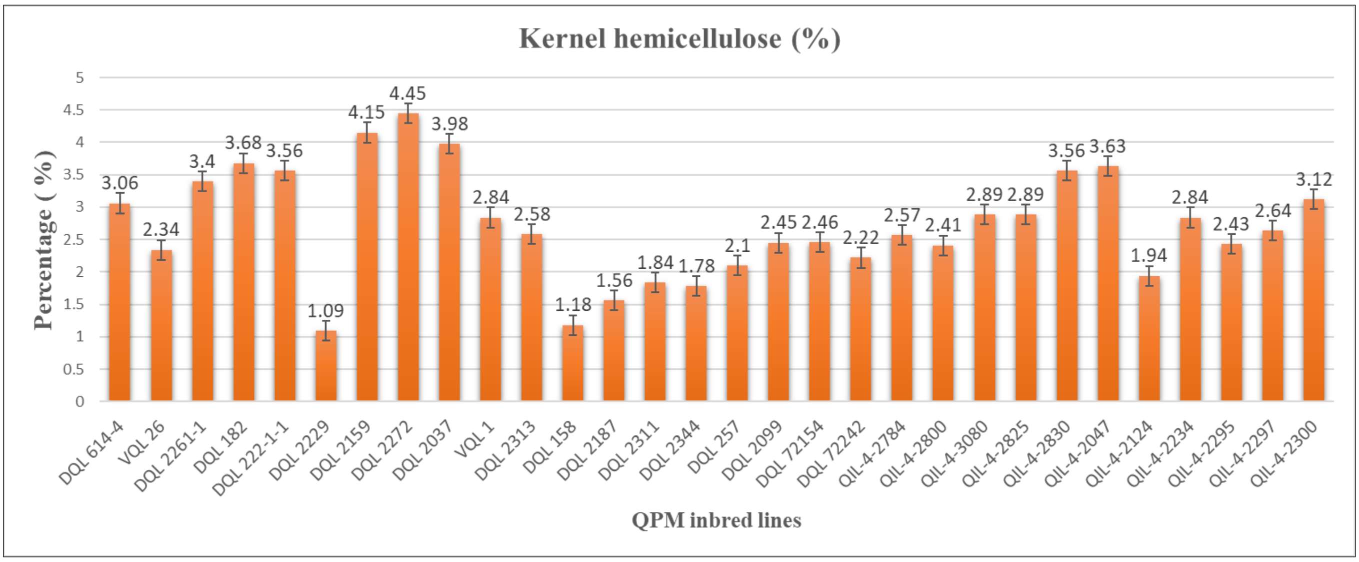

Fig. 3. Estimation of kernel hemicellulose content in maize genotypes.

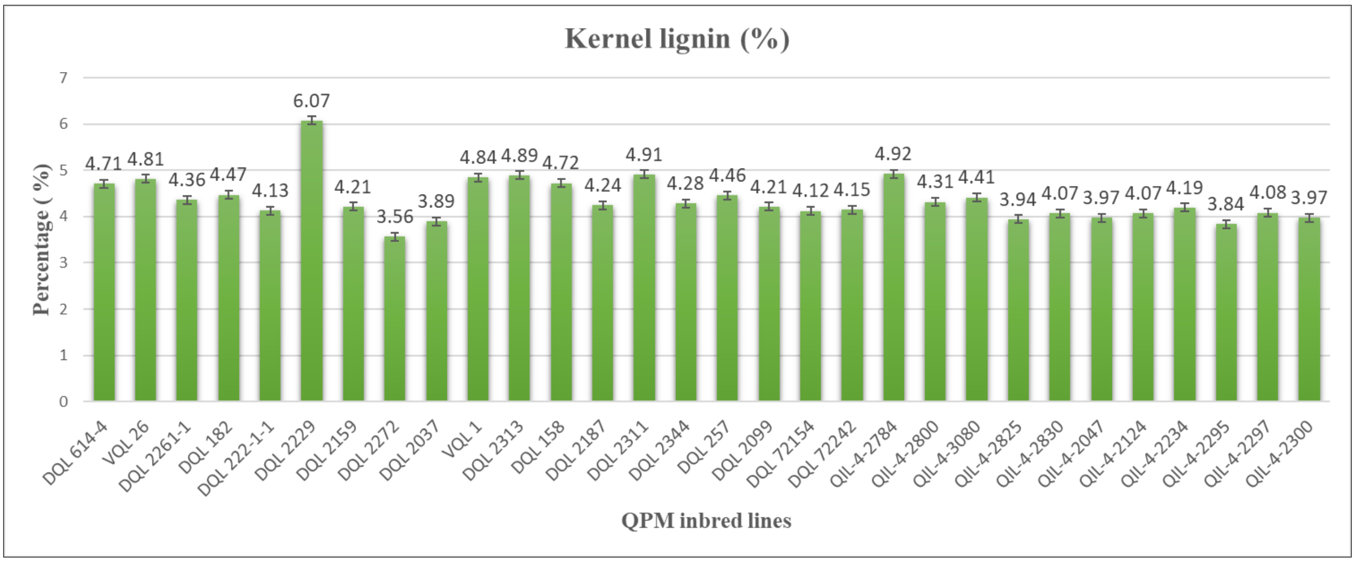

Fig. 4. Estimation of kernel lignin content in maize genotypes.

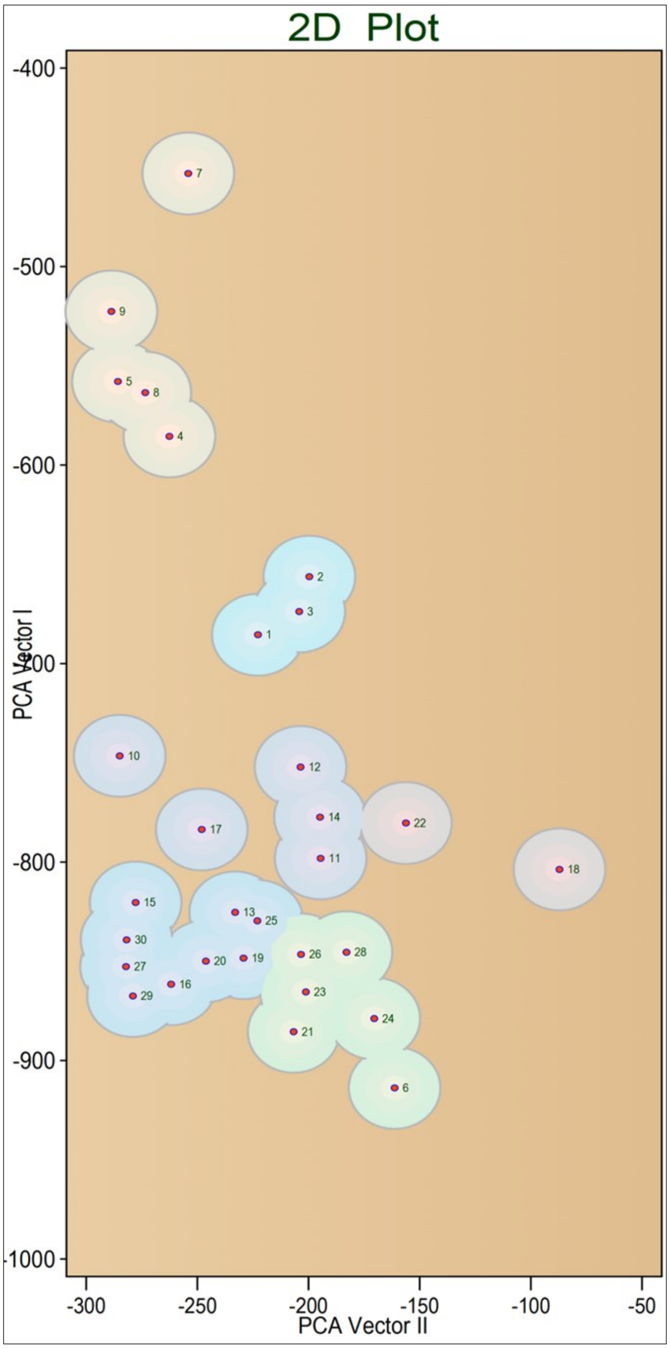

Fig. 5. Two dimensional (2D) scatter plot diagram representing the clusters of maize genotype based on genetic divergence.

The Biplot analysis (PCA) provided a clear visual representation of complex relationships between genotypes and traits. It helped understand correlations between different traits, thus, summarizing large datasets effectively, making it easier to identify key genotypes. The genotype by attribute biplot results provide an excellent estimate of the total variation of the standardisation data, explaining 64.83 % of the variation overall. According to PC1 and PC2, the following variables had relatively long vectors: stem cellulose content, stem lignin content, kernel cellulose content, kernel hemicellulose content and kernel lignin content. These variables comprised height of the plant, area of leaf, leaves per plant, stover weight per plant and 100-seed weight. These variables indicate that there was a significant variation among genotypes. Stated otherwise, they demonstrate significant variation across the 30 genotypes examined, indicating that they are highly discriminating of the morpho-biochemical data being examined. Conversely, based on PC1 and PC2, the least discriminating factors were stem hemicellulose concentration, stalk diameter and grain yield per plant (Table 3 and Fig. 6).

The biplot is divided into 4 quadrants each showing 4 sets of characters versus 4 sets of genotypes (Fig. 6). Quadrant I exhibited high values for height of the plant, area of leaf, leaves per plant, stalk diameter, stover weight per plant and 100-seed weight while quadrant IV exhibited high values for grain yield per plant, stem cellulose content, stem hemicellulose content, kernel cellulose content, kernel hemicellulose content. The trait pairs (shoot dry weight & stalk diameter; kernel cellulose and kernel hemicellulose) had an angle almost equals to zero, indicating a perfect positive correlation (+1). Due to the presence of acute (< 90°) angles among the traits of each quadrant, the variation present in them is also comparable and the recorded correlation is in between 0 to 1 (positive correlation). Trait pairs 100 seed weight vs kernel cellulose and kernel hemicellulose had (90°) right angle and hence, were not correlated with each other (zero correlation). However, the lignin content of both stem and kernel shared negative correlation (>90°), with almost all the traits under study.

Table 3. Principal components, eigenvalues (Latent root), percent variability, variability and component loading of different morpho-biochemical characters of QPM inbred lines

Sl. No.		PC 1	PC 2	PC 3	PC 4
	Eigen value (Root)	7.27129	1.74335	1.02033	0.94992
	% Var. exp.	55.93301	13.41039	7.84867	7.30711
	Cum. var. exp.	55.93301	69.34339	77.19205	84.49917
1	Plant height (cm)	0.31378	0.04749	0.37879	0.16818
2	Leaf area (cm^2)	0.33067	0.08809	0.37679	-0.06393
3	Number of leaves per plant	0.12632	0.33861	-0.14282	-0.66976
4	Stalk diameter (cm)	-0.19878	0.21592	-0.57785	-0.16763
5	Shoot dry weight (g)	0.27331	0.35216	0.1168	-0.01323
6	100 seed weight (g)	0.20322	0.4946	0.11128	-0.13951
7	Grain yield per plant (g)	0.24459	-0.1789	-0.14738	-0.17324
8	Stem cellulose (%)	0.29198	-0.33689	-0.23724	0.09137
9	Stem hemicellulose (%)	0.02668	-0.52211	0.22077	-0.59273
10	Stem lignin (%)	-0.3425	0.01128	0.29477	-0.18181
11	Kernel cellulose (%)	0.35147	-0.0446	-0.13863	0.00822
12	Kernel hemicellulose (%)	0.34303	-0.19939	-0.14059	-0.12675
13	Kernel lignin (%)	0.34122	0.00974	-0.28565	0.1801

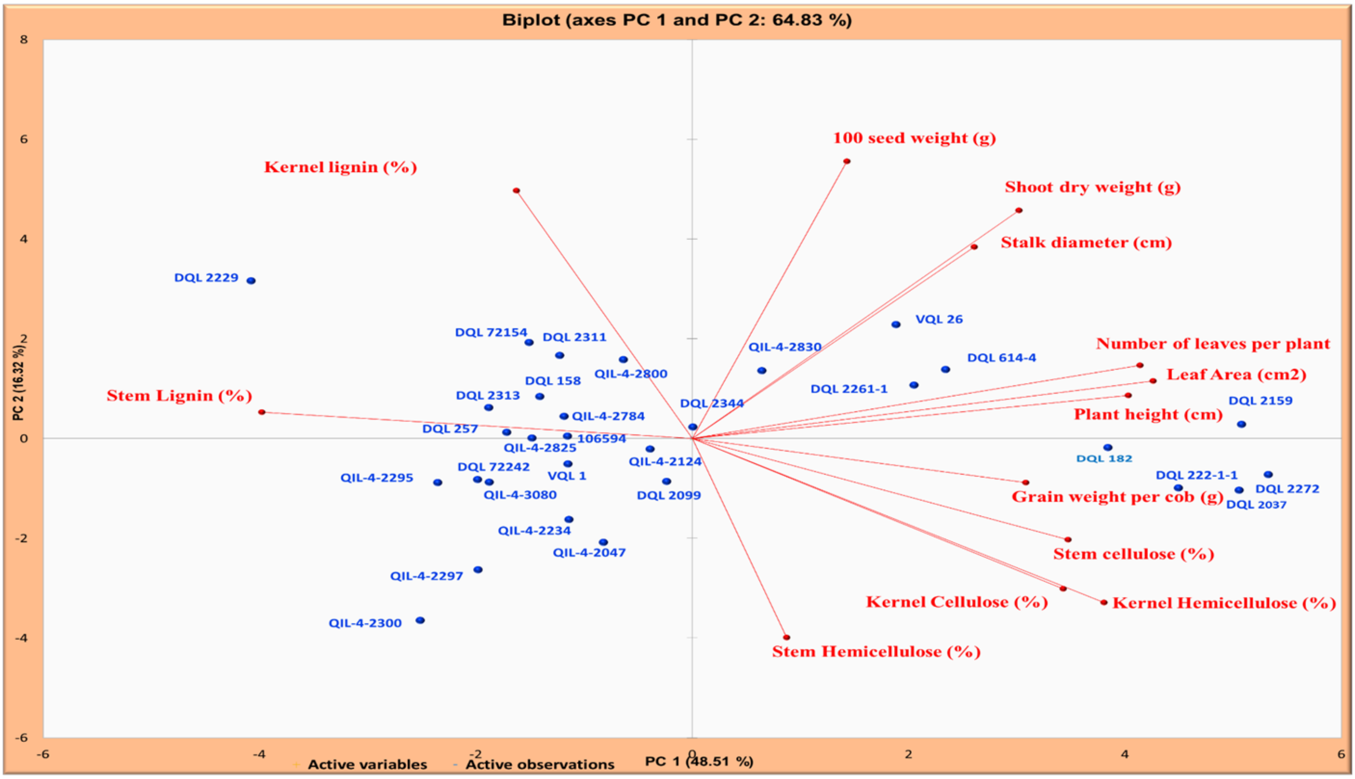

Fig. 6. Genotype by trait biplot depicting the association between PC1 and PC2 for thirty maize inbred lines and its morpho-biochemical traits.

Correlation matrix

The correlation matrix among the morpho-biochemical traits of maize revealed a strong association between these traits (Fig. 7). There was exist a significant ($p<0.001$) negative correlation between the stover cellulose and stover lignin content (r^2= -0.92). Also, the lignin content had negative correlation between all morphological characters of maize. Strong positive correlation was observed in between kernel cellulose and kernel hemicellulose content (r^2= 0.90). Overall, there was significant positive correlation exist between the stover cellulose with plant height (r^2= 0.57) and leaf area (r^2= 0.58) of maize.

Discussion

The biochemical composition of the biomasses has a direct bearing on the pyrolysis behaviour and ultimately, the bioenergy potential of rice genotypes (9-11). Pyrolysis is considered an important thermos-chemical conversion process for generation of various greener products like bio-oils. Considering the above concept, all the thirty maize genotypes were biochemically characterized for cell wall polymers i.e., cellulose, hemicellulose and lignin accumulation. The estimated values of cellulose, hemicellulose and lignin accumulation are in line with previous experiments conducted in maize (12). Various valuable green products including bioethanol can be harnessed from the biomass possessing higher level of cellulose and hemicellulose, whereas a high lignin presence increases biomass recalcitrance, making conversion more difficult (13). However higher lignin content can be useful in production of biochar which is a useful byproduct used in fertilizer industry (14).

The relationship between biochemical composition and biofuel yield is governed by several key mechanisms that

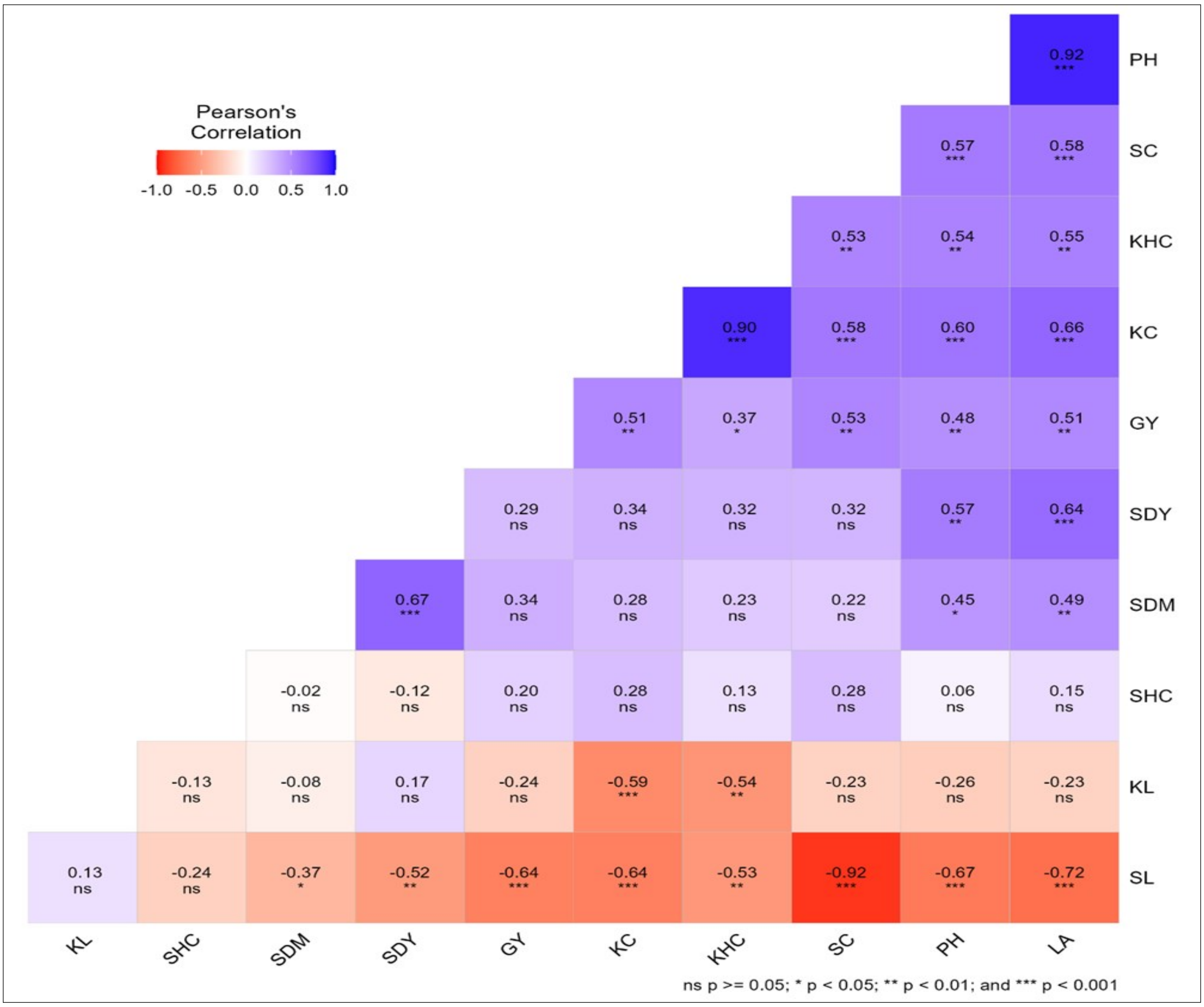

Fig. 7. Correlation matrix of morpho-biochemical traits of maize (PH: plant height; LA: leaf area; SDM: stem diameter; SDY: stover dry yield; GY: grain yield; SC: stover cellulose; SHC: stover hemicellulose; SL: stover lignin; KC: kernel cellulose; KHC: kernel hemicellulose; KL: kernel lignin)

influence the efficiency of biomass conversion into bioenergy. The composition of feedstock, particularly its cellulose and lignin, plays a crucial role in determining biofuel production potential. High starch and sugar concentrations in crops such as 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. In the case of QPM, modifications in biochemical composition, including altered cellulose and lignin profiles, may impact fermentation efficiency and overall biofuel yield. Optimizing biochemical traits through genetic improvement, enzymatic hydrolysis efficiency and process engineering can enhance biofuel production while maintaining sustainability and economic viability (14).

The biochemical profiling of QPM inbred lines presented in this study reveals significant potential for improving biofuel production efficiency. The variation observed in the lignocellulosic composition specifically in cellulose, hemicellulose and lignin content indicates that certain QPM inbred lines possess favourable characteristics like higher cellulose and lower lignin content for enhanced biofuel production (5). These traits are crucial for optimizing biomass conversion to biofuels, as high cellulose and hemicellulose content, combined with lower lignin levels, are desirable for enhanced fermentation efficiency and reduced biomass recalcitrance.

In our analysis, several QPM inbred lines exhibited superior biochemical profiles, demonstrating that not only can maize be a potential biofuel crop, but QPM can also be strategically utilized due to its dual-purpose value for both nutrition and bioenergy. The lines with higher cellulose and hemicellulose content are particularly promising, as they may provide higher fermentable sugar yields, a key factor in bioethanol production (6). Conversely, the reduction in lignin content observed in some inbreds could improve the efficiency of biomass breakdown during pretreatment processes, thus, reducing the energy inputs required in biofuel production (8).

The morphological traits analysed in parallel further support these findings, with biomass-related characteristics such as plant height, stem diameter and leaf area positively correlating with higher cellulose and hemicellulose content in certain inbreds. Some inbred lines including DQL 2037, DQL 2272, DQL 2159, DQL 222-1-1 and DQL 182 showed considerably good performance with respect to key biofuel related morphological traits *viz*., plant height, grain yield per plant and shoot dry weight per plant. These genotypes exhibiting both high grain yield and stover yield per plant will be key in breeding dual purpose genotypes of maize. This suggests that QPM lines that excel in both biomass yields could be ideal candidates for biofuel breeding programs. While inbred lines like DQL 2037, DQL 2272, DQL 222-1-1, DQL 2159 and DQL 182 are having considerably higher level of cellulose and hemicellulose with relatively lower level of lignin accumulation. These inbred lines can be further be used in the breeding programme to develop biofuel efficient maize varieties (15, 7).

Moreover, use of PCA has provided valuable insights into the relationships between these traits, enabling the identification of genotype groups that offer a balanced combination of desirable morphological and biochemical characteristics. PCA proved effective in identifying genetically diverse genotypes through a 2-D scatter plot, with four principal components (PCs) having eigenvalues greater than 1 contributing to 84.50 % of the total variability among the genotypes (16-19). For biplot visualization, the principal components PC1 and PC2 were scaled to ensure a symmetrical distribution between genotype and trait scores. The genotype-by-trait biplot captured 64.83 % of the total variation, providing a reliable representation of the standardized data.

Based on PC1 and PC2, traits such as stem cellulose content, stem lignin content, kernel cellulose content, kernel hemicellulose content and kernel lignin content exhibited relatively long vectors. These variables, which included plant height, leaf area, leaves per plant, stover weight per plant and 100-seed weight, revealed significant variability among the genotypes and served as the traits that contribute the most to distinguishing the genotypes in the morpho-biochemical dataset (20-24). The biplot divided the data into four quadrants, each representing a distinct set of traits and genotypes. From this analysis, it was concluded that genotypes in quadrant I can be selected for superior morphological traits, while those in quadrant IV are suitable for improving lignocellulosic characteristics. Certain trait pairs, such as shoot dry weight and stalk diameter, as well as kernel cellulose and kernel hemicellulose, exhibited a perfect positive correlation (+1). Conversely, traits like stem lignin and kernel hemicellulose content showed a perfect negative correlation (-1). Acute angles (< 90°) observed between traits within the quadrants indicate comparable levels of variation, with correlations ranging from 0 to +1 (positive correlation). Traits such as 100-seed weight versus kernel cellulose and kernel hemicellulose showed no correlation (90° angle), while lignin content in both the stem and kernel displayed negative correlations (> 90°) with most of the traits studied (20, 23). This identification of negative correlation between cellulose and lignin content is helpful for selection of biofuel efficient genotype of maize (23). The similar pattern of associations was also observed from the correlation matrix as well (Fig. 7).

The biochemical profiling of QPM inbred lines suggests strong potential for optimizing biofuel production. The variation observed in key lignocellulosic traits provides valuable insights into the potential of QPM as a dual-purpose crop for both nutrition and renewable energy. Based on mean performance and genetic diversity analysis using PCA, eight QPM inbred lines DQL 2037, DQL 2272, DQL 2159, DQL 222-1-1, DQL 182, DQL 2261-1, VQL 26 and DQL 614-4 were identified as superior, exhibiting higher grain and stover yield along with elevated cellulose and hemicellulose content. These lines can be widely utilised as parents in hybridisation programs or be used directly to produce biofuel. While this study serves as an important step forward in identifying superior genotypes with enhanced biofuel-related traits, further research is necessary to validate these findings under diverse environmental conditions and through advanced breeding techniques. The integration of morphological, biochemical and agronomic data, combined with a deeper understanding of genetic factors, will be essential for developing high-performing QPM cultivars tailored for biofuel applications (25-27). Ultimately, this research contributes to the broader goal of advancing sustainable biofuel production while maximizing the potential of QPM as a renewable and versatile resource.

The trade-offs between food and feed use versus biofuel production highlight the complex challenge of optimizing the multifunctionality of QPM. As a nutritionally enhanced maize variant, QPM plays a crucial role in addressing protein deficiencies in human diets and improving livestock productivity. However, its potential as a biofuel feedstock offers economic incentives and contributes to renewable energy goals (26). The primary trade-off arises from competition for land, water and other resources, which can influence food security and market dynamics. Prioritizing QPM for biofuel production may drive up prices, reducing accessibility for food and feed purposes, particularly in regions reliant on maize-based nutrition. Conversely, dual purpose maize varieties can be developed through strategic breeding programmes, where grain can be used as food/feed and stover can be diverted to biofuel production (28). Moreover, integrating sustainable practices such as crop rotation, precision agriculture and policy-driven allocation strategies can help balance these competing demands (28). A holistic approach that considers socioeconomic factors, environmental impacts and long-term sustainability is essential to maximizing the benefits of QPM across multiple sectors while mitigating adverse consequences.

In context of efficient biofuel recovery from stover, biochemical composition and biomass yield primarily plays crucial roles. Cellulose and hemicellulose serve as the primary fermentable sugars, where higher cellulose content enhances enzymatic hydrolysis efficiency, leading to increased ethanol production. Hemicellulose, although partially fermentable, contributes additional sugars that can improve biofuel yield. In contrast, lignin acts as a major barrier, reducing enzymatic accessibility and decreasing overall conversion efficiency (28). A lower lignin-to-cellulose ratio improves biomass digestibility, facilitating better saccharification and fermentation. Additionally, the presence of structural carbohydrates and lignin-associated phenolics influences pretreatment efficiency, which is crucial for breaking down complex polymers into fermentable sugars. Thus, optimizing QPM inbred lines with high cellulose, moderate hemicellulose and low lignin content enhances bioethanol yield while ensuring efficient biomass utilization (29).

The present study on the biochemical profiling of QPM inbred lines for optimized biofuel production provides valuable insights into the potential of QPM as a bioenergy feedstock. The study primarily focuses on biochemical composition. However, extensive evaluation of fermentation efficiency, enzymatic digestibility and process optimization are required which are critical for assessing economically feasible biofuel yield. Future research should be undertaken to explore large-scale fermentation trials, life cycle assessments and genetic modifications aimed at enhancing starch and sugar content while minimizing lignin interference (29). Advanced molecular and biotechnological approaches, such as metabolic engineering and enzyme optimization, could further improve conversion efficiency, making QPM a viable and sustainable bioenergy resource.

Conclusion

This study highlights the potential of QPM inbred lines for optimized biofuel production. Significant variability among the lignocellulosic traits were observed, with inbred line DQL 2037 and DQL 222-1-1 excelling in biomass-related morphological traits. While inbred lines DQL 2159, DQL 222-1-1 and DQL 2272 exhibited superior biochemical characteristics with higher cellulose and hemicellulose content and lower lignin levels. Biplot analysis and correlation matrix reveals that the negative correlation between cellulose and lignin content is useful for selecting maize genotypes with enhanced biofuel efficiency. Estimation of mean performance of morpho-biochemical traits and PCA analysis facilitated genotype selection, identifying eight high-performing inbred lines DQL 2037, DQL 2272, DQL 2159, DQL 222-1-1, DQL 182, DQL 2261-1, VQL 26 and DQL 614-4 for either direct use for biofuel production or as parents for hybridization. Further breeding programs should aim to assess their general combining ability and specific combining ability to develop hybrids with higher heterosis for biofuel related traits, thus, enhancing biomass yield and biofuel efficiency for commercial use. The use of QPM for biofuel production offers significant economic and environmental benefits. Economically, QPM provides a dual-purpose advantage, supporting both food security and biofuel production, thereby enhancing farmer's profitability and promoting energy independence. Its higher biomass yield and improved digestibility increase bioethanol production efficiency, reducing processing costs. Environmentally, QPM-based biofuels help lower greenhouse gas emissions, mitigate reliance on fossil fuels and promote sustainable land use by integrating food and energy production. Additionally, its efficient nutrient-use traits reduce the environmental footprint of cultivation, supporting a greener bioeconomy. Future advancements in genetic improvement, multi-environment testing, process optimization and sustainable farming practices will be crucial in maximizing QPM's role in a circular bioeconomy, fostering a balanced approach to 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 SRP and AM. 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.

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