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RESEARCH ARTICLE

Molecular diversity and transgressive segregation in F_2 population based on SSR markers and phenotypic evaluation in quality protein maize (*Zea mays* L.)

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Abstract

The present study was conducted during the *Zaid* (February-June, 2023) at the P.G. Research Farm, MSSSoA, Paralakhemundi, Gajapati, Odisha aimed to identify superior segregants in the (CML149 × CML330) F_2 population and assess their potential for genetic improvement using molecular markers. Among the segregants, F_2-3 and F_2-26 exhibited the highest grain yield per plant (720 g and 719.5 g, respectively) and showed transgressive segregation for yield-contributing traits such as ear length, ear girth, kernel rows per cob and kernels per row. Additionally, variations in protein content, oil content, chlorophyll content and membrane stability index were observed, highlighting their potential for nutritional enhancement and heat stress tolerance. Molecular diversity analysis was conducted in quality protein maize (QPM) using two parent genotypes (CML149 and CML330) and F_2 72 population of individuals. Initially, 91 simple sequence repeats (SSRs) markers were screened in the parent genotype and 10 polymorphic markers were selected for further analysis in the F_2 population. These 10 markers revealed significant polymorphic ranging from 0.141 (p-umc1798) to 0.812 (p-phi053) with an average polymorphic information content (PIC) of 0.373. Cluster analysis grouped the F_2 individuals into 10 clusters. The genetic variability observed in the F_2 population of QPM provides valuable insights for exploiting heterosis and developing genetically diverse breeding lines aimed at improving QPM traits.

Keywords: diversity; molecular diversity; nutrition; QPM; SSR; transgressive segregation

Introduction

Maize (*Zea mays* L.) is one of the world's most important cereal crops also used in human diets and livestock as fodder as well as a source of edible oils and bio-fuels (1). It is the primary staple food in many parts of the world grown under tropical, sub-tropical and temperate agroclimatic conditions (2). Therefore, maize serves a multifaceted and dynamic role in global agri-food systems, contributing significantly to food and nutritional security (3). Therefore, further improving the nutrient content of maize, one of the most widely consumed staple foods could serve as a practical and sustainable strategy for addressing global malnutrition, benefiting approximately 900 million people worldwide (4). For the development of superior maize varieties, it is essential to understand the genetic mechanisms governing the inheritance of various traits in breeding (5).

Quality protein maize (QPM) is one of the alternatives proposed to reduce malnutrition associated with essential amino acids as well as growth and development at different stages of life. These maize varieties were obtained through improved maize grain with higher yield and vitreous appearance by combining the *opaque-2* gene and genetic modifier systems using backcross and recurrent selection. QPMs are nutritionally described as having a higher content of lysine and tryptophan along with a high biological value and a higher protein content compared to native maize (6).

Inbred lines serve as fundamental genetic resources for maize improvement efforts. Understanding the extent and distribution of genetic diversity among maize inbred lines is crucial for effective breeding strategies to develop promising hybrids and introgression favorable alleles to enhance crop productivity and resilience (7). Molecular genetic diversity is pivotal for rapidly identifying robust hybrids without testing every possible parental combination in breeding initiatives (8). This diversity encompasses variations in morphological, agronomic traits and molecular markers reflecting genomic differences. Thus, elucidating the genetic diversity of maize inbred lines is indispensable for developing new high-yielding hybrids to sustain maize production and food security (9-11). Recent advances in DNA markers play a crucial role in revealing the genetic distances between maize inbreds. Simple sequence repeats (SSRs) are highly valued for their informativeness, multi-allelic nature, co-dominance and reproducibility (12). The evaluated maize inbred lines under study were hypothesized to demonstrate significant genetic variability, laying a foundation for developing superior maize hybrids under low nitrogen conditions in arid environments (13). The current experiment aimed to identify superior segregants in the (CML149 × CML330) F$_2$ population and assess their potential for genetic improvement using molecular markers.

Materials and methods

Plant material and DNA extraction

The experimental materials comprising two parents CML149, CML330, F$_1$ hybrid (CML149 × CML330) and the corresponding F$_2$ populations were evaluated using a compact family block design (CFBD) in two replications during the *Zaid* (February-June, 2023) at the P.G. Research Farm, MSSSoA, Paralakhemudi, Gajapati, Odisha.

Observations were recorded on a whole plot basis for days to 50 % tasseling and days to 50 % silking. Additional observations were recorded for characters such as plant height (cm), ear height (cm), ear length (cm), ear girth (cm), number of kernels row^{-1}, number of kernels cob^{-1}, 100-grain weight (g), grain yield plant^{-1}(g), canopy temperature (°C), SPAD meter, membrane stability index (%), protein content (%), oil content (%), catalase activity (U mg^{-1}) and peroxidase activity (U mg^{-1}). Leaf firing, tassel blast, root lodging was recorded based on scoring (14, 15). To evaluate predominant gene effects in maize, analyses were carried out by fitting the data into a six-parameter model to assess the additive and non-additive types of genetic effects.

The plant materials consisted of two parents CML149 and CML330 maize genotypes and 72 F$_2$ population individuals of (CML149 × CML330). Genomic DNA was extracted from fresh leaf tissue using the CTAB method (2). The quantity and quality of the extracted DNA were assessed using 0.8 % agarose gel electrophoresis. Following quantification, the DNA samples were diluted to a final concentration of 40 ng/µL for subsequent SSR analysis.

Statistical analysis

Transgressive segregation

Statistical analysis was performed according to Panse and Sukhatme (14). Following the methodology recommended by Falconer and Mackay (15). The data collected on individual plants were subjected to the statistical analysis. The data on individual plants for each character were pooled together and means, standard deviations, standard error of means, variances and standard varieties were obtained.

SSR analysis

A total of 91 SSR markers (Table 1) were initially screened to study genetic polymorphism between the parental lines CML149 and CML330. Among these only 10 markers displayed polymorphism, while the remaining markers were monomorphic. The polymorphic markers were selected for further analysis. These 10 polymorphic markers were subsequently utilized to screen 72 individuals from the F$_2$ population derived from the cross CML149 × CML330. PCR amplification was performed in a 10 µL reaction volume containing PCR buffer, dNTPs, primers, Taq DNA Polymerase and template DNA using a thermal cycler. Touchdown -PCR included an initial denaturation at 94 °C, followed by 10 cycles with decreasing annealing temperatures, 25 cycles at 55 °C annealing and a final elongation step at 72 °C. Amplified products were resolved on a 2 % agarose gel stained with ethidium bromide dye and visualized it under UV light and documented using a gel documentation system.

Band scoring and data analysis

In the study, SSR primers were used to amplify clear and distinct bands across CML149 and CML330 QPM genotypes. The bands were scored visually for their presence [1] or absence [0] generating a binary matrix for further analysis. The Polymorphism Information Content (PIC) values for each primer were calculated using Power Marker 3.5, reflecting the genetic diversity at each SSR locus.

Table 1. List of 91 SSR primers used in the study.

S. No.	primers	S. No	primers	S. No	primers	S. No	primers	S. No	primers	S. No	primers	S. No	primers
1.	p-umc2652	14.	p-umc1058	27.	p-umc1545	40.	p-bnlg118	51.	p-umc1144	66.	p-umc1562	79.	p-phi073
2.	phi120	15.	phi050	28.	bnlg391	41.	phi127	54.	p-phi059	67.	p-phi065	80.	p-phi014
3.	p-umc1546	16.	p-umc1019	29.	p-umc1415	42.	p-umc1094	55.	p-umc2019	68.	p-umc1771	81.	p-phi061
4.	p-phi036	17.	p-phi076	30.	p-phi116	43.	p-phi064	56.	phi053	69.	p-phi034	82.	p-nc130
5.	p-phi001	18.	p-umc2038	31.	p-phi071	44.	p-umc2290	57.	p-umc1515	70.	p-umc1433	83.	p-phi083
6.	p-phi076	19.	p-phi047	32.	p-umc2088	45.	p-umc1056	58.	p-umc2363	71.	p-umc1421	84.	phi054
7.	p-umc1798	20.	P-phi053	33.	p-phi295450	46.	p-umc1021	59.	p-bnlg2191	72.	p-umc1073	85.	p-umc2047
8.	p-umc2006	21.	P-umc1169	34.	P-phi085	47.	p-umc1433	60.	p-umc1019	73.	phi073	86.	phi054
9.	bnlg391	22.	nc130	35.	nc133	48.	phi002	61.	phi006	74.	phi011	87.	phi014
10.	phi024	23.	phi029	36.	phi032	49.	phi034	62.	phi046	75.	phi050	88.	phi053
11.	phi056	24.	phi059	37.	phi062	50.	phi063	63.	phi064	76.	phi065	89.	phi073
12.	umc1353	25.	umc1128	38.	phi064	51.	phi079	64.	umc1078	77.	umc2332	90.	umc2373
13.	umc1056	26.	umc2200	39.	umc2017	52.	umc2139	65.	umc2298	78.	umc1555	91.	umc1178

A similarity matrix was generated using the SIMQUAL function in NTSYS-pc and the Jaccard similarity coefficient was employed to assess genetic relationships among the genotypes. Cluster analysis was performed using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) and a dendrogram was constructed through the SAHN clustering function in NTSYS version 2.02 (16). This analysis revealed the genetic relationships and diversity among the maize genotypes based on the SSR markers used.

Results

Morphological and biochemical trait variation for F2 transgressive segregation

The evaluation of maize genotypes in the F_2 generation derived from the CML149 × CML330 cross revealed significant phenotypic variation and a high degree of transgressive segregation (T.S.) across both morphological and biochemical traits (Fig. 1). This observation underscores the effectiveness of the cross in generating novel genetic combinations through recombination. Ear girth averaged 8.628 cm, with a transgressive segregation of 3.44 %, indicating the presence of favorable alleles that enhance cob development beyond parental levels. The number of kernel rows per cob (NKPR) averaged 12.952, with a T.S. of 5.68 %, suggesting recombination-induced improvement in kernel distribution. The 100-grain weight exhibited the highest T.S. value at 9.6 %, with an F_2 mean of 32.88 g, which exceeded both parental means and the F_1 hybrid. This strong response in grain weight is reflective of polygenic inheritance and the additive effect of favorable alleles. Grain yield per plant, a key performance indicator, averaged 215.396 g and impressively, 132.32 % of the segregants demonstrated transgressive performance, highlighting the significant yield potential embedded within the population. Notably, lines F_2-3 and F_2-26 were among the top performers. Chlorophyll content in the F_2 population was 25.0512 SPAD units, with 13.64 % T.S., indicating superior photosynthetic efficiency in a subset of the segregants. Canopy temperature, an indirect marker for transpiration efficiency and stress response, averaged 32.404 °C with 13.768 % T.S., reflecting differential stomatal behavior among the lines. Protein content ranged from 6.8863 % on average, with 3.736 % T.S., reinforcing the nutritional enhancement goal of QPM breeding. Oil content showed a mean of 4.528 %, with 1.704 % T.S., again validating the nutritional potential in select recombinants. Biochemical stress-response traits exhibited varied but substantial transgressive segregation. Membrane stability index (MSI) averaged 45.98 %, with a notable 21.976 % T.S., identifying genotypes with superior membrane integrity under heat stress conditions. Catalase activity measured at 0.2058 U mL^{-1} (0.1056 % T.S.) and peroxidase activity was 0.355 U mL^{-1} (0.2024 % T.S.), indicating enhanced enzymatic ROS-scavenging capacity in certain lines.

Traits like leaf firing, tassel blast and root lodging exhibited minimal T.S. (0.08 %), suggesting low variability and limited scope for improvement through this cross alone for those traits.

Overall, the trait variability confirms the impact of recombination and segregation of complementary parental alleles. This presents a valuable reservoir for selecting lines combining yield, stress tolerance and nutritional superiority. Additional statistical analyses including variance coefficients and heritability estimates (data not shown here) also supported moderate to high heritability for grain yield and associated traits, further validating their amenability to selection. Principal component analysis (PCA) highlighted grain yield, 100-grain weight and MSI as key contributors to phenotypic divergence within the F_2 population.

Furthermore, correlation analyses revealed strong positive relationships between grain yield and ear girth ($r = 0.74$), 100-grain weight ($r = 0.78$) and kernel rows per cob ($r = 0.69$), indicating the possibility of indirect selection using these secondary traits. These findings highlight the effectiveness of

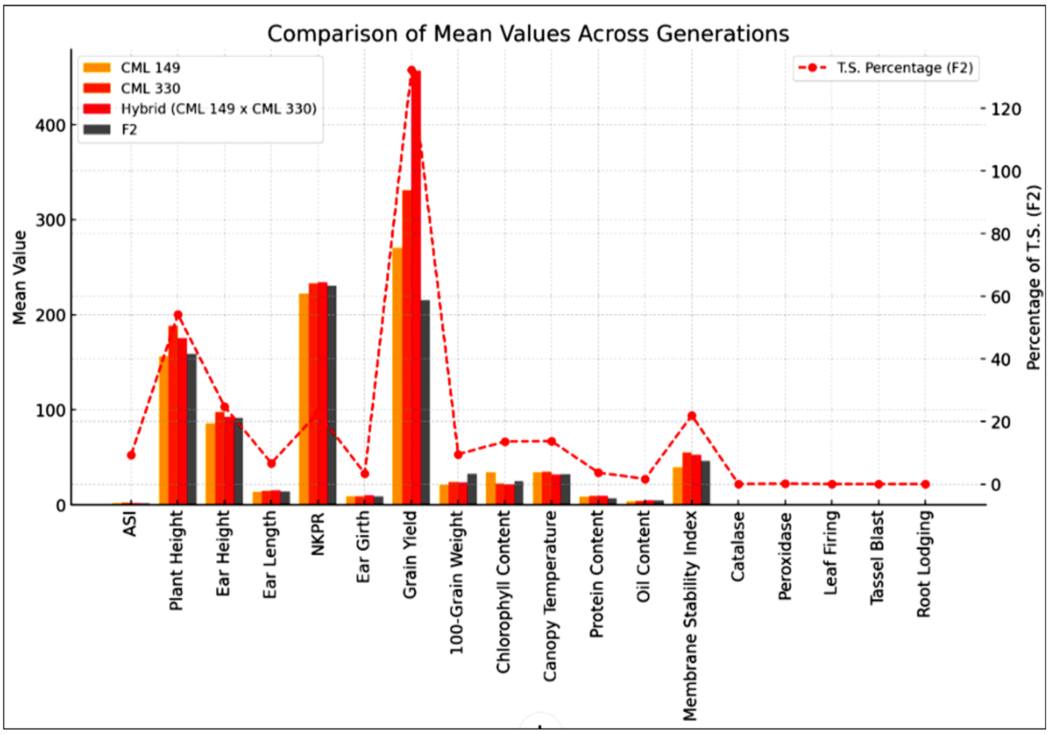

Fig. 1. Means, percentage of desirable transgressive segregants (T.S.) in different generations of the cross CML 149 × CML 330 for the traits.

combining physiological and biochemical indicators in early-generation selection strategies, thereby increasing the likelihood of identifying stress-resilient, high-yielding lines.

Molecular diversity analysis using SSR markers in F2 population

A set of 10 polymorphic SSR markers revealed a total of 20 alleles among 72 F_2 genotypes, with the number of alleles per locus ranging consistently at 2, reflecting a moderate level of genetic variability inherent in the biparental population. This consistency aligns with expected segregation ratios in F_2 generations, especially those derived from homozygous inbred lines. The average number of alleles per SSR marker was 2.0, which, while limited compared to natural populations, was sufficient for delineating genetic divergence among progeny. This demonstrates that even with a narrow marker panel, substantial differentiation can be achieved in structured populations.

DNA polymorphism

The polymorphic information content (PIC) values, a measure of marker informativeness, ranged from 0.141 (p-umc1798) to 0.812 (p-phi053) (Table 2). An average PIC of 0.373 across the panel suggests moderate informativeness, sufficient for detecting genetic polymorphism across the F_2 individuals.

Marker p-phi053, with the highest PIC value, emerged as the most informative and reliable marker, demonstrating strong utility for genotype discrimination and potentially associated with key traits (Fig. 2).

In contrast, p-umc1798, the least informative marker, may be less useful in future analyses (Fig. 3). These results confirm the robustness of SSR markers for molecular screening in early breeding generations, facilitating the identification of genotypes with diverse genomic backgrounds. It is noteworthy that several of the SSR markers used in this study have been previously mapped to QTL regions associated with grain yield, stress response and protein content, further reinforcing their relevance in marker-assisted selection.

Molecular diversity among 72 F2 inbred lines

Genetic relationships among the 72 F_2 genotypes and their two parents were analyzed using Jaccard's similarity coefficient and visualized via an unweighted pair group method with arithmetic mean (UPGMA) dendrogram (Fig. 4).

This clustering approach enabled robust grouping based on molecular distances. The dendrogram categorized the genotypes into 10 distinct clusters (Clusters I-X), confirming the presence of considerable genetic variation (Table 3). Cluster III was the largest, containing 42 genotypes, suggesting that this group has the most common allelic combinations inherited from both parents. Cluster I included 17 genotypes, potentially representing a moderately distinct subset. Clusters IX and VI contained 4 and 3 genotypes respectively, while clusters II and V included 2 genotypes each. Interestingly, clusters IV, VII, VIII and X each contained a single genotype, indicating the presence of rare or unique allelic combinations. These singleton clusters are

Table 2. Gene diversity, amplified alleles and PIC values obtained with SSR primers.

S. No	Primer name	Chromosome number	No. of alleles	Major allele frequency	Gene diversity	PIC value
1	p-phi053	3	2	0.316	0.782	0.812
2	p-phi295450	4	2	0.356	0.721	0.77
3	p-umc1021	1	2	0.556	0.563	0.472
4	p-umc2006	6	2	0.683	0.312	0.367
5	p-bnlg2191	6	2	0.672	0.371	0.345
6	p-umc1073	1	2	0.784	0.412	0.282
7	p-umc2047	1	2	0.824	0.238	0.196
8	p-phi085	5	2	0.832	0.195	0.175
9	p-umc1169	1	2	0.912	0.152	0.172
10	p-umc1798	2	2	0.941	0.162	0.141

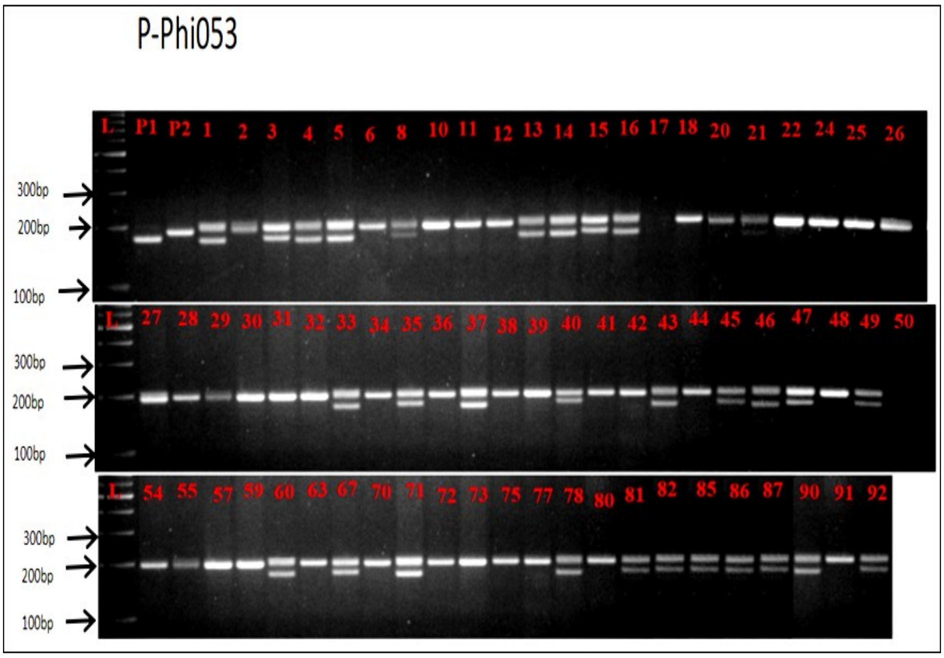

Fig. 2. Amplification pattern of primer P-Phi053.

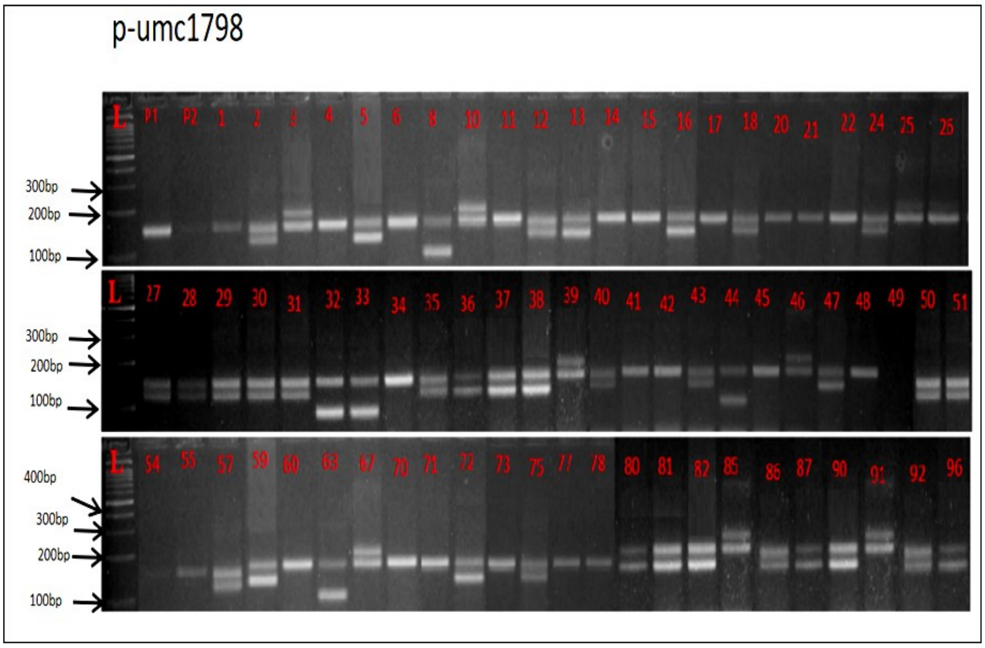

Fig. 3. Amplification pattern of primer P-umc1798.

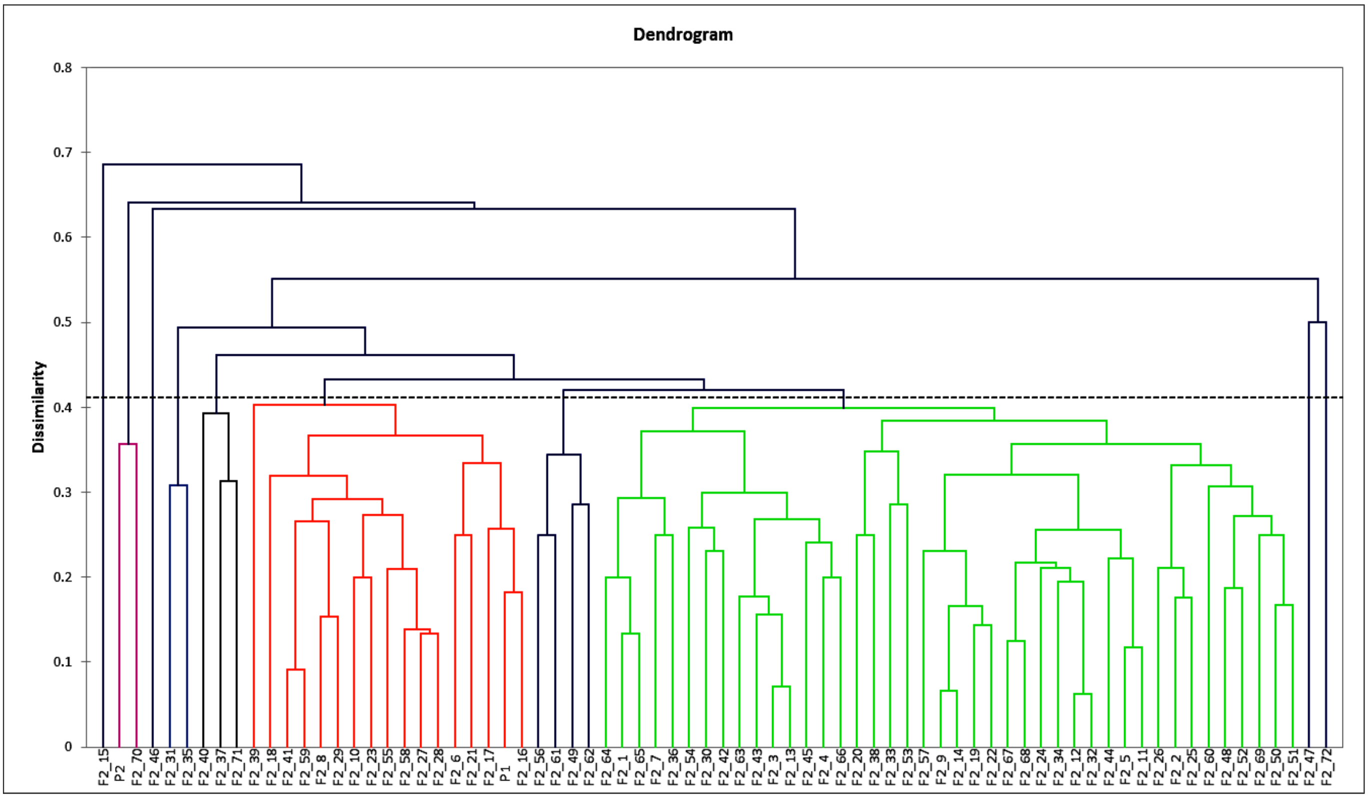

Fig. 4. The UPGMA dendrogram of 72 maize inbred lines based on SSR markers analysis.

Table 3. The UPGMA dendrogram grouped 72 inbred lines.

S. No.	Major cluster	No of genotype	Genotypes
1	Cluster I	17	CML 149, F_2 6, F_2 8, F_2 10, F_2 16, F_2 17, F_2 18, F_2 21, F_2 23, F_2 27, F_2 28, F_2 29, F_2 39, F_2 41, F_2 55, F_2 58, F_2 59
2	Cluster II	2	CML 330, F_2 70
3	Cluster III	42	F_2 1, F_2 2, F_2 3, F_2 4, F_2 5, F_2 7, F_2 9, F_2 11, F_2 12, F_2 13, F_2 14, F_2 19, F_2 20, F_2 22, F_2 24, F_2 25, F_2 26, F_2 30, F_2 32, F_2 33, F_2 34, F_2 36, F_2 38, F_2 42, F_2 43, F_2 44, F_2 45, F_2 48, F_2 50, F_2 51, F_2 52, F_2 53, F_2 54, F_2 57, F_2 60, F_2 63, F_2 64, F_2 65, F_2 66, F_2 67, F_2 68, F_2 69
4	Cluster IV	1	F_2 15
5	Cluster V	2	F_2 31, F_2 35
6	Cluster VI	3	F_2 37, F_2 40, F_2 71
7	Cluster VII	1	F_2 46
8	Cluster VIII	1	F_2 47
9	Cluster IX	4	F_2 49, F_2 56, F_2 61, F_2 62
10	Cluster X	1	F_2 72

especially valuable, as they may possess rare recombination events or minor alleles of interest for novel trait expression. Such lines should be prioritized for further evaluation.

Furthermore, genetic distances revealed through the dendrogram suggest considerable potential for heterotic group formation and cross-combination strategies. Selection of lines from genetically distant clusters can serve as a basis for hybrid development with enhanced heterosis. Future marker-trait association analysis could further validate these clusters by linking them to observed phenotypic traits, offering a genotype-to-phenotype bridge. Integration of this clustering data with phenotypic selection will enhance breeding precision and resource efficiency.

Discussions

The identification of superior segregants in the (CML149 × CML330) F_2 population underscores the potential for genetic enhancement through transgressive segregation. Notably, F_2-3 and F_2-26 exhibited the highest grain yield per plant (720 g and 719.5 g, respectively) along with favorable traits such as increased ear length, ear girth, kernel rows per cob and kernels per row (Fig. 5). This suggests the accumulation of beneficial alleles influencing multiple yield components (17-19). Similar transgressive effects have been observed in other cereal crops such as rice, where the accumulation of complementary alleles led to enhanced grain yield and plant vigor (18). In wheat, transgressive segregants have been reported to outperform parental lines for both grain yield and component traits, supporting the polygenic basis of such traits (29). These findings align with the theoretical framework that emphasizes the role of additive and non-additive gene actions in transgressive segregation, suggesting both dominance and epistasis contribute significantly to such expression (15).

Beyond yield traits, variations in protein content, oil content, chlorophyll content and membrane stability index were observed among segregants, particularly in F_2-3 and F_2-26, indicating their potential for nutritional quality improvement and heat stress tolerance. Additionally, enzymatic activity analysis revealed that F_2-26 had high catalase and peroxidase activity, further supporting its ability to withstand environmental stress conditions, consistent with previous findings (20, 21). The elevated antioxidant enzyme activity in QPM lines as a mechanism of enhanced thermotolerance, linking enzymatic response directly with sustained productivity under stress, was similarly demonstrated in earlier study (6). Catalase and peroxidase play key roles in scavenging reactive oxygen species during abiotic stress, thereby maintaining cellular integrity and function (8). These studies collectively support the current findings and validate the association between biochemical defense and transgressive performance in maize.

Assessing genetic diversity and similarity among genotypes is crucial for crop improvement. This study demonstrated the utility of SSR markers in detecting genetic diversity among maize genotypes. Out of 91 SSR markers screened, 10 polymorphic markers were used for further analysis revealing alleles ranging from 125 to 480 bp in size. The PIC values ranged from 0.141 to 0.812, with an average of 0.373. These values highlight the efficiency of SSR markers for genetic differentiation (21, 22). SSR marker polymorphism ranging from 0.287 to 0.693 in low-nitrogen conditions, supporting the use of SSRs in stress-related trait analysis was reported in previous study (13). Similar PIC distributions in SSR-based diversity analysis for leaf blight resistance in maize, reinforcing the effectiveness of this marker system for genotype profiling and trait tagging was observed in previous study (19).

The marker p-phi053 exhibited the highest PIC value (0.812), confirming its reliability for genotype discrimination. A high PIC value of 0.367 for p-phi053 has also been reported, consistent with the present observations (19). Comparable results were obtained where p-phi053 distinguished the maize hybrid DHM-117 from its parental lines (17). PIC values ranging from 0.287 to 0.693 have also been reported, consistent with the present study (23, 24). Highly polymorphic SSRs like p-phi053 are valuable for differentiating even closely related inbred lines, underscoring their importance in germplasm maintenance and

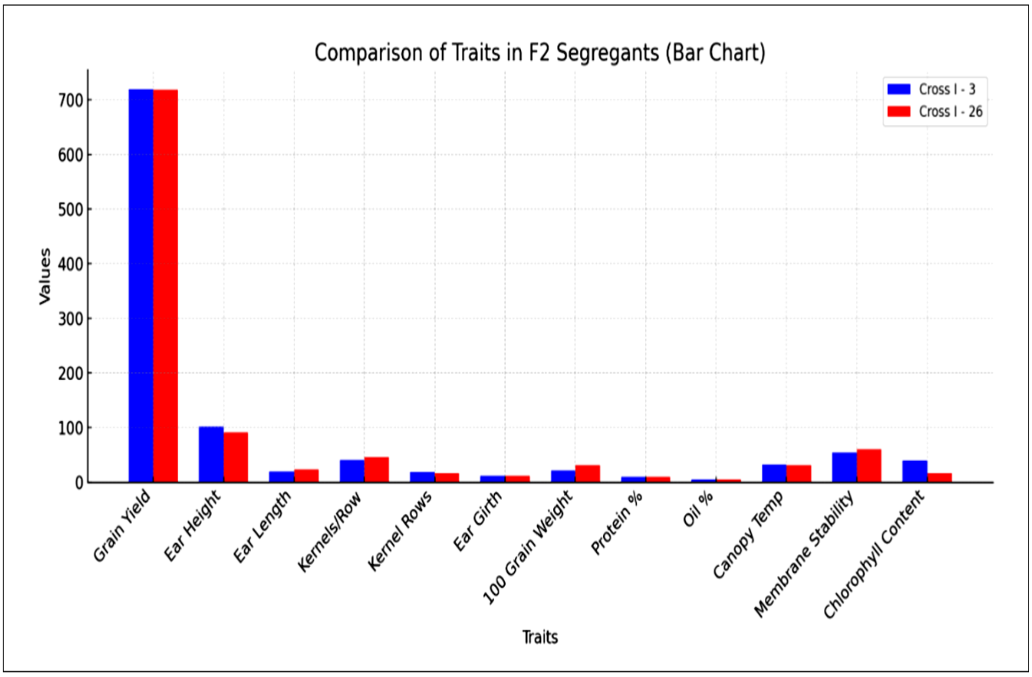

Fig. 5. Potential transgressive segregants.

purity testing (22). The p-phi053 was found to be effective for QPM and normal maize inbred characterization, contributing to efficient parentage verification and line stability (23, 26).

The UPGMA dendrogram grouped the genotypes into 10 distinct clusters, indicating substantial genetic diversity. The clustering pattern suggests that factors beyond geographical origin, such as genetic drift and gene flow, have contributed significantly to the observed diversity. This finding aligns with previous studies highlighting the role of allelic diversity in shaping genetic variability (21, 23). It has been confirmed that population structure in maize is influenced more by historical gene flow, introgression and selection pressure than by geographical separation, which echoes the patterns observed in our F_2 population (12, 22). Similar clustering patterns have been reported in SSR-based studies, supporting the use of dendrograms as powerful tools for detecting underlying genetic relationships within breeding populations (24).

High PIC value and genetic discrimination

p-phi053 exhibited the highest PIC value (0.812), indicating strong polymorphism and reliability for genotype differentiation. This marker has been previously reported to distinguish hybrids from their parental lines, making it useful for genetic purity testing and selection of superior lines (23**,** 25, 26). This finding aligns with previous work where p-phi053 was used to authenticate hybrids and inbred line integrity in QPM programs, thereby ensuring genotypic uniformity and breeding success (23, 26).

Genetic diversity and line selection

The SSR markers identified distinct allelic variations (125-480 bp), highlighting genetic diversity among segregants. The presence of significant genetic variation suggests that these markers can be applied for marker-assisted selection (MAS) to develop genetically stable inbred lines (27, 28). The role of SSR markers in trait pyramid strategies, where markers linked to yield, stress and quality traits are introgressed concurrently into elite backgrounds, expediting breeding cycles (21). The role of SSRs in assessing hybridity and guiding backcross programs in cocoa, a strategy transferable to maize line selection (25).

Association with morphological and stress-tolerance traits

F_2-3 and F_2-26 (high-yielding segregants) exhibited favorable alleles linked to grain yield, protein content and enzymatic activity (catalase & peroxidase), crucial for heat stress tolerance. Using markers linked to these traits can accelerate the selection of lines with superior performance under diverse environments. Similar results have been reported in wheat (13, 29) and noticed the function of non-additive gene effects for the trait. Similar non -additive genetic effects underpinned heterosis in maize crosses, validating the use of F2 progenies to capture dominance and epistasis for trait enhancement (11). Agronomic diversity in maize is largely governed by interaction effects, making early-generation selection critical in the breeding pipeline (9).

Application in line purity and hybrid development

The UPGMA clustering pattern suggests that selected lines can serve as diverse parental sources for hybrid breeding. The markers will help confirm the genetic purity and stability of newly developed inbred lines before hybridization (19, 21, 30). These insights contribute to the global goals of sustainable and climate-resilient agriculture. Improving maize nutritional quality without compromising yield is key to achieving the Sustainable Development Goals (3). Molecular tools such as SSRs will be instrumental in developing regionally adapted, nutritionally superior maize cultivars to address food security challenges across Africa and Asia (4).

Conclusion

The subsequent evaluation of F_2 transgressive segregants, especially those from elite hybrids CML149 × CML330 revealed superior performance for grain yield and antioxidant status. These potential transgressive segregants exhibited adaptability to showcasing their importance as genetic resources for developing high-yielding QPM varieties. This study demonstrates the effectiveness of SSR markers in assessing genetic diversity among maize inbred lines. The markers showed moderate variability with PIC values ranging from 0.141 to 0.812 highlighting p-phi053 (0.812) as the most informative. Clustering analysis revealed significant genetic diversity, providing valuable insights for selecting diverse parents for breeding. These findings emphasize the utility of SSR markers in developing superior maize hybrids through marker-assisted selection. The p-phi053 SSR marker can be integrated into a molecular breeding strategy for inbred line development. Their high discriminatory power and association with key traits make them valuable tools for enhancing maize genetic improvement and hybrid performance.

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Authors' contributions

VT carried out the experiment, took observations and analyzed the data. KR, SN guided the research by formulating the research concept and AR, IV approved the final manuscript. VR, SRN participated in the design of the study and performed the statistical analysis reviewed the. KR, SN. AR, IV, VR, AT, ZAD and SRN contributed by imposing the experiment and helped edit, summarise and revise the manuscript. KR and VR, RK, AAL helped summarize and revise the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None.

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