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Molecular characterization and assessment of genetic diversity in maize parental genotypes using RAPD markers and its relationship with hybrid performance

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Abstract

Molecular characterization provides a useful framework for assessing genetic diversity at the DNA level and can complement conventional approaches to parental selection in maize hybrid breeding. The present investigation evaluated twelve inbred lines and five male testers using Random Amplified Polymorphic DNA (RAPD) markers and examined the relationship between molecular divergence and hybrid performance. Fifteen arbitrary decamer primers produced 123 scorable amplification bands, of which 97 were polymorphic, giving an average polymorphism of 78.86%. The percentage polymorphism ranged from 66.67 to 90.91%, with OPB-05 showing the highest polymorphism (90.91%). Jaccard's genetic similarity coefficients among selected parental pairs ranged from 0.55 to 0.86. The highest similarity was observed between CML-161 and CML-163 (0.86), whereas K25 × DMH 1301 (0.55), BMI-45 × HARITA_VMH 180 (0.57), and BMI-45 × DKC 9144 (0.58) represented highly divergent combinations. UPGMA clustering grouped the seventeen parents into four major molecular clusters, with the five testers forming a distinct cluster. Comparison with hybrid evaluation showed that some highly divergent parental combinations also produced superior hybrid performance; K25 × DMH 1301, for example, recorded 241.63 g seed yield per plant, 92.64% mid-parent heterosis and 69.13% better-parent heterosis. The findings indicate that RAPD markers were effective for detecting molecular diversity and for identifying divergent parental combinations worthy of further evaluation in maize hybrid breeding.

Keywords: Maize, RAPD markers, molecular diversity, genetic similarity, heterosis, parental selection

Introduction

Maize (*Zea mays* L.) is one of the world's most important cereal crops and underpins food, feed, and broader agricultural productivity, sustaining continued demand for high-yielding cultivars and improved hybrids ^[1-4]. In modern maize breeding, heterosis exploitation remains a central route for increasing grain yield, and hybrid development is widely used to improve productivity across diverse agro-ecologies ^[1, 2, 4, 5].

The efficiency of heterosis breeding depends strongly on parental choice because parental genetic divergence and allelic interactions contribute to hybrid performance, combining ability, and the extent to which heterosis is expressed ^[1, 7-9]. Accordingly, characterization of genetic diversity among candidate inbred lines is an important component of hybrid breeding and helps identify parental combinations with a greater probability of producing superior F₁ hybrids ^[9-11]. Combining ability analysis further assists breeders in identifying superior general and specific combiners and narrowing the number of crosses requiring detailed field evaluation ^[1, 7, 6].

1.1 Diversity Assessment

Genetic divergence in maize has traditionally been assessed through morphological, biometrical, and multivariate procedures, including Mahalanobis D² clustering of agronomic traits ^[9, 10, 12, 13]. These phenotypic approaches remain informative for classifying germplasm, but molecular and phenotypic groupings may differ because phenotypic expression is partly influenced by environmental and developmental factors ^[9, 10, 14]. DNA-based markers therefore provide a complementary means of estimating genetic relationships at the genome level ^[9, 15, 16, 14].

Molecular markers can improve discrimination among parental genotypes before extensive field testing [9]. SSR and other marker systems may provide robust estimates of genetic relationships [14, 17]. Integration of molecular and conventional information can support more informed hybridization planning [9, 15].

1.2 RAPD Utility

Among PCR-based marker systems, RAPD markers have been widely used in maize diversity studies because they can detect polymorphism across the genome without prior sequence information and can generate multiple fragments from a single primer [16, 17]. RAPD has been applied to genotype identification, purity assessment, phylogenetic grouping, and heterotic classification, making it a practical low-cost diversity tool, although newer marker systems can provide greater resolution in some applications [16, 18, 19].

Table 1: Reported relationships between molecular genetic distance and hybrid performance in maize

Comparison	Main finding	Citation
RAPD distance vs hybrid productivity	Positive, significant relationship reported	[8]
SNP distance vs F_1 mean and SCA	Positive, significant for grain yield	[7]
SNP distance vs grain-yield heterosis	Non-significant in one maize study	[7]
SSR distance vs most traits	Often weak or inconsistent	
RFLP distance in tropical maize	Positive with F_1 , SCA, MPH and HPH	[11]

The literature therefore supports molecular diversity estimates, including RAPD-based measures, as a useful complement rather than a standalone substitute for conventional combining ability and heterosis analyses in maize parent selection [7, 1, 8, 15].

Therefore, the present investigation was undertaken to characterize twelve maize lines and five testers at the molecular level using RAPD markers, with the following objectives:

To estimate DNA-level polymorphism and genetic diversity among parental genotypes using arbitrary decamer primers.

To group the parental lines and testers into molecular clusters using UPGMA hierarchical analysis.

To examine the relationship between RAPD-based genetic similarity and hybrid performance, combining ability, and heterotic response for grain yield.

2. Materials and Methods

2.1 Plant Material and Genomic DNA Extraction

The experimental material comprised the seventeen parental genotypes used in the Line $\times$ Tester crossing programme: twelve inbred lines (BMI-45, CML-161, CML-163, CML-165, CML-224, CML-465, CML-468, HKI-163, HKI-323-B, K25, LM-14 and POP-61) and five male testers (DKC 9144, DKC7044, DMH 1301, HARITA_VMH 180 and PAC 751). Fresh, healthy leaf samples were collected from young seedlings. Genomic DNA was isolated using a modified CTAB extraction protocol. DNA quality and integrity were assessed by 0.8% agarose gel electrophoresis and spectrophotometric analysis before RAPD amplification.

2.2 RAPD-PCR Amplification

PCR amplification was performed using fifteen random decamer RAPD primers under standardized reaction and thermal-cycling conditions. Amplified products were separated by agarose gel electrophoresis and visualized

RAPD has detected high polymorphism in maize lines and cultivars [16]. RAPD has been used to separate elite maize lines into distinct genetic groups [8]. RAPD can support fingerprinting and purity assessment of hybrids and inbreds [16].

1.3 Genetic Distance and Heterosis

An important objective of molecular diversity analysis is to determine whether parental genetic distance predicts hybrid performance, heterosis, or combining ability [11, 15, 7]. Evidence is not uniform. Some studies have reported positive relationships between molecular genetic distance and hybrid productivity, F_1 performance, or SCA, whereas other studies have found weak or non-significant relationships with grain-yield heterosis itself [7, 8, 11, 17]. Thus, the predictive value of molecular distance depends on the germplasm, marker system, trait, and environment.

using a gel documentation system.

2.3 Electrophoresis and Scoring

Amplified PCR products were resolved on 1.5% agarose gels and scored manually for the presence (1) or absence (0) of clear and reproducible amplification fragments across the seventeen parental genotypes.

2.4 Statistical and Cluster Analyses

Polymorphism percentage was calculated from the total and polymorphic bands generated by each primer.

Binary banding matrices were used to calculate Jaccard's genetic similarity coefficients among parental genotype pairs.

UPGMA hierarchical clustering was used to construct a dendrogram showing molecular relationships among lines and testers.

Molecular similarity was compared with corresponding hybrid performance, SCA and heterosis information obtained from the Line $\times$ Tester evaluation.

3. Results and Discussion

3.1 RAPD Polymorphism and Marker Efficiency

All fifteen RAPD primers produced clear and reproducible amplification profiles across the seventeen parental genotypes. The primers generated 123 bands, of which 97 were polymorphic and 26 were monomorphic. The average number of bands per primer was 8.20, while average polymorphic bands per primer was 6.47. Mean polymorphism was 78.86%, with a range of 66.67-90.91%. OPB-05 generated the maximum number of bands (11) and the highest polymorphism (90.91%), followed by OPC-05 (90.00%) and OPB-03 (88.89%). OPA-04 and OPC-04 showed the lowest polymorphism (66.67%). These results demonstrate substantial molecular variability among the parental material.

Table 2: Summary of RAPD polymorphism detected among maize parental genotypes

Parameter	Observation
Total RAPD primers screened	15
Primers showing amplification	15
Total bands amplified	123
Total polymorphic bands	97
Total monomorphic bands	26
Average bands per primer	8.20
Average polymorphic bands per primer	6.47
Average polymorphism (%)	78.86
Maximum bands amplified by primer	11 (OPB-05)
Minimum bands amplified by primer	6 (OPA-04 and OPC-04)
Highest polymorphism (%)	90.91 (OPB-05)
Lowest polymorphism (%)	66.67 (OPA-04 and OPC-04)
Range of polymorphism (%)	66.67-90.91

3.2 Genetic Similarity among Parental Genotypes

Jaccard's similarity coefficients ranged from 0.55 to 0.86, indicating the coexistence of closely related and highly divergent parental combinations. The highest similarity (0.86) was observed between CML-161 and CML-163,

whereas K25 × DMH 1301 (0.55), BMI-45 × HARITA_VMH 180 (0.57), and BMI-45 × DKC 9144 (0.58) represented highly divergent combinations. The similarity estimates obtained from the result chapter are summarized below.

Table 3: Selected Jaccard's genetic similarity coefficients among maize parental genotypes

Genotype pair	Similarity coefficient	Genetic relationship
BMI-45 × CML-465	0.82	Closely related
CML-161 × CML-163	0.86	Highly similar
HKI-163 × HKI-323-B	0.79	Moderately similar
K25 × POP-61	0.61	Genetically diverse
BMI-45 × DKC 9144	0.58	Highly diverse
CML-224 × PAC 751	0.63	Genetically diverse
CML-468 × DMH 1301	0.67	Moderately diverse
LM-14 × HARITA_VMH 180	0.65	Moderately diverse
HKI-323-B × DKC7044	0.60	Genetically diverse
K25 × DMH 1301	0.55	Highly diverse
BMI-45 × HARITA_VMH 180	0.57	Highly diverse
CML-465 × PAC 751	0.62	Genetically diverse

The relatively low similarity coefficients observed for K25 × DMH 1301, BMI-45 × HARITA_VMH 180 and BMI-45 × DKC 9144 indicate wide molecular divergence and distinct genetic backgrounds. Conversely, the high similarity between CML-161 and CML-163 indicates a comparatively close molecular relationship.

3.3 UPGMA Cluster Analysis

UPGMA analysis grouped the seventeen parental genotypes into four major molecular clusters. Cluster I consisted

primarily of BMI-45, CML-224 and CML-468. Cluster II included CML-161, CML-163, CML-465, LM-14 and POP-61. Cluster III comprised CML-165, HKI-163, HKI-323-B and K25, with K25 occupying a distinct sub-branch. Cluster IV consisted exclusively of the five male testers—DKC 9144, DKC7044, DMH 1301, HARITA_VMH 180 and PAC 751. The grouping was consistent with the molecular similarity estimates, particularly the close placement of CML-161 and CML-163.

Table 4: UPGMA molecular cluster composition of the seventeen maize parents

Cluster	Genotypes
I	BMI-45; CML-224; CML-468
II	CML-161; CML-163; CML-465; LM-14; POP-61
III	CML-165; HKI-163; HKI-323-B; K25
IV	DKC 9144; DKC7044; DMH 1301; HARITA_VMH 180; PAC 751

The separation of the five testers into a distinct molecular cluster indicates marked differentiation between the female line pool and tester pool. Such differentiation provides a

useful molecular basis for examining the performance of crosses involving contrasting parental groups.

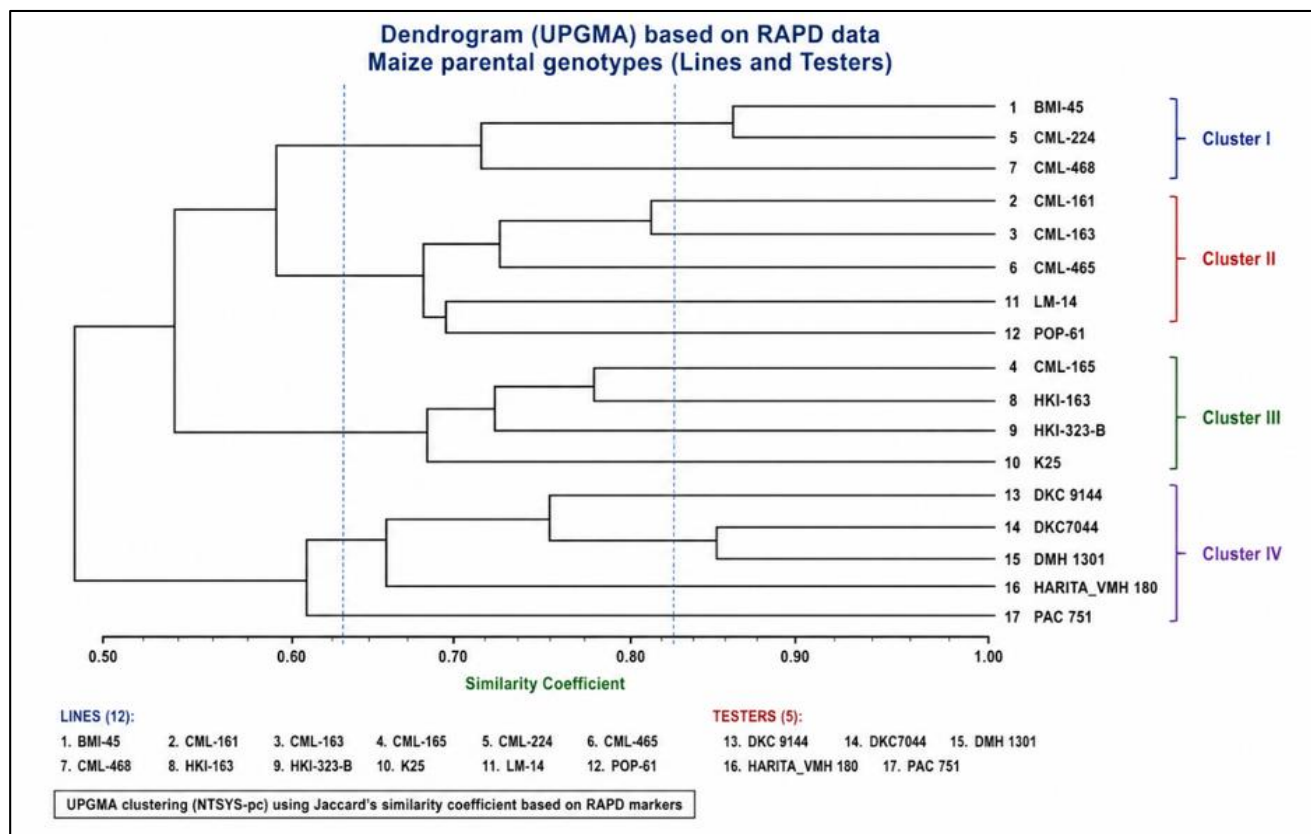


Fig 1: UPGMA dendrogram depicting molecular diversity among the seventeen maize parental genotypes. (Insert the final dendrogram image from the molecular analysis at this position.)

3.4 Relationship Between Molecular Diversity and Hybrid Performance

A major objective was to examine whether molecularly divergent parental combinations were also associated with superior hybrid performance. The results indicate that several highly divergent parental combinations identified through RAPD analysis produced hybrids with strong seed yield and combining ability. K25 × DMH 1301 had the lowest similarity among the highlighted pairs (0.55) and recorded the highest seed yield among the five top heterotic hybrids (241.63 g plant⁻¹), together with 92.64% mid-parent heterosis and 69.13% better-parent heterosis. BMI-45 ×

HARITA_VMH 180 also showed high molecular divergence (0.57) and a strong positive SCA effect for seed yield (+ 50.23) and biological yield (+ 56.24).

The comparison nevertheless should be interpreted as evidence of association within the present germplasm rather than proof that molecular distance alone determines heterosis. Hybrid performance is also influenced by parental combining ability and specific interactions. The result chapter itself shows that the most successful crosses combined molecular divergence with strong SCA and high heterotic expression.

Table 5: Top heterotic hybrids for seed yield identified in the Line × Tester evaluation

Rank	Cross combination	Seed yield (g/plant)	MPH (%)	BPH (%)	Primary core driver
1	K25 × DMH 1301	241.63	92.64	69.13	Harvest index/hyper-partitioning (+ 112.83% MPH)
2	POP-61 × DMH 1301	231.33	89.98	61.34	Biomass accumulation (+ 64.36% MPH)
3	POP-61 × PAC 751	223.36	89.02	64.64	Sink frame/cob length (+ 43.50% MPH)
4	HKI-163 × DKC7044	237.51	87.80	72.02	True heterobeltiosis over elite tester
5	LM-14 × DMH 1301	234.35	86.73	61.27	Balanced reproductive acceleration

3.5 Molecular Divergence and Specific Combining Ability

The comparison of molecular similarity with combining ability provides additional evidence for the usefulness of divergent parental selection. K25 × DMH 1301, with a RAPD similarity coefficient of 0.55, showed a positive SCA effect of + 55.37 for seed yield. BMI-45 × HARITA_VMH

180, with similarity of 0.57, showed + 50.23 SCA for seed yield and + 56.24 for biological yield, together with + 70.33 for 1000-seed weight. These combinations illustrate that molecularly divergent parents can generate strong specific interactions, although the relationship should not be interpreted as universally deterministic.

Table 6: Selected molecularly divergent parental combinations and corresponding hybrid evidence

Cross	RAPD similarity	Seed yield (g/plant)	MPH (%)	BPH (%)	SCA for seed yield
K25 × DMH 1301	0.55	241.63	92.64	69.13	+ 55.37
BMI-45 × HARITA_VMH 180	0.57	233.96	Not reported in top-5 table	Not reported in top-5 table	+ 50.23
BMI-45 × DKC 9144	0.58	Not reported in top-5 table	Not reported in top-5 table	Not reported in top-5 table	Not reported in extracted SCA summary

The top heterotic combinations identified in the result chapter further included HKI-163 × DKC7044, which recorded 237.51 g seed yield per plant and the highest better-parent heterosis among the five highlighted crosses (72.02%). The molecular similarity information therefore provides a useful additional dimension for interpreting why particular parental combinations performed well, but conventional SCA and heterosis estimates remain essential for final hybrid selection.

4. Conclusions

The RAPD analysis demonstrated substantial molecular diversity among the twelve maize lines and five testers used in the Line × Tester programme. Fifteen primers generated 123 bands, of which 97 were polymorphic, with an average polymorphism of 78.86%. Jaccard's similarity coefficients ranged from 0.55 to 0.86, and UPGMA analysis separated the seventeen parents into four major molecular clusters. The molecularly divergent combinations K25 × DMH 1301 and BMI-45 × HARITA_VMH 180 were also associated with strong hybrid performance and SCA, while K25 × DMH 1301 recorded particularly high mid-parent and better-parent heterosis for seed yield. These results support the use of RAPD characterization as a complementary tool for identifying divergent parental combinations in maize. However, molecular distance should be considered together with per se performance, GCA, SCA, heterosis and field evaluation before advancing hybrids for breeding or commercial testing.

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