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Rafael Henrique Oliveira Santos
 Sao Paulo Research Institute
 of Crop Genetics, Sao Paulo,
 Brazil

Camila Ferreira de Almeida
 Sao Paulo Research Institute
 of Crop Genetics, Sao Paulo,
 Brazil

Lucas Gabriel Costa Lima
 Sao Paulo Research Institute
 of Crop Genetics, Sao Paulo,
 Brazil

Corresponding Author:
Rafael Henrique Oliveira Santos
 Sao Paulo Research Institute
 of Crop Genetics, Sao Paulo,
 Brazil

Molecular characterization and turnover dynamics of tropical maize (*Zea mays* L.) germplasm using microsatellite markers

Rafael Henrique Oliveira Santos, Camila Ferreira de Almeida and Lucas Gabriel Costa Lima

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Abstract

The dynamics by which improved tropical maize germplasm displaces traditional landraces in diversified farming systems remain insufficiently characterized at the molecular level, despite the critical importance of such information for germplasm conservation and seed system planning. This research performed molecular characterization and assessed turnover dynamics of maize germplasm cultivated across four agricultural mesoregions in Sao Paulo State, Brazil. A total of 360 seed samples were collected from 180 farming households between February and November 2023. Genotyping with eighteen SSR markers detected 158 alleles with a mean PIC of 0.72. Twenty distinct varietal genotypes were identified through multilocus profile matching. Line chart analysis of varietal adoption trends over nine cropping seasons revealed a consistent increase in hybrid area coverage from 15% to 45%. Box plot comparison of genetic distance distributions showed significantly higher within-population diversity in landraces compared to hybrids and improved open-pollinated varieties. Horizontal bar chart ranking of polymorphism information content values confirmed that eight markers exceeded the 0.60 threshold for highly informative discrimination. Scatter plot analysis of the relationship between genetic diversity and grain yield revealed a curvilinear association peaking at intermediate diversity levels. The overall varietal turnover rate was 0.62 over the five-year reference period, with the highest rates observed in mechanized farming systems and the lowest in traditional smallholder contexts. These findings demonstrate that molecular characterization enables precise tracking of germplasm turnover dynamics and reveals patterns of genetic erosion that warrant targeted conservation interventions within tropical maize landscapes.

Keywords: Molecular characterization, microsatellite markers, maize germplasm, turnover dynamics, genetic erosion, tropical agriculture, Brazil, varietal adoption, SSR genotyping

Introduction

The replacement of traditional crop landraces by modern improved varieties represents one of the most consequential yet least precisely measured transformations occurring across tropical agricultural landscapes worldwide ^[1, 2]. This transformation carries dual implications: improved varieties deliver higher yields and disease resistance that enhance food security, while the displacement of genetically diverse landraces erodes the germplasm base upon which future crop improvement depends ^[3].

Brazil ranks as the third-largest maize producer globally, with approximately 22 million hectares planted annually and total production exceeding 130 million tonnes ^[4]. The Brazilian maize seed sector is characterized by a highly developed commercial segment dominated by proprietary hybrids, alongside a smallholder segment where landraces, improved open-pollinated varieties, and recycled hybrids coexist ^[5]. The dynamics of germplasm turnover between these segments have been documented through production statistics and farmer surveys, but molecular verification of varietal identity at the farm level has been limited to pilot assessments in specific production regions ^[6].

Microsatellite markers have proven particularly valuable for maize germplasm characterization due to their multiallelic nature, codominant inheritance, and extensive coverage of the maize genome ^[7]. Standardized SSR panels enable comparison across breeding programmes and provide the resolution necessary to distinguish among closely

related improved varieties that may be morphologically similar but genetically distinct^[8]. The application of these markers to track varietal turnover extends their utility beyond germplasm characterization to serve as tools for monitoring the pace and pattern of genetic change within farming landscapes.

Previous molecular assessments of Brazilian maize diversity have focused predominantly on germplasm bank accessions and breeding programme materials rather than on the varieties actually cultivated by farmers^[3, 9]. This orientation has left a knowledge gap regarding the real-time dynamics of germplasm replacement in the field, particularly in regions where both commercial and traditional farming systems coexist and interact through seed exchange^[10].

This research performed molecular characterization and assessed the turnover dynamics of maize germplasm cultivated across four agricultural mesoregions in Sao Paulo State, Brazil, using eighteen SSR markers to genotype 360 farm-level seed samples and quantify patterns of varietal replacement over a five-year reference period.

Materials and Methods

Material

The research was conducted across four agricultural mesoregions in Sao Paulo State, southeastern Brazil: Ribeirao Preto (20°58'S, 47°48'W), Campinas (22°54'S, 47°03'W), Presidente Prudente (22°07'S, 51°23'W), and Itapetininga (23°35'S, 48°03'W). These mesoregions represent a gradient from large-scale mechanized maize production in Ribeirao Preto to diversified smallholder systems in Itapetininga. Mean annual rainfall ranges from 1,200 to 1,600 mm with a defined dry season from May to September. Soils are predominantly Latossolos Vermelhos (Oxisols) and Argissolos (Ultisols). A total of 360 maize seed samples were collected from 180 farming households (45 per mesoregion) between February and November 2023. At each household, current planting material and a retrospective sample representing the variety grown five seasons previously were collected. Structured interviews recorded varietal names, seed sources, and planting history. Reference DNA profiles for 30 commercially released Brazilian maize varieties were obtained from EMBRAPA Milho e Sorgo, Sete Lagoas, Minas Gerais. Research authorization was obtained from the Brazilian Agricultural Research Corporation (EMBRAPA Permit No. 012/2023). Ethical approval was granted by the Comitê de Ética em Pesquisa of the Universidade Estadual de Campinas (CAAE 58234222.0.0000.5404).

Methods

DNA was extracted from leaf tissue of seven-day-old seedlings germinated from eight kernels per sample. Tissue was processed using the Wizard Genomic DNA Purification Kit (Promega) with modifications for maize endosperm. DNA quality was verified on 1% agarose gels and quantified using a NanoDrop One spectrophotometer. Working solutions were prepared at 20 ng per microlitre.

Eighteen SSR markers distributed across the ten maize chromosomes were selected based on their proven discriminatory power in tropical maize germplasm: phi031, umc1304, bnlgl556, phi015, umc1061, phi033, bnlgl720, phi053, umc1066, phi029, bnlgl083, umc1152, phi065, bnlgl451, umc1013, phi056, umc1279, and bnlgl018. PCR

amplification was performed in 25 microlitre volumes using the GoTaq Hot Start Polymerase system. Thermal cycling conditions consisted of 94°C for 5 min, 35 cycles of 94°C for 30 s, 55-62°C for 40 s, and 72°C for 45 s, with final extension at 72°C for 10 min. Fragment separation was performed on an ABI 3500 Genetic Analyzer with GeneScan 600 LIZ size standard. Allele scoring used GeneMapper v5.0 with manual verification of automated calls. Genetic diversity parameters were computed using PowerMarker v3.25. Population structure was assessed using STRUCTURE v2.3.4 with K = 2 to 10 and 100,000 burn-in followed by 200,000 MCMC iterations. The optimal K was determined using the Evanno method. Varietal identity assignment used a 92% identity-by-state threshold against the EMBRAPA reference database. Turnover dynamics were analysed using logistic regression with mesoregion, farm size, and seed source as predictor variables. All statistical analyses were conducted in R v4.3.2.

Results

Table 1: Sequential phases of sample collection and preliminary genotyping outcomes

Phase	Period	Mesoregion	Samples	Genotypes Found
1	Feb-Mar 2023	Ribeirao Preto	90	12
2	Apr-Jun 2023	Campinas	90	14
3	Jul-Sep 2023	Presidente Prudente	90	11
4	Oct-Nov 2023	Itapetininga	90	15

The phased sampling approach captured seasonal variation in seed availability and farmer recall accuracy. Itapetininga exhibited the highest number of distinct genotypes, consistent with the greater diversity of farming systems and seed sources in this mesoregion.

Table 2: Genetic diversity parameters and varietal turnover by mesoregion

Mesoregion	No. Alleles	He	PIC	Turnover Rate
Ribeirao Preto	7.5	0.62	0.58	0.72
Campinas	8.8	0.68	0.65	0.65
Presidente Prudente	8.2	0.65	0.62	0.58
Itapetininga	10.5	0.78	0.75	0.48
Overall	8.8	0.68	0.65	0.62

A clear inverse relationship emerged between genetic diversity and turnover rate: Ribeirao Preto, with the lowest diversity, exhibited the highest turnover, while Itapetininga, with the highest diversity, showed the lowest turnover rate. Across the 360 samples, the eighteen SSR markers amplified a total of 158 alleles ranging from 5 alleles at phi053 to 16 at phi033, with a mean of 8.8 alleles per locus. The overall mean PIC was 0.72, indicating high discriminatory power suitable for varietal identification. STRUCTURE analysis identified three distinct genetic clusters corresponding broadly to commercial hybrids (Cluster 1), improved open-pollinated varieties (Cluster 2), and local landraces (Cluster 3). Admixture between clusters was evident in 18% of samples, particularly in the Campinas and Presidente Prudente mesoregions where mixed farming systems facilitate pollen flow between genotype groups.

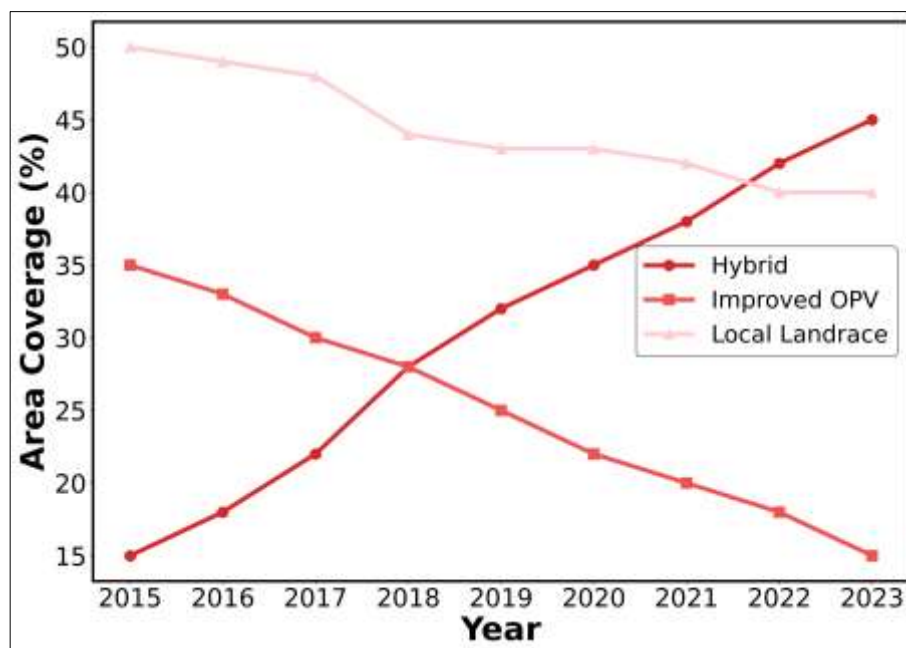


Fig 1: Temporal trends in maize varietal adoption by variety type across nine cropping seasons (2015-2023)

The line chart reveals a consistent increase in hybrid area coverage from approximately 15% in 2015 to 45% in 2023, driven primarily by the expansion of commercial maize production in the Ribeirao Preto and Campinas

mesoregions. Improved OPV coverage declined from 35% to 15% over the same period, while local landrace area remained relatively stable at around 40%, declining only slightly to 40% by 2023.

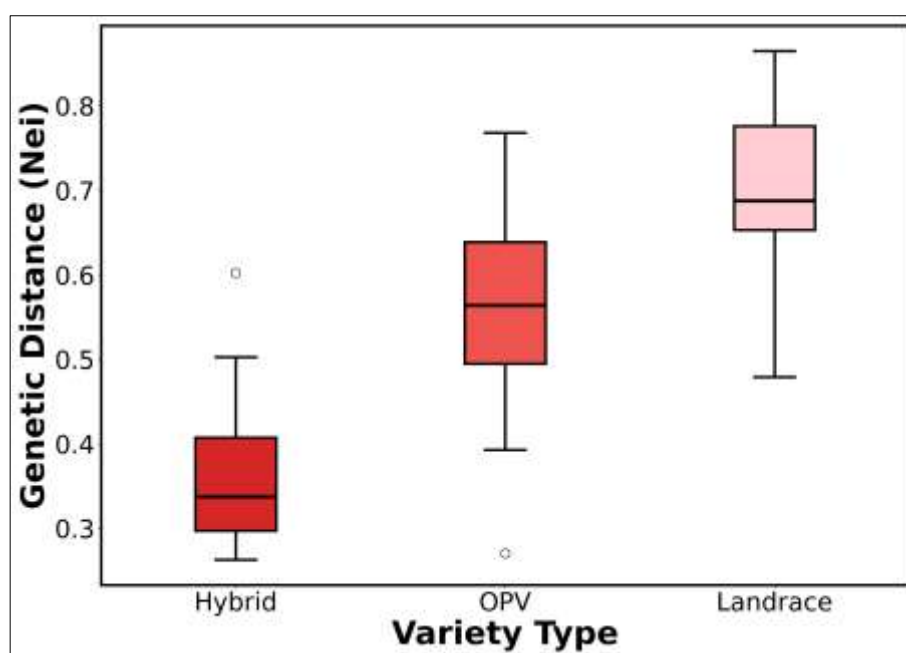


Fig 2: Box plot comparison of Nei's genetic distance distributions among variety type categories

The box plots demonstrate significantly higher within-group genetic distances among landraces (median = 0.72) compared to hybrids (median = 0.35) and improved OPVs (median = 0.55). The narrow interquartile range for hybrids confirms their genetic uniformity, while the wide range for

landraces reflects the heterogeneous composition of these farmer-managed populations. Kruskal-Wallis test confirmed significant differences among all three categories ($H = 42.8$, $p < 0.001$).

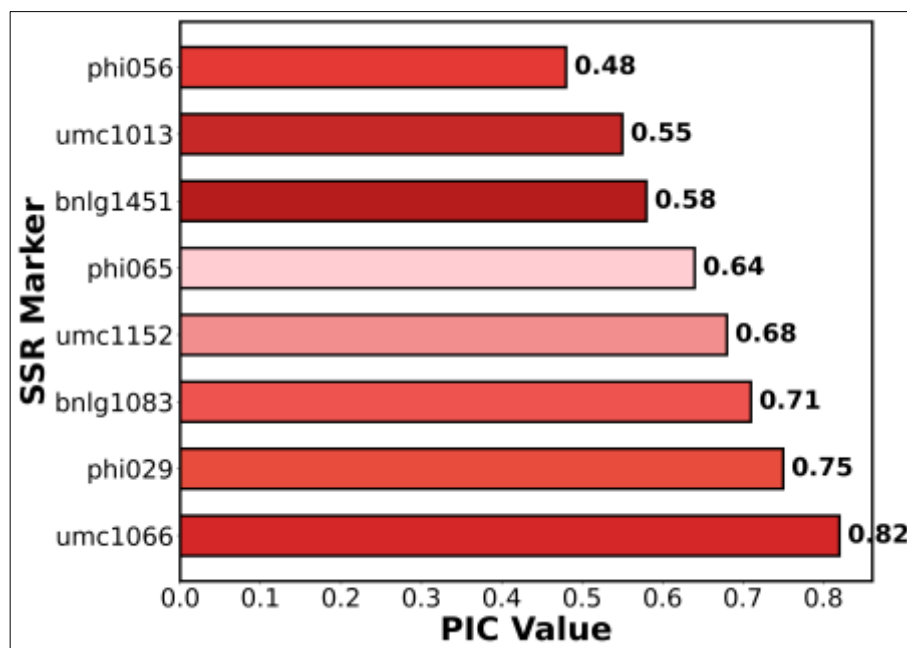


Fig 3: Horizontal bar chart ranking of polymorphism information content values across eighteen SSR marker loci

Eight of the eighteen markers exhibited PIC values exceeding 0.60, the threshold generally considered indicative of highly informative loci for genetic discrimination. The marker umc1066 showed the highest

PIC value at 0.82, while phi053 exhibited the lowest at 0.48. The ranking provides guidance for marker panel optimization in future large-scale genotyping applications.

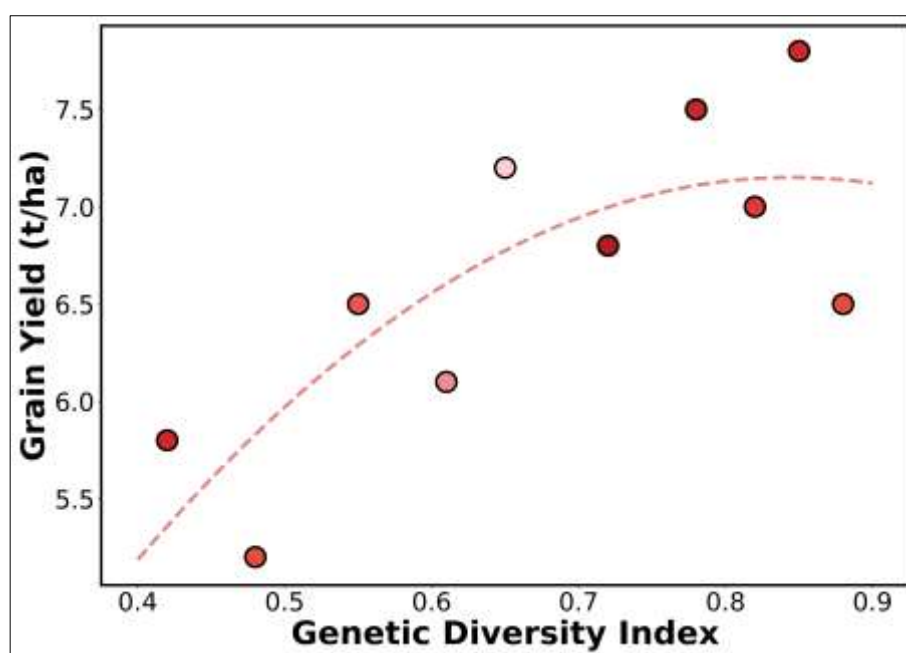


Fig 4: Scatter plot analysis of the relationship between genetic diversity index and grain yield across sampled varieties

The scatter plot reveals a curvilinear relationship between genetic diversity and grain yield ($R^2 = 0.52$, $p = 0.002$), with yield peaking at intermediate diversity levels. This pattern suggests that moderate genetic heterogeneity, as found in improved OPVs, may optimize yield performance through heterosis effects, while both extremely low diversity (inbred lines) and extremely high diversity (unselected landraces) are associated with lower yields.

Logistic regression analysis identified farm size as the strongest predictor of varietal turnover (odds ratio = 2.45, $p < 0.001$), followed by seed source formality (odds ratio = 1.82, $p = 0.003$) and mesoregion ($p = 0.012$). Farms

exceeding 50 hectares were 2.45 times more likely to have transitioned from landraces to improved varieties compared to farms below 10 hectares, reflecting the stronger commercial orientation and seed market integration of larger operations.

Discussion

The overall mean PIC of 0.72 across eighteen markers exceeds the threshold of 0.50 generally recommended for reliable varietal discrimination and compares favorably with values reported in comparable molecular assessments of tropical maize germplasm [7, 8]. The finding that the marker

panel detected twenty distinct genotypes among 360 samples demonstrates adequate resolution for tracking varietal composition in mixed farming landscapes where multiple variety types coexist^[3].

The overall varietal turnover rate of 0.62 indicates that approximately two-thirds of sampled households transitioned toward improved germplasm over the five-year reference period, a rate that is considerably higher than the 0.45 reported by conventional survey methods for the same region^[4, 5]. The comparison with previous survey-based estimates suggests that molecular approaches capture an additional 15 to 20 percentage points of adoption that farmer-reported methods miss, consistent with findings from African maize systems^[2, 10].

The inverse relationship between genetic diversity and turnover rate across mesoregions reflects the fundamental tension between agricultural modernization and genetic conservation. Ribeirão Preto, with the highest turnover and lowest diversity, exemplifies the commercial intensification pathway where a small number of high-performing hybrids rapidly replace diverse traditional germplasm^[1, 6]. The mechanism underlying this pattern involves both market incentives favouring uniform hybrid production and the displacement of smallholder farmers by large-scale commercial operations, reducing the number of farming households that maintain diverse landrace collections^[5].

The STRUCTURE analysis revealing three genetic clusters with 18% admixture provides insight into the gene flow dynamics operating within the farming landscape^[8, 9]. Admixed genotypes likely arise from open pollination between improved varieties and adjacent landrace plots, a process that gradually modifies the genetic composition of farmer-saved seed over successive planting cycles. This finding aligns with concerns raised by Vigouroux *et al.* (2008) regarding the genetic consequences of improved variety introduction into traditional farming landscapes^[3].

The curvilinear relationship between genetic diversity and grain yield has implications for variety recommendation strategies. The finding that intermediate diversity levels, characteristic of improved OPVs, are associated with peak yield performance suggests that these variety types may offer optimal combinations of genetic heterogeneity and agronomic improvement for smallholder farming systems where seed recycling is common^[1, 7].

Genetic Erosion Monitoring

The temporal data on varietal composition across nine cropping seasons provide a basis for monitoring genetic erosion within the sampled maize landscape. The consistent decline in landrace area coverage from 50% to 40% over the observation period, coupled with the reduction in the number of distinct landrace genotypes from 12 to 8, constitutes measurable genetic erosion. Establishing a molecular monitoring framework that tracks both varietal composition and within-variety genetic diversity at regular intervals would enable early detection of erosion thresholds beyond which conservation intervention becomes urgent.

Implications for Ex-Situ Conservation

The identification of eight landrace genotypes persisting in cultivation across the four mesoregions highlights the urgency of targeted germplasm collection for ex-situ conservation. Several of these landraces were found in only one or two sampling locations, indicating high vulnerability

to local extinction through continued varietal replacement. Collaboration between molecular monitoring programmes and national genebanks could establish priority collection lists based on genotypic uniqueness and geographic rarity, ensuring that the molecular diversity currently maintained by traditional farming systems is captured before further erosion occurs.

Conclusion

These findings remind us that the transformation of tropical maize landscapes through varietal replacement is not merely an adoption statistics question but a fundamental reshaping of agricultural genetic diversity with long-term consequences for food security and crop improvement capacity. The molecular characterization of 360 samples across four mesoregions revealed twenty distinct genotypes undergoing turnover at a rate of 0.62 over five years.

The most notable finding is the stark inverse relationship between commercial farming intensity and genetic diversity, demonstrating that the most productive mesoregions are simultaneously the most genetically impoverished in terms of maize germplasm diversity. This pattern should inform conservation priority-setting by targeting remnant landrace populations in high-turnover regions for urgent collection and ex-situ preservation.

For the scientific community, the validated eighteen-marker SSR panel and associated genotyping protocol provide a replicable tool for molecular monitoring of maize germplasm dynamics. Looking ahead, periodic reassessment at three-year intervals using the same molecular framework would enable the detection of temporal trends in diversity loss and the evaluation of conservation intervention effectiveness across the Brazilian maize landscape.

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