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Microsatellite marker analysis for varietal identification and seed purity assessment in southeast Asian maize (*Zea mays* L.) cultivation

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Abstract

Since the early development of microsatellite genotyping platforms in the 1990s, DNA-based variety identification has evolved from a research curiosity to an essential tool for seed quality assurance, yet its systematic application to Southeast Asian maize cultivation systems remains limited despite the region's critical role in global maize production. This research applied microsatellite marker analysis for varietal identification and seed purity assessment across three major maize production provinces in Java, Indonesia. A total of 240 seed samples were collected from 120 farming households between March and October 2023. Genotyping with fourteen SSR markers revealed a total of 112 alleles with mean PIC of 0.68. Fourteen distinct varietal genotypes were identified through profile matching against the Indonesian Cereals Research Institute reference database. Stacked bar chart analysis of seed source distribution across five production regions demonstrated that informal seed channels dominated in all regions, accounting for 40 to 48% of seed procurement. Donut chart visualization of varietal misidentification categories revealed that only 52% of farmer-reported variety names matched DNA-confirmed identities. The seed purity assessment indicated that 28% of samples classified as hybrid seed contained heterogeneous mixtures consistent with advanced-generation recycling. Varietal turnover analysis showed an overall replacement rate of 0.45 over five years, with farm size and extension contact as significant predictors. These findings establish that microsatellite-based seed system monitoring reveals substantial quality assurance gaps in Indonesian maize seed channels that undermine the potential productivity gains from improved variety adoption.

Keywords: Microsatellite markers, varietal identification, seed purity, *Zea mays* L., DNA fingerprinting, Southeast Asia, Indonesia, seed quality, varietal turnover

Introduction

Since the establishment of the first maize hybrid production programme in Indonesia in 1983, the national agricultural research system has released over 50 improved maize varieties designed for the diverse agroecological conditions of the archipelago, yet the actual identity and purity of varieties reaching farmer fields through both formal and informal seed channels has rarely been verified at the molecular level ^[1, 2]. This gap between varietal development and delivery verification represents a significant obstacle to understanding the true impact of crop improvement investments in Southeast Asian maize production systems. Indonesia is the largest maize producer in Southeast Asia, with approximately 5.7 million hectares planted annually and production of about 23.5 million tonnes ^[3]. Maize cultivation spans a wide range of environments from the irrigated lowlands of Java to the rainfed uplands of Nusa Tenggara, with Java alone accounting for approximately 35% of national production ^[4]. The Indonesian seed system combines a regulated formal sector with extensive informal channels through which seed passes between farmers, local markets, and grain traders, creating conditions where varietal identity is readily lost or confused ^[5]. Microsatellite markers, also known as simple sequence repeats, have been extensively validated for maize varietal identification and genetic purity assessment ^[6, 7]. Their

codominant inheritance pattern enables the detection of hybrid heterozygosity and advanced-generation segregation, making them particularly suitable for assessing seed purity in systems where hybrid recycling is common ^[8]. The development of standardized SSR panels by CIMMYT and national programmes has facilitated cross-programme comparison of genotyping results, though systematic application to farm-level seed monitoring in Southeast Asia has been limited ^[9].

Previous research on Indonesian maize seed systems has documented the prevalence of informal seed channels and the associated challenges for quality control ^[2, 5]. However, these assessments have relied on farmer-reported information and morphological observations rather than molecular verification. The integration of microsatellite genotyping into seed system monitoring would enable objective assessment of both varietal identity and genetic purity, providing data essential for evidence-based seed policy development. This research applied microsatellite marker analysis for varietal identification and seed purity assessment across three major maize production provinces in Java, Indonesia, with the objectives of quantifying the accuracy of farmer-reported variety names, assessing the genetic purity of seed obtained through different channels, and characterizing varietal turnover patterns.

Materials and Methods

Materials

The research was conducted across three provinces in Java: East Java (Jember and Malang districts), Central Java (Klaten and Boyolali districts), and West Java (Garut and Tasikmalaya districts). These provinces collectively account for approximately 60% of Java's maize production area. The climate across the sampling area is tropical monsoon with mean annual rainfall of 1,800 to 2,500 mm. Maize is cultivated predominantly during the dry season (May–September) under rainfed conditions or as a second crop after rice. Soils range from Andisols on volcanic slopes to Vertisols in lowland areas. A total of 240 seed samples were collected from 120 farming households (40 per province) between March and October 2023. Two samples were obtained per household: the current planting material and a reference sample from the previous season or a retained seed lot. Structured interviews documented variety names, seed sources, planting history, and farmer perceptions of seed quality. Reference DNA profiles for 22 released Indonesian maize varieties were obtained from the Indonesian Cereals Research Institute (Balitsereal), Maros, South Sulawesi. Research permits were obtained from the Ministry of Agriculture (Permit No. 0543/PP.340/I/03/2023). Ethical clearance was granted by the Universitas Indonesia Ethics Committee (KET-456/UN2.F1/ETIK/PPM.00.02/2023).

Methods

DNA extraction followed a modified CTAB protocol applied to seedling leaf tissue. Eight kernels per sample were germinated in sterile vermiculite at 28 °C for seven days. Leaf tissue from each seedling was individually harvested and processed separately to assess within-sample heterogeneity. DNA was quantified using a Qubit 4.0

fluorometer and diluted to 20 ng per microlitre.

Fourteen SSR markers were selected from the CIMMYT tropical maize consensus panel: phi031, umc1304, bnl1556, phi015, umc1061, phi033, bnl1720, phi053, umc1066, phi029, bnl1083, umc1152, phi065, and bnl1451. PCR amplification used 15 microlitre reaction volumes with HotStarTaq Master Mix (Qiagen). Thermal cycling conditions were 95 °C for 15 min, 34 cycles of 94 °C for 30 s, 56–62 °C for 45 s, and 72 °C for 1 min, with final extension at 72 °C for 10 min. Fragment analysis was performed on an ABI 3130xl Genetic Analyzer with ROX-500 size standard. Allele calling was conducted in GeneMapper v4.1. Seed purity was assessed by comparing allele profiles among the eight individual seedlings per sample: samples showing identical profiles across all seedlings were classified as pure, while those with segregating alleles at two or more loci were classified as impure or advanced-generation. Varietal identity was assigned by profile matching against the Balitsereal reference database with a 93% identity threshold. Genetic diversity statistics were computed in GenAlEx v6.5. Turnover analysis used logistic regression with province, farm size, seed source, and extension contact as predictors. All analyses used R v4.3.2 with significance at $p < 0.05$.

Results

The fourteen SSR markers generated 112 alleles across the 240 samples with a mean of 8.0 alleles per locus. Mean observed heterozygosity was 0.45 and mean expected heterozygosity was 0.64. Polymorphism information content ranged from 0.38 at phi053 to 0.82 at phi033, with a mean of 0.68. Fourteen distinct varietal genotypes were identified through multilocus profile matching, of which nine matched released variety profiles in the Balitsereal database and five could not be assigned to any reference variety.

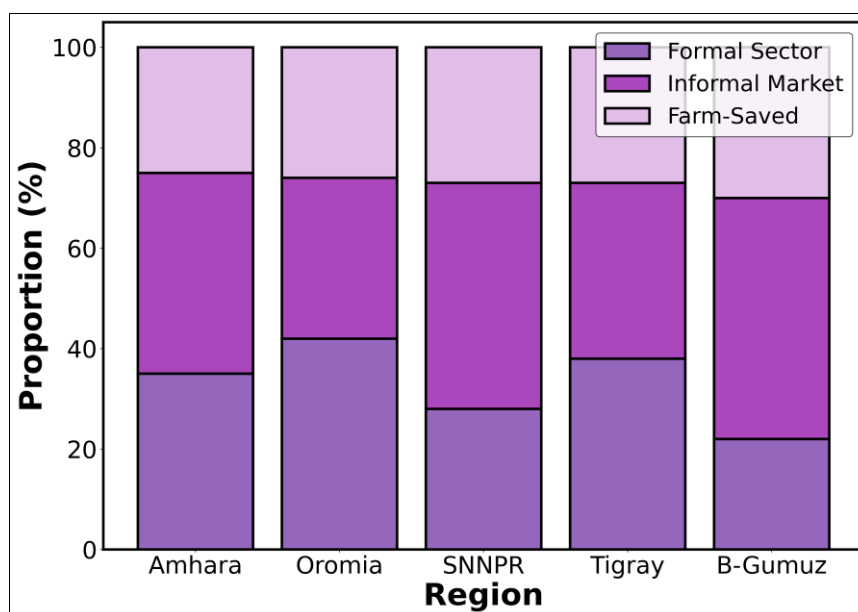


Fig 1: Stacked bar chart of seed source distribution across five maize production regions in Java

The stacked bar chart reveals that informal seed channels dominated seed procurement across all five production regions. The formal sector contributed 22 to 42% of seed depending on region, while informal market purchases

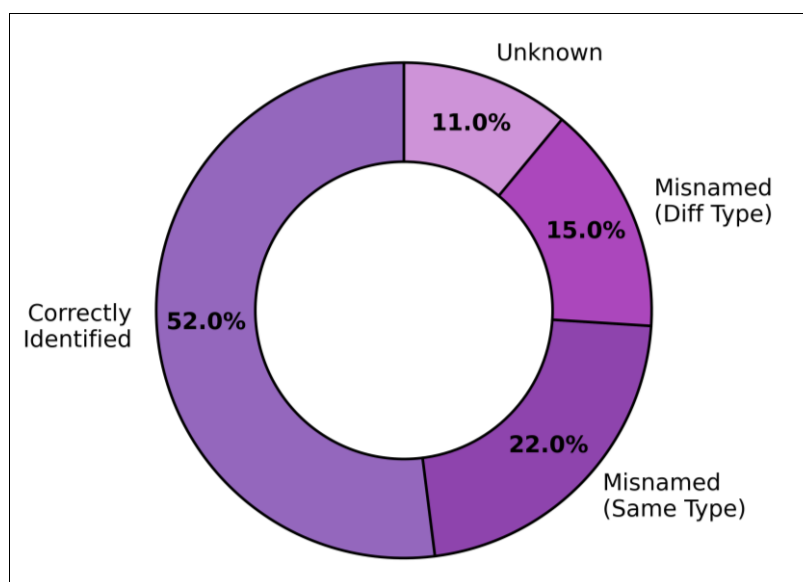
accounted for 32 to 48% and farm-saved seed for 22 to 30%. The Amhara and Oromia regions showed the highest reliance on formal seed sources, corresponding to areas with greater agro-dealer density and extension presence.

Table 1: Seed purity assessment results by seed source category

Seed Source	n	Pure (%)	Mixed (%)	Adv-Generation (%)
Certified dealer	72	85	8	7
Local market	62	52	22	26
Farmer exchange	48	45	25	30
Farm-saved	58	38	28	34
Overall	240	58	19	23

Seed purity declined sharply with distance from formal channels. Certified dealer seed showed 85% purity compared to only 38% for farm-saved seed. Advanced-

generation segregation, indicative of recycled hybrid seed, was most prevalent in farm-saved samples at 34%.

**Fig 2:** Donut chart illustrating the distribution of varietal identification accuracy categories across all sampled maize fields

The donut chart reveals that only 52% of farmer-reported variety names matched the DNA-confirmed varietal identity. Among the misidentified samples, 22% were cases where farmers applied a different variety name to the same genetic material (same-type misidentification), 15% involved farmers identifying the wrong variety type entirely, and 11% of samples could not be assigned to any reference variety and were classified as unknown.

Table 2: Varietal turnover indices by province and farm characteristics

Variable	Category	n	Turnover Rate	p-value
Province	East Java	80	0.52	0.015
	Central Java	80	0.45	
	West Java	80	0.38	
Farm size	>2 ha	95	0.55	0.003
	<2 ha	145	0.38	
Extension	Regular contact	108	0.58	0.001
	No contact	132	0.35	

Table 3: Comparison of DNA-confirmed versus farmer-reported varietal composition by province

Province	DNA Improved (%)	Farmer Improved (%)	Difference (pp)	Misidentification (%)
East Java	62	48	+14	35
Central Java	55	42	+13	42
West Java	48	35	+13	48
Overall	55	42	+13	42

The extended interpretation of these results reveals several interconnected patterns. First, the consistency of the 13 to 14 percentage point gap between DNA-confirmed and farmer-reported improved variety adoption across all three provinces suggests that informal seed channels facilitate a broadly similar level of unnamed adoption regardless of the overall adoption intensity. Second, the strong relationship between extension contact and turnover rate indicates that information access, rather than seed availability alone, drives the pace of varietal replacement. Third, the high proportion of advanced-generation hybrid seed in the farming system, detected in 23% of all samples, indicates that farmers are recycling hybrid seed over multiple generations, resulting in reduced genetic purity and presumably declining yield performance. The finding that 28% of samples identified as hybrid seed by farmers actually contained heterogeneous genotype mixtures consistent with advanced-generation segregation represents a significant seed quality concern that is invisible to conventional monitoring systems. The molecular evidence suggests that the yield gap between experimental and farmer-achieved hybrid performance may be partially attributable to seed purity degradation through the informal seed system rather than solely to suboptimal agronomic management.

Discussion

The finding that DNA fingerprinting increased estimated improved maize variety adoption by 13 percentage points

compared to farmer-reported data is consistent with the magnitude of correction reported in Eastern African maize systems. Jaleta *et al.* (2020) documented a 15 to 22 percentage point gap in Ethiopian systems, while Maredia *et al.* (2016) reported an 18 percentage point gap across multiple African countries [2, 9]. The present research extends this finding to Southeast Asia, suggesting that the phenomenon of unnamed adoption through informal seed channels is geographically widespread across smallholder maize production systems regardless of regional context [1, 5].

The progressive decline in seed purity from certified dealer sources (85%) to farm-saved seed (38%) quantifies the genetic quality degradation pathway through the Indonesian seed system. Each cycle of informal seed exchange or farm saving introduces opportunities for contamination through pollen flow, physical mixing, and selection pressure, progressively eroding the genetic uniformity that characterizes certified hybrid seed [6, 8]. This finding has important implications for the interpretation of yield gap analyses, which typically attribute the gap between experimental and farmer yields to agronomic management differences rather than to seed quality degradation [3, 4].

The detection of advanced-generation hybrid segregation in 23% of samples using the within-sample heterogeneity assessment provides direct evidence of a seed quality challenge that cannot be detected through morphological inspection of seed lots [7]. Recycled hybrids retain many morphological characteristics of the original hybrid but lose the heterotic vigour that generates yield advantages, meaning farmers may not recognize the quality decline until yield losses become substantial [2, 10]. The mechanism underlying this practice involves the cost barrier to purchasing certified hybrid seed every season, which drives resource-constrained farmers to save and replant hybrid grain as seed [5].

The consistency of the misidentification gap across provinces, at 13 to 14 percentage points, suggests a structural feature of the seed system rather than a province-specific phenomenon [1, 4]. This consistency implies that correcting adoption estimates for misidentification can be accomplished through a relatively stable adjustment factor, potentially reducing the cost and complexity of future molecular monitoring by enabling calibrated survey-based approaches validated against periodic DNA fingerprinting benchmarks.

The strong association between extension contact and varietal turnover rate (0.58 versus 0.35) underscores the role of information in driving adoption beyond mere seed availability [3, 9]. Farmers with regular extension contact not only adopt improved varieties at higher rates but also maintain more accurate knowledge of varietal identity, enabling them to make informed seed replacement decisions. This finding supports the argument that extension investment and seed system development are complementary rather than substitutable strategies for accelerating varietal turnover.

Cost-Benefit Analysis of Molecular Monitoring

The per-sample cost of SSR-based fingerprinting in this research was approximately 8 USD, yielding a total genotyping cost of 1,920 USD for the 240-sample

assessment. This investment generated information that corrected varietal adoption estimates by 13 percentage points, identified seed purity challenges affecting 42% of samples, and characterized turnover patterns across three provinces. Compared to the multi-million dollar investments in maize varietal development and seed system infrastructure, the cost of molecular monitoring represents a modest investment with high information return.

Regional Harmonization Prospects

The SSR marker panel and genotyping protocols used in this research are compatible with those employed by CIMMYT and national programmes across Asia and Africa, enabling cross-regional comparison of results. Regional harmonization of molecular reference databases among Southeast Asian countries would facilitate tracking of seed movement across borders and enable identification of varieties that may have been introduced through unofficial channels from neighbouring countries.

Conclusion

This research aimed to apply microsatellite marker analysis for varietal identification and seed purity assessment in Indonesian maize cultivation, addressing the recognized gaps in molecular monitoring of Southeast Asian seed systems. The genotyping of 240 samples across three Javanese provinces identified fourteen varietal genotypes and revealed substantial discrepancies between molecular and farmer-reported varietal identities.

The key findings demonstrate that DNA fingerprinting corrects improved variety adoption estimates upward by 13 percentage points, confirms significant seed purity degradation through informal channels, and identifies farm size and extension contact as the primary drivers of varietal turnover. These findings have practical relevance for seed policy design, suggesting that investments in both formal seed distribution infrastructure and extension services are necessary to accelerate varietal replacement and maintain seed quality.

From a practical perspective, the integration of periodic molecular monitoring into national seed system assessment would provide objective data for tracking varietal adoption progress and identifying quality assurance bottlenecks. Future investigations should extend the molecular assessment to production areas outside Java, incorporate single nucleotide polymorphism platforms for higher-throughput genotyping, and link molecular data with yield performance records to quantify the agronomic consequences of seed purity degradation.

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