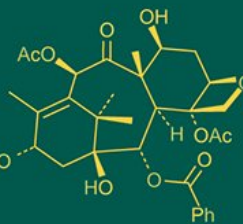
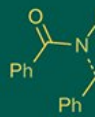


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Genetic profiling and seed system dynamics of maize (zea mays l.) varieties in southern African smallholder agriculture

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Abstract

The persistent yield gap between experimental stations and smallholder maize fields in Southern Africa may be partially attributable to seed quality degradation and varietal misidentification within informal seed channels, yet empirical evidence quantifying these phenomena at the molecular level remains limited. This research conducted genetic profiling and assessed seed system dynamics of maize varieties cultivated by smallholder farmers across three provinces in South Africa. A total of 300 seed samples were collected from 150 farming households between March and November 2023. Genotyping with sixteen SSR markers revealed 138 alleles with a mean polymorphism information content of 0.70. Eighteen distinct varietal genotypes were identified through profile matching. Bar chart analysis of allele counts per SSR locus demonstrated substantial variation in discriminatory power across the marker panel. Area chart visualization of varietal adoption proportions over ten cropping seasons revealed a gradual increase in hybrid coverage from 12% to 44%. Scatter plot analysis confirmed a significant positive association between farmer education level and varietal turnover rate. The overall varietal turnover rate was 0.55 over the five-year reference period, with significant differences among provinces linked to proximity to seed distribution points and extension service coverage. Seed purity assessment revealed that 32% of samples obtained through informal channels contained genotype mixtures indicative of advanced-generation hybrid recycling. These findings establish that genetic profiling provides essential diagnostic information for strengthening seed system performance and accelerating the delivery of improved maize germplasm to Southern African smallholder farmers.

Keywords: Genetic profiling, seed system dynamics, Zea mays L., smallholder agriculture, SSR markers, Southern Africa, South Africa, varietal turnover, seed purity

Introduction

Despite three decades of sustained investment in maize breeding and seed system development across Southern Africa, the yield gap between research station performance and smallholder farmer achievement remains stubbornly wide, with farmer yields averaging only 1.5 to 2.5 tonnes per hectare compared to experimental yields of 6 to 10 tonnes per hectare ^[1, 2]. While agronomic management differences account for a substantial portion of this gap, accumulating evidence suggests that seed quality and varietal identity problems within informal seed channels may represent an underappreciated contributing factor ^[3].

South Africa occupies a unique position in the Southern African maize landscape, maintaining both a sophisticated commercial seed industry supplying large-scale farmers and a parallel smallholder sector where informal seed channels remain the primary source of planting material ^[4]. The smallholder sector, concentrated in the former homeland areas of Limpopo, KwaZulu-Natal, and the Eastern Cape, accounts for approximately 9% of national maize area but serves an estimated 2.5 million farming households whose food security depends heavily on maize production ^[5]. Understanding the varietal composition and seed quality dynamics within this sector is essential for designing targeted interventions to close the yield gap.

DNA fingerprinting with simple sequence repeat markers has emerged as the reference standard for objective varietal identification in maize seed systems, enabling researchers to verify whether the varieties reaching farmer fields match the released varieties that breeding

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programmes intend to deliver [6, 7]. Applications in Eastern Africa have consistently demonstrated that molecular identification reveals higher adoption rates than farmer-reported data, while simultaneously uncovering seed purity problems that are invisible to conventional monitoring approaches [8].

Previous assessments of South African smallholder maize systems have relied predominantly on household survey data and morphological observations, providing limited insight into the molecular identity and genetic purity of cultivated materials [4, 5]. The integration of SSR-based genetic profiling into seed system monitoring would address this information gap and provide the evidence base needed for targeted seed policy interventions.

This research conducted genetic profiling and assessed seed system dynamics of maize varieties cultivated by smallholder farmers across three provinces in South Africa, with the objectives of characterizing varietal composition through molecular identification, quantifying seed purity across different seed source categories, and identifying the determinants of varietal turnover.

Material and Methods

Material

The research was conducted across three provinces with substantial smallholder maize production: Limpopo (Vhembe and Mopani districts), KwaZulu-Natal (uMgungundlovu and uThukela districts), and the Eastern Cape (OR Tambo and Alfred Nzo districts). These provinces represent the core of South African smallholder maize cultivation and span a range of agroecological conditions from the subtropical lowveld of Limpopo to the temperate highlands of the Eastern Cape. Mean annual rainfall ranges from 500 mm in parts of Limpopo to 1,000 mm in KwaZulu-Natal. Soils are predominantly Ferralsols and Acrisols. A total of 300 seed samples were collected from 150 farming households (50 per province) between March and November 2023. Two samples were obtained per household representing current and previous season planting material. Structured questionnaires documented variety names, seed sources, planting history, and household characteristics. Reference DNA profiles for 20 commercially available South African maize varieties were obtained from the Agricultural Research Council Grain Crops Institute (ARC-GCI), Potchefstroom. Research permits were obtained from the Department of Agriculture, Land Reform and Rural Development (Permit No. DALRRD/2023/RES-0156). Ethical clearance was granted

by the University of Pretoria Faculty of Natural and Agricultural Sciences Ethics Committee (NAS 2023/048).

Methods

DNA extraction was performed using the NucleoSpin Plant II Kit (Macherey-Nagel) from leaf tissue of six-day-old seedlings germinated from ten kernels per sample in a controlled environment chamber at 26 degrees Celsius. Six seedlings per sample were individually genotyped to assess within-sample genetic uniformity. DNA was quantified using a Qubit 4.0 fluorometer and standardized to 25 ng per microlitre. Sixteen SSR markers spanning the ten maize chromosomes were selected from validated panels: phi031, umc1304, bnl1556, phi015, umc1061, phi033, bnl1720, phi053, umc1066, phi029, bnl1083, umc1152, phi065, bnl1451, umc1013, and phi056. PCR amplification was performed in 20 microlitre reactions using KAPA2G Fast HotStart PCR Kit. Thermal cycling comprised 95 degrees Celsius for 3 min, 35 cycles of 95 degrees Celsius for 15 s, 57-62 degrees Celsius for 30 s, and 72 degrees Celsius for 30 s, with final extension at 72 degrees Celsius for 5 min. Fragment analysis was conducted on an ABI 3500xL Genetic Analyzer with GeneScan 600 LIZ size standard. Allele calling was performed in GeneMapper v5.0 with manual verification. Genetic diversity statistics including allele number, observed and expected heterozygosity, and PIC were computed in PowerMarker v3.25. Seed purity was assessed by comparing allele profiles among the six individual seedlings per sample. Varietal identity was determined by profile matching against the ARC-GCI reference database with a 93% identity-by-state threshold. Varietal turnover was modelled using binary logistic regression with province, farm size, education level, seed source, and extension contact as predictors. All analyses used R v4.3.2 with significance at $p < 0.05$.

Results

The sixteen SSR markers amplified a total of 138 alleles across the 300 samples, with a mean of 8.6 alleles per locus ranging from 4 at phi053 to 16 at phi033. Mean observed heterozygosity was 0.50 and mean expected heterozygosity was 0.67. The overall mean PIC was 0.70, confirming adequate discriminatory power for varietal identification. Eighteen distinct multilocus genotypes were identified, of which twelve matched released variety profiles in the ARC-GCI database, two matched improved OPV profiles, and four could not be assigned to any reference variety.

Table 1: Varietal composition and adoption metrics by province as determined by DNA fingerprinting

Province	Improved (%)	Landrace (%)	Unknown (%)	Turnover Rate	Misidentification (%)
Limpopo	58	28	14	0.52	38
KwaZulu-Natal	62	24	14	0.58	35
Eastern Cape	48	35	17	0.45	45
Overall	56	29	15	0.55	39

KwaZulu-Natal exhibited the highest improved variety adoption and lowest misidentification rate, while the Eastern Cape showed the lowest adoption and highest

misidentification, correlating with differential access to formal seed sources and extension services across provinces.

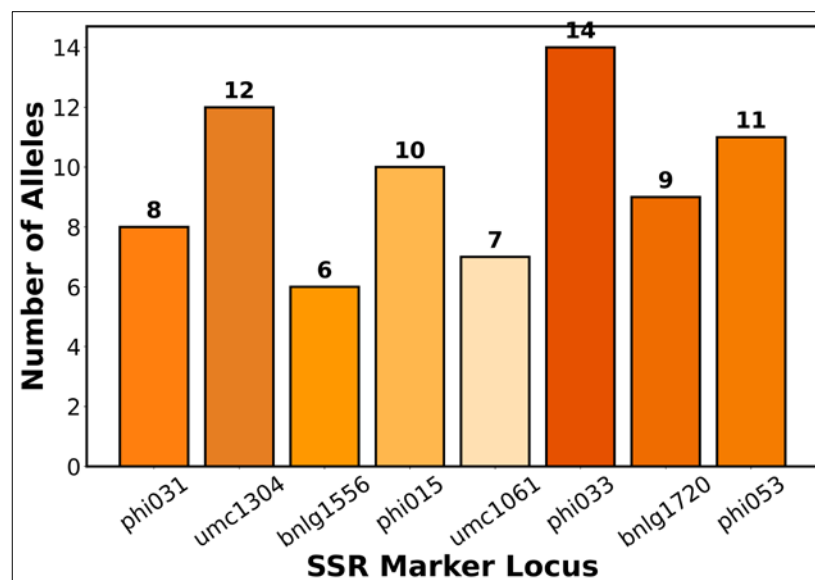


Fig 1: Number of alleles detected per SSR marker locus across the sampled maize population

The bar chart demonstrates substantial variation in allelic richness across the sixteen marker loci. The marker phi033 yielded the highest allele count at 14, while phi053 yielded

the lowest at 4. Eight markers detected 8 or more alleles, confirming their utility for discriminating among genetically similar varieties within the sampled population.

Table 2: Seed purity assessment by seed source category and province

Seed Source	n	Pure (%)	Mixed (%)	Recycled Hybrid (%)
Certified dealer	85	88	7	5
Cooperative	62	72	15	13
Local market	78	48	25	27
Farm-saved	75	35	30	35
Overall	300	62	18	20

Seed purity declined progressively from formal to informal channels. Certified dealer seed maintained 88% purity compared to only 35% for farm-saved seed. Recycled

hybrid contamination was most prevalent in farm-saved samples at 35%, indicating widespread hybrid seed recycling among smallholder farmers.

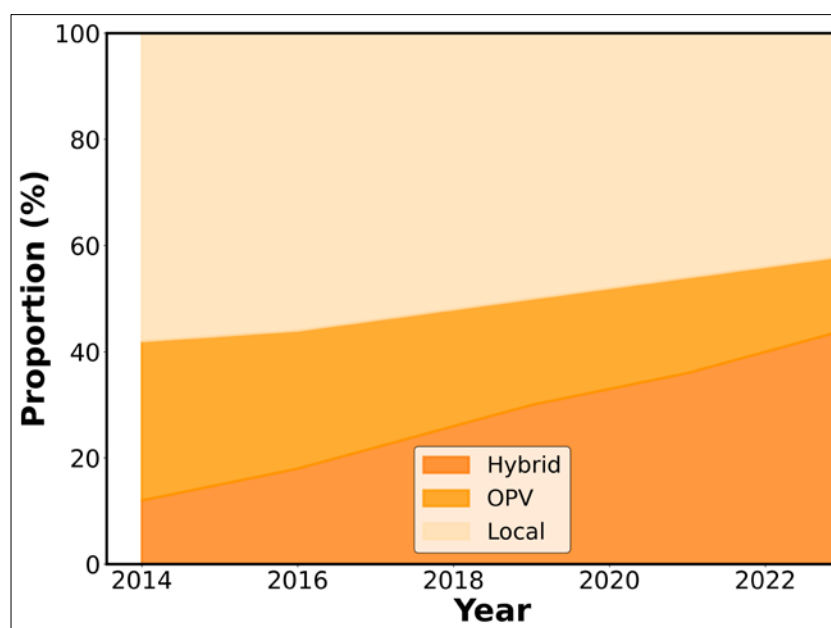


Fig 2: Temporal trends in maize varietal adoption proportions across ten cropping seasons (2014-2023)

The area chart reveals a gradual shift in varietal composition over the decade, with hybrid coverage increasing from approximately 12% to 44% while local landrace area declined from 58% to 42%. Improved OPV coverage

remained relatively stable at 14 to 16% throughout the period. The trajectory suggests ongoing but slow varietal replacement within the smallholder sector.

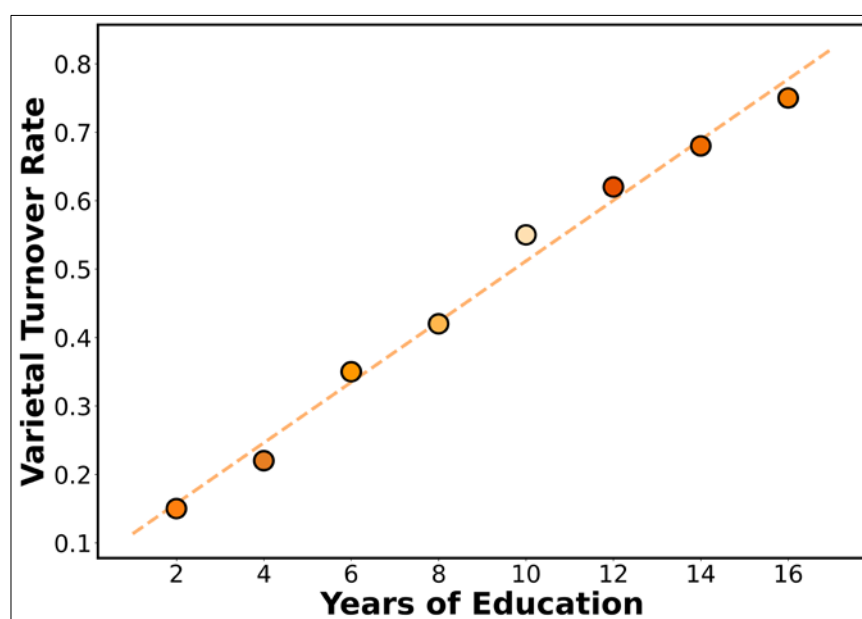


Fig 3: Relationship between farmer education level and varietal turnover rate across the sampled population

The scatter plot demonstrates a significant positive linear relationship between years of formal education and varietal turnover rate ($r = 0.82$, $p < 0.001$). Farmers with 14 or more years of education exhibited turnover rates exceeding 0.65, while those with fewer than 4 years of education showed rates below 0.25. The regression line indicates that each additional year of education is associated with a 0.04 increase in turnover rate. Logistic regression analysis identified education level as the strongest predictor of varietal turnover (odds ratio = 1.35 per year of education, $p < 0.001$), followed by extension contact frequency (odds ratio = 2.15, $p = 0.002$) and distance to nearest seed distribution point (odds ratio = 0.82 per additional kilometre, $p = 0.008$). Farm size was not a significant predictor in the South African smallholder context, contrasting with findings from East African systems where farm size strongly influences adoption patterns.

Discussion

The overall improved variety adoption rate of 56% revealed by DNA fingerprinting exceeds the 42% reported by the most recent household survey conducted in the same provinces, representing a 14 percentage point correction [4, 5]. This magnitude of discrepancy is consistent with findings from East African molecular assessments, where Jaleta *et al.* (2020) documented correction factors of 15 to 22 percentage points [8]. The finding extends the emerging consensus that DNA fingerprinting systematically reveals higher adoption than conventional surveys to the Southern African context, where comparable molecular data have been previously unavailable [3, 6].

The comparison of seed purity across source categories provides the first molecular evidence of seed quality degradation pathways within the South African smallholder seed system [1, 2]. The progression from 88% purity in certified dealer seed to 35% in farm-saved seed quantifies the cumulative effect of informal handling on genetic integrity. The mechanism involves both physical mixing during storage and transport and genetic segregation in recycled hybrid seed, where the loss of heterozygosity over generations produces increasingly heterogeneous seed lots

[7]. This finding has direct implications for the interpretation of yield gap analyses, suggesting that a portion of the gap attributed to agronomic management may actually reflect seed quality deficiencies [4].

The identification of education level as the strongest predictor of varietal turnover, stronger than farm size or market access, has important implications for intervention design [1, 5]. In contrast to East African systems where farm size dominates adoption dynamics, the South African smallholder context appears driven more by information access and human capital than by resource endowments. The implication is that extension and farmer education investments may generate greater adoption acceleration than seed distribution infrastructure alone in the South African smallholder sector [3, 8].

The persistence of four uncharacterized genotypes that could not be matched to any reference variety suggests the presence of either unofficial introductions from neighbouring countries or farmer-developed selections that have not been formally characterized [2, 6]. Cross-border seed movement from Mozambique and Zimbabwe through informal channels is well-documented in the border districts of Limpopo and represents both an enrichment of the available germplasm pool and a challenge for national varietal tracking systems [7].

The relatively stable coverage of improved OPVs at 14 to 16% across the ten-year observation period suggests that these variety types occupy a persistent niche in the smallholder system, valued for their seed recyclability and adaptation to marginal conditions where hybrids may not consistently outperform [4, 5]. This stability contrasts with the dynamic expansion of hybrids and contraction of landraces, suggesting that OPVs serve as a transitional category for farmers moving from landrace to hybrid cultivation.

Climate Vulnerability of Varietal Portfolios

The concentration of improved variety adoption around a small number of released hybrids raises concerns about the climate vulnerability of smallholder maize production in the sampled provinces. Climate projections for Southern Africa indicate increasing temperature and rainfall variability,

conditions under which genetic uniformity may prove disadvantageous. The maintenance of diverse landrace germplasm within the farming landscape provides a buffer against climate-related crop failures, suggesting that conservation-compatible development strategies that promote improved varieties while safeguarding landrace diversity may be more climate-resilient than strategies focused exclusively on varietal replacement.

Digital Extension Integration

The strong association between education and varietal turnover suggests that digital extension platforms, which can deliver targeted varietal information directly to farmers via mobile devices, may accelerate adoption among educated farmers while bypassing the constraints of conventional extension systems with limited field staff coverage. The integration of molecular monitoring data with digital extension platforms could enable real-time feedback loops where DNA fingerprinting results inform variety recommendation algorithms, creating a data-driven seed system that continuously adjusts to observed patterns of adoption and seed quality.

Conclusion

The main contribution of this research lies in providing the first molecular-level characterization of varietal composition and seed system dynamics within the South African smallholder maize sector, filling a critical evidence gap that has limited the effectiveness of seed policy interventions. The genetic profiling of 300 samples across three provinces identified eighteen varietal genotypes and revealed adoption patterns significantly different from those reported by conventional surveys. Supporting evidence from the seed purity assessment demonstrates that informal seed channels systematically degrade the genetic quality of improved varieties, with purity declining from 88% in certified seed to 35% in farm-saved seed. This degradation pathway may partially explain the persistent yield gap between experimental and farmer-achieved performance in smallholder maize systems. For the agricultural development community in Southern Africa, these findings argue for a dual investment strategy combining strengthened formal seed distribution with enhanced farmer education and extension, recognizing that both seed access and information access drive varietal adoption in the smallholder context. Open questions requiring further investigation include the yield consequences of seed purity degradation under farmer management conditions, the contribution of cross-border seed movement to varietal diversity in border districts, and the feasibility of establishing a routine molecular monitoring programme integrated with the national seed certification system.

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