



SSR markers deciphered the genetic purity and identity of maize (*Zea mays L*) varieties

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Introduction: Maize (*Zea mays L.*) is a diverse crop in morphological and molecular characteristics (Zheng et al., 2008). Genetic purity and identity tests are essential to identify purity and conformity of released seeds by companies. Simple sequence repeats (SSRs), or microsatellites, are tandemly repeated nucleotide motifs in the eukaryotic genomes (Lu *et al.*, 2018; Pour-Aboughadareh *et al.*, 2022). Microsatellites are recognized as outstanding molecular markers due to their high amount of allelic diversity, particularly for revealing close relationship of individuals (Ribeiro *et al.*, 2017; Zhao *et al.*, 2023).

Materials and methods: Five maize varieties were used to study molecular characterization at molecular markers Laboratory during 2023. Three hybrids (imported by private companies) and two hybrids (from seed bank) were tested for genetic purity and identity. Genomic DNA extraction was extracted from leaves following the protocol described by Doyle (Doyle, 1991). DNA quantification and qualification were measured by NanoDrop spectrophotometer (ND-1000, Thermo Fisher Scientific) at 260 nm. Twenty-three SSR primer pairs were used to identify the studied maize genotypes varieties. PCR amplification and poly acrylamide gel electrophoresis were run through the highly polymorphic primer sets. Seven out of twenty- three SSR primers showed polymorphisms, as shown in Table 1. Amplified DNA fragments were scored based on the presence or absence of the alleles. The total number of alleles per locus, allele frequency and Principal Coordinates Analysis (PCOA) were calculated using the statistical analysis software GenAlEx version 6.5. The Polymorphic Information Content (PIC) was calculated using POWER MARKER software version 3.23.

Results and Discussion: In this study, twenty-three SSR marker sets were employed to determine genetic purity and identity of three maize varieties introduced by a private company along with two reference varieties from our seed bank. Seven markers demonstrated polymorphism in the maize varieties for the identity test (Table 1). The two SSR markers (SSR-blnl-161, umc-1593) showed high efficiency in identifying maize varieties due to their high PIC values (over 0.7). The highest values for genetic variation parameters were observed in genotype no.2. However, only two markers were polymorphic in the genetic purity test, with SSR umc-1065 reaching the highest PIC value (0.6). The Polymorphism Information Content (PIC) demonstrates the information of allele diversity and allele frequency among the genotypes (Pervaiz *et al.*, 2009). The SSR markers with high PIC value over 0.5 were considered highly informative and proper for presenting the polymorphism level of a marker at a specific locus (Kyi et al., 2022). The PCOA analysis of SSR data grouped the five maize varieties into four main clusters, revealing that two varieties presented similar genetic pattern, while the others were distinct (Figure 1a). Additionally, the introduced varieties showed no complete conformity with our seed bank varieties. However, during the genetic purity test, the varieties were categorized into three groups that showed complete purity for the genotype two and impurity for others (Figure 1b). In this study, we elucidated the potential of three SSR markers, one with higher efficiency in the genetic purity test and two for the genetic identity test.

Conclusion: The present study demonstrated a high level of genetic diversity among varieties that led to determine genetic identity and purity of maize varieties. The SSR markers proved to be reliable genetic tools for variety identification. Besides, more SSR markers would be employed to evaluate differentiation of genotype 1 and 3 in the future research.

Keywords: Genetic purity; Genetic identity; SSR markers; maize; Polymorphism; Genetic variation

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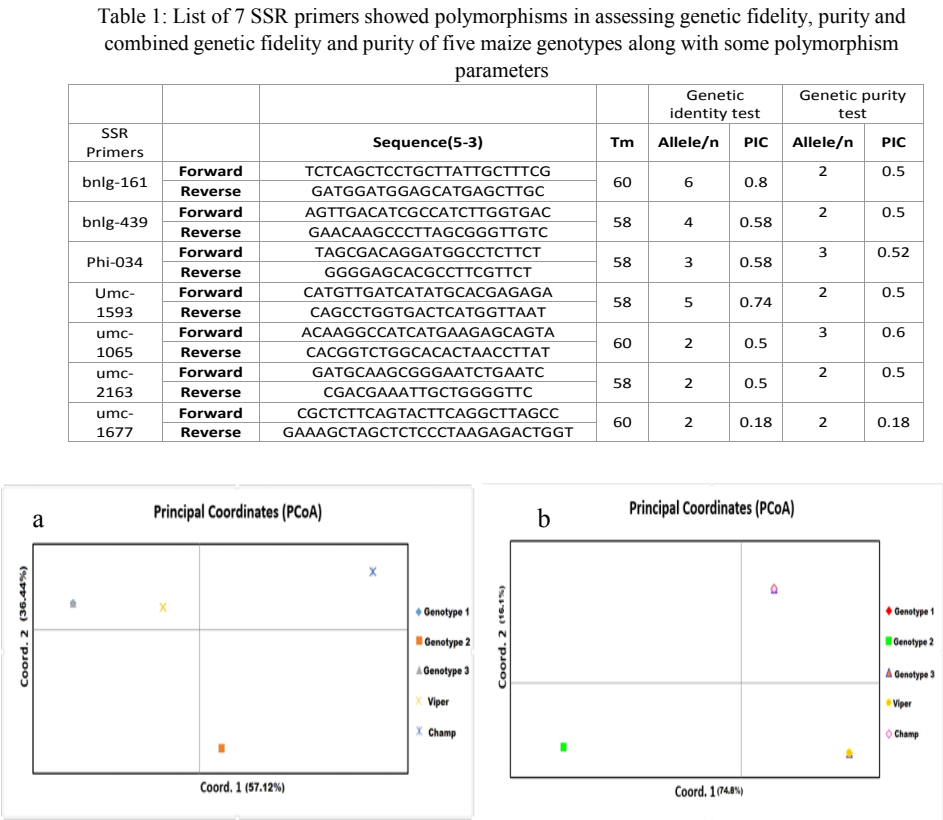


Figure 1: PCoA analysis of genetic identity (a) and purity (b) of five maize genotypes