**DNA-Fingerprinting of microsatellite loci in sesame (*Sesamum indicum* L.): Opportunities and Challenges.**

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ABSTRACT

Sesame is an ancient and multi-utility oilseed crop but has stagnated seed yield levels for quite a long-time and thereby constraining sesame-farming communities in realizing significant profit per unit land area. To circumvent the situation, an increasing number of varieties are being released every year. In this context, establishing a molecular evidence-based technology for ascertaining the distinctiveness of each variety from other is a pertinent yet a challenging task. In the present work, 30 sesame varieties were assessed for DNA fingerprinting of 50 microsatellite loci to establish their genomic distinctiveness.

**Materials and methods:** Sesame varieties were raised in pots under uncontrolled condition. Genomic DNA was isolated using CTAB method with minor modifications. The DNA samples were quantitated, and the quality was ascertained on 1% agarose gel. PCR was carried out using template DNA of 30 genotypes across 50 microsatellite markers, validated and optimized in our laboratory, followed by agarose (3%) gel electrophoresis and gel documentation.

**Results and Discussions:** Polymerization chain reaction-amplicons resolved on agarose gel were compared for their relative sizes (i.e., alleles; Fig. 1). Thirty genotypes were grouped

based on the alleles of each of the 50 marker loci. A panel of 50 SSR markers discriminated 30 sesame varieties, either singly or in combination. The resulting marker-variety matrix was obtained, and it is useful as a starting guide for selecting SSR markers for DNA fingerprinting of new varieties developed. However, none of the markers in the developed matrix may discriminate every new variety. Therefore, it is a challenging task to search a discriminative marker, as it involves a tedious task of trial-and-error method.

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| **Figure 1**: An illustration of amplicon sizes of a microsatellite SIM123 locus among 10 sesame varieties: 1: AKT101; 2: AKT64; 3: Amrit; 4: B67; 5: Chandana; 6: CUMS17; 7: DS5; 8: DS9; 9: E8; 10: GJT5; Mr: 100 bp marker. |

Further, the limitation of the DNA-fingerprinting is that no marker may discriminate a variety, which in fact is a distinct one, for it is difficult to establish similarity unless whole genome is compared base-by-base. The pragmatic solution is to distinguish new variety from its parent(s).

**Conclusions:** Microsatellite marker-based DNA-fingerprinting comes as a useful handy tool for ascertaining distinctiveness of claimed-to-be a new variety and the SSR-Genotype matrix developed in this work is useful as a starting point for DNA-fingerprinting of new varieties. Further, the matrix can be augmented as and when new varieties and their parents are analysed using the studied markers or new marker(s). Enrichment of markers with large number of SNPs and indels require genotyping by sequencing of all the released varieties and their parents to develop a fool-proof DNA-fingerprinting methodology for each variety.

**Keywords:** Sesame; DNA-fingerprinting; Microsatellite Markers; Variety Discrimination