



*Methodology*

## Genetic diversity analysis in Opal cotton hybrids based on SSR, ISSR, and RAPD markers

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**ABSTRACT.** Cotton is one of the most economically important crops in Iran; hybridization is a means to increase the genetic diversity and obtain new elite cultivars in this crop. We examined agronomic characteristics and molecular genetic diversity in the Opal cotton (*Gossypium hirsutum*) cultivar and in F<sub>2</sub> progenies. Ten homo-primers and seven hetero-primers of 26 RAPD primers produced 261 reproducible bands, with an average of 4.18 bands per primer and 22% polymorphism. The OPB12/OPH08 primer gave the highest effective number of alleles ( $N_E$ ), and the largest Shannon index (I), Nei's genetic diversity (H), and polymorphism information content (PIC) values. Some RAPD bands were present in the parental genotypes but were absent in their hybrids. Ten ISSR primers produced 206 reproducible bands, with 49.4% polymorphism. The UBC807 locus gave the highest  $N_E$ , I, H, and PIC values. Some ISSR bands occurred only in the parental genotype, while others were

only present in the hybrid genotypes. Four microsatellite loci produced 12 alleles, ranging from 181 to 236 bp, with 54% polymorphism. The TMB1421 locus, with a monomorphic allele, was digested with three restriction enzymes (CAP-microsatellite) to evaluate sequence variations among samples. Association analysis between molecular markers and agronomic data revealed a significant correlation between ISSR-UBC807-1500 and yield. The Mantel test performed among the genetic distance matrices obtained from RAPD, ISSR and SSR showed a non-significant regression between RAPD versus ISSR and ISSR versus SSR, while RAPD versus SSR showed a significant regression; regression for ISSR and RAPD+ISSR+SSR combined data was also significant. Cluster analysis (UPGMA) based on these three types of molecular markers differentiated cotton genotypes and their progenies. Among the molecular markers, ISSR revealed more genetic variation among the genotypes. However, using all three types of molecular markers provided a better overall view of cotton genome polymorphism.

**Key words:** Agronomic traits; Genetic variation; ISSR; RAPD; SSR; *Gossypium hirsutum* L.