



RESEARCH PAPER

Quality-focused computational approach for SSR marker development in horsegram (*Macrotyloma uniflorum*): A comprehensive assessment framework

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Abstract : Horsegram (*Macrotyloma uniflorum*) represents an underutilized legume crop possessing significant nutritional and therapeutic potential, yet molecular marker resources for this species remain critically limited. Simple Sequence Repeat (SSR) markers serve as essential tools for genetic diversity studies, population genetics analysis, and breeding program development in underutilized crops. This study aimed to develop and comprehensively evaluate SSR markers for horsegram through implementation of a novel multi-parameter quality assessment framework that prioritizes marker reliability over quantity considerations. A total of 91 SSR markers were computationally designed from horsegram genomic sequences utilizing MISA (MIcroSatellite identification tool) and Primer3 software platforms. A comprehensive quality assessment system was developed incorporating six critical parameters: primer structural integrity (20%), thermodynamic compatibility (25%), GC content optimization (20%), SSR polymorphism potential (15%), primer length optimization (15%), and 3' stability (5%). Markers were systematically classified into four distinct categories based on scoring: Excellent (≥ 85 points), Very Good (75-84), Good (60-74), and Poor (< 60). Comprehensive quality assessment revealed that only 2 markers (2.2%) achieved excellent quality status, while 89 markers (97.8%) failed to meet stringent quality criteria established by the framework. Failure mode analysis identified insufficient flanking sequences (27.5%), extreme GC content deviation (19.8%), and suboptimal primer length parameters (16.5%) as primary limiting factors affecting marker quality. The two excellent markers (HG_SSR_001 and HG_SSR_002) demonstrated optimal thermodynamic properties with melting temperatures ranging 58-62°C, balanced GC content between 45-55%, and high polymorphism potential for genetic analysis applications. This study introduces the first comprehensive computational quality assessment framework specifically designed for SSR development in underutilized legumes, emphasizing scientific rigor over inflated marker numbers commonly reported in literature. Despite the relatively low success rate observed, this research establishes a robust methodological foundation for horsegram genetic analysis while demonstrating the critical importance of quality-focused marker development approaches in molecular breeding programs.

Key Words : Horsegram, *Macrotyloma uniflorum*, SSR markers, Computational biology, Quality assessment, Underutilized crops, Molecular breeding

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