

A Novel Molecular Marker for Gel-based Biotyping of Fall Armyworm (*Spodoptera frugiperda*)

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The fall armyworm (FAW), *Spodoptera frugiperda* is a Noctuid lepidopteran pest with a polyphagous feeding habit. The larval stage of FAW feeds on a wide range of crops, including major cereals and vegetables causing significant yield losses globally. The genetic complexities and their migration patterns have resulted two morphologically indistinguishable FAW subpopulations, named “rice strain” (R-FAW) and “corn strain” (C-FAW). Previously, sequence-based analysis, restriction enzyme-based profiles, and dominant marker assays targeting the *mitochondrial cytochrome oxidase 1 (COI)* gene have been used to differentiate the biotypes. In the current study, we developed a gel-based co-dominant temperature-switch PCR (TSP) marker, targeting a single nucleotide polymorphism (SNP) for direct biotyping of FAW. To design the marker, reference *COI* gene sequences of R-FAW (Genbank accession: U72977) and C-FAW (Genbank accession: U72974) were retrieved from the NCBI repository. A 746 bp length sequence of the *COI* gene was aligned using ClustalW feature in Geneious v.7.1.3. The TSP marker was designed using the Primer3 platform on Geneious, with a pair of locus-specific primers defining the region of interest, and a pair of nested locus-specific primers targeting a SNP within the resulted amplicon. A sample each of R-FAW and a C-FAW biotypes (confirmed as the respective biotypes through direct DNA sequencing of the *COI* region and comparing with the FAW reference sequences) collected from Ridimaliyadda amplified the expected 341-bp amplicon, and a 204-bp amplicon, respectively, for the TSP marker assay. Thus, the newly developed TSP marker, named *ABUOP0002* can be recommended as a gel-based molecular marker for the identification of FAW rice and corn biotypes. This marker can be used as a low-cost molecular tool to efficiently diagnose FAW biotypes without DNA sequencing especially in a resource-limited setting.

Key words: Biotyping, *COI* region, Fall Armyworm, Molecular markers, Temperature-switch PCR