Genetic analysis in some citrus accessions using microsatellites - and AFLP-based markers

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ABSTRACT

Twelve ISSR primers, five SSR primer pairs and five AFLP primer combinations were used to estimate the level of polymorphism among 14 Citrus genotypes. The two types of ISSR primers (unanchored and anchored) showed different levels of polymorphism. All of the unanchored primers successfully amplified bands with a total number of 54 of which 44 were polymorphic, representing a percentage of polymorphism of 79.14% and 8.8 polymorphic bands per primer. While, three out of the four 5'-anchored primers amplified scorable bands revealing a total number of 34 bands of which 28 were polymorphic, corresponding to 82.4% polymorphism and an average of 9.3 polymorphic bands per primer. On the other hand, the 3'-anchored primers failed to amplify reproducible patterns even with the modification of the PCR-reaction and temperature profile. Four sets out of five SSR primer pairs amplified a total number of 23 alleles revealing 100% polymorphism, with an average of 5.8 polymorphic alleles per loci. The SSR markers revealed allele frequency ranging from 0.04 to 0.52 . The expected heterozygosity ranged from 0.65 to 0.83 and the effective alleles per locus ranged from 2.9 to 5.9. The five AFLP primer combinations amplified a total number of 311 amplicons, where 304 were polymorphic representing 97.7% polymorphism and 60.8 polymorphic amplicons per primer combination. The microsatellite based-markers revealed genetic similarity ranging from 55.4 [between Ruby red grapefruit (RRG) and Troyer citrange (TC)] to 94.9 [between Washington navel orange (WNO) and Valencia orange (VO)]. While, the highest similarity percentage (73.9%) detected by AFLP markers was between Volkamer lemon (VOL) and Rangpure lime (RL), and the lowest percentage of similarity (8.3%) was observed between Troyer citrange (TC) and Balady sour orange (BSO).Generally, the dendrograms based on the two types of markers (microsatellites and AFLPs) revealed nearly similar topology separating Troyer citrange from the other Citrus genotypes. Moreover, accessions belonging to the same species always clustered together except for the sweet orange group (Valencia orange, Washington navel orange and Robertson navel orange) only in the AFLP dendrogram. All the tested ISSR primer (8) identified ten accessions, and both of Mic4 and Amic1 detected the highest number of identified accessions (4). Moreover, four SSR primer pairs identified six Citrus accessions (Rangpure lime, Willowleaf mandarin, Cleopatra mandarin, Troyer citrange, Robertson navel orange and Abdel Razek mandarin). In addition, SSR markers exhibited species-specific markers. Three species out of eight (C. aurantium, C. deliciosa and C. paradisi) were characterized by specific allele sizes. The AFLP had the potentiality to characterize all the

tested accessions by unique markers. Combination $M_{cag}XE_{aag}$ characterized the highest number of Citrus genotype (10). While, combination $M_{ctc}XE_{agc}$ characterized the lowest number of accession (5). In addition, the genomic DNA of Valencia orange scions grafted onto three different rootstocks (Volkamer lemon, Troyer citrange and Cleopatra mandarin) was analyzed with five different AFLP primer combinations. All the tested combinations revealed new bands (1-6) in the graft-induced variants except for combination M_{ctt} X E_{act} with Troyer citrange and M_{cag} X E_{act} with Cleopatra mandarin. On the other hand, some bands which were present in the scion and (or) stock disappeared in the graft induced variant. The number of these bands ranged from 1 to 7. Moreover, it seems that the examined rootstocks transmitted bands to the graft induced variants, ranging in number from 1 to 6. While, the conserved bands from the scion ranged from 2 (with combination $M_{cac}XE_{acc}$ on both of Troyer citrange and Cleopatra mandarin) to 9 (with combination $M_{ctc}XE_{agc}$ on Volkamer lemon). These results indicated the presence of a genetic relationship between the scion and the stock, however, further studies are needed to elucidate this relationship at the molecular level.

Key words: *Citrus, ISSR, SSR, AFLP, Genotype-specific markers, Scion-stock relationships.*

INTRODUCTION

Genetic analysis including assessment of genetic diversity, relatedness between or within species, population and individuals as well as genotype characterization, are central tasks for many disciplines of biological sciences. Conventionally, genetic analysis was dependent on morphological and/or biochemical markers. During the past few decades, classical strategies of genetic analysis have been increasingly complemented by molecular techniques. The most fundamental of these molecular techniques are DNA markers which portray genome sequence composition, thus, enabling to detect differences in the genetic information carried by the different individuals. Therefore, these markers are powerful tools in genotype identification or fingerprinting and the estimation of relatedness between genotypes. Consequently, they provide the means to utilize our existing germplasm resources to understand fundamental plant processes and mechanisms. Furthermore, marker-mediated genetic analysis elucidates the genetic basis of agronomic characters and leads to their direct manipulation by plant breeders.

Several molecular marker systems have been applied in the last few decades and the development of the polymerase chain reaction (PCR) technology has introduced a considerable number of useful molecular markers. RAPD (Williams *et al.*, 1990) was the first PCR-based marker system used in genetic analysis. Subsequently, a large number of PCR-based marker systems have been developed including microsatellite-based markers and AFLPs.

Microsatellites consist of highly variable tandem repeats of very short motifs (1-6 bp) (Litt and Lutty, 1989). Based on microsatellites two types of DNA markers could be generated, i.e., simple sequence repeats (SSRs) and inter simple sequence repeats (ISSRs). In SSRs, the polymorphism is detected by PCR amplification using primers complementary to unique flanking sequences. While generation of ISSR markers involve PCR amplification of DNA using a