**PRIMER NOTE**

**CITRUS (RUTACEAE) SNP MARKERS BASED ON COMPETITIVE ALLELE-SPECIFIC PCR; TRANSFERABILITY ACROSS THE AURANTIOIDEAE SUBFAMILY**

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- **Premise of the study:** Single nucleotide polymorphism (SNP) markers based on Competitive Allele-Specific PCR (KASPar) were developed from sequences of three *Citrus* species. Their transferability was tested in 63 *Citrus* genotypes and 19 relative genera of the subfamily Aurantioideae to estimate the potential of SNP markers, selected from a limited intrageneric discovery panel, for ongoing broader diversity analysis at the intra- and intergeneric levels and systematic germplasm bank characterization.

- **Methods and Results:** Forty-two SNP markers were developed using KASPar technology. Forty-one were successfully genotyped in all of the *Citrus* germplasm, where intra- and interspecific polymorphisms were observed. The transferability and diversity decreased with increasing taxonomic distance.

- **Conclusions:** SNP markers based on the KASPar method developed from sequence data of a limited intrageneric discovery panel provide a valuable molecular resource for genetic diversity analysis of germplasm within a genus and should be useful for germplasm fingerprinting at a much broader diversity level.

**Key words:** Competitive Allele-Specific PCR; genetic diversity; Rutaceae; single-nucleotide polymorphisms (SNPs).

Single nucleotide polymorphisms (SNPs) are the most frequent type of DNA sequence polymorphism. Their abundance and uniform distribution in genomes make them very powerful genetic markers. Several SNP genotyping methods have been developed. For low-to-medium throughput genotyping, the KBioscience Competitive Allele-Specific PCR genotyping system (KASPar; KBioscience Ltd., Hoddesdon, United Kingdom) appears to be an interesting approach (Cuppen, 2007) that has been successfully applied in animals and plants (Nijman et al., 2008; Bauer et al., 2009; Cortes et al., 2011). For genetic diversity studies with SNP markers, it is very important to determine the representativeness of the discovery panel (Albrechtsen et al., 2010). Ascertainment bias of the SNP makers affects the evaluation of genetic parameters, as was observed for the *Citrus* L. genus using SNP markers mined in a single Clementine cultivar (Ollitrault et al., 2012). Recently, Garcia-Lor et al. (2013) sequenced 27 amplified nuclear gene fragments for 45 genotypes of *Citrus*, which resulted in the identification of 1097 SNPs. Taking advantage of these previously obtained SNP data, the objective of this work was to implement a set of polymorphic SNP markers for systematic germplasm bank characterization within the *Citrus* genus and to investigate their transferability across the Aurantioideae [Engler] subfamily. More generally, the objective was to estimate the usefulness of SNP markers developed using KASPar technology, which were selected from a limited intrageneric discovery panel, for broader diversity analysis at the intra- and intergeneric levels.

**METHODS AND RESULTS**

The 42 SNP markers used in this study were selected from SNP markers identified by Garcia-Lor et al. (2013) in 27 nuclear genes. Most cultivated citrus (except for *C. auranitifolia* (Christm.) Swingle) arose from interspecific hybridization of three ancestral taxa: *C. medica* L., *C. reticulata* Blanco, and *C. maxima* (Burm.) Merr. (Nicolosi et al., 2000; Barkley et al., 2006; Garcia-Lor et al., 2012). Therefore, we selected SNPs between and within these three taxa (based on seven *C. reticulata*, five *C. maxima*, and five *C. medica* accessions). Primers were defined by KBioscience (http://www.kbioscience.co.uk/) from each SNP locus flanking sequence (Appendix S1). Two allele-specific oligonucleotides and one common oligonucleotide were defined for each locus (Table 1). The KASPar system uses two Förster resonance energy transfer (FRET) cassettes, where fluorometric dye is conjugated to the primer but quenched via resonance energy transfer. In this system, sample DNA is amplified in a thermal cycler using allele-specific primers, leading to the separation of fluorometric dye and quencher when the FRET cassette primer is hybridized with DNA (Cuppen, 2007). Normalized signals of each SNP allele (x and y) were provided by KBioscience. Automatic allele calls provided by KlusterCaller software were visually checked with two-dimensional plot representations using SNPViewer software (KBioscience Ltd.).

Eighty-four accessions (Appendix 1) were genotyped for the 42 SNP markers. The sample set included representatives of the two tribes of the Aurantioideae (Clausenae and Citreae). In Clausenae, the subtribe Clausenae was represented by four genotypes (three genera). Within the Citreae, three subtribes were represented: Triphasilinae (one genus was included), Balsamocitri-nae (represented by six genera), and Citreae (11 genera represented). For the Citreae, we adopted the subdivision of this tribe into three groups (as proposed by Swingle and Reece, 1967), namely the primitive citrus fruit group (four accessions of four genera), the near citrus fruit group (three accessions of two