

or horticultural traits, but trueness-to-type can now be confirmed using molecular markers, thereby limiting inadvertent cultivar misidentification. However, following routine testing the reported parentage of two mandarin hybrids is now questioned. 'Orri' mandarin is derived from 'Orah' mandarin hybrid by induced mutation, and was bred by Spiegel-Roy and Vardi (The Volcani Center, Israel) with 'Temple' tangor and 'Dancy' mandarin reported as the parents. 'Orri' is prized in many markets for its excellent organoleptic qualities, and other favorable traits. With 'Dancy' as the pollen parent, at least some degree of susceptibility of 'Orri' to *Alternaria* Brown Spot (ABS) might be expected, but more likely to have inherited a high degree of susceptibility; yet it appears to be immune to ABS. SSR analysis provided evidence that excludes 'Dancy' as the pollen parent of 'Orah'. Further testing has revealed the likely candidate as the pollen parent. 'Fortune' mandarin was a popular late-maturing mandarin cultivar until the high incidence of ABS rendered the cultivar uneconomical to produce. This exceptionally late, high quality, attractive cultivar is reported to have originated from a clementine mandarin x 'Dancy' cross made by Furr (USDA, Indio, California). 'Fortune' is used as a female parent in breeding programs. However, two independent molecular marker studies revealed that the pollen parent of 'Fortune' is actually 'Orlando' tangelo and not 'Dancy'. In both cases, the pollen parent of 'Orri' and 'Fortune' mandarin hybrids appears to not be 'Dancy' as published. This information will assist geneticists and breeders to re-interpret heritability studies on ABS susceptibility of mandarins and their hybrids.

S02P02

Simple PCR-based tools provide molecular identification of the main rootstock used in Sao Paulo State, Brazil.

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Distinguishing between 'Sunki' and 'Cleopatra' mandarins as well as between 'Carrizo' or 'Troyer' citranges and 'Swingle' citrumelo very early in the nursery based only based on their respective leaf morphology could result in erroneous interpretation. Horticultural advantages of 'Swingle' rootstock are incomparable to 'Carrizo' under our conditions (Sao Paulo State, Brazil). The crescent diversification of citrus rootstock varieties in SP state has demanded, in particular situations, for identification of rootstock varieties very early in the nursery, sometimes as a consequence of accidental mixture of seeds. To help growers and nurseryman accurately identify rootstock varieties, we developed PCR-based tools that identify most of rootstocks used in SP state. Information from three-selected RAPD 10mer primers (M04-ggcggttgc, M15-ctggcgtgc, and AB18-gacctaccac) can be successfully used to identify and genetically separate citrange, citrumelo, and *Poncirus trifoliata*. Another 10mer primer (R04-cccgtagcac) identifies 'Rangpur' lime (*Citrus limonia*) and separates it from 'Volkameriano', and 'Siciliano' lemons. From the fingerprints generated by those 10-mer primers, repeatable and consistently well-amplified DNA fragments are used as variety-specific markers. Also, 'Sunki' and 'Cleopatra' mandarins are identified by the microsatellites CCSM-6R (cctctattaatgtgcctg) and CCSM-6F (atctgtgtgaggactgaa) primers. These simple PCR based protocols have been used to avoid significant economic losses by the early and correct identification of rootstocks in SP state nurseries.

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S02P03

Assignment of SNP allelic configuration in polyploids using Competitive Allele-Specific PCR: application to triploid citrus progenies

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Estimation of DNA allele doses in polyploid genotypes is essential to assign the allelic configuration for the different types of heterozygosity for parents and segregating progenies. It is of special interest in citrus with the implementation of extensive triploid breeding programs to develop new seedless varieties. Single nucleotide polymorphisms (SNPs) are becoming the most important class of molecular markers and we have tested the potential of KASPar technology (based on competitive allele-specific PCR) to infer allele doses of SNP markers in

polyploid citrus. DNAs from haploids *Citrus clementina* and *Citrus maxima* were mixed at different proportions to test the accuracy of the technique for quantitative analysis. All analyzed 7 SNPs provided clear clustering related with allele doses. DNA mixes at intermediary proportions revealed high correlation coefficients between observed and expected data (mean = 0.9796; sd = 0.0094). For all SNPs, separated cluster analyses and ANOVA from mixed DNA data formed all expected homogeneous groups, with correct assignation for practically all samples. Moreover, two triploid populations were easily genotyped and results were in agreement with expected segregations. KASPar technology is a routine and a cost-effective technique to assess the allele doses at the DNA level, which is especially interesting in citrus triploid breeding programs. Moreover, it could be also used to correlate genomic and transcriptomic doses in allele specific expression analyses.

S02P04

The parentage analysis contributes to the validation of high throughput SNP genotype calls of citrus

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High-throughput genotyping is an important breeding tool to provide genome wide genotyping of germplasm for breeding and the selection of markers through linkage mapping. However, the validation system for genotyping calls from the automatic analysis has not been adequately developed to apply the high-throughput genotyping system in a practical fashion, because the marker data sets derived from DNA markers often contain missing or questionable genotype calls. Therefore, the objective of this study was to develop validation procedures using SNP data sets from a previously developed 384 multiplexed SNP array, named *CitSGA-1*, for the genotyping of *Citrus* cultivars. For this purpose, we initially used the manufacture's criterion that included: (1) the call frequency scores (over 0.9) for SNPs and (2) the GC10 and GC50 scores of samples. Thereafter, the following validation procedures were investigated: (3) removal of monomorphic SNPs genotype calls, (4) removal of No Call SNPs, and (5) removal of SNPs with discrepancies in the parentage analysis. In these procedures, the parentage analysis could detect the genetic discrepancy between parents and progeny in the reliable criteria. The obtained reliable SNP types were also tested for the reproducibility of calls by the replicated genotyping of accessions on the same SNPs. The results confirm these the validation procedures, which include the design of samples for parentage assays and the replicates necessary to obtain the reliability of large genotype calls.

S02P05

Comparative values of SSRs, SNPs and InDels for citrus genetic diversity analysis

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SSRs have long been considered as almost ideal markers for genetic diversity analysis. With the increasing availability of sequencing data, SNPs and InDels become major classes of codominant markers with genome wide coverage. We have analyzed the respective values of SSRs, InDels, and SNPs for intra and interspecific *Citrus* genetic diversity analysis. Moreover, we have compared the diversity structure revealed by markers mined in a single heterozygous genotype (the clementine) and markers mined in a large interspecific survey. A random set of 25 markers was selected for each marker class to genotype 48 citrus accessions. SSRs were the most polymorphic markers at the intraspecific level allowing complete varietal differentiation within basic taxa (*Citrus reticulata*, *Citrus maxima*, *Citrus medica*). However, SSRs gave the lowest values for interspecific differentiation, followed by SNPs and InDels, that displayed low intraspecific variability but high interspecific differentiation. A clear effect of the discovery panel was observed for SNPs and InDels. The ascertainment biases associated with the clementine heterozygosity mining resulted mainly in an over estimation of within *C. reticulata* diversity and an underestimation of the interspecific differentiation. Therefore SSRs are very useful for intraspecific structure analysis while SNPs and InDels mined in large discovery panel will be more powerful to decipher the interspecific mosaic structure of secondary cultivated species.