S03P13

Fast and cost-effective DNA marker typing method with accuracy for citrus by direct PCR and multiplexed post-labeling

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DNA marker typing has been used in citrus for many purposes such as linkage mapping, marker-assisted selection, and cultivar identification. However, DNA marker typing is time-consuming and costly in citrus because of requiring highly purified DNA to eliminate compounds that strongly inhibit PCR. Fragment analysis with capillary DNA sequencer enables highly accurate genotyping analysis but it requires expensive fluorescently labeled primers that are another cost factor. We developed direct PCR method for citrus in order to achieve fast and cost-effective genotyping. This method consists of two steps; (1) pricking a leaf sample with a toothpick, and (2) dipping the toothpick into a PCR mixture or a TE buffer solution directly to prepare a DNA sample. The prepared DNA sample was then provided for PCR analysis. We were able to process 95 samples within 1.5 h, in contrast to 23 h by conventional DNA extraction method. Subsequent PCR amplification using five different STS markers was highly successful (96–100%) except for the 1200 bp marker using the DNA samples dissolved in TE buffer solution (86%). We combined the direct PCR method with multiplexed post-labeling method for fragment analysis with DNA sequencer, which enables multiplexed post-labeling of DNA markers (up to 6) in a single tube without laborious labeling step. As a consequence, total cost was decreased to one tenth or less than those using pre-labeled primers.

S03P14

Data mining and systems biology for identifying key genes involved in citrus quality

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Quality in citrus is mainly characterized by fruit and juice colour, fruit and skin size, juice percent, total soluble solids, titrable acidity, and carotenoid/flavonoid contents. Moreover, studies of biosynthetic pathway of the metabolites/proteins involved in quality at transcriptional and translational levels may give relevant information for subsequent functional studies and quality improvement. Data mining of ESTs from HarvEST database allowed the selection of 17 cDNA libraries from albedo, flavedo, peel, pulp and juice sac of different orange, mandarin, clementine and grapefruit varieties. In order to select key genes involved in quality we used systems biology that offers mathematical tools that include the analysis of the structure, clustering and centralities of the network. In order to have information regarding physical protein-protein interactions (PPPI) from citrus sequences, orthologous sequences of *Arabidopsis thaliana* were used (BLASTX; reciprocal BLASTP). Literature data mining was performed, and PPPI network design was obtained using the Cytoscape software. The interactome networks thus obtained were analyzed with MCODE. Gene ontology clustering analysis was performed using BiNGO. Specific algorithms were applied to identify modules and central nodes within the citrus libraries associated network. The obtained results will be used as a guideline to select specific genes/proteins from citrus for further functional studies as gene expression or plant transformation.

S03P15

Genotyping and mutation scanning by high resolution melting (HRM) analysis of citrus EST-SNPs and SSRs

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Citrus taxonomy is very complex mainly due to specific aspects of its reproductive biology. A number of studies have been performed using various molecular markers in order to evaluate the level of genetic variability