Hybridization (CGH), global gene expression/transcriptomic profiling, metabolite and proteomic profilings and phenotypic description. Two examples are reported, the use of aCGH for the characterization of deletions in fast-neutron mutants and the utilization of transcriptomic analyses for the identification of gene function. We report the successful identification of genes included in hemizygous deletions induced by fast neutron irradiation. Microarray-based CGH allowed the identification of underrepresented genes in a mutant showing color break delay. Subsequent confirmation of gene doses and comparison of hits against annotated genomes enabled the prediction that these genes were clustered into a 700 kb fragment, rendering thus a partial physical map of the deletion. Microsynteny and local gene colinearity with Populus were higher than with the phylogenetically closer Arabidopsis genome. In addition, the transcriptome of several selected citrus genotypes has been investigated to identify gene functions that in citrus are hardly accessible through genetic approaches. Gene expression profiles and parallel metabolite analyses highlighted key genes involved in physiological processes of high agronomical relevance. For example, a gene coding for a terpene synthase was highly repressed in fruits of alf mutant with reduced emission of aromatic compounds, mainly the sesquiterpenes linalool and caryophyllene. Furthermore, specific anion and cation channels and active transporters were implicated in salt sensitive genotypes. In conclusion, a combined strategy including genomics tools and induced citrus mutations has been proved to be a successful approach to identify genes with major roles in citrus fruit growth stress tolerance. This approach also allowed new insights in the structural analysis of mutations contributing novel evolutionary conclusions for the genus Citrus.

[99]

New Insights into Plant 'stay-green' Phenotypes through Integrated Transcriptomic, Proteomic and Metabolite Profiling of Navel Negra (nan) Citrus Mutant

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Chlorophyll (Chl) degradation is central to the degreening process that is common in senescing leaves and ripening of many fruit. However, despite its importance, the basic steps of the Chl catabolic pathway have only recently been elucidated and remarkably little is known about its regulation. Important insights are likely to be provided through the characterization of 'stay-green' mutants from several species that have been shown to exhibit unusual Chl retention. To this end, we identified Navel negra (nan) as a spontaneous *Citrus sinensis* mutant whose fruits develop a dark brown external colour upon ripening, rather than the characteristic orange of the wild type. Analysis of pigment composition in the nan flavedo suggested that typical ripening-related Chl degradation, but not carotenoid biosynthesis, was impaired. Several lines of evidence further suggested that the nan mutation is distinct from those in previously described stay-green mutants. In order to better understand the basis and consequences of the stay-green phenotype, we applied integrated large-scale transcriptome, proteome and metabolite profiling to obtain a holistic view of the nature and consequences of the phenotype. A summary of this 'systems biology' approach will be presented, highlighting the potential of citrus as a model for genome scale analyses of complex physiological phenomena.

[100]

Molecular and Cytological Characterization of Homozygous Plants of *Citrus clementina* Hort. ex Tan., Candidates for Ctrus Genome Sequencing

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One of the main goals of the International Citrus Genome Consortium (ICGC) is to have a freely available, high quality reference genome sequence for *Citrus*. It was concluded that the best assembled sequence would be derived from the most homozygous genome available, and such materials were sought from the international citrus research community. Tissue and DNA samples from three homozygous plants of *Citrus clementina* Hort. ex Tan., cv. Nules, obtained by gynogenesis or by pollen embryogenesis and freely provided by researchers in Spain, France and Italy, were analyzed and characterized by laboratories in several institutions worldwide employing various methods. Specifically, chromosome numbers were determined, and the evaluations of homozygosity and of integrity of the genome through genomic and EST-derived SSR markers, and using microarray technology, were carried out. Plant materials from Spain and France were determined to be haploid, and the accession from Italy was found to be trihaploid, by chromosome counts. Nearly 180 SSR markers were selected, in many cases from previous mapping exercises, to represent as broad and unbiased coverage of the citrus genome as possible, and plant materials were genotyped. Only one SSR locus revealed anomalous results in the French haploid, while the remainder revealed only a single allele product at all other loci surveyed. The microarray results using the Spanish cDNA array revealed anomalous results from only 7 genes of the more than 20, 000 represented on the array. The results obtained by the different screening teams regarding the first step of the *Citrus* genome sequencing project will be reported in detail.

[101]

EST-SSRs in Citrus: Features, Polymorphism and Mappability

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From a clementine EST sequences dataset we investigated the presence, occurrence and repartition of different type of SSR in EST and compared the results obtained with other plant species. Among 11000 unigenes from 37000 ESTs, we have found at least one microsatellite sequence (repeated units size ranged from 2 to 6 nucleotides) in 1500 unigenes (13.6%). This frequency of ESR-SSR (one in 5.2 kb) seems higher than in other species such as rice, barley, wheat etc. Nevertheless, the observed differences can be partially explained by the number of repeat units considered as limit for SSR selection in computational screening. As for many plant species, di and trinucleotides are dominant in citrus ESTs (88% of total SSRs). Trinucleotides are the most abundant (52%) and hexanucleotides, only 2% being under represented when compared to other plants. Dinucleotide microsatellites were preferentially (50%) concentrated in the 5' 100th nucleotides and a large majority (75%) is positioned in untranslated regions (UTR), since trinucleotide microsatellites were equitably distributed inside and outside the translated region (TR) of the ESTs (48 % and 52% respectively). To optimize the use of EST-SSR we assessed their polymorphism among a citrus species set (50 genotypes) representing the genetic diversity. More than 90% of EST-SSR markers were polymorphic. Furthermore, dinucleotide microsatellite markers were more polymorphic than trinucleotide ones, probably related to their distribution that was more often located in the 5'-UTR. We obtained a good agreement of diversity relationships between the citrus species and relatives assessed with EST-SSR markers with the established taxonomy and phylogeny. To end, the heterozygosity of each genotype and all dual combinations were studied to evaluate the percentage of mappable markers. Higher values (> 45%) were observed for putative *Citrus* inter-specific hybrids (lime lemon, or sour orange) than for *Citrus* basic true species (mandarin, pummelo and citron) (<30%). Most favorable combinations for genome mapping were observed in those involving interspecific hybrid genotypes. Those gave higher levels of mappable markers (>70%) with a significant proportion suitable for synteny analysis. Whatever the position of the SSR in the ESTs, the EST-SSR markers are powerful to investigate genetic diversity and genome mapping in citrus.

[102]

Transcriptome Analysis of a Spontaneous Red-flesh Mutant in Sweet Orange (*Citrus sinensis* [L.] Osbeck) During Fruit Development

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Bud mutations arise often in citrus. The selection of mutants is one of the most important breeding channels in citrus. However, the molecular basis of bud mutation has rarely been studied. To identify potential important or