[P126]

Genetic transformation of the main commercial citrus cultivars in Hunan

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A protocol for regeneration and genetic transformation using seedling and mature internode stem segments as explants were developed. 'Xupu', 'Dahong',, 'Bingtang' and 'Succari' sweet orange (*Citrus sinensis* (L.) Osbeck), 'Xinnu' ponkan (*C. reticulata* Blanco) and Shatianyou pumelo (*C. grandis* Osbeck) were transformed by using seedling explants, while a mature material transformation protocol was established for 'Newhall' navel orange, 'Bingtang' and 'Succari' low acid oranges and ponkan. By *Agrobacterium* infection, *phy*B, *rol*ABC, *chit*42, *terf*1 (tomato ethylene responsive factor 1), *pth*A –NLS (nuclear localization signals of the pathogenesis factor pthA of *Xanthomonas axonopodis* pv.*citri*) and the ScFv gene of the monoclonal antibody against to PthA were transferred into the tested cultivars and about 900 transmants with various function genes were obtained. The transginc sweet orange plants with *terf*1 were showed resistance to citrus canker and citrus anthracnose diseases. The transgenic 'Succari' sweet orange with *pth*A-NLS and 'Dahong' sweet orange plants with ScFv gene demonstrated high resistance to citrus canker disease in the in vitro assays. The plant transformed with *phy*B were improved their photosynthesis and those with *rol*ABC dwarfing character.

[P127]

Establishment of a high frequency plant regeneration system for Ponkan mandarin by using orthogonal experiment

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In this paper, 5 factors influencing the efficiency of adventitious buds inducting of Ponkan mandarin (*Citrus reticulata* Blanco.), including the basic medium, α-naphthalene acetic acid (NAA), 6-benzyl adenine (6-BA), kinetin (KT), and zeatin (ZT), were evaluated by using the orthogonal experiment design. The explants from seedlings included young leaves, cotyledons, epicotyls and hypocotyls. The results showed that, (1) the optimum explant was epicotyl. (2) The frequency and quantity of shoot regeneration as high as 73.3% and 1.32 shoots/explant were obtained on MT medium supplemented with 0.1 mg.l-1 NAA, 2.5 mg.l-1 KT, and 2.0 mg.l-1 ZT, respectively. (3) Among 5 experimental factors, the order of influencing the efficiency of bud inducing was 6-BA>NAA>KT>basic medium>ZT.

[P128]

International Effort toward a SSR-based Linkage Map for *C. clementina*

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Following the difficulties encountered for assembling a 1.2 x sequencing of the highly heterozygous sweet orange genome, the International Citrus Genomic Consortium (ICGC) decided to establish the first reference sequence of a whole nuclear citrus genome from a haploid Clementine. A saturated genetic linkage map of Clementine based on sequence-characterized markers was considered by the ICGC as an important tool for genome sequence assembly. In this framework, CIRAD proposed to use an interspecific population *C. maxima x C. clementina* to implement the reference Clementine genetic map. A population of 250 hybrids of Chandler pummelo x Clementine was established in Corsica and 190 hybrids were used in this first phase of mapping. Collaboration was established between two French organizations (CIRAD and INRA), two groups from United States (UF-CREC and UCR), one Spanish institute (IVIA), INRA Morocco and Cukurova University from Turkey. Forty markers were found heterozygous in Clementine among a previous set of 90 SSR markers developed by CIRAD from microsatellite-enriched genomic libraries. With the objective to integrate the physical and genetic maps of Clementine, CIRAD and IVIA have developed new SSR markers from microsatellite sequences identified in BAC End Sequences (BES) of diploid Clementine. On hundred and 10 of these new markers were found heterozygous for Clementine or Chandler pummelo and were used for genotyping. INRA France developed 500 SSR markers from ESTs databases and found 170 markers heterozygous for Clementine. INRA Morocco contributed to the genotyping of 112 SSR markers developed from EST databases and genomic libraries, while 50 ESTs SSR were analysed by Cukurova University. SSR markers mainly

developed from EST databases and already mapped for sweet orange were genotyped by UF-CREC (70 markers) and UCR (60 markers) to allow comparisons among the *C. sinensis, C. maxima* and *C. clementina* maps. Indeed, taking advantage of the important allelic differentiation between Clementine and Chandler, two parallel linkage maps can be developed from this population. As perspective, in the framework of the global haploid Clementine sequencing project, a collaboration between the French and Spanish groups plans: (i) to extend the population size to 380 hybrids between Clementine and pummelo, and (ii) to develop an array from SNPs identified in Clementine BES for High-Throughput Genotyping. All genotyping data will be stored in the online TropGene database (http://tropgenedb.cirad.fr/). Additional international groups are very welcome to join the project, using these progenies for genotyping their own markers. This should contribute to a very high density map of Clementine and to comparative mapping studies between citrus species.

[P129]

Functional Study of Genes Potentially Involved in Juvenile-to-adult Transition in Citrus Plants Identified by Gene Expression Profiling

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In citrus the juvenile phase can be as large as 5-20 years depending on the variety, what is a very serious constraint for breeding of citrus varieties. With the aim of identifying regulatory genes involved in the process of juvenile-to-adult phase transition we have developed a citrus transcription factor (CTF) microarray, and used it to screen for transcription factors differentially expressed between juvenile and adult plants. The CTF microarray was generated with a specific oligonucleotide set for 1152 putative citrus transcription factors. Meristems of juvenile and adult plants were harvested from sweet orange (*C. sinensis* (L.) Pineapple), Tangor Murcott (*C. reticulata* x *C. sinensis*), grapefruit (*C. paradisi* Macf. Duncan) and rough lemon (*C. jambhiri* (L.)), and samples were hybridized to the oligonucleotide microarray. Several transcription factors were identified as differentially expressed between adult and juvenile plants in all four species and this phase-specific regulation was validated by quantitative RT-PCR. To analyse the potential involvement of such TFs in the studied process, the putative function of one of them, CTF607, was further investigated by generating transgenic plants of the annual herbaceous plant Arabidopsis thaliana over-expressing and silencing the encoded TF. Over-expression of the TF caused shortening of the flowering time in transgenic plants while the CTF607 silencing plants showed a late flowering phenotype. Although developmental processes in annuals and woody perennial trees may share some genetic factors they differ in their life cycle. Arabidopsis has a short juvenile developmental phase followed by the production of flowers and seeds at the reproductive adult phase. Our results seem to indicate that transition to flowering may be interconnected with the juvenile to adult phase transition and therefore, these and future studies based on this approach could help us to decipher the molecular mechanisms involved in the juvenile-to-adult transition in citrus plants.

[P130]

Identification of SNPs in Clementine by Denaturing High-Performance Liquid Chromatography Analysis as a Useful tool for Varietal Characterization

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Molecular identification of cultivars of some citrus comercial species as clementine mandarins (*Citrus clementina* Hort. Ex Tan.) or sweet oranges (*Citrus sinensis* (L.) Osb.) has always been particularly difficult. In deed intra-specific phenotypic variability of these species is only based on bud-sport mutations or variation. The possibility to differentiate citrus cultivars, which mostly differ from each other in mutations, is of paramount importance for protecting the rights of citrus breeders, growers and nurseries. Single-nucleotide polymorphisms (SNPs) are the most abundant type of DNA sequence polymorphisms. Their higher availability and stability when compared to other markers provide enhanced possibilities for genetic and breeding applications. Denaturing High-Performance Liquid Chromatography (DHPLC) represents a highly sensitive and automated method for DNA variant detection. In the present work the DHPLC was applied to a systematic search of SNPs in 56 mandarin clementine varieties. The selection of the genes to be analysed was based on the DNA sequence information obtained from the citrus EST database (http://bioinfo.ibmcp.upv.es/genomics/cfgpDB/) and in previous results of our group. Primers were designed and 20 PCR fragments (averaging 350 bp in length) amplified and analysed for the presence of polymorphisms. So far, this analysis has enabled us to identify more than 20 SNPs with an average of 200 bp/SNP that clustered the studied clementines in five groups: one main group with 34 varieties, three groups of two, nine and nine clementine varieties and one of 3 varieties with the greatest amount of discriminative changes. Some of these SNPs allow the discrimination between some parental varieties and its direct derivatives originated by spontaneous mutation. To our knowledge, this is the first time this approach is used in plant varietal characterization and the results demonstrate