

Self-incompatibility reaction in *Citrus* is assumed to be of gametophytic control, with pollen tubes that arrest after they have grown some distance through the pistil, and genetically controlled by the *S*-locus. In contrast to what is known in other species, very little is known about molecular regulation of *Citrus* self-incompatibility. Pollen tube growth behaviour of two near isogenic clementine varieties, 'Comune' (self-incompatible) and 'Monreal' (self-compatible mutation), was analysed by histological assays. Pollen tubes in self-pollinated 'Comune' arrested their development in the upper part of the style while in self-pollinated 'Monreal' they grew down to the ovary. To identify genes involved in pollen-pistil interaction and in self-incompatibility response, a research based on cDNA-AFLP technique was carried out to compare transcript profiles between the two clementine varieties and search for differentially expressed genes. RNA was extracted from emasculated non-pollinated pistils and from self-pollinated pistils four days after pollination. cDNAs of the two varieties were amplified using 43 *EcoRI*/*MseI* primer combinations, and more than a hundred differentially expressed or polymorphic Transcript Derived Fragments (TDFs) were successfully cloned and sequenced. Among the TDF showing homology with known genes, BLASTX search identified several genes related to reproductive biology such as calcium modulated proteins, receptor-like kinases, thioredoxins, a histone deacetylase gene, a putative cleavage and polyadenylation specificity factor, transcription factors and genes related to hormone metabolism and signaling. Moreover, a high percentage of active retrotransposons were identified among the TDFs, which may indicate their possible role in the regulation of self-incompatibility genes. Some of the TDFs were analysed through RT-PCR and showed differences in their expression patterns.

[P95]

Identification of Zygotic and Nucellar Seedlings in Citrus Crosses using SSR Markers

Golein B and Fifaei R

Department of Seed and Plant Improvement, Iran Citrus Research Institute, Ramsar, Iran, bgoleincitrus@yahoo.com

A series of reciprocal crosses designed between Minneola tangelo + Changsha and Page, Changsha and Ponkan mandarins and Marss and Hamlin oranges to develop Minneola tangelo + Changsha with a reduced number of seeds and early fruiting characteristics. A total of 303 plantlets were obtained and subjected to screening experiments, in order to distinguish zygotic seedlings from nucellars. The results showed that SSRs are very efficient and reliable to identify the sexual origin of citrus seedlings.

[P96]

Regeneration by Stigma/Style Somatic Embryogenesis of Citrus Genotypes in Algeria: Preliminary Results

Meziane M¹, Messaoud B², Frasher D³, Carra A⁴, Carimi F⁴, and D'Onghia AM³

¹Institut National Agronomique 16200 Hacène Badi, El Harrach, Alger, Algeria; ²Laboratoire des Cultures in vitro. Ens Kouba, Alger, Algeria; ³Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM) /Mediterranean Agronomic Institute, Via Ceglie 9, 70010 Valenzano (BA), Italy; ⁴Institute of Plant Genetics/CNR, Research Division of Palermo, Corso Calatafimi 414, 90129 Palermo, Italy. donghia@iamb.it

Stigma/style somatic embryogenesis proved to be an excellent method for the regeneration of most Citrus spp, except the Clementines, without inducing somaclonal variations in regenerants. This method was applied in this study on a Citrus collection at ITAF, Algeria. The main citrus local and international varieties were chosen for regeneration by stigma/style somatic embryogenesis. Explants were cultured in medium I and II to induce somatic embryos formation. In both media MS basal salts, vitamins, sucrose and malt extract were used. In medium II the growth regulator (6-benzylaminopurine 3 mg/l) is also added. All explants of *C. limon* and *C. sinensis* produced callus at the cut end of the styles, about 8 days after culture initiation. Most of the tested genotypes proved to regenerate somatic embryos in a different period of time (25-90 days), which were cultured in a single tube before *in vivo* acclimatization.

[P97]

Friables and Embryogenic Callus Induction from Embryo, Ovule and Style of Citrus Rootstocks

Benyahia H¹, Talha A¹, Arsalan N¹, Handaji N, Beniken L¹, Benazzouze A¹ and Ollitrault P²

¹National Institute of Agronomic Research (INRA), Unit of plant improvement and germplasm conservation, laboratory of citrus improvement and biotechnology, BP: 257, kenitra Maroc; ²CIRAD, UPR 75, TA50/PS4 Avenue Agropolis, TA A-75/02, 34398 Montpellier, Cedex 5, France. hamidbenyahia2002@yahoo.fr

The incorporation of new technologies such as in vitro selection and protoplast fusion methods into citrus improvement programs offers new opportunities to facilitate, expedite, and fully utilize germplasm variability for both scion and rootstock. Somatic embryogenesis plays a central role in these methods. In citrus, the main factors influencing callus induction and somatic embryogenesis are genotype, composition of culture medium and kind and stage of explants. The aim of the study was to develop protocols adapted for embryogenic callus induction of different citrus rootstocks. Embryos of mature seeds, style from flower buds collected during full bloom, and ovule prelevé 8 week after anthesis were used as explants. All explant material comes from the citrus germplasm collection of INRA EL Menzeh. The basic nutrient medium of Murashige and Skoog (1962) or MS medium was used.

Three medium were compared M1 = MS. M2 = MS + kinetin 1mg/l and M3 = MS + 2-4 D 1 mg/l + BAP 0.5 mg/l. Cultures were incubated at a temperature of 26- 28°C under 70% relative humidity, in the dark. After culturing of embryo, ovule or style of 37 citrus rootstocks, calli were induced. The percent of friable callus induction varied between explants, culture medium and rootstock genotypes. Detailed results are given and best combinations of explants and culture medium are discussed for the different genotypes.

[P98]

STG (Shoot tip Grafting) a Biosafe Technique for Sustainable Maintenance of Biodiversity of Exotic Citrus Germplasm

Vijayakumari N, Ghosh DK, Das AK and Singh S
National Research Centre for Citrus, PB No.464, Shankar nagar P.O., Nagpur-440010 Maharashtra, India.
vasu91098@yahoo.co.in

The basis for any fruit tree industry is to start with high quality trees. Introduction of Citrus germplasm is highly desirable to take advantage of quality selections existing in other areas and to span the citrus production throughout the year. Inevitably, movement of germplasm poses the risk of accidentally introducing plant quarantine diseases along with the host material which may be devastating for the citrus industry. To minimize this risk, NRCC, Nagpur had come up with promising therapy procedure based on shoot tip grafting to ensure distribution of clean/healthy material and to provide a safe mechanism for introduction of promising citrus cultivars in India for research, variety improvement or commercial production. The STG based citrus quarantine programme which is being implemented at the Centre since 2002 to 2006 of *in vitro* grafting of shoot tips taken from plants grown in green house/ *in vitro* cultured budwood, which is also being followed in advanced citrus growing countries viz. Spain and USA for germplasm introduction and exchange (for quarantine). Out of 20 micrografted exotic cultivars 18 were indexed and 14 micrografted exotic cultivars declared free from major graft transmissible pathogens and recommended for field evaluation. The objective of this investigation was to establish clean nucleus stock of diverse citrus repository through shoot tip grafting to utilize for future breeding and germplasm exchange programs after indexing for major citrus pathogens.

[P99]

Application of STG Technique for Production Healthy Trees and Free of CTV in Cultivar of Washington Navel Orange

Ghasemi M, Fifaei R and Sajadi MH
Iran Citrus Research Institute, Ramsar, Iran,
malek_ghasemi@yahoo.com

This study was carried out for producing CTV free plants. Shoot-tip of infected Washington navel orange by

tristeza containing meristem and two leaf primordia was excised (0.2 – 0.4 mm). It was grafted on Troyer Citrange and Citrumelo seedlings by two methods, inverted T-budding and in contact with the vascular ring. These seedlings had 3-5 cm length. Grafted seedlings were planted on liquid medium and incubated on growth chamber in 27 °c and 16 hours photoperiod with 1000 lux illumination. If grafting was successful, plants were transplanted on suitable soil. Shoot-tip grafted plants were tested by DAS ELISA against tristeza virus. All of them were not infected. In this research, we used factorial design based on complete randomized design with four treatments and four replications. Each treatment had 10 plantlets. The results showed that inverted T- budding was more successful. There were no significant differences between used root stocks.

[P100]

Somaclonal Variation with Early Granulation Obtained from Interspecific Protoplast Fusion Between Navel Orange (*Citrus sinensis* [L.] Osbeck) and Red Tangerine (*C. reticulata* Blanco)

Ding J¹, Deng XX¹, Zhang HY², Cheng YJ¹, Xu Q¹, and Liu Q¹

¹ National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, PR China, xxdeng@mail.hzau.edu.cn; ² College of Horticulture and Forestry, Huazhong Agricultural University, Wuhan 430070, China

Six plants with early juice sacs granulation trait, derived from the interspecific protoplasts fusion between Skaggs' Bonanza navel orange (*Citrus sinensis* [L.] Osbeck) embryogenic callus and Dahongpao red tangerine (*C. reticulata* Blanco) mesophyll cell, were analyzed via flow cytometry (FCM), molecular markers including random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), cleaved amplified polymorphism sequence (CAPS) and restriction fragment length polymorphisms (RFLPs). Results indicated that all six plants were diploids; they inherited the DNA from the embryogenic suspension parent Bonanza navel orange. But morphological, fruit characters analysis and measurement of the components of cell wall in juice sacs showed that they were not true-to-type Bonanza navel orange, especially the fruit traits like juice sacs granulation and the navel structure. The results verified that these plants were not hybrids; they are more likely somaclonal variants from the regeneration of navel orange protoplasts. These plants are good materials for study the mechanism of granulation of juice sac, a common phenomenon during the storage of fruits of pummelo and other citrus.

[P101]

Distribution and Influence of Ca²⁺ in Pollen and Pollen Tube of *Citrus sinensis* Osbeck