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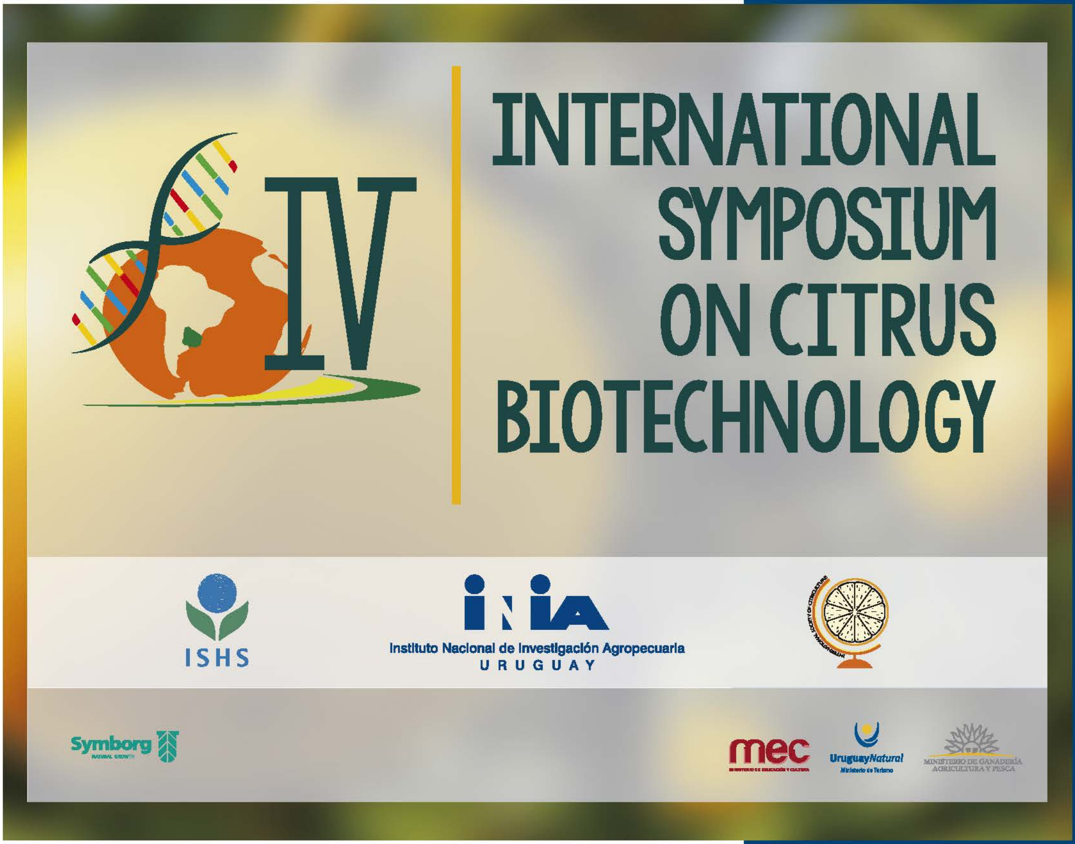

IV INTERNATIONAL SYMPOSIUM ON CITRUS BIOTECHNOLOGY: BOOK OF ABSTRACTS

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Prologue

The Organizing Committee welcomes you to the IV International Symposium on Citrus Biotechnology (ISCB) held in Uruguay from April 16th to 18th, 2018.

This Symposium is organized by International Society for Horticultural Science (ISHS), International Society of Citriculture (ISC) and the National Agricultural Research Institute (INIA) of Uruguay.

The ISCB has been organized by the ISHS since 1998. The first ISCB took place in Eilat, Israel by the efforts of PhD. R. Goren, PhD. E. E. Goldschmidt, PhD. M. Davidzon and PhD. Y. Erner. The second International Symposium was held in Catania, Italy in 2009 organized by of PhD. A. Gentile and PhD. E. Tribulato. The third Symposium was held in Shizuoka, Japan in 2014 by PhD. Shimizu and PhD. Tominaga.

The IV ISCB aims to gather knowledge in a wide range of fields where Biotechnology is applied in order to improve the Citrus Industry worldwide. The applications and approaches based on biotechnology are widely used in all research fields for greatly reducing time span and improving reliability in citrus studies for breeding, genomics and genetics, physiology and fruit quality, pests and diseases management and the advance and application of new emerging technologies. Therefore, we are confident that this ISCB will greatly contribute to strengthen the development of your research as well as the Citrus Industry.

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Keynote Lectures of the IV ISCB

Dr. Manuel Talón

Session I: Breeding, Genomics and Genetics

“A new evolutionary framework for the genus *Citrus*”

Dr. Manuel Talón is Head of the Centro de Genómica at IVIA, (Valencia). He received his Ph Degree in Biology at the University of Valencia (Spain). He holds the “Antonio José Cavanilles” and the “Manuel Alonso” first awards to the scientific research on the Agrifood sector. He is the scientific leader of the Citruseq/Citrusgenn Consortium, a private-public initiative that is participating in the study of the genomes of the genus Citrus. This information is being used to elucidate the relationships between genomic variants and agronomic traits, to authenticate varieties and to build specific markers for breeding. He has developed 3 plant patents that are being exploited by the agricultural sector and demanded by multinational companies. He is author of more than 200 publications, out of which about 120 are SCI Journals, including Nature Biotechnology and Nature. He has acted as editor of 3 university textbooks, is author of 2 technical books and has wrote 16 book chapters. According the WOS, he has been cited 11.630 times, possesses an h-index of 48 and has delivered more than 450 presentations. He is member of several Editorial Committees and has acted as reviewer in not less than 30 Scientific Journals.

Prof. Frederick G. Gmitter Jr.

Session I: Breeding, Genomics and Genetics

“Fruit color, flavor and quality: Understanding genetic control and devising strategies for improvement”

Dr. Frederick G. Gmitter Jr. is a University of Florida (UF) Research Foundation Professor in the Horticultural Sciences Department, located at the UF Citrus Research and Education Center in Lake Alfred, Florida. He received BA and MS degrees from Rutgers University and the PhD degree from UF. His research focuses on citrus breeding and cultivar development, with specific emphasis on more fundamental studies of host-pathogen interactions (particularly Huanglongbing tolerance), development and application of genomics-based breeding approaches, and unravelling the complex genetic basis of fruit quality attributes while tying these traits to consumer preferences. He has published well over 150 refereed manuscripts in international scientific journals, including Nature, Nature Biotechnology, Food Chemistry, BMC Plant Biology, Plant Science, among others. He has served as an Associate Editor for three different scientific journals. The Florida Fruit and Vegetable Association recognized him as Researcher of the Year in 2011. He led the International Citrus Genome Consortium, which made the first citrus genome sequences publicly available in 2011. With colleagues at UF, he has released more than 30 new scion and rootstock cultivars in the past 10 years. More than 1.6

million U trees of UF-CREC cultivars have been planted since 2015, many of which demonstrate enhanced levels of HLB-tolerance.

Prof. Ji-Hong Liu

Session II: Physiology and Fruit Quality

“Transcriptional regulation of abiotic stress-responsive genes in *Citrus* and its related genera: from mechanism elucidation to gene exploitation”

Prof. Dr. Ji-Hong Liu graduated from Huazhong Agricultural University in 1990, and continued his master and PhD degree study there. In 1999, he earned his PhD degree and got the position in the same university. Since 2003 until now he has been working on stress physiology and molecular biology in citrus and its related genera, such as Poncirus and Fortunella. He has been supported by Japanese Society for the Promotion of Science to visit the National Institute of Fruit Tree Science, Japan, as a post doc or a visiting scholar (four times). In addition, he is also a visiting scholar (Tang Scholar) at Cornell University from 2013-2014, under the support of Tang Cornell-China Scholars Program. At present he has three major research highlights in his laboratory, including elucidation of the physiological and molecular mechanisms underlying biotic or abiotic stress tolerance, isolation and functional characterization of agronomically valuable genes involved in stress response, creation of novel germplasms with enhanced stress tolerance via genetic engineering. So far, he has published nearly 70 papers in international peer-reviewed journals, including Plant Physiology, Plant Cell Environment, Journal of Experimental Botany, among others. He acts as an Academic or Associate editor for Gene, PLoS One, and Acta Physiologiae Plantarum

Prof. Nian Wang

Session IV: Development of Emerging Technologies and their Applications

“*Citrus* improvement via CRISPR technology”

Dr. Nian Wang is an Associate Professor in the Department of Microbiology and Cell Science at the Citrus Research and Education Center, University of Florida (UF). He is a UF Research Foundation Professor and Director of China-USA Citrus Huanglongbing Joint Laboratory. He received his B.S. degree in Plant Protection at Shandong Agricultural University, and his M.S. degree in Plant Pathology at China Agricultural University, followed by a Ph.D. degree in Plant Pathology and Microbiology at Texas A&M University. Dr. Wang then conducted postdoctoral research at University of California, Berkeley. His research interests include molecular genetics and functional genomics of plant pathogenic bacteria, molecular plant-microbe interactions, and management of plant bacterial diseases. He has published more than sixty refereed papers including recent papers in Annual Review of Phytopathology, PLoS Pathogens, ISME, MPMI, Plant Biotechnology, and PNAS. He has served as Chair of the Bacteriology Committee of American Phytopathological Society, Chair of Pierce’s Disease Research Scientific Advisory Panel, Senior Editor for Plant Disease, Associate Editor for Phytopathology and Guest Editor for PLOS Pathogens. He will serve as a Senior Editor for Phytopathology starting from January 2018.

SESSION I: Breeding, Genomics and Genetics

KEYNOTE LECTURES

SI-KL-1

A NEW EVOLUTIONARY FRAMEWORK FOR THE GENUS *Citrus*

Manuel Talón[1]*, Francisco-Ramón Tadeo[1], Guohong Albert Wu[2], Javier Terol[1], Victoria Ibáñez[1], Antonio López-García[1], Estela Pérez-Román[1], Carles Borredá[1], Daniel Ventimilla[1], Concha Domingo[1], Fred G. Gmitter[3], Daniel S. Rokhsar[2]

In the genus *Citrus*, the long history of cultivation has arrested during the past decades the reliable development of basic disciplines such as taxonomy, genealogy and phylogeny. Fortunately, the recent irruption of comparative genomics has provided evidence to discriminate “pure” species of citrus from hybrids and admixtures. Genomic, phylogenetic and biogeographic analyses support the proposal that the center of origin of citrus was the Southeast foothills of the Himalayas, in a region including the eastern India, northern Myanmar and western Yunnan. Citrus evolved during late Miocene through a rapid Southeast Asian radiation correlating with a dramatic weakening of the monsoons. The Australian limes and Tachibana mandarin split later from mainland during the early Pliocene and Pleistocene. Taken together, these findings draw a new evolutionary framework for these fruit crops, a scenario that challenges current taxonomic and phylogenetic thoughts and points towards a reformulation of the genus *Citrus*.

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SI-KL-2

FRUIT COLOR, FLAVOR AND QUALITY: UNDERSTANDING GENETIC CONTROL AND DEVISING STRATEGIES FOR IMPROVEMENT

Frederick G. Gmitter, Jr.

Citrus breeders historically have focused on traits of importance to producers, such as disease resistance, stress tolerance, and yield. However, consumer expectations and demands are another aspect requiring breeders' attention to ensure the future competitiveness and viability of the world's citrus industries in an ever-expanding field of purchase options. External fruit appearance and color are the first attractants to the eyes of prospective fresh fruit customers facing a myriad of visual stimuli in the market. Many times, such visual stimuli may link to memories of flavors and aromas, and these attributes serve as the second attractant enticing consumers to purchase, and purchase again. The same sensory characteristics also can appeal to consumers for citrus juice purchases. On a more cerebral level, nutrient content and potential functional health benefits likewise can motivate consumers to purchase citrus. Research to understand the underlying genetic control of the consumer-centric fruit traits has begun to take breeding for fruit quality from a somewhat random process to one of greater precision by targeting specific genes and alleles. This understanding and new knowledge can be applied in traditional breeding programs to enhance the efficiency of selection for fruit quality improvements, in the development of new citrus cultivars. Likewise, such understanding can inform new strategies to manipulate and improve the fruit quality of existing commercial cultivars through genome editing and other emerging technologies. This presentation reviews the basis for focus on product quality, some recent advances made in understanding the genetic control of critical traits, and applications of the information in practical genetic improvement programs.

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ORAL PRESENTATIONS

SI-O-1

A MODEL FOR DOMESTICATION AND DIVERSIFICATION PROCESSES OF MODERN CITRUS VARIETIES

Tokurou Shimizu[1]*, Akira Kitajima[2], Keisuke Nonaka[1], Terutaka Yoshioka[1], Satoshi Ohta[1], Shingo Goto[1], Eli Kaminuma[3], Yasukazu Nakamura[3]

Diverse varieties of genus *Citrus* have been recognized to be derived from the natural hybridization of founder varieties in four basic taxa; mandarin, pummelo, citron, and papeda. However, our knowledge about the process for domestication and diversification of modern citrus varieties from the founder varieties is quite limited. Our recent study used with high precision DNA marker for nuclear and organelle genomes inferred the parents or a parent of 67 citrus varieties. We confirmed those inferred parentages by genome-wide genotyping analysis of 1,841 SNP markers. These studies identified that a few citrus varieties, Kishu, kunenbo, yuzu, koji and sweet orange were the key varieties of modern citrus varieties. Those inferred parentages clarified three unique aspects for the occurrence of modern citrus varieties: 1) a few varieties served as a parent of those modern varieties, 2) repetitive hybridization events between same varieties occurred, and 3) some of them were backcrossed with the parent variety. The first point suggests the process for diversification from a few varieties. Furthermore, the second point implicates the prolonged cultivation of those varieties, and it could explain the third point. Consequently, a model for domestication and diversification processes of diverse modern varieties was hypothesized. 1) A few key citrus varieties were cultivated in the same region closely. 2) Attractive seedlings were selected consciously from many open-pollinated seeds and then maintained for production. 3) The selected seedlings that had been maintained at the same region occasionally backcrossed with a parent variety. Though further verification of these processes is demanded, old documents described that those key varieties had been produced in wide regions in Japan. Those descriptions coincided with this model.

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SI-O-2

HIGH RESOLUTION CHROMOSOME CONFIGURATIONS OF SOME KOREAN LANDRACE CITRUS BY CMA BANDING AND rDNA LOCI

Kwan Jeong Song[1]*, **Kyung Uk Yi**[2], **Ho Bang Kim**[3]

Citrus is the major agricultural crop in Korea as well as Jeju with a long history of cultivation. Despite its long cultivation history and the immense clout of agricultural and economical value, ecological, evolutional, and phylogenetical taxonomic investigations of citrus based on phenotypes or genotypes are scarcely conducted in Korea. Korean landrace citrus presents broad and natural genetic variability, which are important and valuable as genetic material. In this study, metaphase chromosomes of five Korean landrace citrus were analyzed to understand the phylogenetic relationship among them and to compare these characteristics with those of other citrus species at a cytogenetic level using CMA banding patterns and rDNA loci. The CMA banding patterns of the five Korean landrace citrus were 1A+2B+2C+6D+7E in dong-geongkyul (*C. erythrosa*), 3B+1C+7D+5E+2F in hongkyul (*C. tachibana*), 2A+1B+3C+4D+8E in sadoogam (*C. pseudogulgul*), 1A+3B+1C+7D+6E in dangyooza (*C. grandis*), 1A+1B+1C+9D+6E in jigak (*C. aurantium*). All types of chromosome bands were present in all accessions except in hongkyul (*C. tachibana*), in which type A chromosome is absent, but two of type F chromosomes were observed. The numbers of type A, B, and C chromosomes were lower in all accessions. In contrast, the type D and E chromosomes were remarkably constant and predominantly observed in all accession. The distributions of 5S and 45S rDNA loci by FISH were heterogeneous among the five. All accessions possessed one 5S rDNA locus except hongkyul (*C. tachibana*), which displayed two 5S rDNA loci. And they always co-localized with 45S rDNA locus. All 45S rDNA loci were homotopic to CMA-positive regions. Every type A and B chromosomes possessed one 45S rDNA locus in the centromere near the proximal region of the chromosomes. There was no type C and E chromosome with rDNA observed. The chromosome configurations of Korean landrace citrus analyzed here suggest that all accessions in this study are hybrids that have relationships more or less with mandarin and pummelo. This study provides high resolution of chromosome configurations, which could complement previous studies, and elucidated phylogenetic relationships of Korean landrace citrus at the cytogenetic level.

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SI-O-3

LOSS OF SELF-INCOMPATIBILITY IN *Citrus*

Rafael Montalt[1]***, Mari Carmen Vives**[1]**, Patrick Ollitrault**[2]**, Luis Navarro**[3]**,
Pablo Aleza**[3]

Self-incompatibility (SI) concerns around half of all species of flowering plants. It limits endogamy and contributes to increase genetic diversity, but might also hamper genetic studies and plant breeding programs. Soost (1968) reported gametophytic self-incompatibility system in *Citrus* that stops pollen tube development in the style. Bud pollination, temperature stress and polyploidization can induce the loss of SI in several species. In this work, we have investigated how the SI reaction can be broken in Citrus. Four self-incompatible diploid *Citrus* genotypes were used: 'Fortune' [*Citrus clementina* x (*C. paradise* x *C. tangerina*)] and 'Moncada' [*C. clementina* x (*C. unshiu* x *C. nobilis*)] mandarins; 'Clemenules' clementine (*C. clementina*); and 'Chandler' pummelo (*C. grandis*). Three experiments were performed for the evaluation of (i) the impact of bud pollination on the SI loss in all four genotypes; (ii) the effect of temperature stress on the SI reaction in 'Fortune' mandarin; and (iii) the effect of polyploidization by comparison of the SI reaction in diploid and doubled-diploid 'Moncada' mandarin. The observation of pollen tubes growth along the pistil was used to measure the loss of SI. We observed that SI reaction can be overcome by bud pollination, temperature stress and polyploidization. Plants obtained in each experiment were analyzed with molecular markers (SSR and SNP), confirming that all of them resulted from self-pollination. Regarding the effect of temperature stresses, our results confirm previous evidences that a loss of SI can be induced by high temperatures and show for the first time that low temperatures can also avoid the SI reaction in *Citrus*.

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SI-O-4

APPLICATION OF A MITE *Citrus* APOMIXIS MARKER IN THE AUSTRALIAN ROOTSTOCK BREEDING PROGRAM

Malcolm Wesley Smith[1]*, Matthew Webb[2], Debra Gultzow[3], Toni Newman[3], David Innes[4], Natalie Dillon[5], John Owen-Turner[6], Qiang Xu[7]

Australia's *citrus* breeding efforts are small by international standards, and unashamedly focused on conventional approaches. Molecular markers had yet to be used in the program because they failed to meet our four essential criteria of being: linked to a trait of economic significance; technically difficult to phenotype by conventional methods; temporally difficult to phenotype by conventional methods and; likely controlled by a simple genetic mechanism. This situation changed dramatically with the 2017 publication of a miniature inverted-repeat transposable element (MITE) marker that co-segregated with the *Citrus* apomixis trait. This met all of the above four criteria and was quickly verified on local germplasm. Application of this MITE marker is now a standard screening procedure in our rootstock breeding research. An extensive network of field rootstock trials is used to identify parents for rootstock breeding, and the resulting segregating populations are nursery-screened within 18 months of sowing for tolerance to phytophthora, resistance to CTV, and salt exclusion using conventional screening techniques. Hybrids that survive this screening are then assessed for apomixis using the MITE marker. Monoembryonic and polyembryonic hybrids are both useful for future breeding, but those with putative apomixis have more immediate commercial application. Consequently, putative apomictic hybrids are propagated in great numbers (via cuttings) to maximise replication and data precision in rootstock field trials. Use of the MITE marker has enabled maximum replication of putative apomictic hybrids, dramatically reducing the size and cost of field trials and hastened the establishment of seed-source trees. We consider it to be the first useful molecular marker in citrus breeding.

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SI-O-5

MECHANISMS OF UNREDUCED POLLEN AND OVULE GAMETES IN A DIPLOID HYBRID BETWEEN CLEMENTINE AND SWEET ORANGE AND IN TWO CULTIVARS OF LEMON, `Eureka Frost´ AND `Fino´

**Pablo Aleza[1]*, Houssem Rouiss[1], Jose Cuenca[1], Luis Navarro[1],
Patrick Ollitrault[2]**

Unreduced (2n) gametes have played a pivotal role in polyploid plant evolution and are useful for sexual polyploid breeding in new seedless citrus varieties. Recently the mechanisms of 2n female gametes formation in mandarins and mandarin hybrids have been revealed (predominantly SDR) but it remains unknown in other citrus species like lemon. Moreover, it was not known if this phenomenon occurs in pollen. In this work we identified the meiotic mechanisms of 2n female gametes in `Eureka Frost´ and `Fino´ lemons, and 2n pollen produced by a diploid tangor `CSO´ (clementina x sweet orange). We analyzed 48 triploid and tetraploid lemon hybrids recovered via 2x x 2x and 2x x 4x hybridizations and 72 tetraploid plants recovered in 4x x 2x hybridizations using `CSO´ tangor as male parent. The use of complementary methods, including individual LOD analysis from centromeric and telomeric loci genotyping, and the analysis of parental heterozygosity restitution patterns along a linkage group, allowed us to distinguish among the different mechanisms of 2n gamete formation. In lemon tree meiotic mechanisms were detected, 88% of 2n female gametes were obtained from SDR, 7% from FDR or Pre-meiotic genome doubling (PRD), and 5% from Post-meiotic genome doubling (PMD). In the case of the `CSO´ tangor, we identified the production of 2n pollen gametes, 77% originated by FDR and 23% by SDR. To our knowledge, this is the first report of (i) the production of a large number of lemon progenies from 2n gametes and the identification of a new mechanism, PMD, which had never been observed in citrus and rarely been described in other herbaceous or woody species and (ii) the first description of the large progenies of citrus tetraploid hybrids arising from 2n pollen gametes and the coexistence of two meiotic restitution mechanisms (SDR and FDR) producing 2n pollen gametes.

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SI-O-6

A PIPELINE FOR PHYLOGENOMIC INFERENCE IN LARGE DIPLOID AND POLYDIPLOID POPULATIONS FROM GENOTYPING BY SEQUENCING (GBS) DATA

Franck Curk[1]*, Patrick Ollitrault[2], Dalel Ahmed[1], Amel Oueslati[3], François Luro[1], Gilles Costantino[1], Hélène Vignes[4], Pierre Mournet[4], Yann Froelicher[1], Raphaël Morillon[2]

Four ancestral taxa (*Citrus. maxima*, *C. reticulata*, *C. medica* and *C. micrantha*) are the ancestors of all the cultivated *Citrus* species. The genomes of most modern varieties and therefore of recent hybrids from breeding programs are a kind of mosaic of large genomic fragments inherited from these ancestors. The genomic structure of several varieties representative of the most important horticultural groups has been recently deciphered by WGS. Despite the rapid evolution of NGS, its cost remains too high to be applied to large populations. The application of NGS on a reduced genome representation open the way for pangenomic studies of large populations. The objective of this work was to validate a GBS approach on citrus and to identify a pangenomic panel of diagnostic SNP markers (DSNPs) of each ancestor to be used for the analysis of the phylogenomic structures of diploid and triploid recombining populations. A set of 29 representatives of the four basic taxa was used for DSNP identification. Genotype calling of ancestor representatives was performed with Tassel and DSNPs searched by GST analysis. Diversity structure analysis was consistent with the previous molecular marker studies, validating our GBS approach. A set of 1,4926 DSNPs were identified. The phylogenomic inference along the genomes of diploid and polyploid hybrids was then based in a maximum likelihood test on the DSNP allele read numbers, working by windows of 10 DSNPs for each ancestor DSNPs set. It was successfully applied to a di-ancestor (*reticulata* / *maxima*) diploid germplasm population and a triploid segregating population implying the four ancestors. This work demonstrates the potential of GBS for deciphering the phylogenomic structures of the modern citrus varieties and hybrids of segregating diploid and polyploid populations. It open the way for genetic associations studies and QTLs analysis based on phylogenomics and further for genomic selection.

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SI-O-7

NMR METABOLOMICS AS A PREDICTION TOOL FOR CONSUMERS' ACCEPTANCE OF MANDARINS

Ignacio Migues[1], Veronica Cesio[1], Adriana Gámbaro[1], Guillermo Moyna[2], Cecilia Rodriguez[1], Joana Lado[3], Fernando Rivas[3], Horacio Heinzen[1*]

Aiming to correlate flavor and consumers' acceptance of different mandarin varieties to their metabolic profile, an untargeted metabolomics approach was applied. Choi's broad polarity extraction method was followed to cover the widest portion of the mandarins' metabolome. New hybrid mandarin varieties from INIA's Citrus Research Program were selected and studied with their parental varieties. The extracts were analyzed through ^{1}HNMR (400 MHz). The acquired spectra were processed, aligned and binned to allow the comparison between samples. Finally, we used statistical metabolomic tools to correlate the spectral data with consumers' acceptance to identify the signals that weighted the most in the differentiation between samples. The consumers' acceptance data was gathered using the CATA method from 100 individuals tasting peeled mandarins. The consumers' preference was obtained in a 1 to 9 scale. A simple correlation between NMR signals and consumers acceptance could be established. The relationship among proton signals due to sucrose, fructose and glucose properly weighted and those of the alpha protons of the carboxylic acids from the krebs cycle correlates point by point with the acceptability scale established by consumers. The results show that this analytical approach, initially thought for disease identification in human samples, is also useful for natural products analysis and their correlation with different types of bioactivity, in this case for taste and flavor, from which objective tools for their evaluation are not usually described. These results could be applied in breeding programs to select new cultivars based in chemical data that correlates with consumers' acceptance without bias or subjective opinions, speeding up the development of new *Citrus* varieties.

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POSTER PRESENTATIONS

SI-P-1

IDENTIFICATION OF ZYGOTIC AND NUCELLAR SEEDLINGS IN *Citrus limon*: SEARCHING OF MOLECULAR MARKERS

Olaya Pérez-Tornero*, Nuria Navarro-García, Fernando Córdoba, Antonio López-Pérez, Yolanda Jiménez Alfaro

All the Spanish lemon cultivars are polyembrionic. Thus, when a breeding program in lemon tree (*Citrus limon*) is carried out, identification of zygotic seedlings with molecular markers is a critical stage. The aim of this study was to obtain a group of molecular markers able to identify zygotic plants from the crossing of two Spanish lemon cultivars 'Verna 51' and 'Fino 49'. We tested fourteen SSRs markers developed for different citrus species and sixteen SSRs markers used in not related to citrus species but neither of the two SSRs trials was successful. Some SNPs developed for other lemon tree cultivars were also tested but none of them were useful for our study. After these results, we decided to look for specific molecular markers type SNP for 'Verna 51' and 'Fino 49'. Samples were collected from both cultivars, the DNA was extracted and two libraries were constructed and sequenced with Illumina technology. After carrying out the bioinformatics discrimination process on the nearly 5 million variants SNP type initially detected, we selected 35 homozygous variants that differed between both lemon varieties: 25 SNPs in coding regions and 10 SNPs from non-coding regions. Primers to amplify these SNPs were designed and tested at our laboratory. Four of these SNPs, two from coding regions and two from non-coding regions, successfully distinguished hybrids from the nucellar plants. In order to obtain more SNP molecular markers, the raw data from the sequencing of 'Verna 51' and 'Fino 49' were used to perform a new SNP calling. Thirty six new molecular markers were detected in homozygosis. They will be tested at our laboratory to confirm that are useful to identify our hybrid plants.

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SI-P-2

THE INCIDENCE OF PLOIDY ACCORDING TO EMBRYO TYPES IN CITRUS SEED

Jin Yeong Kim*, Young Chul Park, Jong Hun Kang, Sang Hun Kang, Tea Hyeon Heo

In citrus breed, triploids are rarely naturally occurred and can be induced by crossing diploid and tetraploid. The induced triploid citrus fruits have seedless characteristics. In this study, we investigated the incidence of ploidy in citrus hybrids by 2013 ~ 2015. A total of 6,546 one year old seedlings, including 24 cross combination using mono-embryo cultivar parents, 13 cross combination using poly-embryo cultivars and 5 cross combination using mixed embryo cultivars, were selected for this experiment. Ploidy level test was performed by cutting to 1 cm x 1 cm size of green leaf of new shoots, using flow cytometry, and confirmed the chromosomes by observing the growth point of root tips. As a result, triploids were occurred 183 plants from 18 combinations, and tetraploids were occurred 86 plants from 17 combinations. 98.4% of the triploids were occurred in mono-embryo cultivar parents and 77.9% of the tetraploids occurred in poly-embryo cultivar parents. When ‘Wilking’ was used as a parent, the incidence of triploids was 18% higher than that of other mono-embryo cultivar, followed by also higher in ‘Kinokuni’ cultivar. In particular, the incidence of triploids reached 20.6% in the combination of ‘Kinokuni’ (mono-embryony) × ‘Wilking’ (mono-embryony). Among the poly-embryo cultivars, tetraploids are occurred in the order of ‘Mihocore’ 5.4%, ‘Ougonkan’ 3.8%, and ‘Encore’ 3.3%. And the rate of tetraploids was high 5.4% when crossing ‘Okitsu No. 46’ (mixed-embryony) and ‘Page’.

As a result of this study, additional studies will be necessary to determine whether the mono-embryony × mono-embryony hybridization affects the incidence of triploidy. And, in order to obtain tetraploidy citrus which has high value as a breeding material, it is effective to use poly-embryo cultivars. And in order to acquire tetraploid resources from mono-embryony cultivars it is effective.

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SI-P-3

MOLECULAR CHARACTERIZATION BY MICROSATELLITES OF CULTIVARS FROM THE CUBAN CITRUS PROTECTED GERMPLASM BANK

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The genetic certification of the species and cultivars present in the Citrus Protected Germplasm Bank (BGP) is a task of the first order to continue improving the Production System of Certified Citrus Material of Cuba. The objective of this work was to perform a preliminary characterization, with microsatellite markers (SSR), of a representative group of citrus species and cultivars to determine those useful for the identification of characteristic band patterns. Twenty-seven species and cultivars, representative of the diversity present in the BGP, were chosen, and young leaves were taken from them to proceed to DNA isolation and molecular characterization using nine microsatellite molecular marker primers. The evaluation of these accessions with the SSR combinations used allowed to differentiate a group of cultivars but not all analyzed, so it is necessary to extend the number of primers, or complement the study with another type of molecular marker, in order to achieve certification genetics of the accessions under study.

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SI-P-4

IDENTIFICATION OF MOROCCAN SWEET ORANGE VARIANTS WITH SSR AND ISSR MARKERS

Samia Lotfy[1]*, Abdel Kader Benazzouz[1], Driss Ezzoubir[2]

Within the framework of a sanitation and rejuvenation program of sweet orange (*Citrus sinensis* L. Osbeck) collection by nucellar way in the Afourer domain of INRA, the plants coming from seeds resulting from open pollination of different varieties of orange, had been subjected to field trials for agronomic and quality characteristics evaluation running the 1980s compared to the original mother plants. Among these potential nucellar plants, 34 variants in 9 varieties of Orange (Salustiana, Sanguinelli, Pineapple, Person brown, Moro, Navel, Grosse sanguine, Cadenera, and Hamlin) proved to be distinct from the parent plants on the basis of phenotypic and pomologic traits. These variants selected by INRA in collaboration with the citrus profession, have subsequently been subjected current the 1990s, to new field trials in different INRA and profession areas and in different regions of Morocco (Gharb, Souss, Tadla). At the level of the Gharb region, one of the tests had been installed in 1995 at the El Menzeh station of INRA Kénitra and clones with agronomic and commercial interests had been registered current 2000s in official catalogue of new varieties. To determine the genetic origin of all of these 34 orange variants, we analyzed their genome with SSR and ISSR markers by comparison to that of corresponding mother plants. Several variants have proved to be different from mother plants and would be potential spontaneous hybrids arising from crosses with other groups of citrus than orange trees. Indeed, these spontaneous hybrids contain alleles not present in sweet orange. Prospection and selection of new variants among potential nucellar plants remain one of the interesting ways of diversification of citrus germplasm. This selection originally based only on phenotypic and agronomic criteria in Morocco is currently completed by the genome analyses using molecular markers effective for resolving phylogenetic questions among citrus, as shown in this study.

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SI-P-5

FRIABLE CALLUS INDUCTION AND PLANT REGENERATION BY ORGANOGENESIS IN TWO STRAINS OF TRIFOLIATE ORANGE (*Poncirus trifoliata*)

Hamid Benyahia[1]*, B. Ait Al ouad[2], O. Chetto[2], R. Benkirane[3], H. Benaouda[2]

Citrus species are the most widely produced fruit crops in the world. *Citrus* rootstock *Poncirus trifoliata*, is characterized by cold hardiness and resistance to *Phytophthora*, nematodes and *Citrus tristeza virus*. These characteristics can be exploited in somatic hybridization using protoplast fusion, which enabled to produce promising somatic hybrids. Nevertheless, the *P. trifoliata* genotypes are highly recalcitrant to friable callus formation, hence the need to optimize the conditions and improve the efficiency of tissue culture. The objective of this study is to evaluate the effect of hormonal composition of the medium on friable callus induction, rate of growth, and regeneration of plants by organogenesis from *P. trifoliata* calluses. Mature embryos of two *P. trifoliate* genotypes were isolated from seeds of ripe fruits, then cultured on MS medium supplemented with 30 g/L of sucrose and plant growth regulators (2,4-dichlorophenoxyacetic acid [2,4-D] and 6 - benzylaminopurine [BAP]) at different concentrations. Comparison of the two genotypes was reported with regard to the efficiency of callus induction from mature embryos. In all media, both genotypes of *Poncirus* responded by forming friable callus. Some media have resulted in the plant neoformation depending on their composition. Furthermore, the study has revealed that callogenesis is conditioned by the nature of the plant regulator used and its interaction with the genotype. The protocol used in this research will pave the way for the development of an in vitro regeneration system for these cultivars and will therefore favor the application of plant tissue culture in the resistance improvement programs.

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SI-P-6

PRODUCTION AND MOLECULAR CHARACTERIZATION OF NEW CITRUS HYBRIDS USING SOMATIC HYBRIDIZATION COUPLED WITH NUCLEAR AND MITOCHONDRIAL MICROSATELLITE MARKERS

Hamid Benyahia[1]*, Ouiam Chetto[2], Pascal Barantin[3], Patrick Ollitrault[3], Dominique Dambier[3]

Somatic hybridization is a powerful biotechnology technique which helps to create new somatic genotypes combining desirable traits from both parents. Nowadays, its use has become an integral part of plant breeding programs. In this study, plants derived from five protoplast fusion combinations, i.e. «*Citrus macrophylla* + Valencia orange (*C. sinensis* (L.) Osbeck)», «*C. macrophylla* + Kinnow mandarin (*C. nobilis* x *C. deliciosa*)», «Carrizo citrange (*C. sinensis* x *Poncirus trifoliata*) + Willow leaf mandarin (*C. deliciosa* Ten.)», «Volkamer lemon (*C. volkameriana*) + Willow leaf mandarin» and «Nasnaran mandarin (*C. amblycarpa*) + Willow leaf mandarin» were subjected to molecular analysis of nuclear and cytoplasmic genome. The use of SSR markers confirmed the formation of hybrids and somatic cybrids within this population. Flow cytometry analysis has further shown that 12 of the 14 regenerated plants were diploid, while two, which originated from the 'Macrophylla + Valencia orange' combination, were tetraploid. The analysis of cytoplasmic genome using universal primers revealed that chloroplast DNA (cpDNA) of these genotypes was inherited randomly, while their mitochondrial genomes were inherited from the embryogenic callus parents.

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SI-P-7

PRODUCTION OF SEEDLESS TRIPLOID CITRUS FROM CROSSES BETWEEN DIPLOID FEMALE AND TETRAPLOID MALE PARENTS

Minju Kim*, Suhyun Yun, Sukman Park

One of the main purposes of citrus breeding is to produce seedless fruit. In recent years, triploid cultivars have been widely used having characteristics that produce almost all of the desired traits and produce seedless fruits. Triploid cultivars do not form normal chromosome segregation during meiosis. So, seeds cannot be developed even after fertilization by forming pollen and embryo sac that have completely lost their fertility. Therefore, to produce a seedless triploid, use a model of diploid female C. hybrid ‘Kiyomi’ which is widely used as a cross - breeding model with good fruit quality and use a model of tetraploid male parents formed by the protoplast fusion. To prevent the embryo from being easily atrophied, immature embryos were isolated 3~4 months after pollination and cultured *in vitro*. When immature embryo becomes plant, it was determined triploid through flow cytometry. In the future, identified triploid citrus plant can be used to seedless cultivar.

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SI-P-8

GENETIC DIVERSITY USING MOLECULAR MARKERS IN CITRUS FRESH FRUIT MARKET CULTIVARS

Luana Maro*, Adriana Pereira, Keny Henrique Mariguele

To quantify the degree of similarity between genotypes is important to know the genetic variability available and the formation of heterotic groups. For from that, making crossings to obtain segregating population or the formation of hybrids. Thus, the goal of this work was to study genetic diversity using ISSR and SSR of 20 citrus fresh fruit market cultivars in Santa Catarina, Brazil. It was used 13 SSR and 11 ISSR markers. The Jaccard coefficient was used to estimate the genetic similarity between genotypes and the phenogram was obtained based on UPGMA, through the NTSYS 1.7 software. Genetic analysis showed that the similarity coefficient ranged from 0.40 – between ‘SCS454 Catarina’/‘Champanha’ – up 1.00 – ‘SCS456 Sigmar’/‘SCS455 Reinaldo’/‘Cara cara’, ‘Hamlin’/‘Bahia’ and ‘Ponkan’/‘Oota Ponkan’. Through generated dendogram it was observed the formation of seven groups. Group I consisted of all genotypes of *Citrus sinensis* (L.) Osbeck: ‘SCS454 Catarina’, ‘SCS457 Souza’, ‘SCS456 Sigmar’, ‘SCS455 Reinaldo’, ‘Cara cara’, ‘Moro’, ‘Hamlin’, ‘Bahia’, ‘Sanguinelli’, ‘Blood’, ‘Valência’; Group II – ‘Clemenules’ (*C. clementina*) and ‘Fallglo’ [*C. reticulata* Blanco x (*C. paradise* Macf. x *C. reticulata* Blanco)] x (*C. reticulata* x *C. sinensis*); Group III – ‘Tankan’; Group IV – ‘Champanha’; Group V – *C. unshiu* (‘SCS458 Osvino’ and ‘Okitsu’); Group VI – *C. reticulata* (‘Oota Ponkan’ and ‘Ponkan’) and Group VII – ‘Mexerica Rio’ (*C. deliciosa*). With these results, we can conclude that there is genetic variability among genotypes and it is possible to produce promising interspecific hybrids.

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SI-P-9

SCS458 OSVINO: EARLY TANGERINE CULTIVAR, HIGH PRODUCTIVE POTENTIAL, COLD TOLERANCE AND SEEDLESS FOR SANTA CATARINA STATE, BRAZIL

Luana Maro*, Osvino Leonardo Koller, Keny Henrique Mariguele

Tangerines generally are highly appreciated by the consumers for being usually easy to peel, have segments that easily separate from each other and have unparalleled flavor and aroma. In addition, organoleptic characteristics have nutritional function and allow economic return in small areas of cultivation. The offer of tangerines during the period from October to February – that is off-season period in Brazil, is an interesting strategy to obtain higher profitability. The adoption of early cultivars with the use of microclimates that exert influence during the maturation are factors to be considered on tangerines growth. In this sense, 'SCS458 Osvino' was selected in a ´Okitsu` orchard due to precocity, good fruit quality, absence of seeds and high productive potential. This cultivar produces flattened fruit shapes (mean diameter of 7.24 cm) and light yellow color when ripe. The mean values for juice yield and soluble solids / acidity ratio are 46.15% and 11.22, respectively. However, ratio values from 8 can be used to establish the beginning of the harvest. Plants presented means values: 2.8 m to height; 3.30 m to canopy diameter; 62.43 m^3 to canopy volume; 6.68 kg m^{-2} to productive efficiency and can reach 60.8 Kg plant $^{-1}$. 'SCS458 Osvino' is the earliest cultivar among the main tangerines cultivated in Santa Catarina/Brazil and can contribute to supply the demand at a time when there is lack of tangerines.

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SI-P-10

SOMATIC EMBRYOGENESIS THROUGH *IN VITRO* ANTHER CULTURE OF *Citrus sinensis* (L.) Osbeck CULTIVAR 'MORO'

Maria Antonietta Germanà[1]*, Nicolò Iacuzzi[1], Pablo Aleza[2], Andres García-Lor[2]

In many crops, anther culture is the most used method to induce gametic embryogenesis, aimed to regenerate homozygous plants. However, also somatic embryogenesis can be obtained by this method, when somatic tissue is involved in regeneration process. Many factors can affect this procedure, such as genotype, pre-treatments applied to floral buds, pollen developmental stage, donor plant states, culture media composition and growth culture conditions. Anthers of *Citrus sinensis* (L.) Osbeck cv. Moro, were collected at the vacuolate stage, and after a chilling (4 °C) pre-treatment of 7 days, were placed on the same medium, evaluating different temperature stresses applied after the placing in culture. In this study, the effect of three thermal treatments, compared with direct *in vitro* culture of the anthers (after the pre-treatment to the floral buds at 4 °C for 7 days), was observed in a *Citrus* genotype. Regeneration of embryos has been obtained and their characterization, through ploidy analysis and molecular analysis, showed that they were heterozygous tetraploids.

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SI-P-11

DEVELOPMENT OF TRIPLOIDY PROGRAM FOR CITRUS MOROCCAN CULTURE

Hamid Benyahia*, Najat, Handaji, Najat Arsalane, Karim Mahmoudi, Kawtar Label, Tarik Aderdour, Rajae Yacoubi, Hanae Naciri, Hassan Benaouda

Morocco is one of the major exporters of small citrus fruits, such as mandarin and Clementine. Seedlessness is a major criterion for this horticultural group. So, the present study focuses on selecting best triploid mandarin hybrids ($2n=3x=27$) characterized by seedless fruits. A series of cross between Sidi Aissa clementine (female parent) and seven mandarins' varieties (Lee, Wilking, Osceola, Carvalhal, Satsuma Frost, Satsuma Owari, Chienka) was performed by the National Institute for Agricultural Research, with the main aims obtaining citrus triploid. Forty triploids mandarins were obtained and planted since 2002 in an experimental field at El Menzeh. Varietal evaluation was focused on quality traits during seven years of study. Statistical analyzes showed that there is a significant difference for all studied characters and between hybrids. The number of seeds per fruit is the main criterion which differentiates between triploids mandarin hybrids and their diploid parent "clementine Sidi Aissa". The best hybrids selected are: HT11, HT13, HT27, HT43, HT44, and HT49. The best crosses are *C1* (Sidi Aissa X Wilking) et *C2* (Sidi Aissa X Osceola).

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SI-P-12

CONSTRUCTION OF GENETIC MAPS OF CLEMENTINE AND STAR RUBY GRAPEFRUIT BASED ON SNP DETECTED FROM GENOTYPING BY SEQUENCING (GBS) DATA

Francois Luro[1]*, Gilles Costantino[1], Helene Vignes[2], Sylvain Santoni[3], Pierre Mournet[4], Patrick Ollitrault[5]

Elaborating genetic map with traditional markers is time consuming as it requires the selection of useful markers for the specific progeny and then the genotyping with these markers. Sequencing of reduced parts of the genome is an efficient and fast alternative method for, at the same time, detect SNPs useful for the considered progeny and genotype the segregating progeny. The objective of this work was to develop a workflow for the selection of robust SNPs from data obtained by Genotyping by Sequencing (GBS) and to establish high density genetic maps of Clementine and Star Ruby grapefruit. A GBS library was established using ApekI restriction enzyme and a selective PCR to improve the depth of the analysis, from 90 (Commune clementine x Star Ruby grapefruit) hybrids and their parents. The library was sequenced in a single ILLUMINA HISeq 2000 lane (single reads) and SNPs were detected with Tassel 4.0 pipeline. A workflow was implemented to select robust SNP loci using parameters on missing data, allele frequency and ratio between reads with reference and alternative alleles at each locus to select the heterozygous markers. The GBS approach revealed initially 1 142 763 SNPs in the clementine x grapefruit progeny. After the filtration pipeline 4 428 and 4 777 heterozygous markers were respectively selected for grapefruit and clementine. After discarding the hardly skewed markers ($<10^{-4}$), about 3 000 SNP were used for the construction of the two genetic maps. Maps were compared with the reference map and with the genome sequence of clementine. Collinearity was respected for more than 90% of the markers on the GBS clementine map. Mapping of SNP markers on grapefruit was subjected to few gaps.

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SESSION II: Physiology and Fruit Quality

KEYNOTE LECTURE

SII-KL-1

TRANSCRIPTIONAL REGULATION OF ABIOTIC STRESS-RESPONSIVE GENES IN CITRUS AND ITS RELATED GENERA: FROM MECHANISM ELUCIDATION TO GENE EXPLOITATION

Ji-Hong Liu

Citrus is frequently challenged by a variety of biotic and abiotic stresses. Therefore, elucidation of stress response pathways and exploitation of key genes that play key roles in stress tolerance are of paramount significance for genetic engineering for reinforcing stress tolerance in citrus. This is greatly expedited by using some special germplasms with unique stress tolerance capacity. Trifoliate orange [*Poncirus trifoliata* (L.) Raf.] is closely related to citrus and exhibits extremely desirable cold hardiness after a full acclimation. Within the last years, we carried out different high throughput pipelines, including suppression subtraction library screening, RNA-sequencing and microRNA (miRNA)-sequencing, in combination of metabolic analysis so as to decipher the molecular and physiological mechanisms underlying the cold tolerance of trifoliate orange. We found that accumulation of sugar and polyamines is promoted under salt, concurrent with up-regulation of genes associated with sugar and polyamine biosynthesis, such as *BAM* (b-amylase), *ADC* (arginine decarboxylase), and *INV* (invertase). In addition, a number of miRNAs and transcription factors were shown to be cold responsive. We further characterize the function of the cold-responsive genes or miRNA, such *PtrBAM1*, *PtrINV*, *PtrADC*, *PtrbHLH*, *PtrICE1*, *ptrmiR396b*, using transgenic approaches. Meanwhile, regulatory network and pathways associated with these genes/miRNA are also illustrated. Our findings indicate that various transcription modules composed of a transcription factor and its target gene(s) play crucial roles in cold stress tolerance of *P. trifoliata*. Moreover, these results shed light into the molecular network explaining the accumulation of sugar and polyamines and activation of antioxidant enzymes in response to cold stress.

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ORAL PRESENTATIONS

SII-O-1

SUMMER EXPRESSION PATTERNS OF FLOWERING GENES IN BUDS OF *Citrus sinensis* (L.) Osbeck SHOOTS AT PROGRESSIVELY ADVANCED STAGES OF MATURITY

Christopher Drozd, Lisa Tang, Rui Li, Peggy Mauk, Carol J. Lovatt*

The time of floral induction in citrus is equivocal. Proposals suggest all buds on an adult citrus tree are induced to flower as the result of phase transition from juvenile to adult or alternatively, buds transition from vegetative to reproductive development annually. Published evidence suggests annual transition occurs in fall or winter preceding spring bloom. Expression of the floral regulatory genes *FLOWERING LOCUS (FT), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1), LEAFY (LFY), APETALA1 (AP1), APETALA2 (AP2), SEPALLATA1 (SEP1), PISTILLATA (PI),* and *AGAMOUS (AG)* and the floral inhibitor *TERMINAL FLOWER (TFL)* was analyzed in 'Washington' navel orange (*C. sinensis*) buds from vegetative shoots of progressively advanced age and maturity (young feathery, young angular, and mature dormant shoots having no new growth). All buds were collected at the same time in August to eliminate differences related to climate and cultural management. In August, all buds expressed *FT, SOC1, LFY, AP1, AP2* and *SEP1*; no buds expressed *PI* or *AG*. Only buds on feathery shoots expressed *TFL*. Buds on all shoots flowered the following spring: feathery 73%, young angular 93% and mature dormant 93%. For comparison, buds from juvenile (seedling) shoots expressed low levels of *FT, SOC1, LFY* and *AP1* but also expressed *TFL* and did not flower. Taken together, the results suggest annual transition from vegetative to floral development in 'Washington' navel orange occurs as early as summer via an autonomous (developmental) pathway, in which age-dependent down regulation of *TFL* might play an important role.

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SII-O-2

AUXIN POLAR TRANSPORT IS ASSOCIATED WITH THE CONTROL OF ALTERNATE BEARING IN CITRUS

Avi Sadka*, Liron Shalom, Yasmin Levi, Naftali Zur, Lyudmila Shlizerman, Assa Florentin

Many commercial citrus cultivars undergo cycles of heavy fruit load (ON-Crop) one year, followed by low fruit load (OFF-Crop) the following year (alternate bearing, AB). Fruit load might affect flowering at various developmental stages, such as, flowering induction, and subsequent stages of flower development and bud break. It is commonly accepted that the fruit or an organ-sensing fruit presence generates an inhibitory signal that moves into the bud and inhibits flowering. However, the mechanism by which the fruit exerts its affect is still unknown. Identification of regulatory processes modified in the bud in association with altered fruit load might shed light on the nature of the AB signaling process. We previously compared the bud transcriptome of Murcott mandarin at various fruit load states: ON-Crop trees, OFF-Crop trees and ON-Crop trees of which fruits were removed (a treatment inducing next year flowering). Among altered regulatory processes, the induction of Ca^{+2}-dependent auxin polar transport was detected in OFF-Crop- as compared to ON-Crop-buds. Fruit removal from On-Crop trees induced this process within one week. Analyses of IAA level in the buds showed that hormone level in OFF-Crop buds was significantly lower than in ON-Crop buds, with fruit removal inducing relatively rapid decline in its level. By use of auxin-specific antibodies, alternation in hormone levels were detected in the apical meristem of the bud. Polar transport was studied with radiolabeled IAA, demonstrating that fruit load affected directly auxin flow in the stem and its release from lateral buds. Overall, our data showed that auxin polar transport is associated with the perception of flowering-inducing signal by fruit load, thus might play a role in the control of AB.

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SII-O-3

VESICULAR TRAFFICKING IN ABSCISSION ZONE CELLS DURING ETHYLENE-PROMOTED FRUIT ABSCISSION IN CITRUS

**Francisco-Ramón Tadeo*, Paz Merelo, Javier Agustí, Daniel Ventimilla,
Manuel Talón**

Abscission is a cell separation process by which plants are able to shed aerial organs. This physiological process proceeds through a breakdown in adhesion between a group of specialized cells that usually differentiate in particular positions of the plant body named abscission zones (AZs). The occurrence of fruit abscission has special relevance in fruit tree crops such as citrus since may have negative consequences on yield. To identify AZ-specifically expressed genes involved in citrus fruit abscission, we used a combined strategy of laser microdissection and microarray analysis to compare the global expression profiles of calyx abscission zone (AZ-C) cells of citrus fruits and adjacent fruit rind cells during ethylene-promoted fruit abscission. The comparison enabled to identify a set of potentially specific genes including those associated with vesicular transport and intracellular transport vesicles. The first ultrastructural observations on AZ-C cells during fruit abscission (Iwahori and van Steveninck, 1976) showed an increase in rough endoplasmic reticulum profiles and dictyosome stacks with associated vesicles that fused with the plasma membrane to release their contents to the cell wall. In this comparative transcriptomic survey, we highlighted specific over-expression in AZ-C cells of genes belonging to the major transport steps along the secretory and the endocytic pathways of vesicle trafficking. In particular, it is likely that some of the genes identified in this study would represent major candidate genes for further biotechnological approaches aimed at trying to solve or at least mitigate agronomic issues regarding citrus fruit abscission.

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SII-O-4

MOBILIZING Ca TO ENHANCE FRUIT QUALITY - PREHARVEST APPLICATION OF HARPIN αβ (ProActR) IN CITRUS ORCHARDS IN SPAIN

Angel Marín[1]*, Kevin Staska[2], Aoife Dillon[3]

By delaying citrus harvest until later in the season growers can potentially achieve a higher price. However, delaying harvest may increase the risk of fruit peel disorders associated with adverse weather (e.g. creasing and water spot). Susceptibility to these disorders is related to cell wall integrity, which is determined by the level of structural calcium. Harpin αβ proteins are biostimulants which activate plant growth and stress-defense pathways. Harpin αβ proteins enhance mobilization of calcium, and its subsequent absorption into the cell wall. To demonstrate this effect in the field, Harpin αβ protein (ProAct®) was applied to commercially grown citrus in Spain (Mandarin v Queen). Trials were in 2015 (Alicante) and 2017 (Seville). ProAct® (150 g/Ha x 3) was applied as a foliar spray three times between fruit set and harvest. In Alicante, compared to the control, total Ca (mg/100 g fruit) increased by 99% and cell wall Ca increased by 49%. In Seville, total Ca increased by 45% and cell wall Ca by 9%. Fruit harvested from the ProAct® treatment on the Seville site were significantly larger (3%) than fruit from the Grower Standard treatment (72.66 ± 0.66 mm versus 70.45 ± 0.69 mm; t-test $P < 0.05$). These results demonstrate that ProAct® facilitates the mobilization of Ca into the fruit. As elevated Ca in the cell wall enhances structural integrity, growers are better positioned to optimize economic returns.

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SII-O-5

EFFECT OF LOW TEMPERATURE-STORAGE ON THE PROTEOME OF MORO BLOOD ORANGE FLESH

Lourdes Carmona López[1]*, Berta Alquézar[2], Susana Tárraga[2], Leandro Peña García[1]

Anthocyanins are a subclass of flavonoid pigments with important therapeutic properties beneficial to human health. Blood oranges are rich in these pigments, which present synergistic effects (i.e. anti-inflammatory and anti-obesity) with other orange juice phytonutrients. Anthocyanin biosynthesis is cold induced in blood citrus cultivars, requiring a broad day/night thermal range to get purple pigmentation in fruit. This cold-dependency limits geographically a reliable quality for commercial production to only a few regions worldwide. For example, cultivation of blood oranges under tropical/subtropical climates, as those of Brazil, yields fruit with very low or lack of anthocyanins. A feasible alternative in tropical countries to enhance anthocyanin content is cold postharvest storage, as it has been shown that anthocyanin synthesis is induced when fruits are kept below 10 ºC for a few weeks after harvesting. Blood oranges kept at 9 °C showed higher expression levels of anthocyanin biosynthetic genes, increased anthocyanin content and reached a darker purple coloration than those stored at 4 ºC. In order to gain insight on the effect of storage temperature on activation of anthocyanins biosynthesis and accumulation as well as on purple orange coloration, we have investigated the proteome of Moro sweet orange [*Citrus sinensis* (L.) Osbeck] fruit stored either at 4 °C (low temperature) or at 9 °C (moderate temperature). Results on increased or reduced accumulation of specific proteins in the Moro orange pulp upon storage at different temperatures will be presented and discussed.

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POSTER PRESENTATIONS

SII-P-1

IN VITRO ASSESSMENT OF GROWTH CHANGES PRODUCED BY SALT IN CITRUS ROOTSTOCKS MUTANTS TOLERANT TO SALINITY

Fernando Córdoba, Antonio López-Pérez, Nuria Navarro-García, Olaya Pérez-Tornero*

Salinity is one of the main environmental stresses affecting citrus production. The combined use of mutagenesis and tissue culture may facilitate the selection of salinity-tolerant citrus lines. This study evaluated the effect of saline medium on explants from mutant lines of salinity-tolerant citrus rootstock obtained by irradiation of *Citrus macrophylla* seeds and *in vitro* selection. Explants from mutant clones (MM1A, MM3A, MM1B, MM3B, MM4B and MM5B) and explants from ‘Macrophylla’ (control) were cultured in vitro in a proliferation medium with different concentrations of NaCl depending on the mutant: 0 and 60 mM NaCl (MM1B, MM3B, MM4B and MM5B); 0 and 80 mM NaCl (MM1A and MM3A); 0, 60 and 80 mM NaCl (explants from ‘Macrophylla’ control). A better growth of mutant explants was observed compared to control explants in the saline medium. MM1B productivity was similar in 0 and 60 mM NaCl. In MM3B and MM4B, productivity in the salt medium was only 9% lower than in the 0 mM NaCl medium; however, in MM5B the decrease in productivity in the saline medium compared to the unsalted medium was 17%, similar to the control in these conditions. In MM1A and MM3A productivity decreased by only 10% in saline medium (80 mM NaCl) compared to the salt-free medium; however, in control this decrease was 27%. The number of damaged and falling leaves was lower in mutant explants than in control explants in the saline medium in MM1B, MM4B and MM5B, but no significant differences with control were observed in MM3B, MM1A or MM3A. The salinity tolerance of these mutants is currently being tested under *ex vitro* conditions.

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SII-P-2

CHARACTERIZATION OF THE CAROTENOID ACCUMULATION IN DIFFERENT HYBRIDS FROM THE URUGUAYAN CITRUS BREEDING PROGRAM

Ana Arruabarrena*, Pedro Pintos, Eleana Luque, Ana Inés Moltini, Fernando Rivas, Joanna Lado

Citrus family (*Rutaceae*) is a very complex and diverse source of carotenoids. Carotenoid profile and content varies greatly among different species, varieties and even fruit tissues (e.g. flavedo and juice sacs). In Uruguay, the Citrus Breeding Program is focused on obtaining good quality seedless mandarin varieties for fresh market. One of the strategies to improve the nutritional value of citrus, is to increase the accumulation of antioxidant compounds in the fruit pulp. In order to characterize the mechanisms of carotenoid accumulation, we analyzed the carotenoid content in the pulp of five mandarin hybrids through their maturation cycle during the year 2017. Additionally, we analyzed the mRNA expression patterns of key enzymes (PSY, βLCY1, βLCY2, εLCY, βCHX) of the carotenoid biosynthesis pathway in different maturity stages: immature green (IG), mature green (MG), color break (B), mature (M). Taken together, these results allowed the identification of specific hybrid genotypes that have the potential to accumulate higher levels of carotenoids in the pulp and, therefore, are good candidates for genetic engineering the carotenoid biosynthetic pathway.

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SII-P-3

ACTIVITY OF THE ARBUSCULAR MYCORRHIZAL FUNGUS *Glomus iranicum* var *tenuihypharum* var *nova* ON CITRUS DEVELOPMENT IN SOUTH-EASTERN, SPAIN

Félix Fernandez Martín*, Jesus Juarez, Antonio Jose Bernabe,
Francisco García and Jose Miguel Gómez

Arbuscular mycorrhizae (AM) are symbiotic associations between fungi of the Phylum *Glomeromycota* and most plants and may be considered a useful tool to help meet the challenges of modern agriculture. The symbiosis has direct benefits for plant nutrition through an increase in the root system, exploration of the soil and greater nutrient uptake through the hyphae and roots. Selecting a suitable species of mycorrhizal fungus for use in intensive agriculture is not an easy task, as it is necessary to ensure satisfactory profitability, given the high amounts of nutritional salts derived from fertilizer solutions that must be applied under these conditions. *Glomus iranicum* var *tenuihypharum* var. *nova* was selected due to its tolerance of a wide range of soil pH values, ranging from 5 to 9.5, and of high Mg, Ca and Mn concentrations and salinity, all properties that ensure its complete adaptation to fertilization protocols (Fernandez et al., 2014). The main goal was to study the use of the AM fungus *G. iranicum* var *tenuihypharum*, its adaptability and functionality in different varieties and species of citrus. First, a trail was carried out to ascertain the effectiveness of supplying two Arbuscular mycorrhizal fungus strains, *G. iranicum* var *tenuihypharum* and *Rhizophagus irregularis* on the productions of Fino lemon, and to compare the results with those obtained for untreated plants. Several mycorrhizal parameters were measured, including colonization percentage and mycelium production, together with fruit calibers and production. A second battery of trials used different tangerine cultivars/varieties (Oronules, Nadorcott, Clemenrubi, Clemenvilla and Clemenpons) and untreated plants in plantations where *G. iranicum* var *tenuihypharum* at a rate of 3 kg /ha applied was applied through irrigation system. Again, the results were compared with those obtained with untreated plants. Development variables such as Colonization percentage, mycorrhizal evolution, fruit production, caliber, root length and biomass production, were measured. The plants treated with *G. iranicum* var *tenuihypharum* showed a significant increase in fruit production compared with *R. irregularis* (35.67%) and in root system development, to highly the treatment with the *G. iranicum* var *tenuihypharum* strain. In the second trial, the percentage of colonization in every variety inoculated was always significantly higher than the control plants. The production of treated plants increased by 7.20% to 26% in the tangerine varieties studied. In Clemenpons variety, the percentages of Calibers of fruits between 46-56 mm was less in the treated plants than the control, on contrary of percent’s of calibers found between 56-70 mm, that favors to *G. iranicum* var *tenuihypahrum* with a 9.18 % of increment. Advancement of the fruit’s coloration effect also appeared in the sub-plot treated with *G. iranicum* var *tenuihypharum* making possible to harvest more fruit in the first pass. The highest response of mycorrhizal productions through to the increment of production was reached in the early variety Clemenrubi Prim 23 (16%), followed by Oronules (12%), Clemenvilla and Clemenpons (8%) and finally Nadorcott with a 7 % increase. In this case, it should be noted that, despite the differences observed, in all cases they were indirectly proportional to total production.

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SII-P-4

EFFECT OF FRUIT SIZE AND POLYETHYLENE BAG WRAPPING ON THE STORAGE ABILITY OF CITRUS HARUMI

Fumitaka Takishita[1]*, Hikaru Matsumoto[1], Masaya Kato[2]

Fruit of citrus ‘Harumi’ usually is harvested from Dec. to Jan. to avoid cold injury on the peel. After the storage period from Jan. to Feb., fruit are shipped until middle of Mar. But the elongation of shipping period until late Mar. or Apr. is expected. In this experiment, the effects of fruit size and polyethylene bag wrapping on the storage ability were investigated. Fruit size was linked to Brix and specific gravity, i.e. small fruit had high Brix and high specific gravity. Citric acid content was not correlated with fruit size. Polyethylene bag wrapping reduced fruit weight loss but didn’t affect Brix after 40 days storage at ambient temperature. The effect of wrapping to reduce fruit weight loss was greater in small fruit than in middle or large-sized fruit. In the wrapped middle or large-sized fruit, the decrease of specific gravity, the occurrence of peel puffing, granulation or dehydration of juice sacs were remarkable. However, fruit quality of non-wrapped middle or large-sized fruit was kept high until Apr. On the other hand, the occurrence of peel wrinkle and fruit weight loss was detrimental to non-wrapped small fruit. Consequently, by adjusting wrapping period in accordance with fruit size, the storage ability would be improved, and the shipping period elongated.

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SII-P-5

VARIABILITY LEVELS OF SELECTED AMINO ACIDS AMONG MANDARINS PRODUCED IN URUGUAY

Sofía Rezende[1]*, Natalia Besil[2], Sabrina Banchero[3], Ignacio Migues[1], Verónica Cesio[1], Fernando Rivas[4], Joana Lado[4], Horacio Heinzen[1]

The characterization of the nutraceutical properties of mandarins, besides their high Vitamin C content and antioxidant flavonoids, is of great interest to promote the consumption of *Citrus*. Additionally, it provides a qualitative differential trait since the demands for functional food is a growing trend in the world. The presence of amino acids (AAs) in Uruguayan mandarins could be identified through ^{1}H NMR data. To learn more about its occurrence and variability between species, a “targeted metabolomics” study in three mandarins’ varieties (‘Ellendale’, ‘Willowleaf’ and ‘Page’) was performed. The AAs studied through ion exchange LC (ESI-)-MS/MS were: phenylalanine, glutamic acid, glutamine, threonine, tyrosine, proline, asparagine, histidine and methionine. The extraction methodology was adapted from Choi. The differences between two consecutive seasons (2015 and 2016) were evaluated, and the influence of fruit maturity in the AAs content of ‘Page’ variety collected in two periods within the same year (May and June/2015) as well. The analytical methodology was validated (linearity between 10-1000 μg/L for each AA, LOQs between 10-500 μg/L). The concentration levels of the AAs for the mandarin varieties were analyzed through Principal Component Analysis (PCA). A clear differentiation could be observed between the varieties harvested in 2016 and 2015 according to their AAs profiles. The PCA analysis comparing the AAs levels at different harvesting periods of cv. ‘Page’ in 2016 indicated a clear similarity between the observed profiles during 2015 and 2016 exclusively with samples harvested in July, winter time. The different profiles in the AAs content of mandarins are due to the genetic diversity among varieties, but the climatic conditions could influence them. This is quite interesting since these profiles could be used to add value to each product, according to the AAs content.

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SII-P-6

POLYPHENOLS AND LIMONOIDS CHARACTERIZATION IN MANDARIN CULTIVARS AND ITS HYBRIDS

Cecilia Rodriguez Ceraolo[1]*, Veronica Cesio[1], Ignacio Migues[1], Natalia Besil[2], Joana Lado[3], Fernando Rivas[3], Horacio Heinzen[1]

Breeders always seek for new mandarin-type cultivars with distinctive and valuable characteristics. Among the pursued properties new mandarins should have, are the absence of seeds, easy peeling, good colour and flavor, as well as early maturity and remarkable nutritional properties. Secondary metabolites such as polyphenols and limonoids are responsible of many of the organoleptic and nutraceutical attributes mandarins have. In the present work, the profile of polyphenols and limonoids in the pulp of phylogenetic related mandarin varieties using LC-MS/MS and HPLC-DAD was studied. The aim was to find cues of on the inheritance of phenotypic characters of interest from parents to hybrids. A total of 19 cultivars were evaluated in the 2015 and 2016 harvests. Four of them gave origin to the remaining 15. All pulp flavonoid profiles were recorded and 10 polyphenolic compounds were quantified, (ferulic acid, eriocitrin, tangeretin, narirutin, didymin, hesperidin, among others), to perform chemometric studies. Limonoids such as nomilin and limonin were detected in different concentrations according to the variety analyzed, showing an overall trend of hybrids to have an intermediate value of concentration in relation to the original progenitor. Page was the variety with the highest content of limonoids, and the lowest content was found in Willow leaf. Page was the only cultivar with grapefruit within its phylogeny. Hesperitin derivatives were the most common flavanones among the different varieties. Only hyperoside glycosides were detected but no neohyperoside glycosides, characteristic for their bitter taste, were found in any of the varieties analyzed. Ripe fruit stability between both years, in terms of flavonoids concentration, was checked. In addition, relationship between the profile of parents and hybrids were found. This study allows the characterization of the cultivars under study from a chemotaxonomic point of view, providing valuable data on their nutraceutical properties.

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SII-P-7

CHEMICAL COMPOSITION AND SENSORY ANALYSIS OF MOROCCAN ORANGE JUICE

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The objective of this study is to determine the chemical composition and sensory analysis of some industrial and natural orange juices consumed in Morocco. The biochemical's tests (sugar, protein, lipid, vitamin C, carotenoid, Brix and salt) were performed using analytical methods and the sensory analysis was also assessed by principal component analysis (PCA). Results showed that natural juices were rich in vitamin C, carotenoids and Brix compared to the industrial juices. Sweet 'Valencia late' (*Citrus sinensis*) juice recorded the highest concentration of vitamin C (68.10 mg/100 ml), carotenoids (0.61 mg/100 ml), and higher concentration of sugar (31.4 g/100 ml). The PCA analysis revealed the presence of two groups; the first group contains the sugar, Brix and salt which are positively correlated, while the second group which is constituted of proteins, lipids and carotenoids were negatively correlated, on the other hand vitamin C contributed positively to PC2. In addition, the correlation between the scores given by the panelists and the results of the physico-chemical parameters shows a good correlation between the nutritional parameters and most of the attributes.

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SII-P-8

FORMATION OF ‘FLYING DRAGON’ AND ‘SWINGLE’ ROOTSTOCKS: SUBSTRATES AND TEGUMENT IN THE EMERGENCE OF SEEDLINGS

Luana Maro[1]*, Vanessa Winter Forest[2], Rosete Pescador[2]

The production of citrus seedlings in Santa Catarina State has grown in the last few years. However, the process of seedlings production needs adjustment in order to optimize the system to get genetic material with high phytotechnical standard that allows greater profitability to the producer. The objective with the present study is to evaluate the effect of different substrates and the removal of tegument in the seedlings formation of ‘Swingle’ and ‘Flying Dragon’ rootstocks. The experimental design was completely randomized in a 2x4x2 factorial design, with 2 rootstocks (‘Swingle’ and ‘Flying Dragon’), 4 substrates (commercial 1 - Rohrbacher®, comercial 2 - Golden Mix®, commercial 3 - Tecnomax®, 4 – formulated from green coconut waste added poultry manure) and absence and presence of tegument. The evaluated parameters were percentage of germination, germination speed index, polyembryony, size of root and aerial part. The formulated presented the lowest value for both germination and germination speed index, for which reason the others evaluation were disregarded. The polyembryony did not show significant statistical difference, when the seeds were submitted to the treatment, not having direct influence of the seeding condition. About the rootstocks, the ‘Swingle’ showed better results with the removal of tegument, for the other hand, seeds of ‘Flying Dragon’ were better with tegument. The commercial substrate 1 and 3 provided higher mean values for both rootstocks in the root length variable, indicating the dependence of soil conditions on the root system. In the aerial part size, genetic characteristics exceed the interaction with the substrate factor, presenting greater development of ‘Swingle’. It was concluded that is not favorable the removal of the tegument because the low cost benefit. The most promising rootstock was ‘Swingle’ and the commercial substrates 1 and 3.

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SII-P-9

DEVELOPMENT OF MOLECULAR MARKERS FOR THE GENOTYPING OF RUBY ALLELES RELATED TO RED-FLESH TRAIT IN CITRUS AND THEIR FUNCTIONAL ANALYSIS

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Hye-Young Ms. Lee[1], Sukman Park[3], Su-Hyun Yun[3], Kwan Jeong Song[4]**

Blood oranges (*Citrus sinensis*) are believed to have significant health-promoting properties, combining the high content of vitamin C, carotenoids, citrus-specific flavonoids, and dietary fiber of sweet oranges with health benefits of anthocyanin pigments. The high anthocyanin content in blood oranges has been generally known to reduce cardiovascular risk factors due to their high antioxidant activity. In a mouse model, blood orange juice consumption has been shown to prevent obesity caused by a high-fat diet. Up to now, six different alleles on *Ruby* locus encoding a MYB transcriptional activator of anthocyanin production were identified. For the marker-assisted selection of citrus cultivars with red flesh trait, we developed PCR-based DNA markers for the genotyping of each *Ruby* allele. During the marker development, we identified the 7th *Ruby* allele from a citrus hybrid, 'Shiranuhi', and traced its origin from the genotyping on parents and grandparents of 'Shiranuhi'. We finally concluded that the 7th *Ruby* allele was originated from *C. unshiu* 'Miyagawa Wase', a grandparent of 'Shiranuhi'. Several *Ruby* alleles were introduced into *Arabidopsis thaliana* to study their functional analysis.

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SESSION III: PESTS AND DISEASES

ORAL PRESENTATIONS

SIII-O-1

DEVELOPING OF HLB RESISTANCE IN CITRUS ROOTSTOCKS THROUGH ANTIMICROBIAL PEPTIDE EXPRESSION

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The disease Huanglongbing (HLB) represents the greatest threat to citrus crop and has resulted in losses of up to 100%. In Argentina, the first diseased plants were detected in Misiones Province in 2012 and currently exceed 200 positive cases including several provinces. The causative agent is a bacterium of the genus *Candidatus* Liberibacter, which is transmitted by an insect vector present in citrus regions, the psyllid *Diaphorina citri*. Among control strategies, genetic engineering techniques appear as very promising, since traditional breeding and alternative management of the Psyllid through application of insecticides has shown to be difficult expensive and inefficient. Citrus plants are mainly propagated by grafting commercial varieties onto rootstocks. The present work shows the generation of transgenic plants of the rootstock *Citrange troyer* with a construct for the expression of the antimicrobial peptide snakin/ GASA that has previously demonstrated to exert direct antimicrobial effects in potato plants. After regeneration, rustication and molecular characterization, the generated transgenic lines will be challenged against bacterial pathogens such as *Pseudomonas syringae*, *Xanthomonas* sp and *Candidatus* Liberibacter. Commercial non-GMO sweet orange scions will be grafted onto resistant/ tolerant transgenic lines for field-testing to determine if the improved tolerance can also be transmitted to the scion.

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SIII-O-2

EFFECT OF *Candidatus* Liberibacter asiaticus ON CENTRAL CARBON METABOLISM IN DIFFERENT CITRUS CULTIVARS

Camila Ribeiro*, Gmitter Jr. Frederick G, Nian Wang

‘*Candidatus* Liberibacter’ spp. is the presumed causal agent of citrus Huanglongbing (HLB). It is transmitted by two species of phloem-feeding citrus psyllids, *Diaphorina citri* and *Trioza erytreae*. HLB is currently considered the most devastating citrus disease in the world. HLB symptoms include yellow shoots, leaf blotchy mottle, lopsided fruit with color inversion, and seed abortion. Moreover, starch accumulation and phloem damage affecting source-sink flow of photoassimilates, are also observed in the infected citrus plants. Even though this disease affects all citrus species, some varieties show less severe symptoms and much slower decline than others. Although substantial research efforts have been made to understand the pathogen infection, there is still a lot to unravel on the physiological and biochemical mechanisms of this disease. In the present research, we investigated selected genes and enzymes of central carbon metabolism, using a combined gene expression and enzyme activity quantification approach. The materials chosen for the comparative experiments were tolerant and susceptible siblings of known HLB tolerant cultivars (LB8-9 Sugar Belle® and Siamese pummelo) and a very susceptible sweet orange cultivar (Valencia). By comparisons and correlations among the infection stages of these cultivars, our results aim to understand citrus innate tolerance mechanisms against HLB.

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SIII-O-3

EVALUATION OF THE TOLERANCE OF DIPLOID AND TRIPLOID LIMES INFECTED BY HLB

Raphael Morillon[1]*, Benoit Heuguet[1], Saturnin Bruyère[1], Rosiane Boisne-Noc[1], Pierre Brat[2], Olivier Gros[3], Patrick Ollitrault[1]

World citrus industry faces one of the most devastating disease known Huanglongbing (HLB). HLB is caused by the bacteria *Candidatus* Liberibacter spp. In a short term, this disease causes the dieback and the tree death. HLB is responsible of the synthesis of callose at sieve plate of the phloem sieve tubes leading to the obstruction of the pores that provide connection between adjacent sieve elements thus limiting the symplastic transport of sugars and starch synthesized in leaves to organs of the plants such as fruits or roots. Polyploidy is a frequent occurrence in the plant kingdom. Our results showed that polyploidization leads to an increase in the size of cells and organs in citrus. Also, the use of tetraploid citrus rootstocks was shown to confer increased tolerance to water deficit. Our objective was to decipher the potential traits of tolerance to HLB related to polyploidy. Diploid (2x, *Citrus aurantiifolia*) and triploid (3x, *Citrus latifolia*) limes grafted onto diploid citrumelo 4475 (*Citrus* paradise × *Poncirus trifoliata*) rootstocks were investigated when naturally infected by HLB. The plant physiology, the anatomy of leaf petiole was analyzed using Scanning Electron Microscope to observe callose deposition at sieve plate of the phloem, as well leaf starch content and detoxification enzyme activities in 2x and 3x leaves were investigated. Triploid variety was shown to present less symptoms of chlorosis due to HLB. Photosynthesis, stomatal conductance and transpiration decreased compared to control plants, but values remained greater in 3x than in 2x. Analysis of the petiole sieve plate in control petiole samples shown that pores were about 30% larger in 3x than in 2x. SEM analysis of infected petiole samples shown important deposition of callose onto 2x and 4x pores while biochemical analysis revealed a better behavior in 3x than in 2x.

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SIII-O-4

TRANSCRIPTOME PROFILING OF CANKER RESISTANT Bs2-TRANSGENIC CITRUS PLANT REVEALED THE UP-REGULATION OF DISEASE RESPONSE GENES

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Pepper Bs2 gene confers resistance to *Xanthomonas campestris* pv. *vesicatoria (Xcv)* pathogenic strains containing the avrBs2 avirulence gene in susceptible pepper and tomato varieties. The avrBs2 gene is highly conserved among members of the *Xanthomonas*, and *avrBs2* of *Xcv* shares 96% homology with the *avrBs2* of *Xanthomonas citri* subsp. *citri (Xcc)*, causal agent of citrus canker disease. In our previous work, transgenic plants of *Citrus sinensis* cv. Pineapple expressing *Bs2* under the control of a pathogen-inducible promoter (glutathione S-transferase from potato) were obtained. In *Bs2*-lines, reduction of canker disease symptoms was observed and was dependent on the presence of the *Xcc avrBs2* gene. In order to know the molecular mechanisms underlying the differences between *Bs2*-transgenic and non-transgenic plants after infection with Xcc, transcriptomic profiles of both plants were compared by using RNAseq. A total of 2022 genes were differentially expressed (DEG) in transgenic plant compared with non-transgenic plants, among them, 1356 genes were up-expressed and 666 were down-expressed. Gene ontology (GO) annotation and Singular Enrichment Analysis (SEA) of the DEGs showed that genes related to the biotic stress were the most highly induced in *Bs2*-transgenic (36.7%), some of these were β-1,3-glucanase, PR4 and PR5 proteins. Moreover, several genes codifying receptors like kinases were also induced (15.6%), followed by transcription factors like WRKY, MYB and bHLH family (9.5%). These results show that *Bs2* gene from pepper triggers molecular mechanisms involved in defense response in citrus plants *Xcc*-infected and can be used to confer resistance in other crops of agronomic importance where *Xanthomonas* species are pathogens. Moreover, the identification of these genes could be an important step towards developing different strategies to improve citrus response not only to *Xcc* but also to other diseases affecting this crop, like Huanglongbing.

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SIII-O-5

CHALLENGE OF TRANSGENIC SWEET ORANGE EXPRESSING d4e1 or csd1 GENES TO *Xanthomonas citri* subsp. *citri*

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Currently, plant health management is one of the major challenges in the citrus industry worldwide. Among several diseases affecting citrus, those caused by or associated with bacteria are a major threat. Several strategies may be applied and integrated in order to achieve adequate disease management in citrus, including healthy nursery trees, efficient monitoring of disease incidence and severity, inoculum reduction in affected areas, and genetic resistance. This last approach can be achieved by integration of conventional citrus breeding and modern biotechnological tools, including genetic transformation. This work aimed to perform an initial evaluation of transgenic sweet orange plant events bearing two different gene constructs with potential for bacterial resistance. 'Hamlin' sweet orange plants expressing the D4E1 synthetic antimicrobial peptide driven by the 35S promoter, or expressing a superoxide dismutase (CSD1), from *Poncirus trifoliata*, driven by ubiquitin promoter were propagated by grafting and inoculated, by spraying, with a *Xanthomonas citri* subsp. *citri* suspension, the causal agent of citrus canker. Foliar symptom evaluations were assessed with the use of diagrammatic scales, up to 30 days after inoculation. Transgenic plants from both gene constructs showed reduction in disease incidence and disease severity comparing to the control plants (non-transgenic). Further analyses are underway in order to better characterize the transformation events potentially more resistant to this disease.

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SIII-O-6

CANDIDATE GENES FOR RESISTANCE TO ALTERNARIA BROWN SPOT IN CITRUS AND SNP MARKERS FOR ASSISTED SELECTION

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Alternaria brown spot (ABS) is an important fungal disease affecting susceptible citrus genotypes and represents a strong limitation regarding citrus cultivation in humid areas and also for developing breeding programs. We previously located a 3.3 Mb genome region near the chromosome III centromere, containing the dominant locus responsible for ABS-susceptibility. Through fine-linkage mapping using a 268-segregating diploid progeny derived from a heterozygous susceptible × resistant cross, we have identified nine candidate genes related to pathogen resistance and developed new Single Nucleotide Polymorphism (SNP) molecular markers for effective marker-assisted selection. Fine mapping limited the genomic region containing the candidate ABS-resistance genes to 366 kb, flanked by markers at 0.4 and 0.7 cM. Among the identified candidate genes, eight are resistance (R) gene homologs, with two of them harboring a serine/threonine protein kinase domain. These two genes, along with a gene encoding a S-adenosyl-L-methionine-dependent-methyltransferase protein, should be considered as strong candidates for *ABS-resistance*. Moreover, the closest SNP was genotyped in 40 representative citrus varieties, revealing a very high association with the resistant/susceptible phenotype. This new SNP marker is currently used in our citrus breeding program for ABS-resistant parent and cultivar selection, at diploid, triploid and tetraploid level and has been applied to date to select more than 3000 new resistant hybrids. Moreover, we are currently addressing differential expression analysis of the candidate genes in susceptible and resistant genotypes under inoculation and control conditions. The identification of a unique ABS-susceptible gene will pave the way to address directed mutation of high quality varieties, but susceptible to ABS, using precise techniques such as CRISPR/Cas genome editing.

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SIII-O-7

PROGRESS ON *Citrus tristeza virus* RESEARCH IN URUGUAY: UNRAVELLING THE ENEMY FROM THE INSIDE

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Since its appearance two centuries ago, the disease known as tristeza, is considered the most devastating virus-related illness of the citrus industry worldwide. *Citrus tristeza virus* (CTV), the causing agent of the disease, is transmitted by graft or by aphids. The existence of genetic variants of the virus leading to different outcomes in the infected plant has been reported in all affected citrus regions. Molecular and biological characterization of circulating isolates is especially important since it brings epidemiological information to control the disease. In the past few years, we have been focused on the study of CTV biological and genetic diversity in Uruguay. Biological indexing on five citrus indicator species showed the prevalence of severe CTV isolates. Based on a complete surveillance of Uruguayan citrus orchards harbouring 1200 field samples, we described to co-circulation and co-infection of citrus trees with CTV genotypes VT, T3, T36, RB, as well as a fifth new lineage named NC, with high prevalence in the fields. With the aim of developing a long-term cross-protection program, to be included in the ongoing National Citrus Sanitation schedule, we are trying to obtain an in-depth knowledge about the CTV genotypes prone to cause severe damages to the Uruguayan citrus industry. Here, we report the results of the biological, molecular and evolutionary full-genome characterization of the most relevant genotypes in the country.

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POSTER PRESENTATIONS

SIII-P-1

PERFORMANCE AND REACTION TO HUANGLONGBING OF TAHITI ACID LIME GRAFTED ON CITRANDARINS

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The Tahiti acid lime (*Citrus latifolia* Tan.) has a great economic importance, being the state of São Paulo responsible for 70% of the Brazilian crop. There are no reports in the literature of the use of citrandarins (hybrids of mandarins with *Poncirus trifoliata*) as rootstock (RT) for Tahiti. The RTs were not initially part of the discussion related to the *Huanglongbing* (HLB or greening) studies, one of the main diseases of citrus, however, there is evidence that citrus plants in different RT may differ in incidence or tolerance to the disease. This work aimed to evaluate the reaction of Tahiti acid lime grafted onto Rangpur lime (RL), Sunki mandarin, *Poncirus trifoliata* Flying Dragon (FD) and thirteen citrandarins (TSxPT) to the bacterium *Candidatus* Liberibacter asiaticus, causal agent of HLB, and its vegetative and productive performance. The experiment was conducted at the Paulista Agency of Agribusiness Technology, located in Pindorama, Brazil, in the years 2016 and 2017. The biometrics of the plants, the monthly production of the fruits and the detection and quantification of the bacteria by real-time quantitative PCR were carried out. Tahiti grafted onto citrandarin TSxPT14 presented a positive result for HLB, but no visual symptoms were observed in the field. The most prominent RT in relation to the accumulated production was RL, followed by citrandarins TSxPT10 and TSxPT121. The lowest and highest canopy volume were observed for citrandarin TSxPT14 and RL, respectively. The evaluations made in the sixteen rootstocks allowed to verify that citrandarin TSxPT14 presents a possible tolerance to HLB, however, its canopy volume and production are low. It can be considered as a dwarfing rootstock for Tahiti acid lime.

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SIII-P-2

GLOBAL GENE EXPRESSION OF *Poncirus trifoliata* UNDER INFECTION OF *Candidatus* Liberibacter asiaticus

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Huanglongbing (HLB) or greening have caused enormous damage in all regions where it occurs, it has been considered the most important disease of citrus. The disease is caused by the gram-negative bacterium, *Candidatus* Liberibacter spp (CLas), non-cultured, alpha-proteobacteria, restricted to the phloem sieve vessels. The development of symptoms in plant is considered as a consequence of a series of molecular, cellular and physiological alterations. All commercial citrus species are susceptible to the infection caused by CLas, however, tolerance or resistance is often reported by *Poncirus trifoliata*. This genotype and some hybrids develop less severe HLB symptoms and has a lower CLas titer, for this reason we decided to analyze *P. trifoliata* responses to 'Ca. L. spp.' infection. Three biological replicates of the genotype, inoculated (CLas-infected budwood) and mock-inoculated (health budwood) have the total RNA isolated and the cDNA library was sequencing using the Illumina © HiSeq 2500 platform. For the differential expression genes identification, the package DESeq was used. Considering 0.05 of significance, only one hundred genes showed significantly different expression from the control, 70% of the genes were up-regulated and 30% down-regulated, most of the differential expression genes are involved in the metabolic and cellular process. In this case, results obtained, shows that despite *P. trifoliata* modulates only a few genes when infected by CLas, it is relatively tolerant and in some cases resistant to HLB (does not present CLas titer), for this reason this genotype makes it interesting for the study, and may perhaps help to answer which genes would be involved in resistance to HLB.

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SIII-P-3

PREVALENCE OF *Citrus tristeza virus* (CTV) GENOTYPE T30 IN CUBAN CITRUS AREAS

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Citrus tristeza virus (CTV) (*Closteroviridae: Ampelovirus*) is the pathogen of viral origin that causes more economic losses to the cultivation of citrus fruits. In Cuba, the technological changes introduced in citrus farming because of huanglongbing (HLB) detection could influence the incidence of the disease and the composition of the genotypes in populations of virus isolates. To determine the incidence of CTV and circulating isolates, a survey was carried out in the main citrus regions of the country. The collection of 27 CTV isolates allowed the partial characterization of the majority populations of the virus. The results showed that the incidence of CTV in the plantations has decreased with respect to previous studies and the majority isolates are mild and of T30 genotype, although other evidences suggested the presence of severe isolates in our country. These preliminary results demonstrate the importance of studying tristeza disease situation in the current context of Cuban citriculture due to the threat of the emergence of a new epidemic and to update management strategies for the disease.

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SIII-P-4

SURVEY ON THE PRESENCE OF *Xylella fastidiosa* AND ITS POTENTIAL INSECT VECTORS IN MOROCCAN CITRUS ORCHARDS

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Xylella fastidiosa, a gram negative bacterium belonging to the family *Xanthomonadaceae*, is xylem-inhabiting, devastating pathogen which causes various diseases on more than 300 plant hosts. Until relatively recently, *X. fastidiosa* was primarily limited to North and South America, but in 2013 a widespread infection of olive quick decline syndrome caused by this fastidious pathogen appeared in south-eastern Italy, and several cases of *X. fastidiosa* infection have been reported in other European Countries (France, Germany and Spain). Following these recent confirmed findings of *X. fastidiosa* in the European Union; this bacterium is becoming a serious threat to the Moroccan agricultural sector. A survey was conducted during April-September 2015 on the presence of *X. fastidiosa* and its potential insect vectors in several citrus commercial groves. In a few trees, severe symptoms which could be associated to the bacterium were observed. A total of 410 samples of different varieties and ages from different regions were randomly collected and were tested for the presence of *X. fastidiosa* by using an ELISA commercial kit. ELISA results did not show any positive sample; these preliminary results are taken as good news, considering that *X. fastidiosa* was not found in the Moroccan citrus orchards. As for insect vectors, investigations were focused in the *Auchenorrhyncha* species, particularly leafhoppers, a group that includes known vectors of *X. fastidiosa*. Adults of leafhoppers species belonging to *Empoasca* genera were captured, using yellow sticky traps or a sweeping net, in addition to other already known species.

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SIII-P-5

THE CITRUS CERTIFICATION SCHEME IN MOROCCO

Mohamed Afechtal*, **M. C. Smaili, H. Benaouda, H. Benyahia**

The Certification scheme for pathogen-tested material of citrus has the aim of providing plants that are true-to-type, free from virus and virus-like diseases and substantially free from other pests. Virus and virus-like diseases of citrus cause very important economic losses in Morocco such as decline, loss of vigour, shortening of the commercial life of the infected trees, low yields and poor fruit quality. There is no effective chemical control against these diseases; therefore, preventive measures must be based on the mass production of virus-free planting stocks. The Moroccan Ministry of Agriculture has established, since 1983, a national certification program for citrus nursery plants. This program includes four propagating material categories: Primary source, Pre-basic, Basic, and certified. All plants of each category are periodically tested for the following virus and virus like agents: *Citrus tristeza virus, Citrus psorosis virus, Citrus exocortis viroid, Citrus cachexia viroid* and *Spirolama citri*. Nowadays, more than 5 million certified citrus plants, of different varieties and rootstocks, are produced annually in the country. The organization of the Moroccan citrus certification program through its steps, as well as varietal and sanitary controls are described.

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SIII-P-6

THE USE OF NEXT GENERATION SEQUENCING TO INVESTIGATE THE SUSCEPTIBILITY OF *Murraya* GENUS TO CITRUS CANKER

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Three major bacterial diseases, Citrus Bacterial Canker (CBC) caused by *Xanthomonas citri* subsp. *citri* (Xcc) and *X. fuscans* subsp. *aurantifolii* (*Xfa*), Citrus Variegated Chlorosis (CVC) caused by *Xylella fastidiosa* subsp. *Pauca* and *Huanglongbing* (HLB) associated to three '*Candidatus* Liberibacter' species, could threaten citrus industry in the EU and Mediterranean countries. CBC is a severe disease affecting citrus worldwide, even though Mediterranean area is free. In 2014, EFSA highlighted ornamental rutaceous species (ORS) as possible pathway for CBC entry. In this context, ORPRAMed research project is underway to evaluate the risk of introduction of *Xcc* and *Xfa* through ORS, especially those not covered by 2000/29EC Directive, using phytopatological, molecular, genetic and economic approaches. From a genomic point of view, it was focused the attention on *Murraya* genus, based on previous results highlighting a dual behavior of the *Murraya* species. Specifically, *M. paniculate* showed a resistant response, differently from *M. ovatifoliolata* that has displayed a high degree of susceptibility. For this purpose, we performed the de novo sequencing of *M. paniculate* genome, the first sequenced among Citrus relatives, using HiSeq2500 platform. De novo assembly results support a high robustness of data, in term of N50 (more than one megabase) and N90 (scaffold longer than 300 kbp). Gene prediction and functional annotation was performed through RNAseq starting from different tissues. Moreover, transcriptomic analysis of *M. paniculate* and *M. ovatifoliolata* before and after 24 hours of *Xcc* inoculations will elucidate on genes involved in the response of plant susceptibility in the early and advanced phase of infection. The behavior of *Citrus* species and its relatives regards emerging diseases need to be further investigated. For thus the availability of *Murraya* genome will be useful to scientific community to study transcriptional mechanisms involved in the response to different pathogens.

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SIII-P-7

SEMIOCHEMICALS APPLICATIONS FOR CITRUS PEST MANAGEMENT IN URUGUAY: TWO CASES OF STUDY

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Semiochemicals, such as pheromones and volatiles form plant hosts, provide sustainable and eco-friendly tools to be included in integrated pest management programs of many insects. Uruguayan citriculture is majorly aimed for the international trade of fresh fruit, where the allowed limits of pesticide residues are important limitations. *Diaphorina citri* (Hemiptera: Liviidae) and *Diploschema rotundicolle* (Coleoptera: Cerambycidae) are two citrus pests present in Uruguayan groves. Our group has been working in finding alternatives to the conventional control of these insects. For *D. citri*, a synthetic attractive blend to be used together with yellow-sticky traps has been preliminarily developed. To meet this objective, volatiles from two preferred citrus species (*Citrus paradise* and *Citrus sinensis*) were characterized. Based on the Grapefruit profile, its main compounds (limonene and methyl N-methylanthranilate) and a synthetic blend were tested in different concentrations as lures on yellow sticky traps. In two-choice greenhouse cage experiments, it was observed that the Grapefruit blend at lower concentration, as well as the limonene, enhanced the trap caches of *D. citri* adults. As for *D. rotundicolle*, the aim was the development of a synthetic pheromone-based trap for adults. To study the chemical communication in this system, volatile produced by field-caught males and females were studied. Males produce large amounts of (3R)-hydroxy-2-hexanone and some minor compounds. Both, female and male antennae, responded to the natural compound in electroantennography studies, suggesting that the compound may function as an aggregation pheromone.

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SIII-P-8

CURRENT PEST STATUS AND THE INTEGRATED PEST MANAGEMENT STRATEGY IN THE CITRUS GROVES IN MOROCCO

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In Morocco, the citrus industry plays a very important socio-economic role in the national economy with an area about 124 000 ha and an average production of 2.3 million tons per season. Many efforts have been made to switch from chemical control to Integrated Pest Management Strategy on Moroccan citrus orchards. Actually about 55 phytophagous insects, mites and snails species have been recorded from citrus trees in Morocco. However, few of them are considered as a major pests or occasional pests and may cause an economic damage and their control is necessary. The major pests in all main citrus areas are; they Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), The California red scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), the Oriental red mite (Citrus brown mite) *Eutetranychus orientalis* Klein (Acari: Tetranychidae), the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracilariidae) and the Snails. However, the remaining species may have the status of major pest or occasional pests, depending on the region of citrus production area. This is the case of the armored scale, such as, Chaff scale *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae), the Citrus brown soft scale *Coccus hesperidum* L. (Hemiptera: Coccidae); the *Kelly's* citrus *thrips Pezothrips kellyanus* Bagnall and the Western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) and the Citrus red mite *Panonychus citri* McGregor (Acari: Tetranychidae); the Cottony cushion scale *Icerya purchase* Maskell (Hemiptera: Monophlebidae) and the citrus aphids [e.g. *Aphis spiraecola* Patch, *Toxoptera aurantii* Boyer de Fonscolombe and *Aphis gossypii* Glover (Hemiptera: Aphididae)]. Recently, the outbreak of some secondary pests such as, Whiteflies, Leafhoppers and the recent new infestation the citrus tree by the Ant species, especially during flowering period in some citrus production area, has greatly induced Moroccan citrus producers to enhance the IPM strategy. In addition, the new requirements of the markets importers and local market, related to ship agreed quality of citrus fruits, have allowed to Moroccan citrus producers to use new alternative methods to chemical control to control citrus pests. This study gives the current status of the citrus pests in Morocco, according their importance and economic damage. The role of the natural enemies to control the citrus pests and the IPM strategies used in the citrus groves were also mentioned.

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SIII-P-9

HOST SUSCEPTIBILITY OF CITRUS TO *Ceratitis capitata* (Diptera: Tephritidae): DOES PHYSICO-CHEMICAL CHARACTERISTICS OF THE FRUIT INFLUENCE THE IPM STRATEGY IN THE CLEMENTINE GROVES IN MOROCCO?

Moulay Chrif Smaili[1]*, Mounaim Rouissi[2], Abdeljalil Bakri[3], Laarbi El Kraa[4], Mohamed Fadli[2]

Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) (Medfly), is a key citrus pest in Morocco. During the last years, many arrangements are typically made between markets importers, particularly non UE markets (e.g. Russia and USA markets) and Moroccan exporters to ship agreed quality of citrus fruits. According this arrangement, producing high fruit quality requires the combination of selected control methods against medfly. In this context, in order to replace or reduce organophosphate insecticide applications, using of mass trapping method control with the resistant variety concept to control Mediterranean fruit fly, has currently increased in Morocco as a new alternative control method to chemical control. More field trials are required to identify the role of the main physico-chemical characteristics of the fruit in situ and which period certain clementine cultivars are considered as susceptible to medfly punctures. The aim of this study is to develop an eco-friendly approach for sustainable agriculture. IPM Field trials were conducted in several Clementine varieties orchards (*Citrus reticulata* "Marisol"; "Sidi Aissa"; "Nules", "Nour", "Bekria", "Nules", and others clementine variants) in the Gharb area (North-western part of Morocco) during 2014-2017 period. Samples of fruits were picked randomly from each plot and the percentage of infested fruit by Medfly was recorded. In addition, the physical and chemical parameters of the fruit were assessed (firmness, flavedo essential oils, acidity, total soluble solids and maturity index). There was a significant correlation between clementine cultivars and fruit infestation. The main factors were, particularly, fruit firmness and maturity index. The level of citrus fruit infestation in relation to the tested physico-chemical parameters, the variation of the Medfly population, and also the IPM strategy used was discussed. The practical implication of our study are discussed in relation to the development of an IPM strategy against *C. capitata* in Clementine orchards in Morocco.

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SIII-P-10

EFFECT OF SALINITY ON THE DEVELOPMENT OF *Phytophthora* DISEASES IN CITRUS ROOTSTOCKS

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In the present study, the influence of salinity on the development of citrus *Phytophthora* diseases was investigated through three greenhouse experiments. In the first one, three-year-old trees belonging to six citrus rootstocks were subjected to salt stress for 21 days, then inoculated at stem level with isolates of *P. citrophthora* and evaluated for tolerance to gummosis. Salt stress was applied by adding NaCl to the irrigation solution at different levels (0, 40 and 80 mM). In the second experiment, two-month-old seedlings were similarly exposed to salinity, then evaluated for tolerance to root rot after inoculation with isolates of *P. parasitica* at root level. In the third experiment, the direct effect of salinity on *Phytophthora* inoculum was tested by estimating the density of propagules in water extracts of salinized soil samples. The results obtained highlighted a significant effect of salinity on growth of all rootstocks, whatever their age. In addition, there was a significant increase in the severity of *Phytophthora* attacks with increasing salinity, which further correlated with chloride accumulation in leaves. These observations coupled with the minor effect of salinity on soil colonization by *Phytophthora* spp. suggest that NaCl acts by decreasing plant resistance to *Phytophthora* attacks, rather than stimulating proliferation of the inoculum.

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SIII-P-11

THE EFFECT OF HLB ON THE CITRUS INDUSTRY ECONOMY: A CASE OF NAVEL ORANGE IN THREE COUNTRIES OF SOUTH JIANGXI

Chunjie Qi

HLB is an important quarantine disease that endangering citrus fruit production and affecting the sustainable development of citrus industry, it has caused huge economic losses to citrus industry. Therefore, it is urgent to make quantitative research on direct and indirect economic losses caused by HLB to citrus industry, so as to provide theoretical basis and decision-making reference for effective prevention and control of citrus HLB. Based on the official statistical data and field survey data, this paper uses the direct economic losses accounting method and the indirect economic losses assessment method to analyze. The research shows that from 2014 to 2016, the direct economic losses caused by HLB to three counties of south Jiangxi are 5,138 million yuan, 6,499 million yuan and 4,492 million yuan respectively, and the indirect economic losses are 250 million yuan, 298 million yuan and 199 million yuan respectively. The total annual losses in three counties of south Jiangxi caused by HLB accounts for about 21% of the total GDP in the region, which seriously slows down the economic development speed in the south Jiangxi. Taking all these things into account, it should construct early warning and rapid response mechanisms for HLB, implement the virus-free seedling breeding and seedling transplanting, set up eco-friendly orchards, carry out unified prevention and control measure and so on to promote the sustainable development of citrus industry.

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SIII-P-12

PRELIMINARY RESULTS OF *Citrus tristeza virus* (CTV) POPULATION IN LEMON CULTIVARS GRAFTED ON *Citrus macrophylla* AND SOUR ORANGE ROOTSTOCKS

Beatriz Stein*, María Florencia Palacios, Julia Figueroa, Lucas Foguet, Lucas Sebastián Villafañe

Citrus tristeza disease is endemic in Argentina. First reported in 1930, it caused the death of millions of citrus trees on sour orange. The most efficient vector, *Toxoptera citricida* is present and citrus varieties are only grafted on tolerant rootstocks. Lemon plants are symptomless, and yield and growth are not affected. However, when lemon is grafted on macrophylla rootstock (*Citrus macrophylla*) the rootstock is severely affected by stem pitting and the tree become stunted. Due to CTV strains are host-specific, the aim of this study was to compare the CTV population of different lemon cultivars grafted on sour orange and Macrophylla in a field trial planted in December 2013 and exposed to natural infection. In order to identify genotypes, a reverse-transcription polymerase chain reaction (RT-PCR) was performed using five sets of genotype-specific CTV primers within the open reading frame (ORF)-1a (T30, RB, B165, T3 and VT). T3 and RB genotypes were present in all samples analyzed and T30 genotype was absent. Some lemons grafted on sour orange showed lower concentration of T3 or absence of VT genotypes, both of them considered severe. B165 genotype was found for the first time in lemon cultivars in the region, and was present in 85% of the samples analyzed, in all varieties. The natural infection of CTV was very homogenous, regardless of the cultivar, rootstock or position of the plants within the plot. *C. macrophylla* seems to be sensitive to some of these genotypes, while sour orange does not.

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SIII-P-13

APPLICATION OF THE GENETIC ENGINEERING IN BREEDING FOR CITRUS DISEASE RESISTANCE

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Citrus is one of the most important world fruit crops. The fruit yield, quality and economic benefit of citrus are seriously affected by different diseases, which also hindering the healthy development of citrus industry. Breeding with genetic engineering can effectively improve the traits of plant. Therefore, biotechnologies, such as transgenic and genome-editing techniques, have been successfully applied in citrus disease resistance breeding. Transgenic technique was used most to improve the resistance to citrus canker. Exogenous genes, such as plant resistance genes, insect antimicrobial peptide genes, plant metabolic genes, pathogenic genes have been transformed into the citrus through *Agrobacterium tumefaciens* mediated genetic transformation, and many transgenic lines with excellent resistance to canker were obtained. Moreover, broad spectrum disease resistance gene *AtNPR1* and *Cecropin* B gene were also transformed into the citrus separately, both of which improved the resistance to huanglongbing, a devastating disease of citrus. Resistances to other diseases in citrus, such as fungi and viral diseases, anthracnose, gray mold, scab, black spot, root rot and gummosis, tristeza virus and psorosis, were also enhanced by transgenic technology. TALENs (transcription activator-like effector nucleases) and the emerging CRISPR/Cas (clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins) are potent biotechnological tools used for genome editing, which have been used to rapidly, easily and efficiently modify endogenous genes in a wide variety of crops. In breeding for citrus disease resistance, canker susceptibility gene *CsLOB1* and its homologs were modified by TALENs and CRISPR/Cas9 technologies, and the canker-resistant citrus cultivars were efficiently generated by researchers. Because the related susceptibility genes are still unknown, genomic-edit technology has not been applied to increase the resistances to huanglongbing and other diseases in citrus. As time goes on and the application evolves, the power of new biotechnologies will undoubtedly spur the development of breeding for citrus disease resistance.

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SESSION IV: Development of Emerging Technologies and their Applications

KEYNOTE LECTURE

SIV-KL-1

Citrus IMPROVEMENT VIA CRISPR TECHNOLOGY

Nian Wang

Citrus is one of the most important fruit crops worldwide. New cultivars are needed to meet the need to overcome many biotic and abiotic threats as well as to improve the horticultural traits to create new marketing opportunities. Specifically, citrus industry faces multiple disease challenges, including citrus Huanglongbing (HLB) caused by *Candidatus* Liberibacter asiaticus (Las) and citrus canker caused by *Xanthomonas citri* subspecies *citri* (*Xcc*). Generating disease resistant citrus varieties is an efficient and sustainable term solution to either HLB or canker disease. However, the traditional approach of breeding for resistant plants for citrus is difficult and has not provided a timely solution to control HLB or canker due to the long juvenility, large tree size, and prevalence of polyembryonic seeds in commercial varieties. Here I will overview the scientific and technological progress as well as challenges generating disease resistant citrus varieties via CRISPR technology. The application of different CRISPR tools on citrus genome editing will be presented. I will also present our results on identification of suitable targets for resistance development against either HLB or canker. Generating non-transgenic citrus varieties via CRISPR technology will also be discussed.

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ORAL PRESENTATIONS

SIV-O-1

CRISPR/Cas9-BASED EDITING OF THE DMR6 GENES FOR RESISTANCE TO HUANGLONGBING IN CITRUS

Zhanao Deng[1]*, Saroj Parajuli[1], Heqiang Huo[2], Fred Gmitter[1]

Huanglongbing (HLB), or citrus greening, is the most devastating disease in Florida and many other citrus production areas in the world. In Florida alone, this disease currently causes more than one billion dollar loss each year. Effective, economical, long-term control of this bacterial disease will likely rely on genetic resistance or tolerance in citrus cultivars. Several transgenic approaches have been or are being explored to engineer HLB resistance. This study focuses on using the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9-based gene editing of the DMR6 gene family members for increased resistance to HLB. Knocking out DMR6 genes in apple, tomato, Arabidopsis and tobacco has resulted in increased resistance to several fungal and bacterial pathogens. We sequenced the DMR6 genes from 12 citrus genotypes and designed guide RNAs (gRNAs) targeting the conserved regions in exons 1 and 2. These gRNAs and the Cas9 gene were introduced into ‘Carrizo’ citrange and ‘Duncan’ grapefruit. Several dozens of ‘Carrizo’ and ‘Duncan’ plants were obtained. DNA sequencing of the targeted DMR6 gene region in ‘Carrizo’ and ‘Duncan’ showed variable efficiency and nucleotide changes induced by the introduced CRISPR/Cas9 and gRNA in different grapefruit and Carrizo plants.

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SIV-O-2

TRANSFORMATION OF CITRUS PLANTS WITH CYCLIC NUCLEOTIDE-GATED CHANNEL (CNGC) GENE TO DEVELOP BROAD-SPECTRUM DISEASE RESISTANCE

Madhurababu Kunta*, Zenaida Viloria, Hilda del Rio, Eliezer Louzada

Transgenic plants expressing cyclic nucleotide-gated channel (CNGC) transgene were shown to be resistant to fungal and viral pathogens. A PCR amplification product comprising citrus CNGC (*CNGCcit*) open reading frame (ORF) with *XbaI* and *Eco*RI ends was generated, inserted into pRTL22 plasmid, transformed into E. coli, and sequenced. The 3.2 kb Hind III fragment of pRTL22/*CNGCcit* containing the CaMV 35S promoter with dual enhancer, CNGCcit, and CaMV 35S terminator was inserted into the *Hind* III site of pBin 34SGUS to generate pBin35SCNGCcit construct and transformed into E. coli. This construct was used in *Agrobacterium tumefaciens*-mediated transformation of 'Ruby Red' grapefruit, 'Hamlin' and 'Valencia' sweet orange, and Bitters C-22 rootstock plants. The presence and expression of *CNGCcit* in the transgenic plants was verified by b-glucuronidase histochemical assay, *nptII* enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and Southern blot. Transgenic plants showed an enhanced tolerance to *Xanthomonas citri* subsp. *citri* (Xcc) and *Phytophthora nicotianae*. Expression of citrus cytosolic ascorbate peroxidase (*cAPXcit*) in non-transgenic control 'Duncan', 'Ruby Red', and 'Rio Red' grapefruit was suppressed due to *P. nicotianae* inoculations while transgenic 'Ruby Red' showed an increased expression of *cAPXcit*. No significant difference in lethal freezing temperature (LT50) was observed in transgenic and non-transgenic 'Hamlin' and 'Valencia' plants. The physiology tests displayed an increase in the amount of photosynthesis, stomatal conductance, and transpiration rates coordinated with a lower rate of water use efficiency (WUE) in 'Hamlin' and 'Valencia' transgenic plants in comparison to non-transgenic plants. However, gas exchange in transgenic plants was not altered negatively after transformation. Currently, two hundred 3-year old transgenic plants including 'Hamlin', 'Valencia', 'Ruby Red', and Bitters C-22 rootstock and forty transgenic 5-year old 'Ruby Red' grafted onto non-transgenic sour orange rootstock in the field are being evaluated for their tolerance against citrus diseases including Huanglongbing.

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SIV-O-3

DEVELOPMENT OF MARKER FREE TRANSGENIC PLANTS USING RECOMBINASE MEDIATED CASSETTE EXCHANGE

Eliezer Louzada*, Estephanie Ms. Bernal-Jimenez, Hilda Del Rio, Yessica Cerino, James Thomson

Genetic transformation is a great tool to introduce new genes into an already good commercial variety, but lacks traits such as resistance or tolerance to biotic or abiotic stresses, or other horticultural important traits. Despite the great benefits that genetic transformation can bring to improve citrus, there are still consumer concerns about the consumption of genetically modified plants that should be addressed. One of the concerns is the presence of genes used for selection and identification of transgenes (e.g. antibiotic resistance genes and other screenable markers) used during plant improvement. To address this situation, this project, established a method of Recombinase Mediated Cassette Exchange (RMCE) in citrus, which allows for the production of transgenic plants devoid of any molecular markers or sequences that will affect consumer concerns; the transgenic plants contain only the gene(s) required for improvement. RMCE opens the opportunity for new genes to be added later to the same location as the first gene, inserted in a side by side manner. RMCE cassette was constructed to contain the recombinase gene, induced by estradiol or Dexamethazone and containing the *nptII* gene for kanamycin resistance plus cytosine deaminase gene (*codA*). *Agrobacterium*-mediated genetic transformation was performed; explants were cultivated in media containing kanamycin and recombinase was induced by estradiol for excision of markers. Afterwards, explants were transferred to media containing 5-fluorocitosine (5FC) for negative selection. During this time, in plants still containing markers, the cytosine deaminase protein converts 5FC into 5-fluorouracil (5FU) which is toxic to plants. After positive and negative selection, we were able to recover plants containing only a disease resistance gene and devoid of any molecular markers. Additional details will be provided.

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SIV-O-4

DEVELOPMENT OF SITE-SPECIFIC RECOMBINASE TECHNOLOGY FOR TARGETED CITRUS GENOME INTEGRATION WITH MARKER REMOVAL

James Thomson

Recombinase-mediated genetic engineering provides a favorable direction for enhancing the precision of biotechnological approaches. Technology is rapidly expanding the way genetic engineering can be accomplished, questions asked and applications that can be attained. With these possibilities in genetic manipulation the interest in metabolic engineering for products such as Flavinoids, Astaxanthinor Tocopherols that requires the addition of many genes for proper expression has been renewed. However, the combination of required genes and control elements is often empirically determined and thus requires multiple rounds DNA manipulation. While genetic engineering can be accomplished by nuclease or host cell mediated homologous recombination, the efficiencies for precisely inserting large DNA sequences is low and tends to contain errors. Recombinase-mediated engineering offers a solution for sequential rounds of DNA stacking at high rates of integration and low (to nonexistent) levels of error introduction. Another issue is the limitation of available selectable markers for multiple rounds of engineering. Recombinase technology, when properly designed allows the removal of selection marker genes from the system while integrating the next gene(s) of interest. This in turn allows marker gene recycling between successive rounds of DNA insertion. Due to concerns over the presence of antibiotic resistance genes in the food supply and their escape into the environment, the ability to efficiently remove marker genes prior to marketing or release is highly desirable. Previous studies have documented how site-specific recombination can produce transgenics with stable gene expression over multiple generations and also resolve multicopy transgene inserts, initially silenced for expression, to a single functional genomic copy. Our team has developed a series of novel recombinases and strategies for precise genetic manipulation. Data for a practical combinatorial approach for transgene integration and DNA stacking combined with subsequent excision of the selectable marker will be presented.

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SIV-O-5

DETECTION OF NATURAL AND INDUCED MUTATIONS FROM NEXT GENERATION SEQUENCING DATA IN SWEET ORANGE BUD SPORTS

Giuseppina Las Casas[1], Marco Caruso[1], Davide Scaglione[2], Stefano Gattolin[3], Laura Rossini[4], Gaetano Distefano[5], Francesco Romano[6], Giuseppe Cirrone[6], Giacomo Cuttone[6], Federica Cattonaro[2], Antonino Catara[7], Grazia Licciardello[7], Michele Morgante[8], Concetta Licciardello[9]*

Somatic mutations are a cause of intraspecific diversity in many fruit crops, such as citrus, grapevine and apple. In the case of sweet orange [*Citrus sinensis* (L.) Osbeck], intraspecific variability is determined only by somatic mutations. Tools for clonal fingerprinting are required by breeders and nurserymen and have important implications for traceability. With the aim of studying genomic variability and identifying mutational events responsible for varietal diversification, we deep-resequenced 22 accessions including navel, common and blood oranges using an Illumina platform. We also resequenced 4 induced mutants of Tarocco 'Scirè D2062' obtained using different mutagens (ion beams and gamma rays) at different doses and dose rates. A robust and reliable set of single nucleotide polymorphisms (SNPs), structural variants (SVs) and indels, private of each accession or common to varietal groups, was identified both in natural and induced mutants. A subset of SNPs, mobile element insertions and indels was validated by Sanger sequencing, PCR amplification and high resolution melting analysis, confirming the results of the bioinformatics analysis. Moreover, to identify a reliable marker set for traceability of specific cultivars, we collected leaf and juice samples from many Italian citrus growing areas and used a KASP platform for their fingerprinting. These tools will be useful to prove true-to-typeness of specific sweet orange varieties and will potentially provide the consumers with a guarantee on the quality and origin of juices, avoiding eventual frauds.

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SIV-O-6

AN OVERVIEW OF ESTABLISHED METHODS AND EMERGING NEW TECHNOLOGIES FOR THE BIOTECHNOLOGY MEDIATED IMPROVEMENT OF CITRUS

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Citrus is considered a wholesome part of the daily diet and one of the major fruit crops cultivated in most sub-tropical regions of the world. In recent years, Huanglongbing (HLB), a serious bacterial disease that is usually fatal has gained a foothold in several citrus production regions around the world and resulted in a significant decline in production. This disease is caused by different species of the *Candidatus* Liberibacter bacterium. Most commercially cultivated citrus cultivars lack the resistance to combat HLB. While improved production techniques can keep an infected tree alive, the only promising long-term strategy to manage this disease is to utilize resistant trees that can thrive in an endemic HLB environment. To combat HLB, biotechnology has played an important role in our overall citrus improvement strategy. We have several somatic fusion derived primary tetraploid and secondary 'tetrazyg' rootstocks that can survive better under endemic HLB conditions. In many cases, enhanced nutritional supplementation to these trees has resulted in increased fruit yields from heavily infected trees. In addition to somatic fusion derived tetraploid plants, genetically engineered citrus is also an important component in the citrus improvement toolkit and offers promise of durable HLB resistance. Systemic Acquired Resistance (SAR) is being utilized to develop transgenic scions and rootstocks that can immunize the citrus tree against *Candidatus* Liberibacter asiaticus (*C*Las). Transgenic Carrizo citrange containing the *CFT3* (*FLOWERING LOCUS T (FT)*) gene are being utilized to develop early flowering citrus that could assist in rapid evaluation of plants from both our conventional breeding and genetic engineering pipelines. This would result in rapid evaluation of putative HLB tolerant germplasm. Other newer methodologies being developed in our program include nanoparticle mediated transformation of citrus, a visual selection system for cell suspension and callus cell derived transformation and evaluation of several citrus derived promoters, genes and terminators to create an all citrus transformation vector. In addition, CRISPR and RNAi techniques are being explored and constructs are being incorporated into sweet oranges and mandarins by conventional *Agrobacterium* mediated transformation and our unique protoplast transformation methods.

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