XIIth EUCARPIA MEETING ON CUCURBIT GENETICS AND BREEDING

24-28 May 2021, Spain

Book of Abstracts

Edited By:

María Luisa Gómez-Guillamón; Manuel Jamilena; María Belén Picó; Ana M. Pérez-De-Castro

edual

CUCURBITACEAE 2021

EUCARPIA CUCURBITACEAE 2021

Abstracts of the XII Eucarpia Meeting on Cucurbit Genetics and Bree-ding 24-28 May 2021

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FOREWORD

Dear colleagues,

Welcome to the XIIth Meeting on Cucurbit Genetics and Breeding. The Organizing Committee people have done our best to host virtually this meeting. Hopefully it will provide, as always, an excellent opportunity for scientists and plant breeders from both, the public and private research to show and discuss their latest results and ideas.

Despite the global situation created by COVID19, we have received many contributions from colleagues around the world, which have done an important effort helping us to promote this virtual congress. Thank you very much.

We warmly thank the speakers, chairpersons, authors of the works an all the attendants in general. We are especially indebted to the institutions and privates companies that have provided financial support in spite of the difficult global economic situation.

It is the first time that an Eucarpia Meeting on Cucurbits is organized under a virtual format. It was not an easy task, we should say, but thank to all of you we sincerely hope you enjoy it.

The Organizing Committee

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ORGANIZING COMMITTEE

Manuel Jamilena Quesada

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SCIENTIFIC AND SOCIAL PROGRAM

MONDAY, 24TH MAY

13:40-14:00 **Opening Ceremony:**

Prof. Margarita Paneque Sosa, Consejo Superior de Investigaciones Científicas

Prof. Diego Luis Valera Martínez, Universidad de Almería

Prof. Alberto San Bautista Primo, Universidad Politécnica de Valencia

Prof. Yuling Bai, Eucarpia

Session 1.- Genetic Resources

Chairpersons: **Susanne Renner** (Washington University in St. Louis, Missouri, USA) and **Mª José Díez** (Polytechnic University of Valencia, Spain)

14:00-14:45 **Invited lecture:**

Overview of the history of the five major cucurbit crops: Some issues for genomic analysis of archaeological specimens. *Harry S. Paris*

Oral communications:

14:45-15:00 Why did simultaneous 2020 studies of bitter gourd domestication arrive at drastically different conclusions? ***Susanne S. Renner***

15:00-15:15 Pre- and post-zygotic interspecific barriers control reproductive isolation in Cucumis***.*** ***María Ferriol****, Unzué Simó, Alejandro Torres, Belén Picó, Antonio J. Monforte,* and *Carlos Romero*

15:15-15:30 A global conservation strategy for Cucurbitaceae family crops*. Andreas W. Ebert, Emily B. M. Drummond,* ***Peter Giovannini,*** and *Marteen van Zonneveld*

15:30-15:45 Importance of the American resource of Cucurbitaceae conserved by CATIE Germplasm Bank and its potential for genetic improvement. ***Daniel Fernández Rivera*** and *Carlos Cordero Vargas*

15:45-16:00 The Mexican Cucurbita project: advances and perspectives. *Luis E. Eguiarte,* ***Erika Aguirre-Planter****, Josué Barrera-Redondo, Helena S. Hernández-Rosales, Guillermo Sánchez de la Vega, Jaime Gasca-Pineda,* and *Rafael Lira-Saade*

16:00-16:15 Break

Session 2.- Tolerance to abiotic stress

Chairpersons: **Daniel Leskovar** (Texas Agrilife Research and Extension Center, USA) and **Ana Garcés-Claver** (CITA-Universidad de Zaragoza, Spain)

16:15-17:00 **Invited lecture:**

Breeding melons for vine decline resistance, nutritional value and flavor. ***Kevin M. Crosby****, John L. Jifon and Daniel I. Leskovar*

Oral communications:

17:00-17:15 RNA-seq base analysis of zucchini fruit transcriptome in response to exogenous abscisic acid and cold storage. *Álvaro Benítez, Yesica Iglesias-Moya, Fátima Carvajal, Francisco Palma, Cecilia Martínez, Juan Luis Valenzuela, Dolores Garrido,* and *Manuel Jamilena*

17:15-17:30 Insights into *Cucumis sativus* drought stress tolerance using RNA sequencing. ***U. Kłosińska****, M. Nowakowska, W. Szczechura, K. Nowak,* and *M. Nowicki*

17:30-17:45 Screening of a Zucchini mutant collection for abiotic stress tolerance. ***Sonsoles Alonso****, Gustavo Cebrián, Jessica Iglesias, Keshav K. Gautam, Alicia García, Cecilia Martínez,* and *Manuel Jamilena*

17:45-18:00 Evaluation of grafting traditional snake melon "alficoz" under abiotic stress: effects on agronomic performance and fruit quality. ***Alejandro Flores-León****, Santiago García-Martínez, Raúl Martí, Alicia Sifre, Ana Pérez-de-Castro, María José Díez, Carmelo López, María Ferriol, Carmina Gisbert, Juan José Ruiz, Jaime Cebolla-Cornejo,* and *Belén Picó*

18:00- **Poster Session**

TUESDAY, 25TH MAY

Session 3. Genomic Resources-1

Chairpersons: **Amnon Levi** (USDA-Charleston, South Carolina, USA) and **Cecilia Martínez** (Almería University, Spain)

14:00-14:45 **Invited lecture:**

Melon genetic resources in the genomics era. *Maria José Gonzalo, Belén Picó, Carlos Romero* and ***Antonio José Monforte***

Oral communications:

14:45-15:00 Dissecting melon fruit ripening using CRISPR. ***Andrea Giordano****, Miguel Santo Domingo, Marta Pujol, Ana Montserrat Martín-Hernández,* and *Jordi Garcia-Mas*

15:00-15:15 Panning the Melon Genome. ***Elad Oren**, Galil Tzuri, Evan R. Rees, Baoxing Song, Arthur Schaffer, Yaakov Tadmor, Joseph Burger, Edward Buckler,* and *Amit Gur*

15:15-15:30 A Multispecies SNP Array for High-Resolution Genotyping of Melon, Cucumber and Watermelon. ***Martin Ganal,** Andreas Polley, Joerg Plieske,* and *Eva-Maria Graner*

15:30-15:45 Development of Double Haploid melon lines for its use as founders of a MAGIC population. ***Pau Bretó**, L. Olmos, José A. Esteban, Giuliano S. Pechar, María José Clemente-Moreno, Carlos García-Almodovar, Elena Sánchez, Yolanda Hernando, and Miguel A. Aranda*

15:45-16:00 Genome-wide association analysis of downy mildew resistance in a pre-breeding watermelon (*Citrullus amarus*) collection. ***Dennis N. Katuuramu**, Sandra E. Branham, Amnon Levi,* and *W. Patrick Wechter*

16:16:15 Break

Session 4. Genomic Resources-2

Chairpersons: **Mara Ercolano** (Napoles University, Italy) and **Cristina Esteras** (Polytechnic University of Valencia, Spain)

16:15-17:00 **Invited lecture:**

Application of genomic tools for mapping and analysis of disease resistance traits in cucurbits: The CucCAP experience. *Rebecca Grumet, Zhangjun Fei, Sandra Branham, Amnon Levi, W. Patrick Wechter, Yiqun Weng, Yuhui Wang, Ben N. Mansfeld, Ying-Chen Lin, and Stephanie Rett-Cadman***

Oral communications:

17:00-17:15 QTL mapping and pyramiding resistance to *Fusarium oxysporum* f. sp. *niveum* (races 1 and 2) and potyviruses in watermelon. *Sandra E. Branham, W. Patrick Wechter, Kai-Shu Ling, Dennis Katuuramu,* and ***Amnon Levi***

17:15-17:30 Editing the melon genome to attain broad spectrum virus resistance. ***Giuliano Sting Pechar**, Blanca Gosálvez, Carlos García-Almodóvar, Pau Bretó, M. Amelia Sánchez-Pina, Verónica Truniger, Livia Donaire,* and *Miguel A. Aranda*

17:30-17:45 A potyvirus-based vector for transient gene expression in cucurbit plants and fruits. *Belén Picó, Fakhreddine Houhou, Teresa Cordero, Verónica Aragonés, Maricarmen Martí, Raúl Martí, Arcadio García, Ana Pérez-de-Castro, Carmelo López, Jaime Cebolla-Cornejo, Manuel Rodríguez-Concepción,* and ***José-Antonio Daròs***

18:00- **Poster Session**

WEDNESDAY, 26TH MAY

Session 5. Resistance to Pest and Diseases-1

Chairpersons: **Cécile Desbiez** (INRA-Montfavet, USA) and **Ana Pérez-de-Castro** (Polytechnic University of Valencia, Spain)

14:00-14:45 **Invited lecture:**

Disease resistance in Cucurbits: recent progress and future perspectives on the use of plant susceptibility genes. *Lei Cui, Lampros Siskos, Chen Wang, Henk J. Schouten, Richard G. F. Visser,* and ***Yuling Bai***

Oral communications:

14:45-15:00 Resistance to Cucumber Mosaic Virus: a proteomic approach. ***Núria Real Tortosa*** and *Ana Montserrat Martín-Hernández*

15:00-15:15 Syntenic regions control resistance to *tomato leaf curl New Delhi virus* (ToLCNDV) in cucurbit crops. ***Cristina Sáez****, Cristina Esteras, Alicia Sifres, Cecilia Martínez, Alejandro Flores-León, Narinder Dhillon, María Ferriol, Carmelo López,* and *Belén Picó*

15:15-15:30 Adaptation of GWAS models for plant virus resistance: from rediscovering major genes to highlighting of new complex traits. ***Séverine Monnot****, Laurence Moreau, Tristan Mary-Huard, Mélissa Cantet,* and *Nathalie Boissot*

15:30-15:45 New Sources of Resistance to Powdery Mildew in Squash and Pumpkin. ***Andrew Ogden****, Iago Hale, and J. Brent Loy*

15:45-16:00 Deciphering the genetic basis of CYSDV resistance in melon PI 313970. ***Prabin Tamang****, Kaori Ando, William M. Wintermantel,* and *James D. McCreight*

16:16:15 Break

Session 6. Resistance to Pest and Diseases-2

Chairpersons: **Jim McCreight** (USDA-Salinas, California, USA) and **Montse Martín-Hernandez** (Crag Genómica, Barcelona, Spain)

16:15-17:00 **Invited lecture:**

Molecular epidemiology of cucurbit-infecting potyviruses: a rapid turnover of viral strains with a potential impact for resistance breeding. ***Cécile Desbiez****, Catherine Wipf-Scheibel, Pauline Millot, Gregory Girardot and Hervé Lecoq*

Oral communications:

17:00-17-15 Germplasm release of gummy stem blight resistant lines from a watermelon **× citron population**. ***Luis A. Rivera-Burgos,*** *and Todd C. Wehner*

17:15-17:30 The Amino Acid Permease (AAP) genes *CsAAP2A* and *SIAAP5A/B* are required for oomycete susceptibility in cucumber and tomato. *Jeroen A. Berg, Freddy W.K. Hermans, Frank Beenders, Hanieh Abedinpour, Wim H. Vriezen, Richard G. F. Visser, Yuling Bai, and* ***Henk J. Schouten***

17:30-17:45 Downy Mildew Resistance and Fruit Quality in a Cucumber Recombinant Inbred Line Population derived from Coolgreen x PI 197088. ***Emily J. Silverman*** *and Todd C. Wehner*

17:45-18:10 Charcoal rot (*Macrophomina phaseolina*): From melon and watermelon to other hosts, studying phytopatholocial and genetic aspects in the global warming era. ***Roni Cohen****, Meital Elkabetz, Amit Gur, Harry Paris, and Stanley Freeman*

18:10- **Poster Session**

THURSDAY, 27TH MAY

Session 7. Floral and Fruit Development

Chairpersons: **Jinjing Sun** (Chinese Academy of Agricultural Sciences, Beijing, China) and **Pedro Gómez** (IFAPA-La Mojonera, Almería, Spain)

14:00-14:45 **Invited lecture:**

Genome selections drive the evolution of delicious fruit in watermelon. *Shaogui Guo, Honghe Sun, Yi Ren, Jie Zhang, Haiying Zhang, Guoyi Gong, Jinfang Wang, Maoying Li, Yongtao Yu, Zhangjun Fei, and* ***Yong Xu***

Oral communications:

14:45-15:00 A unique chromosome translocation disrupting *ClWIP1* leads to gynoecy in watermelon. ***Jie Zhang****, Shaogui Guo, Hong Zhao, Honghe Sun, Yi Ren, Shouwei Tian, Maoying Li, Haiying Zhang, Guoyi Gon, and Yong Xu*

15:00-15:15 Two induced EMS mutations conferring parthenocarpy in *Cucurbita pepo. Gustavo Cebrián,* ***Alicia García****, Jessica Iglesias-Moya, Jonathan Romero, Cecilia Martínez, Dolores Garrido, and Manuel Jamilena*

15:15-15:30 Validation of the differential expression of zucchini genes during fruit formation. ***Alejandro Ayala****, S Fernández-Rubio, T Pomares-Viciana, J Die, Belén Román, and Pedro Gómez*

15:30-15:45 *ETHQV8.1*, a new player in melon fruit ripening. ***Miguel Santo Domingo****, Lara Pereira, Marta Pujol, and Jordi Garcia-Mas*

15:45-16:15 Break

16:15-17:15 **Virtual Visit:**

SYNGENTA España. ***José Manuel Zapata, José Ignacio Álvarez y Jesús Abad***

17:15-17:45 **Announcements**

17:45-18:00 **Award's Ceremony**

18:00- **Poster session**

FRIDAY, 28TH MAY

Session 8. Production and Quality

Chairpersons: **Grzegorz Bartoszewski** (Warsaw University of Life Sciences, Poland) and **Flor Cocaliadis** (BASF España, Spain)

14:00-14:45 **Invited lecture:**

Genomic resources applied to understand melon fruit quality. *Jordi Garcia-Mas*

Oral communications:

14:45-15:00 Fine mapping of the *Mt-2* gene controlling mottled rind in melon. ***Liu Bin**, Valentino Ruggieri, Lara Pereira, Marta Pujol, and Jordi Garcia-Mas*

15:00-15:15 Underground Heterosis for Melon Yield. *Asaf Dafna, Ilan Halperin, Elad Oren, Tal Isaacson, Galil Tzuri, Ayala Meir, Arthur A Schaffer, Joseph Burger, Yaakov Tadmor, Edward S. Buckler and **Amit Gur***

15:15-15:30 Identification of fruit-associated QTL in winter squash (*Cucurbita maxima* Duchesne) using recombinant inbred lines. *Karolina Kaźmińska, Ewelina Hallmann, Aleksandra Korzeniewska, Katarzyna Niemirowicz-Szczytt, and **Grzegorz Bartoszewski***

15:30-15:45 Breeding quality melons with resistances derived from African accession TGR1551. ***María López-Martín**, Ana Pérez-de-Castro, Ana Garcés-Claver, Mercedes Valcárcel, Jaime Cebolla-Cornejo, Belén Picó, and María-Luisa Gómez-Guillamón*

15:45-16:15 Break

Session 9. New cultivars

Chairpersons: **Emilio Sarria-Villada** (Rijk Zwaan Ibérica, Spain) and **Matthijs Groot** (Enza Zaden España, Spain)

16:15-17:00 **Invited lecture:**

Main typologies and markets of melon, cucumber and watermelon: major traits of interest for breeding new varieties. *Jamila Chaïb, Zahi Paz and **David O´Donnell***

Oral communications:

17:00-17-15 Selection programme of a 'Muscat'-type variety of *Cucurbita moschata* for improved performance and uniformity. *Maria R. Figàs, Arnau Bertomeu, Cristina Casanova, Vicente Bataller, Armando Bataller,* ***Jaime Prohens****, and Salvador Soler*

17:15-17:30 New promising mini melon lines from different genetic backgrounds. ***Cristina Esteras****, Gorka Perpiñá, Gabriel Castro, Antonio J. Monforte, and Belén Picó*

17:30-17:45 CMV-resistant melons for the western United States. *Kaori Ando, Mikyeong Kim, Prabin Tamang, Shaonpius Mondal, Michael Mazourek, William M. Wintermantel, and* ***James D. McCreight***

17:45-18:00 Development of multi-disease resistant melon (*Cucumis melo*) cultivars through marker-assisted selection. ***Sandra E. Branham****, Shaker Kousik, Amnon Levi, Venkata Ganaparthi, and W. Patrick Wechter*

18:00- **Poster Session**

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P1-2. Assessment of Chromosomal Diversity in Indian Cucurbit Species by Fluorescent Karyotype Analysis. *Biplab Kumar Bhowmick* and *Sumita Jha*

P1-3. Characterization of *Cucurbita* spp. germplasm to broaden squash and pumpkin genetic background. *Miguel Leiva-Brondo, Ana Garcés-Claver, Vicente González, María López-Martín, Belén Picó,* and *Cristina Esteras*

P1-4. Analysis of Genetic Diversity in Indian snake melon (*Cucumis melo* L. var. *flexuosus*) using horticultural traits and start codon targeted (SCoT) markers. *Keshav K. Gautam, DR Bhardwaj, DP Moharana, SP Kashyap, AK Singh, PM Singh, Manuel Jamilena,* and *J Singh*

P1-5. Morphological characteristics of winter squash (*Cucurbita maxima* Duchesne) accessions collected at the Polish National Genebank. *Karolina Kaźmińska, Aleksandra Korzeniewska, Dariusz Gozdowski,* and *Grzegorz Bartoszewsk*

P1-6. Screening cucumber (*Cucumis sativus* L.) germplasm for ability to germinate under low temperature. *Emilia Olechowska, Renata Słomnicka, Karolina Kaźmińska, Aleksandra Korzeniewska,* and *Grzegorz Bartoszewski*

P2-1. Evaluation of traditional melon varieties for their water deficit response. *María José Clemente-Moreno, Pau Bretó, José A. Esteban, Yolanda Hernando, and Miguel A. Aranda*

P2-2. Correlation between ABA and chilling tolerance in crosses of *Cucurbita pepo* varieties with contrasted behavior to cold storage. *Alejandro Castro-Cegrí, Francisco Palma, Jessica Iglesias-Moya, Fátima Carvajal, Raquel Jiménez-Muñoz, Manuel Jamilena,* and *Dolores Garrido*

P2-3. The enhanced salt tolerance of the squash *etr2b* mutant is mediated by ABA. *Jessica Iglesias-Moya, Sonsoles Alonso, Gustavo Cebrián, Jonathan Romero, Alicia García, Cecilia Martínez* and *Manuel Jamilena*

P3-1. A point mutation resulting in a 13 bp deletion in the coding sequence of Cldf leads to a GA-deficient dwarf phenotype in watermelon. *Chunhua Wei, Li Yuan,* and *Xian Zhang*

P3-2. GBS characterization of watermelon germplasm and breeding against fungal pathogens. *Cristina Esteras, Ana Garcés-Claver, M. Luisa Gómez-Guillamón, Vicente González, Alejandro Flores-León, Gorka Perpiñá, Eva M. Martínez-Pérez, M. José Díez, Ana Pérez-de-Castro,* and *Belén Picó*

P3-3. Genome-wide association study (GWAS) of seed agronomic traits in cucurbits. *Alba López, Alicia García, Cecilia Martínez,* and *Manuel Jamilena*

P3-4. Cloning and expression of CmROR2 gene in melon and its application in screening broad-spectrum resistant germplasm of powdery mildew. *Cheng Hong, Kong Wei-Ping, Lü Jun-Feng*

P5-1. Resistance to cucumber green mottle mosaic virus (CGMMV) in cucumber. *Esperanza Gea-Caballero, Almudena Castillo, Jesús Abad,* and *Miguel A. Aranda*

P5-2. Melon genome editing with CRISPR-Cas tools to produce varieties resistant to pests and pathogens. *José-Antonio Daròs, Verónica Aragonés, Begoña García-Sogo, Carlos Ribelles, Benito Pineda, Ana Pérez-de-Castro, Carmelo López, José Riado, Belén Picó, and Vicente Moreno*

P5-3. First Report in Spain of Cucurbit chlorotic yellows virus in Cucumber plants. *Alejandro Carralero-González, Ana Crespo-Sempere, Robert Chynoweth, Daniel Jim**é**nez, Daniele Liberti, Daniel Bell**ó**n-Dona,* and *Maria R. Albiach-Marti*

P5-4. Incidence of cucurbit viruses in Spain during 2019 summer season. *María López-Martín, Alicia Sifres, Alejandro Flores-León, Cristina Sáez, Mercedes Valcárcel, Carmelo López, María Luisa Gómez-Guillamón, Belén Picó, and Ana Pérez-de-Castro*

P5-5. Resistance to different Cucumber green mottle mosaic virus strains in melon. *Leticia Ruíz, Carmelo López, Belén Picó,* and *Dirk Janssen*

P5-6. Resistance to *Cucumber mosaic virus* (CMV) in Near Introgression Lines (NIL) containing two and three Quantitative Trait Loci (QTL) in melon. *Lorena Areco* and *Ana Montserrat Martín-Hernández*

P5-7. Genetic Variability of Tomato Leaf Curl New Delhi virus in Algeria. *Amina Kheireddine, Alicia Sifres, Cristina Sáez, Ayoub Hadjeb, Belén Picó,* and *Carmelo López*

P5-8. Inheritance of Resistance to *Papaya ringspot virus* in *Cucurbita moschata* Duchesne. *Wilfredo Seda-Martínez, Linda Wessel-Beaver,* and *Angela Linares-Ramírez*

P5-9. Introgression of resistance to ToLCNDV from WM-7 and PI 414723 into traditional backgrounds of *Cucumis melo. Clara Pérez Moro, Cristina Sáez, Alejandro Flores-Le**ón, Alicia** Sifres, Narinder Dhillon, Carmelo López, Belén Picó,* and *Ana Pérez-de-Castro*

P6-1 Further assessment of ToLCNDV seed transmission in cucurbits. *Arcadio García, Amina Kheireddine, Alicia Sífres, Alejandro Moreno, Mª Isabel Font-San-Ambrosio, Belén Picó, Carmelo López and Cristina Sáez*

P6-2. Methods for detection and quantification of four whitefly-transmitted viruses of cucurbit crops during mixed infections. *Shaonpius Mondal, Laura Jenkins Hladky, and William M. Wintermantel*

P6-3. BSA-seq reveals QTLs associated to ToLCNDV resistance in Cucurbita moschata. *Jonathan Romero, Cecilia Martínez, Encarnación Aguado, Alicia García, Jessica Iglesias-Moya, Gustavo Cebrián, and Manuel Jamilena*

P6-4. Screening of melon germplasm resistant to *Fusarium oxysporum* f. sp. *Melonis. Aejin Hwang, Jaejong Noh, Ju-Hee Rhee, Ho-Sun Lee, On-Sook Hur, Na-Young Ro, Jung-Yoon Yi, Jae-Eun Lee, Bichsaem Kim, Tania Afroz, and Ji Hyeon Kim*

P6-5. Evidence of physiological races of *Podosphaera xhantii* in watermelon in Southern Europe. *Juan A. Tores (+), Dolores Fernández-Ortuño, Daniel Jiménez, Samantha Guiderdone, and Daniel Bellón-Doña*

P6-6. Cucurbit-associated taxa of the *Fusarium solani* species complex not previously detected in Spain. *Vicente González, Alejandro Flores-León, Santiago García-Martínez, María López-Martín, Gorka Perpiñá, Ana Pérez-de-Castro, Belén Picó, María Luisa Gómez-Guillamón and Ana Garcés-Claver*

P6-7. Initial studies on transcriptional response of cucumber to *Pseudomonas syringae* pv. *lachrymans* infection. *Renata Słomnicka, Helena Olczak-Woltman, Mirosław Sobczak, and Grzegorz Bartoszewski*

P6-8. Genetic loci associated with resistance to zucchini yellow mosaic virus in Cucurbita moschata. *Swati Shrestha, Yuqing Fu, Vincent Michael, and Geoffrey Meru*

P7-1. Screening of a mutant collection of *Cucurbita pepo* for valuable flower and fruit agronomic traits. *María Segura, José Javier Regalado, Alicia García, Cecilia Martínez, and Manuel Jamilena*

P8-1. Genetic dissection of aroma production in a RIL population in *Cucumis melo. Carlos Mayobre, Ali Eltahiri, Lara Pereira, Marta Pujol and Jordi Garcia-Mas*

P8-2. Use of molecular markers in *Cucurbita pepo*: quality assessment for hybrid production. *Maria Lucia Prazzoli, Paolo Passeri, Alice Brunazzi, and Marina Malatrasi*

P8-3. Deciphering fruit flesh colour in melon. *Laura Valverde Carvajal, Manuel Rodríguez-Concepción and Jordi Garcia-Mas*

P8-4. A Dudaim introgression line collection onto Piel de Sapo background: A tool for the analysis of aroma compounds in melon**.** *Gorka Perpiñ**á**, Cristina Esteras, Gabriel Castro, Antonio J. Monforte and Belén Picó*

P9-1. New Watermelon Cultivars with High Contents of Lycopene and Citrulline. *Oak Jin Lee, Tae Bok Kim, Sang Gyu Kim, and Eun Su Lee*

SESSION 1.
GENETIC RESOURCES

O1-1 Invited

Overview of the history of the five major cucurbit crops: Some issues for genomic analysis of archaeological specimens

Harry S. Paris

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Five cucurbit crops are cosmopolitan: pumpkin, squash, watermelon, melon, and cucumber. For years, the origins and history of these crops have generated much interest among scientists and the general public. The accumulated evidence indicates that pumpkins and squash originate from the Americas, watermelons from Africa, and melons and cucumbers from Asia. Sources of information on the history of cucurbit-crop cultivation consist of ancient plant remains, iconography, literature, and living wild or primitive relatives, but all four of these categories have some limitations. Advances in genomics are contributing to a better understanding of cucurbit-crop history as well, but genomics too has its limitations, notably in the need for judicious selection of germplasm to be used for comparative analysis and correct, detailed botanical and horticultural identification of this germplasm. Advances in genomics now allow analysis of ancient DNA in archaeological plant remains, opening the new field of archaeogenomics. This new technology could identify to the species level *Cucumis* seeds in central and northeastern Europe that date to the ninth century. Next-generation sequencing applied to ancient DNA of plant specimens should soon be capable of distinguishing subspecific groups. Some major issues involve more precise identification of the 10,000-year-old seed, peduncle, and rind remains of *Cucurbita pepo* from Oaxaca, Mexico and 3,500-year-old Egyptian and Sudanese remains of *Citrullus* seeds, foliage, and fruit. Further advancements in archaeogenomics might even allow inferring phenotypic features of archaeological specimes and tracking over time and space the order and occurrence of selection for desired horticultural traits in cucurbit crops.

Key words: Ancient cucurbits, archaeogenomics, crop history, cucurbit genetic resources

O1-1

Why did two 2020 studies of bitter gourd (*Momordica charantia*) domestication arrive at drastically different conclusions?

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In 2020, Matsumura et al. (PNAS 117: 14543-14551) reported a chromosome-level genome assembly of *Momordica charantia* and used re-sequencing to infer the divergence between 'wild samples with var. *muricata*-type morphology' and cultivated samples (var. *charantia*). They dated the initial domestication to 6,000 y ago in Asia. A parallel study by Cui et al. (Horticulture Research 7: 85, 2000), with partly overlapping authorship, instead inferred that divergence between wild populations, called 'TR', and the lineage that gave rise to var. *muricata* & var. *charantia* occurred already ~1.9 Mya, while the split between the two cultivated varieties in Asia was again dated to ~6000 y ago. What might have caused these contrasting inferences? Matsumura et al. included 44 cultivated bitter gourds from Asia and one from Belize, as well as 15 'wild' accessions from Taiwan, Thailand, and the Philippines that have <30 g fruit weight. Some accessions appear admixed, reflecting the difficulty of distinguishing feral from domesticated forms. Cui et al., by contrast, included 187 accessions from Africa, South America, and Asia, arguing that African material was important because wild *M. charantia* populations have been reported from there and because the sister species of M. *charantia* all occur in Africa. In my talk I will explain what caused the different conclusions, clarify the correct application of the name 'var. *muricata*,' and focus on which open questions remain to be answered.

Key words: *Momordica charantia* var. *muricata*, currently unnamed wild *Momordica* populations, seeds as key traits in cucurbits

O1-2

Pre- and post-zygotic interspecific barriers control reproductive isolation in *Cucumis*

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All *Cucumis* species are self-compatible, but interspecific reproductive barriers (IRBs) are known that contribute to reproductive isolation. Particularly, cultivated melon (*Cucumis melo*) cannot be successfully crossed with any other *Cucumis* species and therefore these barriers hinder access to the useful genetic variation available in wild germplasm. This study is aimed to characterize phenotypically IRBs present in *Cucumis* in order to establish bases for future dissection of the genetic control and facilitate overcoming. With this purpose, a set of wild African *Cucumis* species (i.e. *C. dipsaceus*, *C. ficifolius*, *C. anguria* subsp. *longipes*, *C. pustulatus* and *C. zeyheri*) and cultivated melon were crossed all against all. Pre-zygotic IRBs were evaluated by identifying pollen tube rejection at stigma, styles and ovaries using fluorescence microscopy. Post-zygotic IRBs were also assessed by measuring fruit set, seed abortion, seed germination and viability of plantlets, and by determining the production of female and male flowers and androsterility in the interspecific hybrids obtained from viable crosses. In addition, phylogenetic relationships were inferred from clustering analysis based on GBS data of 13 *Cucumis* species. In the light of the results of phenotypic and genotypic characterizations, the correlation between crossability and phylogeny in *Cucumis* is discussed. Specific crosses have also been selected to develop segregating populations for mapping *loci* underlying both pre- and post-zygotic IRBs.

Key words: interspecific reproductive barriers, *Cucumis*, crossability, clustering analysis, GBS

O1-3

A global conservation strategy for Cucurbitaceae family crops

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As part of a new initiative led by the Global Crop Diversity Trust, and funded by the German Government, a Conservation Strategy has been developed for crops in the Cucurbitaceae family. The Strategy aims to promote the rationalization of conservation efforts at national, regional, and global scales, and to identify priority actions to strengthen the conservation of cucurbit genetic resources. The content of the Strategy is derived from a thorough literature review combined with three activities: (i) an expert meeting and stakeholder consultation workshop in Thailand (December 2019); (ii) a detailed online survey sent to some 50 genebanks around the world; and, (iii) analysis of data from Genesys, WIEWS, and other online germplasm databases. In the Strategy document, background information is provided for a variety of economically important Cucurbitaceae crops; and an overview is given for each crop and its wild relatives (CWRs). The Strategy discusses the origins, domestication, and centres of crop genetic diversity, highlighting important CWRs, and provides up-to-date information on crop taxonomy and genepools. Current ex situ holdings for the major crops are summarized, indicating vulnerabilities and gaps in existing collections. Within the Strategy, survey data collected from genebanks inform a discussion of current germplasm and genebank management, highlighting research gaps and outlining ways to improve the efficiency and effectiveness of the conservation of Cucurbitaceae family crops genetic resources. Some of the priority actions identified are: developing a global registry of Cucurbitaceae collections held ex situ, safety duplications in regional genebanks, and collecting threatened and missing genetic diversity.

Key words: plant genetic resources, ex-situ conservation, genetic diversity

O1-4

Importance of the American resource of Cucurbitaceae conserved by CATIE Germplasm Bank and its potential for genetic improvement

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CATIE retains 2332 accessions of Cucurbitaceae (*Cucurbita* = 2119, *Lagenaria* = 147, *Cucumis* = 25, *Citrullus* = 9, *Momordica* = 9, Sicana = 9, *Cionosicyos* = 5, *Cyclanthera* = 3, *Luffa* = 4, *Benincasa* = 2). 93% of the germplasm comes from America and 99% are traditional crops. Different morphological characterizations have reported great variability in the accessions analyzed. In a molecular characterization performed at 218 accessions, 24 haplotypes and 9 unique haplotypes were found. These results confirm the conserved genetic diversity and its potential for genetic improvement, evaluation and research. The Cucurbita genus is the most numerous in the collection, and brings together some of the most important crops of the American continent, where important archaeological findings for this genus have been reported. The Cucurbita collection of CATIE is the second in global importance and the most important in America. 99.66% of the germplasm comes from the Americas, and a global level, with 7% of the 39,583 accessions conserved in the world. This work is a compilation of information generated during 44 years of conservation of Cucurbitaceae. Databases, research, thesis, scientific articles and other sources were reviewed, in order to analyze the global importance and potential of the Cucurbitaceae collection protected by CATIE.

Key words: Cucurbita, Cucurbitaceae, variability, traditional varieties

O1-5

The Mexican *Cucurbita* project: advances and perspectives

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Mexico is the center of origin and diversification of the *Cucurbita* genus. Within its geographical limits we can find 15 taxa of the genus, of which five are endemic to Mexico and another four generally considered that were domesticated in this country. In Mexico, little research has been previously conducted in the genus, in particular with a genetic or evolutionary perspective. In this talk we describe our recent collaborative project between two departments of the National Autonomous University of Mexico (UNAM, Instituto de Ecología and FES-Iztacala) and INIPAP agronomic institute of Mexico, with CONABIO funding. Recently we started an ambitious program to sample all the Mexican territory, collecting representative accessions of both the wild and cultivated taxa, paying special attention to the cultivated landraces. We analyzed the collected material using molecular DNA sequences of mitochondrial and chloroplast regions, nuclear microsatellites, and single nucleotide polymorphisms (SNPs) obtained through genotyping by sequencing (GBS) to evaluate the genetic resources and explore their phylogeographic patterns. We have also investigated their phylogenetic relationships, calibrated molecular clocks and studied their distribution pattern and their relationships with climatic variables. Additionally, genomes of both domesticated and wild taxa were sequenced and analyzed, and this information has proved to be critical to annotate and interpret the GBS data. We are in particular interested in understanding local adaptation and the changes that happened during the independent domestications in the genus. We describe our project and the main analyzes and discuss future studies and possible collaborations.

Key words: landraces, genetic resources, wild relatives, genomics, domestication

P1-1

Characterization of traditional snake melon "alficoz" (*Cucumis melo* l. subsp. *melo* var. *flexuosus* (L.) Naud.) cultivars grown under organic farming conditions

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Since roman times non-sweet snake melon "alficoz" (*Cucumis melo* subsp. *melo* var. *flexuosus* (L.) Naudin) has been cultivated in Spain. Cultivation of snake melon has been declining, although it is still found in local markets in Southeastern Spain. One possible way to revitalize this crop would be organic farming, as demand for sustainable traditional crops is increasing. Selecting the best cultivars adapted to these conditions and with qualitative traits preferred by consumers is of great importance. For this reason, the Valencian Government (CEICE, Generalitat Valenciana) has financed a project for excellence groups (PROMETEO 2017/078) to select Spanish traditional melon cultivars adapted to organic farming conditions. For this study, 5 snake melon cultivars from the UPV-Genebank were assayed. The study was performed in 2018 in Moncada (Valencia), in a field with no previous melon cultivation history. Pests and diseases affecting the plants were surveyed. The main pest detected were aphids, which are virus vectors. Some cultivars were affected by viruses, but not by powdery mildew. No soilborne pathogens were detected. Yield was measured and fruits were characterized and analysed for metabolites. A sensorial analysis with potential consumers was performed. Variability was observed in traits like fruit weight, shape or colour, and in metabolite content and consumer perception. This study will allow the selection of cultivars suited for organic farming. Acknowledgements to the Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana) for funding PROMETEO project 2017/078 (for excellence groups). The FEDER/Ministry of Science and Innovation for funding the project AGL2017-85563-C2-1-R.

Key words: traditional cultivars, *flexuosus*, fruit quality, organic farming, viruses

P1-2

Assessment of Chromosomal Diversity in Indian Cucurbit Species by Fluorescent Karyotype Analysis

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India is a centre of diversification of several agriculturally important cucurbits. However, there is inadequate genomic information on many species. Chromosome analysis paves an easy way to get foundational data on the genomic features of a species. Considering the dearth of study, we had attempted karyotype analysis following EMA method and fluorescence banding with CMA3 (GC-specific) and DAPI (AT-specific) in three Indian species of *Luffa* (2n=26) and two species of *Trichosanthes* (2n=22) of Sicyoeae. Fluorochrome banding pattern in dioecious *Trichosanthes dioica* was studied in comparison with *Coccinia grandis* (2n=24) having X-Y sex determination. Chromosomes with nucleolar CMA^{+ve} bands are differently conserved in the species of *Luffa* and *Trichosanthes*. Although rare, non-nucleolar $DAPI^{+ve}$ bands constituted inter- and infra-specific differences. DAPI bands were found in two species of *Luffa* and in *Trichosanthes* except the Anguina cultivar of *T. cucumerina*. Distribution of the heterochromatin landmarks elucidated affinities between *Luffa* and *Trichosanthes*, congruent with early reports of phylogenetic proximity. The female plants of *T. dioica* showed six CMA^{+ve} bands while male plants lacked such bands altogether. This was the first report of chromosomal distinction between genders of this crop. In addition to the Y chromosome in male plants, sex specific CMA banding pattern in the autosomes of the dioecious *Coccinia grandis* were obtained. Sex chromosome differentiation may be related to autosomal heterochromatin in dioecious cucurbits, subject to further confirmation. The fluorescent chromosome database highlights karyotype specialization in the Indian genomic resources to benefit cucurbit breeding programs and lays a platform to complement genomic analyses in Sicyoeae.

Key words: *Luffa*, *Trichosanthes*, *Coccinia*, EMA, Fluorescence banding

P1-3

Characterization of *Cucurbita* spp. germplasm to broaden squash and pumpkin genetic background

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Cucurbita spp. (gourds, squashes, and pumpkins) are important genetic resources to broaden not only the genetic background of the commercially important summer squash types like Zucchini, but also to develop new rootstocks to manage and control soil-borne diseases in other cucurbits like watermelon and melon. The characterization of landraces and wild *Cucurbita* accessions searching for resistances to the main pathogens affecting cucurbit crops is of great interest, mainly with the aim to adapt and reintroduce highly appreciated local varieties in more sustainable production systems and to introduce new resistant rootstocks. In the framework of the project presented, a screening for resistance to Zucchini Yellow Mosaic Virus (ZYMV) and for two emerging species from *Fusarium solani* species complex (FSSC), *Neocosmospora falciformis* and *N. keratoplastica*, is being carried out. Among a germplasm collection previously characterized by RNA-seq, a first subset of genotypes (including *Cucurbita moschata*, *C. maxima*, *C. pepo*, *C. cordata*, *C. argyrosperma*, *C. foetidissima*, *C. pedatifolia*, *C. ecuadorensis*, *C. lundelliana* and *C. okeechobensis*) have been selected based on the genetic relationships obtained with the Neighbour Joining cluster analysis carried out with the 96-accessions whole collection. Until now, a total of thirty-four and twenty accessions have been assessed based on symptom severity after artificial inoculation with ZYMV (isolate Courgette) and with N. *falciformis* and *N. keratoplastica* (isolates MYC-1450 and MYC-1256), respectively. Despite the high susceptibility found, some accessions belonging to *C. moschata*, like the well-known virus-resistant Nigerian Local accession, and to *C. ecuadorensis* have been considered of interest for future studies about the genetic control and mechanisms of their resistance/tolerance that will enable their introgression in other genetic backgrounds.

Key words: *Cucurbita* genus, germplasm collection, screening for genetic resistance, ZYMV, FSSC, *Neocosmospora*

P1-4

Analysis of Genetic Diversity in Indian snake melon (*Cucumis melo* L. var. *flexuosus*) using horticultural traits and start codon targeted (SCoT) markers

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Snake melon is a commercially grown, short duration salad vegetable crop. The genetic potential of the crop germplasm has not been fully exploited which needs systematic characterization with molecular interventions to identify and evaluate the important horticultural traits. Genetic diversity and multivariate analysis in the 31 diverse accessions of snake melon were studied using horticultural traits and 41 start codon targeted markers (SCoT). Variance and multivariate analysis for horticultural traits indicated that accessions reveal significant variability among all characters studied. The traits like fruit length and number of fruits/plants showed most genetic variation among the studied traits. In molecular diversity analysis, a total of 202 bands were produced, of which 177 bands were polymorphic with high level of polymorphism (87.62%). The number of polymorphic bands varied from 1 to 9, with an average of 4.31 bands per primer. The polymorphic information content (PIC) value ranged from 0.14 (SCoT54) to 0.48 (SCoT2), with an average value of 0.34 per primer. The average of MI and RP were 6.31 and 1.37, respectively representing the effectiveness of primer set for genetic discrimination between different accessions. The dendrogram was constructed to establish genetic relationship among 31 different accessions using Jaccard's coefficient. The distinguishable genetic background and a high degree of variation in the snake melon genotypes successfully exhibited by the SCoT markers. Quick, reliable and effectiveness of SCoT technique for genetic characterization in this study can be helpful in amplifying the genetic base, evolutionary assessments, and selection of specific traits for breeding programs in snake melon.

Key words: *Cucumis melo* L. var. *flexuosus*, genetic diversity, SCoT markers, snake melon

P1-5

Morphological characteristics of winter squash (*Cucurbita maxima* Duchesne) accessions collected at the Polish National Genebank

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Winter squash (*Cucurbita maxima* Duchesne) is one of the *Cucurbita* species of worldwide economic importance. This species is well-adapted to agroecological conditions and is characterized by a large phenotypic diversity of the fruits. Polish National Genebank holds collection of winter squash (*Cucurbita maxima* Duchesne) that includes in majority cultivars and landraces originated from Central and Eastern Europe. Over last few years morphological properties of 188 *C. maxima* accessions were evaluated. Accessions were characterized phenotypically in the field experiments in terms of qualitative and quantitative traits using 27 descriptors: 4 of them related to plant morphology, 20 related to fruit morphology and 3 related to seed traits. Traits related to plant morphology were determined in the field in July and August. Evaluation of fruit and seed related traits was performed after fruit harvesting in October and dry matter content was measured after few weeks of fruit storage. Morphological variability among accessions was described revealing phenotypic similarities and differences. %). The phenotypic characterization of *C. maxima* accession showed the great variation for most of the examined traits. Some of the most variable traits were the fruit weight, ranging from 0,44 kg to 23,04 kg (mean 7,12 kg), fruit length ranging from 9,3 cm to 47,17 cm (mean 23,81 cm) and dry matter content with variation ranging from 3,13 % to 26,4 % (mean 7,5 %). A large variation found within accession displayed the potential of these collection in breeding program. This study is the first step to rationalize Polish Genebank collection of *C. maxima* winter squash.

Key words: *Cucurbita maxima*, germplasm, morphological traits

P1-6

Screening cucumber (*Cucumis sativus* L.) germplasm for ability to germinate under low temperature

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Cucumber (*Cucumis sativus* L.) is sensitive to low temperature during seed germination and early growth of the plants. In this study 168 cucumber accessions from the Polish National Gene Bank were selected and evaluated for ability to germinate under low temperature. For each accession 30 seeds in 3 replications were placed on moistened with water filter paper, rolled-up and incubated in the growth chamber (MLR-352, Sanyo/Panasonic, Osaka, Japan). After 14 days of incubation in 13°C seed germination was evaluated as percentage of germinating seeds. Two germination experiments were performed. For majority of the accessions seeds did not germinate or germinated at low percentage. Seeds of 6 out of 168 accessions were capable of germinating at 13°C with germination ability of 65% or higher. Accessions identified in this study that germinate under low temperature will be further characterized and used to investigate genetic basis of cold tolerance in cucumber.

Key words: *Cucumis sativus*, germplasm, seed germination, cold tolerance

SESSION 2.
TOLERANCE TO ABIOTIC STRESS

O2-1 Invited

Breeding melons for vine decline resistance, nutritional value and flavor

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In south Texas and other warm regions of the world, vine decline of melons is incited by a complex of soilborne fungal pathogens. Melon cultivars resistant to three races of Fusarium wilt have been developed at Texas A&M University, AgriLife Research at Weslaco and deployed since the late 1960's. However, the most serious vine decline pathogens for most of south Texas production regions are currently *Monosporascus cannonballus* and *Didymella bryoniae*, causing root rot and gummy stem blight, respectively. The melon breeding program at TAMU has devoted extensive resources to screening germplasm, verifying resistance and developing novel breeding lines with improved quality during the past 22 years. We have identified resistance to Monosporascus root rot after screening nearly 1000 melon accessions and cultivars from around the world, utilizing both infested field plots and controlled inoculations. The most resistant PI lines: 140632, 165449, 124104, 212210, 20488 and 20598 have been used as parents to cross with more than 30 elite western shipper lines or F1 cultivars. A recurrent selection program has been conducted to create resistant cantaloupe lines. Additionally, backcrossing up to 4 times has been conducted, followed by selfing and selection for resistance at each generation, to introgress resistance loci. Currently, we are developing SNP markers linked to a dominant resistance gene for *Monosporascus cannonballus* utilizing genotyping by sequencing technology. Elite muskmelon lines with this resistance and good quality fruit are being tested as parents for F1 hybrid cultivar development. We have measured beta-carotene, ascorbic acid, total sugars and aroma volatiles in multi-location trials of these hybrids, and also conducted taste panels to identify the best ones for release as cultivars.

Key words: *Monosporascus*, SNP markers

O2-1

RNA-seq base analysis of Zucchini fruit transcriptome in response to exogenous abscisic acid and cold storage

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Zucchini is a non-climacteric immature fruit that is very sensitive to postharvest chilling injury (CI), which reduces the commercial quality of the product and increases postharvest losses under cold storage conditions. Exogenous application of abscisic acid (ABA) has proven to be an effective postharvest treatment for improving the tolerance of zucchini fruit to CI. In this work we have performed a high throughput sequencing of RNA (RNA-seq) to study the transcriptomic changes that were associated with fruit CI tolerance in response to such treatment. Around 251 million raw-reads were sequenced from ABA-treated and -untreated fruits at 1, 5 and 14 days of cold storage. After a trimming and quality checking process, 229 millions of high-quality reads remained. Differential expression analysis was conducted using the edgeR package, finding that most of the differentially expressed genes (DEGs) were found in both control and ABA-treated fruits, indicating that cold storage is the main factor altering fruit transcriptome profile. However, a total of 852, 793 and 1120 DEGs were specifically found in response to ABA application on the 1st, 5th and 14th day after treatment and cold storage, respectively. These genes were further analysed by GO enrichment, KEGG pathway mapping and Gene Expression Profiling, revealing the occurrence of different GO terms like “response to stimulus” and “signal transduction pathways”, KEGG pathways related with plant hormonal transduction and MAPK signalling pathway, and differential expression profiles of tens of transcription factor genes for MYC, MYB, DREB, DREB2, bZip and AP2 families. Results not only provide insight into how C. pepo prevents postharvest CI but it prepares the ground for future comprehensive studies covering multiple CI tolerant treatments.

Key words: ABA, cold tolerance, chilling injury, postharvest fruit quality, RNA sequencing

O2-2

Insights into *Cucumis sativus* drought stress tolerance using RNA sequencing

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Drought is one of the major factors that limit cucumber quality and yield. Mitigation of drought impacts requires a substantial change in the metabolism of plant cells. This is reflected in the extensive transcriptome changes upon the occurrence of the stress. In this study, a comparative time-course RNA-sequencing analysis was performed in two cucumber genotypes with contrasting reactions to water deficiency. Samples of leaves from 5-week-old plants were taken at 0, 2, 6, 24, and 30 h after the imposition of drought stress. A total of 944 identified differentially expressed genes (DEGs) formed 9 clusters with various courses throughout the experiment duration. With the drought-sensitive line as the baseline, the genes overexpressed in the tolerant line were twice as numerous as the repressed ones. Among the biological processes enhanced in the drought-tolerant line as per the GeneOnthology (GO) classes were gene-expression, protein turnover, signal transduction, lipid metabolism and transport, and various metabolic-processes. The DEGs repressed in the drought-tolerant line included those related to DNA duplication, cell division, response to stresses, and response to hormones. This research evidences how broad the drought stress impacts are on the cucumber metabolism across the stages of the imposed stress.

Key words: cucumber, gene expression, transcriptomic analysis, water deficiency

O2-3

Screening of a Zucchini mutant collection for abiotic stress tolerance

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Salinity is one of the most limiting abiotic stresses affecting plant growth and development, and salt tolerance is becoming a priority objective in many current breeding programs to mitigate the harmful effects of climate change. In this work, we have screened 2,751 independent M2 lines of an EMS mutant collection of *Cucurbita pepo* by using two phenotyping approaches: delayed germination in presence of either ABA or NaCl. Five different concentrations of ABA (5-500 µM) and four of NaCl (85-300 mM) were tested for germinating the line MUCU16, the genetic background of the mutant collection. 500 µM of ABA and 300 mM of NaCl were finally selected to discriminate between sensitive and tolerant mutant families in the collection. The germination rate was assessed in 10 seeds per M2 family, selecting those mutants whose seed germinated before that of MUCU16 under the same conditions. The ABA and salt tolerant phenotypes were then confirmed in the M3 offspring obtained after selfing ABA- and NaCl-sensitive and -insensitive M2 seed. In this way we have selected 23 and 46 mutant lines that segregate for ABA insensitivity and salt tolerance, respectively. Six of the selected mutant families showed both ABA insensitive and salt tolerant phenotypes, demonstrating that the salt tolerance of several mutants was dependent on ABA.

Key words: ABA, salt tolerance, EMS mutants

O2-4

Evaluation of grafting traditional snake melon “alficoz” under abiotic stress: effects on agronomic performance and fruit quality

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Snake-melon “alficoz” (*Cucumis melo* subsp. *melo* var. *flexuosus* (L.) Naudin) is a declined traditional crop in Spain, although it is still cultivated in the South-eastern regions. Climate change will affect the cultivation of this neglected crop, forcing its cultivation in stressful conditions such as salt stress. The use of grafted plants could help adapt snake-melons to these agro-ecosystems. Selecting the best scion-rootstock combination to both adapt and minimize or improve certain quality parameters is of great importance. The Valencian Government (CEICE, Generalitat Valenciana) has financed a project for excellence groups (PROMETEO 2017/078) to select Spanish traditional melon cultivars adapted to organic farming conditions. A local snake-melon cultivar “Alficoz valenciano”, was grafted onto five different rootstocks (an interspecific *C. melo* subsp. *agrestis* x *C. melo* subsp. *melo*, two wild *Cucumis* hybrid and two commercial hybrid *Cucurbita maxima* x *C. moschata*). The study was conducted for 2 years in 2 locations: The Natural Park of Carrizales (Alicante, saline water irrigation) and “La Punta” (Valencia, no saline irrigation). Yield was measured and fruits were characterized and analysed for metabolites. A sensorial analysis with potential consumers was performed. Salinity improved the SSC of grafted and ungrafted plants, and also varied the flesh colour. Grafting negatively affected acceptability, especially with *Cucurbita* rootstocks. Salinity affected fruits of *Cucurbita* grafted plants, resulting in a lower acid and sugar content. *Cucumis* rootstocks had a higher effect on the VOCs profile than on sugar and acid profile. Thus, these rootstocks are a good alternative, as their impact on consumer perception was lower.

Key words: traditional cultivars, *flexuosus*, fruit quality, grafting, salinity

P2-1

Evaluation of traditional melon varieties for their water deficit response

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Water stress is one of the main factors limiting crop yields worldwide, especially in the Mediterranean basin, threatening the viability of our primary sector. Improving the drought tolerance of our crops is an urgency in the context of climate change expected for the coming years. Melon (*Cucumis melo* L.) is one of the main Mediterranean vegetable crops. Currently, melon cultivation is mainly carried out under irrigation. However, in the region of Murcia, local varieties have been traditionally cultivated in rainfed land. Local varieties adapted to the cultivation environment where they have evolved are an important source of variation and could provide interesting traits for improving the melon water use efficiency. ABIOPEP, within the PRIMA VEGADAPT project (CDTI reference IDI-20190384), has carried out a screening of 20 traditional melon varieties maintained by the BAGERIM Germplasm Bank of IMIDA (Murcia, Spain), in order to assess their vegetative drought tolerance. The plants were grown under greenhouse conditions for three weeks after transplanting. Three water treatments were established and maintained for 15 days: control (irrigation to field capacity); moderate drought (30% water deprivation) and severe drought (50% water deprivation). Vegetative growth and water use efficiency were evaluated at experiment ending, and 4 varieties showing a better agronomical behavior under water deficit were selected. Our results will be used as the basis for identifying markers of water stress tolerance, and in the long run, for the development of new melon genotypes with a better agronomic behavior under water deficit conditions.

Key words: melon, drought tolerance, water use efficiency

P2-2

Correlation between ABA and chilling tolerance in crosses of *Cucurbita pepo* varieties with contrasted behavior to cold storage

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Abscisic acid (ABA) is a key phytohormone in the regulation of many stress responses, specifically ABA is involved in the response to drought, salinity, and low temperature. In a previous study, the implication of ABA in the response to cold during the postharvest storage of zucchini fruit was investigated using two varieties with different tolerance to cold storage. The cold-tolerant variety 'Natura' increased the content of ABA during the first days of exposure to low temperature, in contrary to the variety 'Sinatra' (a cold sensitive variety). Besides, the inhibition of ABA biosynthesis induced cold sensitivity in the 'Natura' variety. Thus, the aim of this study has been to investigate the relation between ABA production and tolerance to cold storage. To test this relation, backcrosses of the varieties 'Natura' and 'Sinatra, and crosses between these two varieties have been made. The seeds obtained with these crossings were planted in a greenhouse in the installations of the University of Almería until fruit set. After growing, fruit at commercial stage were harvested from each plant individually and then stored in a temperature-controlled chamber and in permanent darkness at 4ºC and 85-90% RH (relative humidity) for 5 days to quantify the endogenous abscisic acid. From a different set of fruit, postharvest quality was analyzed after 14 days of cold storage. Results showed that a positive correlation could be stablished among ABA content and resistance to cold storage. These results and their possible applications will be discussed in this presentation.

Key words: Abscisic acid, *Cucurbita pepo,* chilling tolerance

P2-3

The enhanced salt tolerance of the squash *etr2b* mutant is mediated by ABA

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The improvement of tolerance mechanisms against abiotic stresses has become a priority objective in current plant breeding programs. The phytohormones ethylene and ABA play key roles in mediating the biochemical and physiological tolerance responses of plants to environmental stresses. We have recently demonstrated that three gain-of-function mutations affecting the ethylene receptors *CpETR1B*, *CpETR1A* and *CpETR2B* confer salt tolerance in *Cucurbita pepo*. To study whether this salt tolerance is mediated by ABA, we have compared ABA content and ABA sensitivity in WT and *etr2b* during germination and vegetative plant development. Exogenous ABA application delayed germination and radicle growth of WT seed, but did not significantly affect germination and radicle growth of *etr2b* seed, suggesting that *etr2b* is partially insensitive to ABA. The dry and water-soaked seed of the *etr2b* mutant shows a lower endogenous ABA content, and this content is not induced in response to salt or ABA external treatments as occurs in the WT seed. The enhanced root and shoot growth rates of *etr2b* plants under salt conditions was found to be associated with a higher upregulation of ABA biosynthesis and signaling genes, including *CpNCED3A*, *CpNCED3B, CpPYL8* and *CpPP2c*, but no significant change was found in the expression of ethylene biosynthesis and signaling genes between WT and *etr2b* plants. Taken together these results indicate that the enhanced salt tolerance of *etr2b* plants is mediated by ABA. The early germination of *etr2b* under salt stress is likely the result of a reduced ABA content and sensitivity of mutant seed, and the enhanced salt tolerance of *etr2b* plants is mediated by a higher induction ABA production and response in the leaves of the mutant under salt stress. This work was co-funded by projects AGL2017-82885-C2-1-R y UAL18-BIO-B017-B.

Key words: ABA, ethylene, salt tolerance, mutant

SESSION 3.
GENOMIC RESOURCES-1

O3-1 Invited

Melon genetic resources in the genomics era

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Melon germplasm shows an impressive phenotypic diversity. Horticultural classifications have been proposed to group varieties, landraces and wild genotypes. In parallel, molecular markers, starting with RAPDs and ISSRs, followed by SSRs, SNPs and, finally, whole genome re-sequencing, have been also used to study and classify the genetic diversity. Combination of both approaches has allowed the identification of at least two independent centers of domestication in Africa and India. Traditionally two subspecies have been proposed: *Cucumis melo* subsp. *melo* (including Mediterranean and Near-East cultivars) and *C. melo* subsp. *agrestis* (Oriental Asian cultivars). A new subspecies has been proposed recently (*Cucumis melo* subsp. *meloides*) that includes African melons. India is the center of diversity for the modern cultivated melons. Oriental and Occidental cultivars were developed by divergent selection, likely from independent ancestral gene pools. A second diversification was produced during the travel from India to the Mediterranean basin, generating two different types of cultivars: cantaloupe-like (aromatic and climacteric) and inodorus-like (non-aromatic and climacteric). Current genomics resources are allowing to track this history and to find the genomic regions that were subjected to selection and would include the genes involved in the cultivar diversification.

Key words: SNPs, genome sequence, domestication, diversification

O3-1

Dissecting melon fruit ripening using CRISPR

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Fruit ripening has a high impact on the organoleptic quality and post-harvest durability of fleshy fruits. Fruit can be classified into climacteric, characterized by the increase in respiration and ethylene production at the onset of ripening and non-climacteric, presenting low levels of both ethylene production and respiration rate. Melon (*Cucumis melo* L.) has emerged as a model to study fruit ripening due to the coexistence of climacteric and non-climacteric varieties.

The ripening behaviour of a recombinant inbred line population derived from the climacteric variety Védrantais and the non-climacteric ´Piel de Sapo´ showed that a major QTL *ETHQV8.1* is sufficient to trigger climacteric ripening. The characterization of the QTL genomic interval allowed the identification of a negative regulator of ripening CTR1-like (MELO3C024518), and a demethylase *ROS1* (MELO3C024516), the orthologue of *DML2*, a demethylase regulating fruit ripening in tomato.

In this study, CRISPR knockout mutants of CTR1 and ROS1 were generated in a climacteric genetic background (Védrantais). The homozygous CRISPR lines for the editions (T2) were evaluated for ripening-associated traits and ethylene production was measured during the ripening.

The climacteric behaviour was altered in both CRISPR lines compared to Védrantais. A significant advance in the appearance of the abscission layer formation, aroma production and chlorophyll degradation along with a different ethylene production profile was found in the CRISPR mutants suggesting a role of both genes in climacteric ripening in melon. Further experiments, including the increase of the genetic resolution of the interval, will be used to determine the gene responsible of *ETHQV8.1.*

Key words: fruit ripening, CRISPR, *Cucumis melo*

O3-2

Panning the Melon Genome

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The first plant genome was assembled in the year 2000 and marked the beginning of the genomic era in plant biology. A major advancement occurred a few years later with the introduction of parallel sequencing technologies (NGS) that presented an exponential increase in throughput accompanied by thousands-fold reduction in sequencing costs, and enabled the sequencing and assembly of the first melon genome in 2012. Since then, in conjunction with NGS-based genotyping, the availability of this reference genome has proven to be a powerful tool for comparative genomics, trait mapping and gene discovery for this species. The recent introduction of third generation, single molecule sequencing technologies now put *de novo* assemblies of small and medium size genomes within reach. In the current study, we will present *de novo* genome assemblies of 25 diverse melon accessions from the Newe-Ya'ar collection, using a combination of short (Ilumina) and long (Nanopore) read sequencing data. Technical aspects regarding the sequencing and assembly will be presented. The concept of Pan-genome will be discussed through recent examples from other plant species. Preliminary comparative analyses of the 25 assembled genomes will allow us to discuss the following questions: how much variation is there in genome structure across melon diversity? What are the benefits of building multiple independent genome assemblies? What can we learn about variation in gene content in melon (PAV), and what are the challenges? In addition, what is the potential of adding structural layer to saturated SNP variation maps? We will also propose what will future genetic maps may look like and what will be the potential impact of pan-genomic era on trait mapping and breeding in melon.

Key words: Pan-genome, *Cucumis melo*, genetic mapping

O3-3

A Multispecies SNP Array for High-Resolution Genotyping of Melon, Cucumber and Watermelon

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With the availability of a set of sequenced cucumber, watermelon and melon genomes in public databases, very large sources of SNPs are now available for these important Cucurbitaceae species. We have used these resources of molecular markers for the development of a multispecies genotyping array using the Axiom genotyping platform. The array has been set up with 29,961 melon, 58,238 watermelon, and 56,204 cucumber SNP markers. The markers have been selected for each of the three species based on (i) general allele frequency in all sequenced lines; (ii) chromosomal distribution along the physical length of the chromosomes with a higher marker number towards the end of the chromosomes reflecting the distribution of crossing overs; and (iii) expected marker functionality over a wide range of material.

This Cucurbitaceae genotyping array has been used for the characterization of large sets of cucumber, melon and watermelon breeding lines and varieties. The results show a very high level of individual marker functionality, quality, and polymorphism. With many functional and polymorphic markers and no need for additional data processing as required in full genome sequencing, this array provides a genotyping solution for these Cucurbitaceae species that is lower in costs per sample than genome sequencing including the necessary associated bioinformatics analysis. Furthermore, markers identified with this array can easily be converted into single or low-plex marker sets.

Key words: *Cucumis melo*, *Cucumis sativus*, *Citrullus lanatus*, genetic analysis, breeding

O3-4

Development of Double Haploid melon lines for its use as founders of a MAGIC population

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Traditional local varieties have an outstanding aptitude for adaptative and quality traits, although they often lack high yielding potential. Therefore, combining them with modern germplasm which carries disease resistance and yield traits could generate novel and improved breeding lines. To this aim, and as part of the RIS3Mur project MELOMUR, we decided to set up a MAGIC population of melon, whose founders include several traditional Spanish cultivars, expecting to generate an array of genotypes displaying a wide range of variation in fruit quality and appearance parameters, phytosanitary response, vigor, precocity, and drought stress tolerance. Such collection will be used both for selection of superior materials and for analysis of the genetics underlaying the traits of interest. But heirloom germplasm usually fails to exhibit a complete genetic homogeneity, which impairs its usefulness for genetic studies; thus, we have launched and optimized the protocol for obtaining Double Haploid (DH) lines from melon accessions. As a result, a set of DHs of different characteristics and origin (mainly from the BAGERIM Germplasm Banc of IMIDA, Murcia, Spain), have been generated and evaluated, and six of them are included among the eight parental lines of a MAGIC population of melon currently under development.

Key words: melon germplasm, Double Haploids, MAGIC populations, variability

O3-5

Genome-wide association analysis of downy mildew resistance in a pre-breeding watermelon (*Citrullus amarus*) collection

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Cultivated sweet watermelon (*Citrullus lanatus*) is an important vegetable crop for millions of people around the world. There are limited sources of resistance to economically important diseases within *C. lanatus*, whereas *Citrullus amarus* has a reservoir of traits that can be exploited to improve *C. lanatus*. Downy mildew, caused by *Pseudoperonospora cubensis,* is an emerging threat to watermelon production. We screened 122 *C. amarus* accessions for resistance to downy mildew over two tests (environments). The accessions were genotyped with 2,126,759 single nucleotide polymorphic (SNP) markers. A genome-wide association study approach was deployed to uncover marker-trait associations and identify candidate genes underlying resistance to downy mildew. Our results indicate the presence of wide phenotypic variability (1.1 - 57.8%) for leaf area infection, representing a 50.7-fold variation for downy mildew resistance across the *C. amarus* diversity panel. Broad-sense heritability estimate was 55%, implying the presence of moderate genetic effect for resistance to downy mildew. The peak SNP markers associated with resistance to *P. cubensis* were located on chromosomes Ca03, Ca05, Ca07, and Ca11. The significant SNP markers accounted for up-to 30% of the phenotypic variation and were associated with candidate genes including disease resistance proteins. This information will be useful in understanding the genetic architecture of the *P. cubensis*-*Citrullus* spp. patho-system as well as development of resources for genomics-assisted breeding for resistance to downy mildew in watermelon.

Key words: downy mildew, watermelon, *Citrullus amarus*, disease resistance breeding

P3-1

A point mutation resulting in a 13 bp deletion in the coding sequence of Cldf leads to a GA-deficient dwarf phenotype in watermelon

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The dwarf architecture is an important and valuable agronomic trait in watermelon breeding, which has the potential to increase fruit yield and reduce labor cost in crop cultivating. However, the molecular basis for dwarfism in watermelon remains largely unknown. In this study, a recessive dwarf allele (designated as *Cldf*) was fine mapped in a 32.88 Kb region on chromosome 9 using F_2 segregation populations derived from reciprocal crossing of a normal line M08 and a dwarf line N21. Gene annotation of the corresponding region revealed that the gene *Cla015407* encoding a gibberellin 3-beta-hydroxylase functions as the most possible candidate gene for *Cldf*. Sequence analysis showed that the fourth polymorphism site (a G to A point mutation) at the 3' AG splice receptor site of intron leads to a 13 bp deletion in the coding sequence of *Cldf* in dwarf line N21, and thus results in a truncated protein lacking of the conserved domain for biding of 2-oxoglutarate. In addition, the dwarf phenotype of *Cldf* could be rescued by exogenous GA_3 application. Phylogenetic analysis suggested that the small multi-gene family *GA3ox* in cucurbit species may originated from three ancient lineages in *Cucurbitaceae*. All these data support that *Cldf* is a GA-deficient mutant, which together with the co-segregated marker can be used for breeding new dwarf cultivars.

Key words: Watermelon, dwarfism, gibberellins

P3-2

GBS characterization of watermelon germplasm and breeding against fungal pathogens

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Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is the most important cucurbit in terms of world production. Genomic tools and genetic resources for an efficient genetic breeding are of great importance in this crop, especially to develop cultivars resistant to pathogens and adapted to different growing conditions. Fungal diseases, such as powdery mildew, *Fusarium* wilt, *Monosporascus* root rot, and charcoal root, caused by *Macrophomina phaseolina,* cause important losses in this crop. The screening of large germplasm collections, searching for wild types or landraces bearing resistant alleles is the first step in genetic breeding programs. In the framework of the project CONMESAN (AGL2017–85563-C2–1, 2), the molecular characterization of 54 Spanish *C. lanatus* accessions from two Genebanks (BGHZ and COMAV) has been performed, using the genotyping-by-sequencing (GBS) strategy, also including some international references and genotypes belonging to the related species *C. amarus* and *C. colocynthis.* More than 12,000 SNPs were identified after trimming, cleaning raw reads, and mapping them to Charleston Grey genome assembly v.2. Principal Coordinate and Cluster Analyses performed, including GBS data of 57 USDA-NPGS accessions from Spain, showed that our germplasm presents new alleles regarding the Spanish USDA collection, being an interesting source for breeding. Spanish accessions were compared with 243 USDA accessions selected based on the GBS-based clustering to represent the variability of these three *Citrullus* species, and including most of the accessions previously reported with resistances to highlight the potential value of the collection presented. The analyzed Spanish collection is being phenotyped for resistance to fungi.

Key words: diversity, GBS, *Citrullus*, biotic stress

P3-3

Genome-wide association study (GWAS) of seed associated traits in cucurbits

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Genome-wide association studies (GWAS) are not only clearing the way to understand the genetic bases of complex traits but have also proven highly useful to identify markers for important agronomic traits. In this work we seize the wealthy of genomic information generated in cucurbit species in the last years to analyse seed traits of potential agronomic value in watermelon and zucchini. GWAS for seed size and colour was performed in a panel of 623 accessions of *Cucurbita pepo*, 182 of *Cucurbita maxima,* and 713 accessions of the genus *Citrullus*. SNP variants were obtained by mapping GBS data of each accession to reference genomes: *C. pepo* v4.2, *C. maxima* v1 and *C. lanatus* Charleston Gray v2. The genotypic data was then combined with phenotyping data from ten individuals of each accession. Q-Q plots drive to the selection of the most suitable method, GLM or MLM, to identify genomic regions associated with seed size and seed colour in *C. pepo*, *C. maxima* and *Citrullus* spp. In *Cucurbita pepo* no association was found in relation to seed coat colour, but seed weight is associated with a genomic region of chromosome 01. The most relevant region for seed weight in *C. maxima* is in chromosome 09, while markers of chromosome 11 were found to be associated with seed colour. Finally, in *Citrullus* spp. one region in chromosome 02 and two regions in chromosomes 03 and 06 were detected for seed weight and seed colour, respectively. Synteny between species allow to discuss the conservancy and relevance of the detected genomic regions in the control of seed weight and colour. This work was co-funded by projects AGL2017-82885-C2-1-R y UAL18-BIO-B017-B.

Key words: GWAS, *Cucurbita pepo*, *Cucurbita maxima*, *Citrullus* spp., accessions

P3-4

Cloning and expression of *CmROR2* gene in melon and its application in screening broad-spectrum resistant germplasm of powdery mildew

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Penetration resistance is a well-recognized plant defense process, in which SNARE proteins have impotent roles in membrane fusion processes. A melon material whose resistant to powdery mildew was used to clone the SNARE-like protein in this study. It was obtained by homologous cloning method. *CmROR2* is 1468 bp in length and encodes 307 amino acids. The average homology of the SNARE-like gene with other plants (SYP121, PEN1) is about 73-95%. Q-PCR analysis showed that *CmROR2* expression level in resistant varieties was 4.1 times higher than susceptible varieties, indicating that *CmROR2* is related to the resistance of powdery mildew. Fluorescence localization results showed that CmROR2 was mainly distributed on the cell membrane, and it was accumulated in the mastoid site after powdery mildew invasion, resulting in decreased penetration of powdery mildew. Strong focal accumulation of these proteins at the site of attack by powdery mildew fungi has been considered important for their function. The research results have certain significance for the breeding of powdery mildew resistance, and provide a mark gene to select broad-spectrum resistant melon germplasm materials.

Key words: melon, powdery mildew, CmROR2, GFP localization, expression analysis

SESSION 4.
GENOMIC RESOURCES-2

O4-1 Invited

Application of genomic tools for mapping and analysis of disease resistance traits in cucurbits: The CucCAP experience

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The past decade has seen an explosion in genomics capacity for cucurbits driven by international efforts in Asia, Europe, and North America. In the U.S., the CucCAP project has developed genomic tools including the cucurbit genomics database (http://cucurbitgenomics.org/) and associated analysis and visualization tools, and genetic characterization of the USDA National Plant Germplasm System (NPGS) plant introduction (PI) collections to establish molecularly-informed core populations representing >95% of the genetic diversity present for watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), cucumber (*Cucumis sativus*), and squash (*Cucurbita pepo*). These tools are, in turn, are being used for bi-parental population and GWAS analyses to map traits, identify QTL, and develop markers for resistance to important diseases. Among the disease resistance-associated QTL identified are QTL for *Fusarium oxysporum* f. sp. *niveum* races 1 and 2, bacterial fruit blotch, *Alternaria*, gummy stem blight, *Phytophthora capsici*, powdery mildew and *Papaya ringspot virus* in watermelon and powdery mildew, downy mildew, angular leaf spot, anthracnose and *P. capsici* in cucumber. The genomic and bioinformatic tools also have been utilized to identify candidate genes for several fruit quality traits, including fruit size, shape, and epidermal features of cucumber. This talk will describe identification of the QTL and marker development for the various diseases, along with our work combining genomic and transcriptomic approaches to characterize young fruit and age-related resistance to Phytophthora fruit rot of cucumber and epidermal traits in cucumber fruit.

Key words: disease resistance, germplasm collections, *Cucumis*, *Citrullus*, *Phytophthora capsici*

O4-1

QTL mapping and pyramiding resistance to *Fusarium oxysporum* f. sp. *niveum* (races 1 and 2) and potyviruses in watermelon

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The USDA *Citrullus* spp. germplasm collection is a valuable resource for enhancing modern watermelon cultivars (*Citrullus lanatus*) with resistance to fungal and viral diseases. We have generated genetic populations segregating for resistance to important watermelon diseases: Fusarium wilt (caused by the fungus *Fusarium oxysporum* f. sp. *niveum*; *Fon* races 1 and 2) and potyviruses, including *Zucchini yellow mosaic virus* (*ZYMV*) and *Papaya ringspot* virus-watermelon strain (*PRSV-W*). QTL mapping of *Fon* races 1 and 2-resistance identified several significant quantitative trait loci (QTL). A single QTL was associated with resistance to ZYMV and PRSV-W, adhering to expectations of a previous study which indicated a single-recessive gene inheritance in watermelon. We have been developing kompetitive allele specific PCR (KASP) markers tightly linked to resistance loci. We are using the KASP markers together with phenotyping assays to pyramid the resistance loci into watermelon cultivars. This study is in part supported by the “National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2020-51181-32139” (CucCAP2).

Key words: Fusarium wilt, ZYMV, PRSV-W, watermelon

O4-2

Editing the melon genome to attain broad spectrum virus resistance

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Host genes encoding factors that viruses use to complete their infection cycles (susceptibility factors) are obvious targets for the design of new alleles conferring virus resistance. Melon is a horticulturally important crop for which viruses represent a significant threat. The work that we present here was aimed at setting up a gene editing technology based on the CRISPR/Cas system for editing the melon genome. Using this technology, we have generated melon lines that carry new versions of the genes encoding the eukaryotic translation initiation factors (eIF) 4E and 4G, which are well-known susceptibility factors to viruses. Melon is a species recalcitrant to genetic transformation, therefore we first carried out experiments to improve Agrobacterium tumefaciens-mediated melon transformation using constructs that express fluorescent protein markers to facilitate the identification of transformants, as well as an efficient system for the early identification of editing events. Using the developed technology, we obtained melon lines that carry mutations in the targeted genes. Interestingly, a male sterility phenotype was detected in eIF4E deficient plants. We identified different stages of melon flower development and performed optical and scanning electron microscopy observations to compare eIF4E mutant and wild type (WT) floral primordia at each stage. We also carried out a RNA-seq analysis using flower RNA to identify differences in gene expression between eIF4E knocked-out and WT plants during androgenesis. T0 plants homozygous for a mutation knocking-out eIF4E were crossed by WT plants and their F1 and F2 progenies were obtained. The F2 progeny was biased against the eIF4E mutation in homozygosity. Individual F2 plants were inoculated with moroccan watermelon mosaic virus, a potyvirus known to depend on eIF4E for melon infection. The results of the virus susceptibility assay and further phenotypic characterization of the eIF4E knocked-out mutant plants will be presented and discussed during the conference.

Key words: melon genome, virus resistance, CRISPR/Cas9

O4-3

A potyvirus-based vector for transient gene expression in cucurbit plants and fruits

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Cucurbits host many viruses that frequently challenge crop production by causing different diseases. Among them, more than ten species that belong to the genus Potyvirus, a prolific group of plus strand RNA viruses transmitted by aphids, have been reported to naturally infect cucurbits. Potyviruses have a particular genome expression strategy in which a large polyprotein, representing most of the viral genome, is expressed and subsequently processed in the different mature viral proteins by three virus-encoded proteases. This expression strategy, in which extra genes can be easily inserted at different positions in the polycistronic viral RNA, and the elongated and flexuous nature of the potyviral particles, which allow packaging a substantial extra cargo, make potyviruses excellent gene expression vectors. We built a recombinant infectious clone, derived from zucchini yellow mosaic potyvirus, which expresses a phytoene synthase from bacterial origin. Synthesis of the colorless phytoene is the first committed step of carotenoid biosynthesis. We observed that tissues from different plant species, infected with this recombinant virus, turn yellow as a consequence of phytoene triggering accumulation of downstream colored carotenoids, such as beta-carotene and lutein. We wondered whether this viral vector could be used to induce carotenoid accumulation in cucurbit fruits. Our work showed how the inoculation of particular leaves of zucchini plants, which nurture pollinated flowers, results in fruits with yellow-orange rind and flesh. Metabolite analyses showed a substantial enrichment in health-promoting carotenoids, such as α- and β-carotene (pro-vitamin A), lutein and phytoene, in both rind and flesh. Considerably higher accumulation of α- and γ-tocopherol was also detected, particularly in fruit rind. This work supports that potyvirus vectors are an alternative to labor-intensive and time-consuming stable genetic transformation for transient expression of foreign and endogenous genes in adult plants and fruits.

Key words: viral vector, potyvirus, ZYMV, carotenoid, gene expression

SESSION 5.
RESISTANCE TO PEST AND DISEASES-1

O5-1 Invited

Disease resistance in Cucurbits: recent progress and future perspectives on the use of plant susceptibility genes

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Cucurbit crops are challenged by a wide range of diseases caused by various pathogens and pests, including fungi, oomycetes and viruses. While tomato leaf curl New Delhi virus (ToLCNDV) is a recent emerging disease causing severe epidemic outbreaks in both greenhouse and open-field cucurbit crops, powdery and downy mildew (PM and DM) are two established important diseases. Although breeding of cucurbit crops, like cucumber, with resistance to both PM and DM started already in the 1940s and 1950s, complete and durable resistance is still not around. Many studies have revealed recessively inherited resistance to PM and DM in cucurbits and numerous quantitative trait loci (QTLs) have been mapped across the entire genomes. Recently, causal genes underlying some of these QTLs were cloned and shown to be loss-of-function mutations of plant susceptibility (S) genes, which explains the recessive inheritance of these resistances to both PM and DM. Currently, we are preparing a review paper in which we summarize the different mapped QTLs and candidate genes for resistance to both mildews and to ToLCNDV, as well as breeding strategies that have been applied so far. By presenting such an overview, we hope to provide opportunities and future perspectives to achieve durable broad resistance in cucurbits against these and possibly other diseases by deploying (edited) plant S-genes.

Key words: Downy mildew, Effector, powdery mildew, ToLCNDV, susceptibility factor

O5-1

Resistance to Cucumber Mosaic Virus: a proteomic approach

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*Cucumber mosaic virus (*CMV) is one of the plant viruses with the broadest host range, infecting important crops such as the Cucurbitaceae family. In *Cucumis melo L*., the Spanish cultivar Piel de Sapo (PS) is susceptible to CMV, while the Korean cultivar Songwhan Charmi (SC) is resistant. The gene *cmv1* confers total resistance to CMV subgroup II (SG II) strains, but not to subgroup I (SG I) strains. CMV SG II strains can replicate and move cell to cell (local infection) but are stopped in the Bundle Sheath Cells (BS) before reaching the phloem, while CMV SG I strains enter the phloem and establish a systemic infection. Therefore, a Near Isogenic Line (NIL) with parental background from PS and an introgression from SC with *cmv1* is able to resist CMV SG II strains but is susceptible to CMV SG I strains. Our objective is to examine the mechanisms underlying resistance or susceptibility to CMV through a proteomic analysis. CMV infection caused large perturbation in the melon leaf proteome of susceptible hosts either in systemic or local infection. Different proteins were significantly expressed in local versus systemic infection. In systemic infection there are two patterns of expression of genes which correspond to resistance or susceptibility of melon cultivars. In systemic infection of susceptible cultivars there are abundant stress related proteins, a decrease in photosynthetic activity, carotenoid biosynthesis and translation and increased oxidation-reduction processes, secretory pathway and proteasome components compared to resistant cultivars. In local infection, all SC cultivar (infected or control) present a differential pattern compared to all other cultivars. Interestingly oxidation-reduction processes seem to also be altered in the first stages of infection and not be a by-product of infection. Moreover, specific membrane components were found which seem to be potentially key for viral cell-to-cell transport. Finally, network analysis allowed finding several hub proteins which are central components during either first or late stages of CMV infection.

Key words: *Cucumis melo*, *Cucumber mosaic virus*, resistance, *cmv1*, proteome

O5-2

Syntenic regions control resistance to *tomato leaf curl New Delhi virus* (ToLCNDV) in cucurbit crops

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ToLCNDV (a whitefly transmitted *Begomovirus*, family *Geminiviridae*), firstly limited to Asia, was detected in Spain in 2012 infecting zucchini (*Cucurbita pepo*) crop. It emerged as a major economic threat to cucurbits growers in the Mediterranean basin, and severe ToLCNDV outbreaks continue causing catastrophic yield losses in some specific growing cycles of the main cucurbits. We started a breeding program for ToLCNDV resistance in cucurbits, screening germplasm collections representing the genetic diversity of the main affected species: zucchini, pumpkin (*Cucurbita maxima* and *C. moschata*), melon (*Cucumis melo*) and cucumber (*Cucumis sativus*). Wild Indian relatives within the *C. melo* and *C. sativus* species and two resistant *C. moschata* accessions from USA and India, crossable to *C. pepo,* were identified as resistant. Genetic studies suggest partial-dominance or recessive regulation of resistance. In *C. melo* and *C. moschata*, a major *locus* located in chr11 and chr8, respectively, has been identified in a syntenic region between both species. These regions include common candidates suggesting a common resistance mechanism that is also being confirmed in cucumber. Besides this major region, minor modifiers and genetic background effects modulate the level of resistance. Combining whole genome sequencing, gene expression studies by RNAseq and fine mapping, using recombinant populations, we have narrowed the candidate region and developed molecular markers useful to transfer the resistance for ToLCNDV to elite breeding cultivars. Agreements: Ministerio de Ciencia, Innovación y Universidades, FEDER funds (RTA2017-00061-C03-03[INIA]), PROMETEO project 2017/078 (to promote excellence groups) of Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana).

Key words: resistance, ToLCNDV, mapping, markers

O5-3

Adaptation of GWAS models for plant virus resistance: from rediscovering major genes to highlighting of new complex traits

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Breeding is an efficient tool to control yield losses caused by viruses. Historically, monogenic resistances have been mapped thanks to recombinant inbred lines populations originating from a cross between a susceptible line and a resistant donor. Resistance breakdown is faster for this type of resistance compared with defense mechanism underlaid by complex genetic architecture. Our objective is then to uncover a rapid and efficient method to identify multigenic resistances and enable it to breeding. Genome-Wide Association Study (GWAS) has proved to be a powerful tool to detect loci associated with quantitative traits. Methods to phenotype plant resistance, such as ELISA or symptom severity scales, result in qualitative or semi-quantitative datasets, which can explain why GWAS have not been frequently used to detect pathogen resistance so far. However, GWAS have the advantage to be quick to design since they are based on diversity panels, which do not require to advance multiple generations. GWAS are highly reproductible and valorize germplasm collections. This project aims to adapt GWA models to virus resistance mapping. We gathered a population representing the genetic diversity of several breeding germplasms of *Cucumis sativus*. We screened the panel with different viruses for which the genetic architecture of resistance was known to be different. As expected, classic GWA models resulted in rediscovering major monogenic resistances. Then, by adapting the GWA models to the original population structure and to the data distribution, we have been able to highlight complex genetic architecture, and hopefully durable resistance. These complex resistances could be used for marker-assisted breeding.

Key words: virus resistance, *C. sativus*, GWAS

O5-4

New Sources of Resistance to Powdery Mildew in Squash and Pumpkin

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In North America, the most pervasive annual disease problem in squash and pumpkin is powdery mildew, caused primarily by *Podosphaera xanthii*, but also by *Golovinomyces cucurbitacearum*. Currently, the *Pm-0* gene is the major commercial source of tolerance to PM in varieties of *C. pepo* and *C. moschata* in North America, but this gene gives only intermediate resistance. We are researching two sources of moderately high resistance to PM, one source from an Australian accession of *C. moschata*, designated Aus-PMR, and the other from a Costa Rican landrace of *C. moschata*, designated OSA-PMR. The Aus-PMR resistance has been introgressed into several *C. moschata* breeding lines and appears to be conferred by a single dominant gene. Three populations, including a testcross and an F_2 population segregating for Aus-PMR along with a dihybrid cross segregating for both Aus-PMR and *Pm-0*, were grown and evaluated at the UNH Kingman Research Farm during the summer of 2019. Plants were inoculated with a liquid suspension of powdery mildew inoculum (ca. 10^5 spores per mL applied to abaxial and adaxial surfaces of two mature leaves and adjoining stems), and then evaluated as either resistant or susceptible. The testcross and dihybrid populations supported a single dominant gene model for inheritance of Aus-PMR. In the dihybrid cross, plants carrying the *Pm-0* allele were identified by a SNP marker, revealing that *Pm-0* segregated independently from Aus-PMR. Acknowledgements: This work was supported by the USDA National Institute of Food and Agriculture, Hatch Projects NH00645 and NH00669-R.

Key words: disease resistance, *Cucurbita moschata*, genetic resources, powdery mildew

O5-5

Deciphering the genetic basis of CYSDV resistance in melon PI 313970

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Cucurbit yellow stunting disorder virus (CYSDV) and *Cucurbit chlorotic yellow virus* (CCYV) are devastating to melon (*Cucumis melo* L.) production in the U.S. desert southwest. Their symptoms on melon are nearly identical. Molecular tools are, therefore, needed to differentiate them. Host resistance is regarded as the most effective method of managing these virus diseases, and to that end we previously identified resistance to CYSDV in melon PI 313970. We evaluated a $F_{2:3}$ Top Mark (susceptible to both viruses) x PI 313970 (CYSDV-resistant, CCYV-susceptible) population for disease severity in response to natural, mixed infections by both viruses in an open field test in order to map resistance to CYSDV. Phenotypic data (foliar yellowing) were not useful for mapping CYSDV resistance QTL, as plants resistant to CYSDV exhibited yellowing symptoms from CCYV infection. QTL analysis of the relative titer of CYSDV calculated from RT-qPCR data identified one locus on chromosome 5 that explained 34-38% of the variation in CYSDV titer. Our result confirmed the previous report of a CYSDV resistance QTL on chromosome 5 in TGR 1551 (PI 482420) based on yellowing symptoms and virus titer. Markers flanking this QTL can be utilized in marker assisted breeding of CYSDV-resistant melons. This study showed the utility of differential virus quantification for genetic analysis of resistance to one specific virus when co-infection by a second virus induces identical symptoms. This work was in part supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award numbers 2015-51181-24285 and 2020-51181-32139.

Key words: *Cucumis melo*, virus, host resistance

P5-1

Resistance to cucumber green mottle mosaic virus (CGMMV) in cucumber

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Cucumber cultivation has great economic relevance both nationally and internationally. Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus inducing a disease affecting cucumber crops worldwide and causing large economic losses. Sources of partial genetic resistance to CGMMV have been described, and genes encoding type 1 RNA-dependent RNA polymerases (RDRs) seem to be the most likely candidates to be responsible for the resistance. Specifically, it appears that a RDR1a/1b gene duplication could be related to CGMMV resistance. In order to confirm if the genetic dose of RDR1a/1b associates with CGMMV resistance, we determined the number of copies and the expression levels of the candidate genes (RDR 1a/b) in symptomatic and asymptomatic CGMMV infected cucumber plants from different cultivars, as well as viral accumulation. An inverse correlation between viral accumulation and the presence of a duplication of the candidate genes was found, as well as a positive correlation between duplication and RDR 1b expression. A second objective was to identify new sources of resistance to CGMMV in cucumber. Six to 8 plants from 300 accessions from the USDA Germplasm Bank were inoculated with CGMMV under controlled conditions; for 8 accessions, all inoculated plants did not to show symptoms after 21 days post-inoculation.

Key words: *Cucumis sativus*, RNA-dependent RNA polymerase, Cucumber green mottle mosaic virus (CGMMV)

P5-2

Melon genome editing with CRISPR-Cas tools to produce varieties resistant to pests and pathogens

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Genome editing using the bacterial clustered regularly interspaced short palindromic repeat (CRISPR)-associated nucleases (CRISPR-Cas) has proven a potent strategy to obtain designer varieties of crop plants with improved agronomical traits or resistant to pests and pathogens. However, in plants, a frequent bottleneck of this technology is an initial transformation step to deliver CRISPR-Cas reagents to cells. For this reason, successful editing must frequently go hand in hand with effective procedures for DNA delivery and plant regeneration by tissue culture. We are currently working in the project EDIMELO to obtain new melon commercial varieties resistant to the tomato leaf curl New Delhi begomovirus, the powdery mildew *Podosphaera xanthii* and the leaf miner insect *Liriomyza sativae*. To this aim, using the endogenous reporter gene that encodes the phytoene desaturase, and whose knock-out mutation yields albino or variegated plants, we have developed a pipeline for CRISPR-Cas editing of melon genome. First, we select potential CRISPR-Cas guide RNAs using computational tools. Next, we build the gene constructs to co-express the guide RNAs and the Cas nuclease using the GoldenBraid cloning strategy. Then, we screen for the most effective guide RNAs by transient assays in melon plants. Finally, we use the selected constructs for *Agrobacterium tumefaciens*-mediated transformation of explants derived from melon cotyledons. The project EDIMELO is funded by the Spanish Ministerio de Ciencia, Innovación y Universidades grant RTC-2017-6023-2 (AEI/FEDER UE).

Key words: genome editing, CRISPR-Cas, resistance, ToLCNDV, powdery mildew, Liriomyza

P5-3

First Report in Spain of Cucurbit chlorotic yellows virus in Cucumber plants

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In winter 2018, whitefly population and symptoms of leaf chlorotic spots and leaf interveinal chlorotic symptoms were observed in cucumber (*Cucumis sativus*) plants in several greenhouses at Almeria, South Spain. The symptomology observed was similar to that caused by whitefly transmitted Cucurbit yellow stunting disorder virus (CYSDV, genera Crinivirus, family Closteroviridae). Samples from four different cucurbit plants, in different locations, were collected and tested, using the multiplex and degenerate primer RT-PCR method, for the presence of Crinivirus. Results indicated that CYSDV, Lettuce infectious yellows virus and Beet pseudo-yellows virus were not found in any of the four samples. In 2004, an emergent Crinivirus (Cucurbit chlorotic yellows virus, CCYV), inducing similar symptoms to CYSDV, was described infecting cucurbits in Japan and, in several oriental Mediterranean countries in 2011 and 2014. The molecular detection of CCYV by RT-PCR, using primers specific to the gene encoding the CCYV capsid protein, indicated the presence of CCYV in the four cucurbit plant samples. BLAST analysis of the obtained sequences (around 336 pb) showed 99% identity in the CP gene of CCYV isolates from Greece (LT992911, LT992910), China (KY400633.1, KX118632) and Taiwan (JF502222). To our knowledge, this is the first report of CCYV infecting cucurbits in Spain. Since the yellowing symptomology induced by CCYV in South Spain is similar to that generated by CYSDV, probably CCYV has been spread throughout the Mediterranean basin, masked by CYSDV symptomology. Detection of the emergent virus CCYV represents a new threat for the horticultural area of South Europe.

Key words: cucumber, criniviruses, CCYV

P5-4

Incidence of cucurbit viruses in Spain during 2019 summer season

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Viral infections are the main threat for cucurbits cultivation. Their effect depends on the region and the year. Spain is one of the main European producers of these crops. However, there are not recent studies about the incidence of specific viruses in this country. The main open field producing areas of melon (Cucumis melo), squash (Cucurbita spp.), watermelon (Citrullus lanatus) and zucchini (Cucurbita pepo) were prospected during the 2019 summer season under organic (PROMETEO/2017/078), as well as commercial farming (AGL2017-85563-C2 1R and 2R, cofunded by FEDER). Plants with virus-like symptoms were analyzed by RT-PCR/PCR, hybridization and/or ELISA, to detect Cucumber mosaic virus (CMV), Zucchini yellow mosaic virus (ZYMV), Watermelon mosaic virus (WMV), Moroccan watermelon mosaic virus (MWMV), Cucurbit yellow stunting disorder virus (CYSDV), Cucurbit chlorotic yellows virus (CCYV), Cucumber green mottle mosaic virus (CGMMV) and Tomato leaf curl New Delhi virus (ToLCNDV). Samples were collected from fields in Comunidad Valenciana (eastern Spain), Castilla-La Mancha (central Spain), Murcia (south-eastern Spain) and Andalucía (southern Spain). Al least one of the viruses was detected in a high percentage of the samples. WMV was the most frequently detected, affecting all the crops and regions studied. ZYMV, CGMMV and ToLCNDV were present with lower incidence. ZYMV was mainly detected in Comunidad Valenciana and CGMMV in Andalucía, whereas ToLCNDV had a wider dispersion.

Key words: melon, *Cucurbita* spp., watermelon, zucchini, virus, WMV

P5-5

Resistance to different Cucumber green mottle mosaic virus strains in melon

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Cucumber green mottle mosaic virus (CGMMV) represents a major risk in the production of melon, watermelon and cucumber. It belongs to the genus *Tobamovirus*, family *Virgaviridae,* and causes systemic mottle and mosaic symptoms on leaves and fruits. Worldwide CGMMV isolates are grouped in two clusters based on biological differences and genome sequences: a first cluster constitutes the European strain, and a second is formed by isolates from Asian countries. Spain is currently the first country where both these strains are present. Commercial melon varieties resistant to CGMMV are unavailable, and in view of the presence of two strains of CGMMV in the same territory, the search for varieties resistant to both is a new challenge for breeding programs. We compared symptom expression and viral accumulation of the European (CGMMV-SP) and Asian (CGMMV-16) strain in 23 melon lines from the Cucurbits breeding COMAV-collection. Fourteen melon lines showed mosaic and leaf deformation and had high viral accumulation following mechanical inoculation with both virus strains. Nine accessions also were highly susceptible to CGMMV-16, but showed moderate symptoms and viral accumulation when infected with CGMMV-SP. The results show that certain melon accessions are more susceptible and show higher viral titer with the Asian CGMMV strain than with the European strain. They highlight the importance of evaluating both strains during the screening of melon accessions for CGMMV resistance. Acknowledgement: We acknowledge the financial support from project RTA2017-00061 from INIA and AGL2017-85563-C2-1-R from MICIIU cofunded with FEDER and PROMETEO project 2017/078 by Generalitat Valenciana.

Key words: resistance, CGMMV, melon, viral titer

P5-6

Resistance to *Cucumber mosaic virus* (CMV) in Near Introgression Lines (NIL) containing two and three Quantitative Trait Loci (QTL) in melon

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Cucumber mosaic virus (CMV) can infect more than 1.200 different plant species belonging to *Solanaceae* and *Cucurbitaceae* families, causing severe damage in leaves and strong fruit lesions. Resistance to CMV has been found in several exotic melon accessions, being one of them the Korean accession "Songwhan Charmi" (SC). A doubled haploid line (DHL) collection was previously developed from a cross between SC as resistant parental and the cultivar Piel de Sapo (PS) as a susceptible parental, in order to study the resistance trait. A Quantitative Trait Loci (QTL) analysis revealed a major QTL *cmvqw12.1* located in the linkage group XII. This QTL confer total resistance to subgroup II strain LS. However, for the subgroup I strains M6 and FNY it is necessary but not sufficient. In addition, two more QTLs were described, *cmvqw3.1* located in LGIII and *cmvqw10.1* in LGX. DHLs containing the three QTLs were resistant to M6, whereas DHLs containing two QTLs, being one of them *cmvqw12.1,* were susceptible to M6 strain. In the present work we have developed Near Isogenic Lines (NILs) containing either two or three QTL. These are a NIL with *cmvqw3.1* and *cmvqw12.1*, a NIL with *cmvqw10.1* and *cmvqw12.1* and a NIL with the three QTL. Our aim is to confirm in the NILs the resistance to CMV-M6 observed previously in the DHLs containing two and three QTLs, in order to know whether the results are consistent in these two different genetic backgrounds.

Key words: Resistance, *Cucumber mosaic virus* (CMV), Doubled Haploid Line (DHL), Near Isogenic Line (NIL)

P5-7

Genetic Variability of Tomato Leaf Curl New Delhi virus in Algeria

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Tomato leaf curl New Delhi virus (ToLCNDV), a member of the genus *Begomovirus*, is an emerging virus transmitted in a persistent mode by the whitefly *Bemisia tabaci that* is rapidly spreading and causing economically important losses since 2013 in cucurbit crops of the Mediterranean basin. In this area, the virus was first detected in Spain and later in Italy, Greece, Tunisia, Morocco, and more recently in Algeria. Molecular characterization of the Mediterranean virus populations has shown that are genetically very homogeneous, and clearly different from Asian virus isolates. However, new variants of ToLCNDV could evolve, thereby affecting the genetic structure of the viral population. To investigate the presence and genetic variability of ToLCNDV in Algeria, where the virus was detected in 2019, 78 samples were collected in the northeastern region of the country (state of Biskra) from zucchini (*Cucumis pepo*), cucumber (*Cucumis sativus*), and melon (*Cucumis melo*) crops. The samples were analyzed by dot-blot and PCR and the complete nucleotide sequence of the genomes of some ToLCNDV isolates of the three species were determined. The ToLCNDV Algerian isolates showed identities higher than 99% with the rest of isolates of the Mediterranean basin, but the DNA-B segment had an extra insertion of 17 nucleotides in the intergenic region. The insertion was found in isolates coming from the three species and although the source of this variation remains unknown, this result suggests that genetic diversity in Mediterranean isolates could appear with time. Acknowledgements to the Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana) for funding PROMETEO project 2017/078 (to promote excellence groups). The FEDER/ Ministry of Science and Innovation for funding the projects AGL2017-85563-C2-1-R and RTA2017-00061-C03-03 (INIA).

Key words: ToLCNDV, *Begomovirus*, genetic variability, cucurbits

P5-8

Inheritance of Resistance to *Papaya ringspot virus* in *Cucurbita moschata* Duchesne

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'Nigerian Local' (NL) and 'Menina' (MEN) are sources of PRSV resistance in *Cucurbita moschata*. Studies report resistance in NL to be a single gene; other studies suggest a more complex inheritance. Similar studies are not reported for MEN. The 3rd to 5th leaf of inoculated seedlings were rated for disease severity, then scores were summed for a 0 to 12 scale. F_2 populations with NL had normal distributions with average severities of 5.4 and 6.0. F_2 populations with MEN were skewed towards resistance with average severities of 3.5, 3.3, and 2.8. The NL x MEN F_2 population was strongly skewed toward resistance, with an average severity of 0.8. We grouped plants with a rating of <4 as resistant and plants with a rating of >5 as susceptible. The best fit in F_2 crosses made with NL was to a 7:9 (R:S) model. All three crosses using MEN fit a 3:1 model. NL x MEN fit a 15:1 model. These segregations suggest that at least two genes are involved in resistance from NL while a single dominant gene is responsible for resistance from MEN. At least some of the resistance genes in NL and MEN are non-allelic. The resistance conferred by MEN is greater than that of NL. If the resistance of MEN is a single dominant gene as this data suggests, then it will be easier to identify resistance markers in MEN compared to NL. Supported by Specialty Crop Research Initiative grant no. 2015-51181-24285 and 2020-51181-32139, USDA-NIFA.

Key words: inheritance, potyvirus, virus resistance

P5-9

Introgression of resistance to ToLCNDV from WM-7 and PI 414723 into traditional backgrounds of *Cucumis melo*

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Tomato leaf curl New Delhi virus (ToLCNDV, genus *Begomovirus*, family *Geminiviridae*) is one of the main threats to melon (*Cucumis melo* L.) crops in Spain since 2012. The symptoms are vein clearing, yellow mottling, crinkling, puckering, and upward or downward curling of leaves, and accompanying poor fruit setting and sterility. The best long term approach to control ToLCNDV is the introgression of genetic resistance. In previous works of the research group, accessions WM-7 and PI 414723 (*C. melo* groups *agrestis* and *momordica*, respectively) were identified as resistant to mechanical inoculation with ToLCNDV. Resistance derived from WM-7 was reported as controlled by one major dominant *locus* in chromosome 11 and two additional regions in chromosomes 2 and 12. PI 414723 also carries resistance to other viruses, such as *Watermelon mosaic virus* and *Zucchini yellow mosaic virus*. In the context of research projects AGL2017-85563-C2 1-R (co-financed by FEDER) and PROMETEO/2017/078, the objective of the breeding program here presented is the exploitation of these sources in the introgression of resistance to ToLCNDV into the main melon types cultivated in Spain. The landraces included in the breeding program belong to the *ibericus* group: 'Piel de Sapo', 'Amarillo', 'Rochet' and 'Blanco' melon types. Snake melon (*C. melo flexuosus* group) is also represented. Some F_3 and BC_2 generations derived from the initial crosses between the resistant sources and the selected melon landraces have been obtained. Advanced breeding lines will be developed, with marker assisted selection (MAS), which incorporate the resistance and carry the landrace genetic background.

Key words: *Cucumis melo*, resistance, ToLCNDV, landraces, MAS

SESSION 6.
RESISTANCE TO PEST AND DISEASES-2

O6-1 Invited

Molecular epidemiology of cucurbit-infecting potyviruses: a rapid turnover of viral strains with a potential impact for resistance breeding

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Viruses represent important threats for cucurbit production worldwide, affecting both the yield and quality of the products. Whitefly-transmitted viruses are now emerging in areas where climatic conditions are favorable for their vectors, and aphid-transmitted viruses remain important in numerous countries, notably in temperate climatic conditions. Long-term surveys and field assays in Southeastern France, associated with molecular epidemiology approaches, have helped to understand better the evolution of populations of aphid-borne viruses, with a focus on the potyviruses watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV). Multiple introductions of new strains have been observed in the last 20 years. In the case of WMV, these new strains can be associated with more severe symptoms. They are now present throughout Europe; in France, they have replaced within less than 10 years the “classic” strains present before. Recombinants between “classic” and emerging strains have also been observed, but they do not appear to present a higher pathogenicity so far. Recent studies have revealed that introductions and partial replacement of molecularly divergent variants are still taking place. The high viral diversity and constantly changing populations of cucurbit viruses should be taken into account in breeding programs, since they can affect the efficiency and durability of resistance genes.

Key words: evolution, recombination, resistance

O6-1

Germplasm release of gummy stem blight resistant lines from a watermelon × citron population

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Gummy stem blight caused by *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*) is a major disease of watermelon in the U.S. The lack of progress in development of resistant cultivars led us to a different approach. Three hundred lines were developed by crossing and intercrossing resistant plant introduction accessions, then crossing the progeny with elite cultivars, then intercrossing those progenies, and finally self-pollinating them. The 300 lines were evaluated for disease resistance and fruit quality traits under greenhouse and field conditions in a randomized complete block design with 10 replications and 3 years. The means and correlations for disease severity ratings and fruit quality traits were estimated. Around 200 RILs had disease severity ratings below the mean value of the disease assessment scale (4.5), indicating that they carry one or more genes for resistance to gummy stem blight. All disease severity ratings were correlated with each other ($r=0.67 – 0.98$, $P < 0.001$) but not with fruit quality traits. We are releasing 15 lines with high resistance and good fruit quality for use by interested breeders. The lines have sweet red flesh, elongate or round fruit shape, striped or gray rind, and large, medium or small seeds that are black, brown or tan.

Key words: Gummy stem blight, watermelon, resistance

O6-2

The Amino Acid Permease (AAP) genes *CsAAP2A* and *SlAAP5A/B* are required for oomycete susceptibility in cucumber and tomato

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Cucurbit Downy Mildew (DM), caused by the obligate biotroph *Pseudoperonospora cubensis*, is a destructive disease in cucumber. A valuable source of DM resistance is the Indian cucumber accession PI 197088 that harbors several QTLs contributing to quantitatively inherited DM resistance. With a combination of fine-mapping and transcriptomics, we identified *Amino Acid Permease 2A* (*CsAAP2A*) as a candidate gene for QTL DM4.1.3. Whole-genome and Sanger sequencing revealed the insertion of a *Cucumis* Mu-like element (CUMULE) transposon in the allele of the resistant line NIL DM4.1.3. To confirm whether loss of *CsAAP2A* contributes to partial DM resistance, we performed TILLING on a DM susceptible cucumber genotype in order to identify an additional *csaap2a* mutant, which indeed was partially DM resistant.

In view of the loss of the putative function as amino acid transporter, we measured amino acids in leaves. We found that DM-inoculated leaves of NIL DM4.1.3 (with the *csaap2a* mutation) contained significantly less amino acids than WT cucumber. The decreased flow of amino acids towards infected leaves in *csaap2a* plants compared to wild type might explain the resistant phenotype of the mutant, as this will limit the available nutrients for the pathogen and thereby its fitness.

To examine whether *AAP* genes play a conserved role as susceptibility factors in plant-oomycete interactions, we made targeted mutations in two *AAP* genes from tomato and studied the effect on susceptibility to *Phytophthora infestans*. We conclude that not only *CsAAP2A* but also *SlAAP5A/SlAAP5B* are susceptibility genes for oomycete pathogens.

Key words: Downy mildew (*Pseudoperonospora cubensis*), cucumber (*Cucumis sativus*), nutrient transport, *Amino Acid Permease* (*AAP*), susceptibility gene

O6-3

Downy Mildew Resistance and Fruit Quality in a Cucumber Recombinant Inbred Line Population derived from Coolgreen x PI 197088

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Downy mildew (DM), caused by *Pseudoperonospora cubensis,* is a devastating foliar disease that attacks several cucurbit species in the Southeast US. Annually, spores are transported via air currents to North Carolina (NC) from infected plants in surrounding states. DM is characterized by yellow to brown leaf lesions that are restricted by leaf veins; dark masses of sporangia are visible on the underside of these lesions. Management of DM relies heavily on host resistance, frequent pesticide applications, and avoidance. A biparental cucumber population derived from Coolgreen x PI 197088 was created in effort to improve fruit quality and DM resistance. The permanent population of 132 recombinant inbred lines (RILs) was developed via the pedigree breeding method. Field trials were conducted in 2015 – 2019 at the Horticulture Field Research Station located in Clinton, NC. RILs, susceptible and resistant parents, and checks were evaluated annually under natural disease pressure. DM disease ratings were conducted weekly for 3 to 6 weeks after disease was first observed. A subjective scale for DM severity was used; 0 to 9 subjective scale, 0 = no disease, 9 = completely diseased. Fruit count was recorded for yield comparisons, fruit length and diameter were also evaluated. Ten RILs were identified as highly resistant over five years in the field, and three RILs were found to be highly susceptible. This population will be available to use for molecular genetic studies to identify genes underlying DM resistance.

Key words: Downy mildew, cucumber, host resistance

O6-4

Charcoal rot (*Macrophomina phaseolina*): From melon and watermelon to other hosts, studying phytopatholocial and genetic aspects in the global warming era

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Macrophomina phaseolina is a soil-borne fungal pathogen inciting charcoal rot disease in more than 500 plant species including melon and watermelon. Disease appearance, incidence and severity is affected by multiple plant and environmental factors including host genetic background, plant maturity, soil and air temperature, and water economy.

The symptoms in melon and watermelon are different. In melon there are typical stem lesions, whereas disease symptoms in watermelon are of the "non-specific vine decline" type expressed by partial wilting and poor foliage accompanied by poorly developed fruits. The non-genetic variation in the response of melon to the disease poses a challenge in breeding for resistance, thus there is a need to develop a reliable screening methodology in order to identify resistant melon germplasm and facilitate breeding for resistance to *M. phaseolina*. Plants of 25 melon accessions were inoculated with *M. phaseolina* and tested in glass and plastic greenhouses, in the field, and as detached branches in the laboratory. Combined multi-environment analysis of disease severity enabled us to separate the accessions into resistant, moderately resistant, moderately susceptible, and susceptible to the disease. Disease response of 15 melon hybrids in comparison to their parental means was also evaluated. In most cases, the hybrids exhibited less disease severity than their respective mid-parent values. The interaction between *M. phaseolina* to various hosts is different in almost every perspective. There is a need to study the nature of specific host–pathogen interaction as a basis for fruitful research. The polygenic nature of the genetic background of all hosts and the important effect of the environment on disease incidence and severity makes this approach very challenging. As environmental conditions are highly significant for charcoal rot development, it should be emphasized that the response of a particular host to the pathogen can be different in other parts of the world in which different climates prevail.

Key words: *Macrophomina phaseolina*, melon, watermelon, genetic variation, symptoms, environment, breeding for resistance

P6-1

Further assessment of ToLCNDV seed transmission in cucurbits

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Begomoviruses (family *Geminiviridae*) cause important diseases in a large number of crop families and are considered as a serious economically and profitability limiting factor in cucurbits. Although these viruses are naturally transmitted by the whitefly *Bemisia tabaci*, recent works have reported them as seed transmissible in several cultivated plant species, including the Spanish isolate of *Tomato leaf curl New Delhi virus* (ToLCNDV) in zucchini. After the emergence of this virus in the Mediterranean basin in 2012, ToLCNDV became one of the main threats to cucurbit crops in the region and implied a turning point in management, control and growing techniques. To determine whether or not seed transmission of ToLCNDV occurs is of fundamental importance for understanding the epidemiology of this major plant-pathogenic virus, but also for developing and implementing control strategies. Thus, the objective of this work was to assess by both conventional and quantitative PCR, whether the ToLCNDV can be detected in reproductive organs and seeds of infected plants from cultivated and wild *Cucumis* and *Cucurbita* species, and to evaluate if viral particle can be transmitted from seeds of infected plants to the offspring seedlings. Also, we have evaluated ToLCNDV presence in seeds and seedlings of commercial cucurbits (zucchini, melon, cucumber and watermelon). Even though seedborne of ToLCNDV is confirmed, our results do not support transmission of this virus from contaminated seeds to the progeny. This work was co-funded by projects RTA2017-00061-C03-03 [INIA], PROMETEO/2017/078 [Generalitat Valenciana] and FEDER funds.

Key words: seed transmission, begomovirus, ToLCNDV, seedlings, qPCR

P6-2

Methods for detection and quantification of four whitefly-transmitted viruses of cucurbit crops during mixed infections.

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Viruses transmitted by the whitefly (*Bemisia tabaci*) are an increasing threat to cucurbit production throughout the world. The crinivirus, cucurbit yellow stunting disorder virus (CYSDV), has severely impacted melon production in California and Arizona since its introduction in 2006. A second crinivirus, cucurbit chlorotic yellows virus (CCYV), and the whitefly-transmitted ipomovirus, squash vein yellowing virus (SqVYV) emerged in the region in 2014. CYSDV, CCYV, and the polerovirus, cucurbit aphid-borne yellows virus (CABYV), occur together in the region and produce identical symptoms on cucurbit plants. Mixed infections of these four viruses challenge breeding for virus resistance and disease management. We developed a rapid, multiplex, single-step RT-PCR method to detect, differentiate and quantify virus titers of these viruses in single and mixed virus infections, which enabled us to identify single, double, and triple virus infections. A quantitative multiplex system was also developed and applied to quantify titers of these four viruses in plant samples. We found differences in virus accumulation within melon plants as well as the presence of two- and three-virus mixed virus infections of these four viruses in California and Arizona. This is also the first report of SqVYV in Arizona. The RT-PCR and RT-qPCR multiplex systems can facilitate resistance evaluations, and studies of host range and virus competitiveness in areas with mixed infections and can be adapted for regions throughout the world with different virus combinations. This work was in part supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2015-51181-24285.

Key words: CYSDV, CCYV, SqVYV, CABYV

P6-3

BSA-seq reveals QTLs associated to ToLCNDV resistance in *Cucurbita moschata*

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Tomato Leaf Curl New Delhi Virus (ToLCNDV) is a whitefly-transmitted begomovirus causing significant yield loss in several *Cucurbitaceae* crops, especially in squash. The use of resistant cultivars is the most efficient way to fight against the virus, since cultural practices for managing whiteflies have not proved very effective. The screening of a large germplasm collection of *Cucurbita* using a two-step protocol that included natural infection with whiteflies and mechanical inoculation, resulted in the identification of one C. *moschata* accession (*BSUAL-252*) with a high resistance to the ToLCNDV. The resistant accession was crossed with the high susceptible *C. moschata* accession *BSUAL-265* and F1 and F2 generations phenotyped by symptom scoring after mechanical inoculation. F1 plants were found to be resistant to the virus, and the 185 F2 tested plants segregated 3:1 (resistant: susceptible), indicating that ToLCNDV resistance in *BSUAL-252* is dominant. Bulked-segregant analysis coupled with whole-genome resequencing (BSA-seq) was used to map the resistance. The DNAs from the 10 most resistant and the 10 most susceptible F2 plants were bulked together and sequenced. BSA-seq analysis revealed 939353 SNP markers distributed across *C. moschata* genome. Five QTLs associated to ToLCNDV resistance were detected on chromosomes 3 (*QTLToLCNDV-C03*), 7 (*QTLToLCNDV-C07*), 11 (*QTLToLCNDV-C11*), 15 (*QTLToLCNDV-C15*) and 16 (*QTLToLCNDV-C16*), as well as a deletion of 474 bp in an *RNA-dependent RNA polymerase* gene on chromosome 15. The resequencing data has provided additional SNPs to narrow down the QTL intervals, and to identify valuable markers that can assist the selection of this resistance in squash breeding programs.

Key words: *Cucurbita moschata*, BSA-seq, QTL, ToLCNDV

P6-4

Screening of melon germplasm resistant to *Fusarium oxysporum* f. sp. *melonis*

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Fusarium wilt is a serious disease causing of damping-off, serious wilt symptom or wither to death in melon. The aim of this study was to evaluate resistance of melon germplasm to Fusarium wilt and select promising disease resistant accessions for further applications. Resistance to Fusarium wilt was examined in 308 melon germplasm by root-dipping inoculation method with Fusarium oxysporum f. sp. melonis fungal isolate KACC43206 (race 2). Roots of 1~2 leaf stage melon seedlings were dipped in spore suspension of 1×106 conidia · mL-1 for 20 minutes. And inoculated seedlings were incubated for 4 weeks and evaluated disease index 1 (no symptom) to 5 (plant wither) based on discoloration of underground parts and severity of stunting every 7 days. 44 accessions had shown no symptom of fusarium wilt 4 weeks after inoculation. These accessions could be expected to help to breeding commercial varieties with Fusarium wilt resistance.

Key words: melon, germplasm, resistance, fusarium wilt

P6-5

Evidence of physiological races of *Podosphaera xhantii* in watermelon in Southern Europe

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Thirteen single-spore isolates of *Podosphaera xhantii* collected from the main commercial growing areas of watermelon in Italy, Spain and France, were characterized based on melon differential set. The differential set indicated that 7 of the13 were belonging to race 1, whereas the remaining isolates were belonging to race 5 (one isolate) and race 3.5 (three isolates). Isolates most virulent were preferentially associated with race 1. Based on field observation and genetic information, we defined a differential set for watermelon, represented by one commercial F1 and six BASF breeding lines. The analysis of these watermelon genotypes over the thirteen *Podosphaera xhantii* isolates, indicated a possible host specialization in watermelon. Line X6 was susceptible to all isolates, whereas X1 showed resistance to all isolates. While X2, X3, X4 lines and the commercial F1 showed similar resistance to 7 isolates, they presented a different pattern for the remaining 6 isolates. This experiment was repeated twice in two different laboratories with consistent results. This is the first indication that physiological races are present in Europe in watermelon, two physiological races have been described previously in USA (1W and 2WF).

Key words: Watermelon, Host range, *Podosphaera xhantii*, physiological races

P6-6

Cucurbit-associated taxa of the *Fusarium solani* species complex not previously detected in Spain

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Watermelon and melon crops are affected worldwide by important soil-borne fungal diseases like Fusarium wilt which causes economic damage in a large number of producing areas. Despite *Fusarium oxysporum* is considered one of the main causal agent of this disease, species of other *Fusarium* complexes can be also associated with this disease in cucurbits. In this work, we present the results obtained in several surveys performed to update epidemiological data on these pathogens that affect melon and watermelon cultivation in the main Spanish producing areas, in order to take a current picture of the actual incidence of these soil diseases in our country. Results reveal that several species of the genus *Fusarium* are the most important soil pathogens in the sampled areas. The most frequently found causal agents of Fusarium wilt have been, apart from the previously detected *F. oxysporum*, several taxa of the so-called *F. solani* species complex, like *Neocosmospora falciforme*, *N. keratoplastica,* and *N. petroliphila*, not previously described in Spain. This work was supported by grant AGL2017–85563-C2–1,2 which was partly funded by the ERDF.

Key words: Fusarium wilt, *Neocosmospora*

P6-7

Initial studies on transcriptional response of cucumber to *Pseudomonas syringae* pv. *lachrymans* infection

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One of the factors limiting cucumber (*Cucumis sativus* L.) open-field production is angular leaf spot disease usually caused by *Pseudomonas syringae* pathovar *lachrymans* (Psl). To understand better molecular mechanisms involved in cucumber response to Psl infection we performed transcriptome profiling of two cucumber lines Gy14 and B10 characterized by contrasting response to this pathogen. Plants of Gy14 (resistant line) and B10 (susceptible line) were inoculated with highly virulent Psl strain 814/98 under growth chamber conditions. For RNA isolation plant tissue was collected before inoculation, one- and three-days post inoculation (0, 1, and 3 dpi). Illumina platform was used for RNA-seq. Transcriptional differences between two inbred lines were revealed. Resistant line Gy14 showed massive transcriptomic response to Psl one day after inoculation comparing to susceptible line B10, while similar number of differentially expressed genes (DEGs) was detected for both lines three days post inoculation. Most of the investigated DEGs were classified to metabolic pathway, biosynthesis of secondary metabolism and plant-pathogen interaction. Several transcription factors belonging to different families (e.g. AP2-EREBP, WRKY, NAC and MYB) were differentially expressed. This study provides transcriptomic data for cucumber infected with *P. syringae* pv. *lachrymans* and helps to elucidate angular leaf spot resistance mechanism.

Key words: angular leaf spot, *Cucumis sativus*, RNA-seq

P6-8

Genetic loci associated with resistance to zucchini yellow mosaic virus in *Cucurbita moschata*

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Zucchini Yellow Mosaic Virus (ZYMV), an aphid transmitted potyvirus, causes severe yield losses in Cucurbita production worldwide. Development of virus-resistant cultivars using traditional breeding approaches relies on rigorous and resource-intensive phenotypic assays. QTL-seq, a whole genome resequencing based bulked segregant analysis is a powerful tool for mapping quantitative trait loci (QTL) associated with a trait. In the current study, an F2 population (n =174) derived from a cross between Nigerian Local (resistant) and Butterbush (susceptible) was mechanically inoculated with ZYMV, and disease ratings recorded at 35 days after inoculation (DAI). Whole genome resequencing was conducted for the parents and bulks of resistant and susceptible F2 progeny (each, n = 10). Mapping rate across the samples varied from 94.04 to 98.76 % with final effective mapping depth ranging from 81.77 to 101.73. Alignment of bulks onto the consensus reference genome revealed 1,916,964 SNP's. QTLseq analysis identified four QTLs significantly (P< 0.05) associated with ZYMV resistance on chromosome (Chr.) 2 (QtlZYMV-C02), 4 (QtlZYMV-C04), 8 (QtlZYMV-C08), and 20 (QtlZYMV-C20). Fourteen SNP's and twelve indel markers were genotyped and tested for association with ZYMV resistance in the F2 population. Among these, one SNP on Chr. 8, and three SNP's and one Indel marker on Chr. 20 were significantly (P = 0.05) associated with resistance to ZYMV. Thirty-six resistant gene homologs were found across the four QTL intervals. The outcomes of this study will facilitate marker-assisted selection for ZYMV resistance in Cucurbita moschata.

Key words: squash, disease resistance, potyviruses, breeding, marker-assisted selection

SESSION 7.
FLORAL AND FRUIT DEVELOPMENT

O7-1 Invited

Genome selections drive the evolution of delicious fruit in watermelon

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Watermelon is an important horticultural crop. It has been improved by domestication and breeding from wild watermelons to modern watermelons carrying delicious fruits. Based on the improved reference genome and the resequencing of 414 accessions, genomic loci associated with fruit quality traits such as flesh sweetness, bitterness and flesh color were identified by GWAS. Population genomic analyses reveal that the loci affecting watermelon fruit quality have been under selection during speciation, domestication and improvement. We identified *ClAGA2* gene as the key factor controlling phloem stachyose and raffinose hydrolyzing. Investigation of knockout plants confirmed that *ClSWEET3* and *ClTST2* control fruit sugar accumulation. Selection of the cascade of *ClAGA2*, *ClSWEET3* and *ClTST2* in carbohydrate partitioning during evolution leads to the orchestrated derivation of modern sweet watermelon from non-sweet ancestors. Population genomic analyses strongly suggest a single-base change at the coding region of *ClVST1* as a major molecular event during watermelon domestication, which results in the amino acids truncation and intracellular translocation in sweet watermelons. Red-fleshed watermelons have been selected and domesticated from the pale-fleshed ancestors. Lycopene β-cyclase is the critical node controlling watermelon flesh color. Two missense mutations were selected and largely fixed in domesticated watermelon. Further evidences indicated that the missense mutations within *ClLCYB* contributed to the red flesh color in domesticated watermelon by regulating the stability and abundance of ClLCYB protein. The systematic illuminations of these genome selections and the involved gene mutations provide the novel insights of the evolution of the delicious fruit and further molecular breeding in watermelon.

Key words: watermelon, fruit quality, evolution, sweetness, flesh color

O7-1

A unique chromosome translocation disrupting *ClWIP1* leads to gynoecy in watermelon

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The polymorphism of sex determination in angiosperms is determined by the presence/absence of the three sexual flower types, male, female and hermaphroditic, and their distributions. *Cucurbitaceae* have become model plants in sex determination research because cucurbits can have all seven of the sex forms found in angiosperms. To understand sex determination in watermelon (*Citrullus lanatus*), a spontaneous gynoecious watermelon mutant, XHBGM, was selected from the monoecious wild type XHB. By map-based cloning, resequencing and fluorescence *in situ* hybridization (FISH) analysis, a unique chromosome translocation between chromosome 2 and chromosome 3 was found in XHBGM. Based on the breakpoint location in chromosome 2, a putative C_2H_2 zinc finger transcription factor gene, *ClWIP1* (gene ID: *Cla008537*), an orthologue of the melon gynoecy gene *CmWIP1*, was disrupted. Using the CRISPR/Cas9 system to edit *ClWIP1*, we obtained gynoecious watermelon lines. Functional studies showed that *ClWIP1* is expressed specifically in carpel primordia and is related to the abortion of carpel primordia in early floral development. To identify cellular and metabolic processes associated with *ClWIP1*, we compared the shoot apexes transcriptomes of two gynoecious mutants and their corresponding wild types. Transcriptome analysis showed that differentially expressed genes related to the ethylene and cytokinin pathways were upregulated in the gynoecious mutants. This study explores the molecular mechanism of sex determination in watermelon and provides a theoretical and technical basis for breeding elite gynoecious watermelon lines which should benefit the application of gynoecy in watermelon breeding.

Key words: watermelon (*Citrullus lanatus*), sex determination, gynoecy, *ClWIP1*, chromosome translocation, transcriptome

O7-2

Two induced EMS mutations conferring parthenocarpy in *Cucurbita pepo*

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The application of phytohormones is currently the most widespread method to induce fruit set and development in squash greenhouse productions. This method favours the accumulation of chemical residues, which remain in the field and in the fruit throughout the food chain. The development of parthenocarpic varieties would be a good alternative for fruit setting in absence of pollination and hormonal treatments, promoting so a safer and more environmentally friendly production. A high-throughput screening of an EMS mutant collection of *C. pepo* resulted in the identification of two mutants with a high level of parthenocarpy: *lox* and *aco1*. The two mutations also disrupt the development and maturation of petals, and *aco1* promotes the conversion of monoecy into andromonoecy. For detecting the causal mutation of the phenotypes, M2 mutant plants were crossed twice with the background genotype MUC16, and the resulting BC2S1 segregating populations were subjected to a BSA-seq analysis. Two DNA bulks derived from either 30 WT or 30 mutant plants from each segregating population were subjected to WGS and the resulted reads mapped against the *C. pepo* reference genome. The identified putative causal mutations were then validated in more than 300 plants from segregating populations by using Kompetitive allele-specific PCR (KASP) genotyping technology. A mutation in the coding region of a *LIPOXYGENASE* gene (*LOX*) co-segregated with the *lox* mutant phenotype. On the other hand, the *aco1* mutant co-segregated with a missense mutation in the coding region of *ACC OXIDASE 1* gene (*ACO1*). The mutation resulted in the amino acid substitution P5L in ACO1, an enzyme involved in ethylene biosynthesis. The involvement of ethylene and JA in floral and fruit development, and the possible use of these two mutants in squash breeding programs, is discussed.

Key words: sex determination, parthenocarpy, *LOX, ACO1, C. pepo*

O7-3

Validation of the differential expression of zucchini genes during fruit formation

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Cucurbita pepo L has increased considerably its production in the whole world in the last decades. Spain, the second exporting country worldwide, support this production with cultivation mainly in the province of Almería. Currently, European legislation is aimed at prohibiting hormonal treatment in crops, but the application of synthetic hormones on female flowers is necessary to obtain an economically profitable production. To avoid this problem, it is necessary searching for vegetative parthenocarpic cultivars. However, in zucchini, the genetic factors that induce parthenocarpy are still unknown. An RNA seq approach was focus in the analysis of fruit transcriptome of two cultivars of zucchini, a non-parthenocarpic cultivar and a parthenocarpic cultivar, in an attempt to identify key parthenocarpic genes. A comparison between transcriptome of the unpollinated fruit for each cultivar has been performed determining that 6120 genes were differentially expressed. Analysis of gene annotation of these DEGs revealed that cell cycle, regulation of transcription, carbohydrate metabolism and coordination between auxin, ethylene and gibberellin were enriched biological processes during pollinated and parthenocarpic fruit set. From this panel differential expressed genes, five genes were selected by the homology with significant functional genes involved in fruit formation. The differential expression was confirmed by qPCR of five candidate genes, DVL (ROTUNDIFOLIA/DEVIL), SGR9 (E3 ubiquitin-protein ligase) , EIN3 (ETHYLENE INSENSITIVE 3), EBF2 (EIN3-binding F-box protein 2) and ARL (ARGOS like), is studied under different treatments, non-pollinated fruits, pollinated fruits and fruits treated with synthetic hormones during the process of fruit development, from day zero after anthesis (DPA), until day 6 DPA, when the fruit has already reached its commercial size.This research was done with financial support from project AVA2019-00063 from IFAPA.

Key words: parthenocarpy, fruit development, *Cucurbita pepo*

O7-4

ETHQV8.1, a new player in melon fruit ripening

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Fruit ripening is an essential physiological process in plant development, and has an important impact in fruit quality and post-harvest storage. Fleshy fruits are classified as climacteric, when a peak of ethylene and respiration occurs at the onset of ripening; or non-climacteric, when ripening is not related to an increase of autocatalytic ethylene. Melon (*Cucumis melo* L) is an ideal crop for the study of fruit ripening, due to the coexistence of both behaviors within the species. A major quantitative trait locus (QTL) for climacteric fruit ripening, *ETHQV8.1*, was previously detected in chromosome 8, using a Recombinant Inbred Line (RIL) population derived from "Védrantais" (*cantalupensis*, highly climacteric) and "Piel de Sapo" (*inodorous*, non-climacteric). Using the same cultivars, we developed and evaluated two introgression lines (ILs) carrying reciprocal introgressions covering the *ETHQV8.1* region. The non-climacteric allele in a climacteric background delayed and decreased the production of ethylene. Moreover, the introgression of the climacteric allele in a non-climacteric background caused a weak climacteric behavior. To fine-map *ETHQV8.1*, we crossed the IL in the climacteric background with "Védrantais". After the evaluation of a set of recombinants identified in the F2 population, the QTL was narrowed down to a 114 kb region containing 11 annotated genes. The most likely candidates for *ETHQV8.1* are *MELO3C024518* and *MELO3C024516*, encoding the proteins *serine/threonine-protein kinase CTR1-like* and *ROS1*, respectively. In this work, we present a new QTL involved in climacteric ripening, and provide the basis for the validation of the candidate genes underlying this QTL in future experiments.

Key words: *Cucumis melo*, ripening, QTL, ethylene

P7-1

Screening of a mutant collection of *Cucurbita pepo* for valuable flower and fruit agronomic traits

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One of the main goals of breeding is to identify useful agronomic traits for a changing and demanding market. In this work, we present the result of a direct screening of an EMS mutant collection of *C. pepo* to identify valuable traits for agriculture. One hundred and twenty M2 families with 8 plants each, were transplanted to the greenhouse at seedling stage with 2-3 leaves and visually assessed until 45-50 leaves stage. This evaluation allowed the identification of multiple phenotypes such as albinism, changes in leaf morphology, alterations in plant architecture, and changes in flower and fruit development, confirming the worthy mutation rate previously observed at cotyledon stage in the collection. Three mutants were selected for their agronomic interest in flowering and fruit development. Two of the mutants were disrupted in female flowering transition while the third was characterized by the development of parthenocarpic fruits. The phenotypes were confirmed in M3 and M4 generations. In parallel, M2 mutant plants were crossed with either the background genotype MUCU16 or with the Scallop 'VCU196' line, and then selfed to obtain the BC_1S_1 generations. The analysis of at least 100 plants of BC_1S_1 segregating populations confirmed a recessive inheritance mode of the three mutant phenotypes. These segregating generations are currently being used to identify the causal mutation of the phenotypes by using a BSA-sequencing approach, which will also allow designing suitable markers to introgresa the mutant phenotypes to other materials in *Cucurbita* breeding programs.

Key words: mutant collection, *Cucurbita pepo*, flowering, parthenocarpy

SESSION 8.
PRODUCTION AND QUALITY

O8-1 Invited

Genomic resources applied to understand melon fruit quality

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Morphology (external and internal colour, shape, netting, sutures), aroma, nutritional content, sweetness, acidity, ripening and post-harvest storage are complex traits that contribute to the final fruit quality that is demanded by the consumer. Melon shows a broad, still underexploited diversity in fruit quality. The availability of a wide array of genomic resources in melon is contributing to advance in our understanding of the processes that control fruit quality. In recent years, many loci involved in the genetic control of these traits have been described, information that has started to be implemented in melon breeding programs. Once the genes underlying these traits are identified, the use of natural variation found in germplasm collections or induced variation through genome editing are promising ways to further improve fruit quality.

Key words: Genomic resources, fruit quality, natural variation, fruit ripening

O8-1

Fine mapping of the *Mt-2* gene controlling mottled rind in melon

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As an important vegetable crop worldwide, melon (*Cucumis melo* L.) exhibits a broad diversity in fruit morphology. For example, the mottled to non-mottled rind trait, which somewhat influences the consumer habits, is still underexploited in melon breeding programs. In this study, we found that mottled rind was caused by high density protoplast in fruit skin. Based on our previous studies, a genotyping-by-sequencing (GBS)-based genetic mapping showed that a dominant gene (*Mt-2*) in the distal part of LG II confers the mottled rind phenotype in the "Piel de sapo" *inodorus* melon type. To further characterize this gene, we selected 1000 F_2 individuals of a Ved x PS cross for fine mapping. By screening the F_2 population with two flanking SNPs, chr02_25876579 and chr02_27023404, 86 individuals containing recombination events in the interval were identified. Next, we genotyped in these recombinants by 12 SNPs that distributed in the interval, and with some progeny tests finally delimited *Mt-2* to a ~80kb physical region, flanked by SNP13 and SNP14 and containing 10 candidate genes. Two of these candidate genes were annotated and reported to localize at protoplast. In order to identify the candidate gene, we will look for the genomic variations, as well as genes expression in the ~80kb interval. The identification of the *Mt-2* gene may be of interest to melon researchers and breeders.

Key words: Melon, mottled rind, fine mapping, fruit morphology

O8-2

Underground Heterosis for Melon Yield

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Heterosis, the superiority of hybrids over their parents, is a major genetic force associated with plant fitness and crop yield enhancement. Understanding and predicting heterosis is crucial for evolutionary biology, as well as for plant and animal breeding. We investigated root-mediated yield heterosis in melons (*Cucumis melo*) by characterizing common variety grafted onto 190 hybrid rootstocks resulting from crossing 20 diverse inbreds in a diallel-mating scheme. Hybrid rootstocks improved yield by more than 40% compared to their parents and the best hybrid outperformed the reference commercial variety by 65% under both optimal and minimal irrigation treatments. To characterize the genetics of the underground heterosis we conducted whole-genome re-sequencing of the 20 founder lines, and showed that parental genetic distance was no predictor for the level of heterosis. Through inference of the 190 hybrids genotypes from their parental genomes, followed by genome-wide association analysis, we mapped multiple root-mediated yield QTLs. The yield enhancement of the four best-performing hybrid rootstocks was validated in multiple experiments with four different scion varieties. While root biology is receiving increased attention, most of the research is conducted using plants not amenable to grafting and, as a result, it is difficult to separate root and shoot effects. Here, we use the rich genetic and genomic resources of *Cucumis melo,* where grafting is a common practice, to dissect a unique phenomenon of root-mediated yield heterosis, by directly evaluating in the field the contribution of the roots to fruit yield. Our grafting approach is inverted to the common roots genetics research path that focuses mainly on variation in root system architecture rather than the ultimate root-mediated whole-plant performance, and is a step towards discovery of candidate genes involved in root function and yield enhancement.

Key words: *Cucumis melo*, grafting, GWAS, half-diallel, rootstock, Whole-genome resequencing

O8-3

Identification of fruit-associated QTL in winter squash (*Cucurbita maxima* Duchesne) using recombinant inbred lines

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Cucurbita maxima pumpkins and squashes are commonly grown vegetables produced for fresh market and processing industry. Fruits of *C. maxima* are characterized by great phenotypic diversity including morphological and biochemical aspects. *C. maxima* fruit characteristics and yield are the traits of economic importance and interests for breeders. However, genetic bases of fruit-associated traits in this species are poorly understood. In this study we evaluated fruit-associated traits in *C. maxima* and performed quantitative trait loci (QTL) identification using recombinant inbred lines (RILs). RILs were developed from the cross of two winter squash inbred lines characterized by contrasting fruit types. Using previously developed advanced genetic map we identified over 20 QTL for fruit traits, including fruit weight, length, width, fruit flesh thickness, sucrose and dry matter content. The QTL were found on the 8 chromosomes of *C. maxima*. Major effect QTL for multiple fruit-associated traits were clustered on the lower arm of chromosome 4, suggesting that this genomic region has been under selection during diversification of *C. maxima*.

Key words: *Cucurbita maxima*, fruit traits, recombinant inbred lines, QTL

O8-4

Breeding quality melons with resistances derived from African accession TGR1551

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The *Cucumis melo* ssp. *agrestis* African accession TGR-1551 has been reported as resistant to three of the most important pathogens affecting melon: *Watermelon mosaic virus* (WMV), *Cucurbit yellow stunting disorder virus* (CYSDV) and powdery mildew (*Podosphaera xanthii*). The availability of a population of recombinant inbred lines in the genetic background of the yellow melon 'Bola de Oro' (BO), allowed the mapping of the genomic regions associated to the resistance to these pathogens and the development of molecular markers for marker assisted selection (MAS). In the context of the research projects funded by the 'Ministerio de Ciencia, Innovación y Universidades' cofunded by FEDER (AGL2017-85563-C2 1-R and 2-R) and by the 'Generalitat Valenciana' (project for excellence groups (PROMETEO 2017/078), we have initiated the breeding program for the introgression of the resistance in the genetic background of commercial melon varieties. MAS was used in advanced backcross generations to select plants carrying the regions associated with the resistance. With the aim of recovering the quality traits of the recurrent parent, 'Bola de Oro', selection against the TGR-1551 background was done for the rest of the genome, using previously developed markers uniformly distributed in the 12 chromosomes. The molecular markers associated to the resistance regions were validated by phenotyping for resistance and genotyping selected generations. Moreover, fruits from the resistant plants showed good morphological characteristics and organoleptic quality. Similar work is in progress for the introgression of resistance in the 'Piel de Sapo' background. The generations available will be the basis for the development of quality commercial varieties incorporating TGR1551-derived resistances.

Key words: *Cucumis melo*, organoleptic quality, MAS

P8-1

Genetic dissection of aroma production in a melon RIL population

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Aroma is an essential trait in melon fruit quality, but the metabolic pathways involved are poorly understood. The aim of this study was the identification of quantitative trait loci (QTLs) involved in volatile organic compounds (VOCs) biosynthesis in melon fruit rind and flesh. A Recombinant Inbred Line (RIL) population obtained from the cross 'Piel de Sapo' (PS) x 'Védrantais' (VED), segregating for ripening behavior, was analyzed by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). A total of 82 VOCs and 166 QTLs were identified, showing differences between climacteric and non-climacteric lines. A major QTL cluster in chromosome 8, including 92 QTLs, collocated with the previously described ripening QTL *ETHQV8.1,* which might play an important role in VOCs biosynthesis. Other collocations with previously described QTLs were detected, and interesting clusters were identified in chr02, chr05, chr06 and chr11. An aminotransferase and an aldehyde oxidase were proposed as candidate genes involved in ethyl 3-(methylthio) propanoate and benzaldehyde metabolic pathways, respectively. This work provides the basis for future fine-mapping projects, and for the validation and characterization of candidate genes involved in aroma biosynthesis in melon.

Key words: aroma, climacteric ripening, *Cucumis melo* L., GC-MS, QTL mapping, VOCs

P8-2

Use of molecular markers in *Cucurbita pepo*: quality assessment for hybrid production

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Cucurbita pepo L. is considered the most economically important species within its genus and "summer squash" types rank among the highest-valued vegetables worldwide. The diffusion of summer squash, mainly zucchini, has increased in many countries in the last years and this is mainly due to the ease cultivation, the short crop cycle and the wide adaptability to different climates. The development of summer squash hybrid varieties can be upheld by the use of molecular markers. Morphological differences between true hybrids, self-plants and off-types in grow out test are not always easily detectable because of the laborious screening and environmental influences. Genetic and genomic tools are essential to obtain crops with desirable traits in the market place and to define quality assessment, which is one of the main criteria for obtaining a successful production. Among different markers systems developed to test the purity of hybrids and parental lines, High Resolution Melting (HRM) analysis is becoming one of the best choices in terms of efficiency, speed and costs. In this study we performed a genetic characterization of about 20 zucchini lines which vary in terms of colour and fruit shape, using a panel of different molecular markers (SNPs and SSRs) analysed by HRM technology and we selected a few markers to verify the stability of our parental lines and the related hybrid's purity.

Key words: Cucurbita pepo, purity, molecular markers, HRM

P8-3

Deciphering fruit flesh colour in melon

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Fruit flesh colour in melon (*Cucumis melo*) is a major attribute of fruit quality related to the accumulation of carotenoids and chlorophylls. This character is determined by two *loci, green-flesh (Gf)* and *white flesh (Wf)*, *Gf* being epistatic over *Wf. Gf* is located in chromosome 9 and has already been identified as *CmOr*, a gene that induces an orange phenotype associated to beta-carotene accumulation. *Wf* is in chromosome 8, being responsible of white and green fruit flesh phenotypes. A major quantitative trait locus (QTL) for fruit flesh colour corresponding to *Wf* (LUMQU8.1) was previously mapped in chromosome 8 using a Recombinant Inbred Line (RIL) population derived from the melon cultivars "Védrantais" (*cantalupensis*, highly climacteric, orange flesh) and "Piel de Sapo" (*inodorous*, non-climacteric, white flesh). The QTL interval contained 32 annotated genes. To identify the *Wf* gene in this interval, we searched for candidates with a high and predominant expression in fruit flesh and a functional link to chloroplasts (e.g. presence of a chloroplast transport peptide of predicted role in a chloroplast-associated processes). Gene expression was confirmed by qPCR analyses of melon fruit samples at different developmental stages, whereas chloroplast localization and function was tested by transient expression of "Védrantais" and "Piel de Sapo" alleles in *Nicotiana benthamiana* leaves followed by analysis of chlorophyll and carotenoid content and in photosynthetic activity. The results with some of the candidate genes, including some previously proposed in the literature to be *Wf*, will be presented.

Key words: Cucumis melo, flesh, colour, chlorophyll

P8-4

A Dudaim introgression line collection onto Piel de Sapo background: A tool for the analysis of aroma compounds in melon

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Melon (*Cucumis melo* L.) aroma is an important aspect of fruit quality. This trait has an important role in melon flavor, which influences the consumer's preference. We have developed an introgression lines (ILs) collection, composed of 16 ILs, using the Queen's Pocket melon accession (Dudaim group) of Irak as the exotic donor parent and the Spanish cultivar 'Piel de Sapo' (Ibericus group) as the recurrent parent. Dudaim melons have an ornamentally use, due to their high fragrance, being inedible melons with low sugar content and climacteric behavior. The Volatile Organic Compounds (VOCs) accumulation is slightly higher in their skin than in their flesh, presenting high VOCs accumulation in both tissues compared to other melon groups. The IL population was genotyped by *Agena Bioscience* platform (with 107 SNPs) distributed throughout the melon genome to tag Dudaim introgressions. Several candidate ILs were further genotyped by *Genotyping by Sequencing*. A Dudaim introgression located on chromosome 6 was associated with external aroma production. The carrier IL (DUD_6-2) of this Dudaim genomic region also presented abscission layer, which is linked to a climacteric behavior. Nevertheless, another IL, DUD_3-1, presents abscission layer, but no external fragrance. The observed differences in the ripening behavior and aroma production found within this ILs collection makes it an interesting tool to study the genetics of melon aroma, and to develop prebreeding materials with different VOCs profiles. Acknowledgments: This work was supported by Plant KBBE project (SAFQIM: MINECO/ PIM2010PKB-00691), by the Spanish Ministerio de Ciencia, Innovación y Universidades (grants AGL2017-85563-C2-1-R and RTI2018-097665-B-C2) (jointly funded by FEDER) and by PROMETEO project 2017/078 (to promote excellence groups) by the Conselleria d'Educació, Inves-tigació, Cultura i Esports (Generalitat Valenciana).

Key words: Introgression Lines, *Cucumis melo*, volatile organic compounds, aroma, ripening behavior

SESSION 9.
NEW CULTIVARS

O9-1 Invited

Main typologies and markets of melon, cucumber and watermelon: major traits of interest for breeding new varieties

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Limagrain Group.

Cucurbitaceae is an important plant family in human alimentation and even more when considering the tendency of reducing meat consumption in our diet. *Cucurbitaceae* is represented by various major crops, such as melon, cucumber, watermelon, squash, pumpkins, gourds, etc… which are extensively cultivated all around the world. When talking about breeding for new varieties, several elements must be considered that can be summarized by a single question: what is the demand? If the question is simple, the answer is complex. Indeed, by demand, we are referring to producers, distributors and consumers needs in a wide range of environmental conditions, production methods and socio-cultural behaviours, all together representing a dynamic system that evolves constantly. Plant breeding aims to answer those needs with the best integrated scientific approach in the shortest delays. After a brief presentation of the international agricultural cooperative group Limagrain and its business units HM. CLAUSE/ Hazera / Vilmorin-Mikado, an introduction of the global challenges faced when breeding for new varieties will be presented and an overview of specificities for melon, cucumber and watermelon crops will be illustrated. For each crop, the main typologies according to the value of markets will be introduced and the respective major traits of interest will be described including disease resistances, fruit quality, abiotic stress tolerances and agronomical traits. Doing so, we aim to provide to the audience more insights into Cucurbits private research targets to provide sustainable solutions to answer food challenges, through the release of adapted and performing varieties.

Key words: new varieties, new traits

O9-1

Selection programme of a 'Muscat'-type variety of *Cucurbita moschata* for improved performance and uniformity

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A selection programme of an open pollinated variety of the 'Muscat' varietal type of *Cucurbita moschata* was undertaken to develop materials with better agronomic performance, higher uniformity, high soluble solids content (SSC), and a fruit weight between 1.5 and 2 kg. Starting from six masal selections performed by FlorFruits, 185 plants were grown and 15 of them were selected and selfed. A minimum of 10 plants of the offspring of each selected plant were grown. Twenty cultivars and accessions of *C. moschata* and two of *C. pepo* were used for comparison. All materials were characterized using 24 morphological descriptors and 28 SSR markers. In addition, fruit shape of selections and 'Muscat' type controls was evaluated by using the Tomato Analyzer. A wide diversity was observed among the materials for morphological traits, but the selections, together with some 'Muscat'-type controls clustered together in the multivariate analyses performed. The SSR analyses revealed unique SSR profiles for each of the materials and a differentiation of the 15 selections, which clustered together, from the phenotypically similar controls and from the rest of materials. The genotyping of several plants within selection revealed some heterozygosity, but in general plants from the same selection clustered together. Using the morphological characterization one of the selections with outstanding performance, phenotypic uniformity and conforming to the ideotype was finally chosen. This selection can be differentiated from the controls and other materials both at morphological and molecular levels and has been sent to registration as new cultivar.

Key words: *Cucurbita moschata*, descriptors, selection, SSR markers, new cultivar

O9-2

New promising mini melon lines from different genetic backgrounds

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Piel de Sapo, a non-climacteric melon type of the Ibericus group, and *Charentais*, a climacteric one of the *Cantalupensis* group, are of great economic importance worldwide, but especially in the Mediterranean area. Therefore, the development of new varieties of these market classes with new characteristics in response to consumer demands is one of the most important goals of melon breeders, together with the search of the stabilisation of production using resistance genes. Small-fruited melons are highly desired in current societies, as families tend to be smaller and individual consumption is more frequent. In this sense, the construction of two introgression line collections generated from the Spanish Piel de Sapo (PS) and the French Charentais 'Vedrantais' (Ved) using the exotic germplasm Dudaim and Makuwa as donor parentals respectively, has given rise to the obtention of very interesting pre-breeding lines of small melons (mini melons). Derived lines from DUD_1-2, DUD_2-1 and DUD_4-2 with PS background, and MAK_1-1 and MAK_6-2 with Ved background, bearing few introgressions from the donor parentals based on genotyping-by-sequencing analysis, present a significant decrease in fruit weight compared to the PS and Ved controls. In addition, other quality traits were significantly altered in some of these pre-breeding lines in the environments assayed, such as rounder melons in DUD_4-2 with respect to the PS, which also can attract consumers searching for novel shapes, or the earlier ripening in MAK_6-2 with respect to Ved. Other traits like sweetness, aroma or ripening behaviour remained without differences, retaining most of the characteristics that identify and define the Piel de Sapo and Vedrantais market classes. These lines could be of great interest to develop new consumer-attractive cultivars.

Key words: *Cucumis melo*, new characteristics, fruit quality traits, size and shape, introgression lines

O9-3

CMV-resistant melons for the western United States

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Cucumber mosaic virus (CMV) is a production constraint for cucurbits world-wide and has recently resurged in western U.S. melon (*Cucumis melo* L.) production regions. CMV-resistant cultivars adapted to western U.S. production regions are not available, although resistance to CMV in melon has been known since 1943. CMV resistance from 'Freeman Cucumber' (Group *Conomon*) was partially introgressed to western U.S. melons in the 1990s by Cornell University. CMV and watermelon mosaic virus (WMV) were abundant in the Central Valley, California in 2018 and 2019. Six of 25 Cornell lines exhibited resistance to a CMV subgroup I isolate in mechanically inoculated greenhouse tests in 2018. Offspring from two of the six lines were nearly all resistant to CMV subgroup I (1/16) in a naturally infected field test in 2019; one plant had low virus titer as determined by quantitative triple antibody sandwich ELISA. Offspring from a mostly CMV-resistant line in the greenhouse (1/9) had high virus titer in the field (2/2). An asymptomatic plant in a line that segregated for CMV resistance in the greenhouse (6/9) produced offspring that were CMV-free (0/4) in the field. All plants in the field were WMV-positive. Seven progenies from three Cornell lines showed promise in the field for continued introgression of CMV resistance to elite western U.S. melons; two of them were notable for early fruit set. This work was in part supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2015-51181-24285.

Key words: *Cucumis melo*, *Cucumber mosaic virus,* virus titer, host resistance

O9-4

Development of multi-disease resistant melon (*Cucumis melo*) cultivars through marker-assisted selection

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Melon (*Cucumis melo* L.) is an important agricultural crop that is grown and consumed around the world. Numerous pathogens infect melon reducing potential yield and quality. MR-1, a multi-disease resistant inbred melon line was crossed to the Israeli cultivar 'Ananas Yok'neam' to develop a recombinant inbred line population segregating for resistance to many diseases. The RIL population was genotyped with GBS to map resistance QTL for Fusarium wilt races 1 and 2, powdery mildew race 1 and sulfur phytotoxicity. Kompetitive allele specific (KASP) markers were designed for each major QTL using whole genome resequencing data of both parents. Here, we will present an overview of our melon breeding scheme to develop cultivars resistant to multiple diseases through marker assisted selection. This work was partly supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award numbers 2020-51181-32139 (CucCAP II) and 2015-51181-24285 (CucCAPI).

Key words: host-plant resistance, *Cucumis melo*, marker-assisted breeding, QTL

P9-1

New Watermelon Cultivars with High Contents of Lycopene and Citrulline

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Vegetable is common source for functional substances in daily life. As the house income increases, the awareness of healthy diet has been raised. To follow the consumption patterns, breeding companies focus on developing a cultivar with functional properties and they need to evaluate many fruits in the early stage of breeding. Therefore, we improved evaluation methods of lycopene and citrulline for mass assessment and developed watermelon cultivars with high contents. There are several methods utilize HPLC and UV spectrophotometer with cuvette. However, they require specialized machines and/or lots of time. To save the time and efforts, we improved the evaluation methods with microplate substituted for cuvette. It could shorten the analysis duration 10 and 35 times compared to the method with cuvette and HPLC respectively. Strong correlation was showed between the results from microplate and UPLC with 0.6304(lycopene) and 0.7211(citrulline) as the Pearson's correlation coefficient. Through selection breeding with microplate analysis, we developed high lycopene cultivar, 'Wonye509ho', and high citrulline cultivar, 'Wonye510ho'. We had evaluated functional substances along with other fruit quality traits every generation and selected plants with higher contents for 10 generations each. 'Wonye509ho' was circular shaped fruit with red flesh no stripes. The lycopene content was 57.43mg/kg fresh weight which indicated 1.7 times higher than commercial variety in Korea. 'Wonye510ho' has broad elliptic shape with yellow flesh. The citrulline contents was 5.13mg/g fresh weight which indicated 2.2 times higher than commercial variety. (PJ01421402)

Key words: watermelon, lycopene, citrulline

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