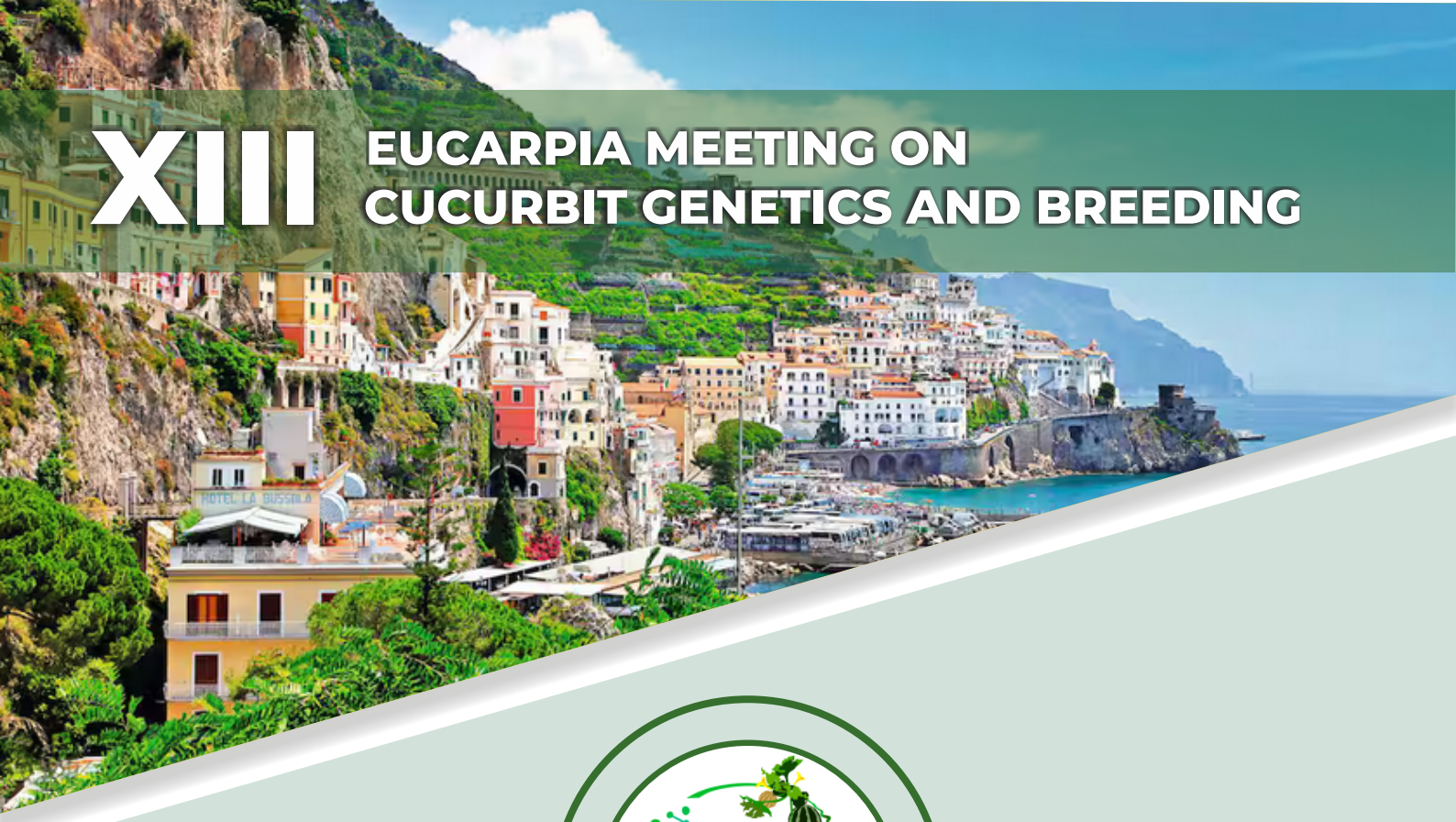

XIII EUCARPIA MEETING ON CUCURBIT GENETICS AND BREEDING

3 - 6 NOVEMBRE 2024

VICO EQUENSE (NA) - ITALY

ABSTRACT BOOK

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We thank Prof. Andolfo for having contributed to the drafting of the abstract book

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SCIENTIFIC PROGRAM

SUNDAY, NOVEMBER 3rd

14:30-18:00	Registration and Poster Setup
18:30	**OPENING CEREMONY**
18:30-18:45	Welcome and Opening of the Meeting
18:45-19:05	**Nadia Ficcadenti** (Invited Speaker): *Melon Genetic Research in Italy*
19:05-19:45	**Antonio J. Monforte (Invited Speaker)**: *Introgression Lines in Melon Genetics Research*
19:45	**WELCOME COCKTAIL**

MONDAY, NOVEMBER 4th

Session 1:
CONSERVATION AND SUSTAINABLE USE OF GENETIC RESOURCES
Chairperson: Rebecca Grumet, Jiaxing Tian

09:00-09:40	**Ulrike Lohwasser (Invited Speaker)** *Plant Genetic Resources of Cucurbitaceae*
09:40-09:55	**Catherine Dogimont** *The selection of new valuable alleles during the melon domestication process in Sudan*
09:55-10:10	**Carlos Romero** *Phenotypic analysis of a Cucumis F2 interspecific population segregating for reproductive barriers*
10:10- 10:25	**Shigita Gentaro** *Museomics-based analyses reveal new crop wild relatives in the genus Cucumis*
10:25-10:40	**Concetta Lotti** *Management and valorization of germplasm of Apulian typical unripened melon*
10:40-11:00	**Harry S. Paris (Invited speaker)** *Southern Italy: Nativity of the Cocozelle Squash (Cucurbita pepo L. subsp. pepo, Cocozelle Group)*
11:00-11:30	**Coffee break and poster viewing (Session 1)**

Session 2:
GENOMICS APPROACHES FOR IMPROVING CUCURBIT CROPS
Chairpersons: **Grzegorz Bartoszewski, Concetta Lotti**

11:30-12:10	**Liu Wenge (Invited Speaker)** *Genetic Analysis of Nutrition, Texture and Flavor of Watermelon Fruits*
12:10-12:25	**Manuel Jamilena** *Development of a TILLING platform as a reverse genetic approach for functional genomics and plant breeding in Cucurbita pepo*
12:25-12:40	**Haibin Wu** *Luffa: Genome Sequencing, Germplasm Innovation, and Functional Gene Cloning*
12.40-12.55	**Shahar Nizan** *Expression of the melon NLR gene complement in response to multiple pathogens*
12:55-14:15	**Lunch Break**

Session 2:
GENOMICS APPROACHES FOR IMPROVING CUCURBIT CROPS (continued)

14:15-14:30	**Shuxia Chen** *Molecular mechanism analysis of aldehyde aroma in cucumber fruit*
14:30-14:45	**Daniele Liberti** *QTL stacking in Cucumis sativus to optimize resistance to ToLCNDV*
14:45-15:00	**Amit Gur** *Pan-genome and multi-parental framework for high-resolution trait dissection in melon (Cucumis melo)*
15:00-15:15	**Marta Pujol** *ETHQV8.1, encoded by ethylene-responsive transcription factor ERF024, regulates chromatin associated proteins before the onset of fruit ripening in melon*
15:15-15:30	**Prerna Sabharwal** *Exploration of Novel Genetic Resistance to Powdery Mildew in Cucurbita pepo Using Genome-Wide Association Studies*
15:30-16:00	**Coffee Break and Poster viewing (Session2)**
16:00-16:15	**Flavia Mascagni** *Comparative genome-wide analysis of repetitive DNA and its structural proximity to functional sequences in the genus Cucurbita*

16:15-16:30 **Jiaxing Tian**
Global identification of fruit-related noncoding RNAs in pumpkin

16:30-16:45 **Gregory Inzinna**
Mapping a Novel Resistance to Powdery Mildew in Cucurbita moschata Development of Markers for Varietal Improvement

16:45-17:00 **Rita Dublino**
Unraveling powdery mildew resistance in Cucurbita pepo: a transcriptomic and genomic exploration of two contrasting cultivars

TUESDAY, NOVEMBER 5th

Session 3:
PLANT RESPONSE TO BIOTIC AND ABIOTIC STRESS

Chairpersons: **Belen Pico, Giuseppe Andolfo**

08:30- 09:10 **Yuling Bai (Invited Speaker)**
Impairing plant susceptibility genes: what did/can we gain in cucurbits for resistance breeding

09:10-09:25 **Henk Schouten**
DNA primase large subunit is an essential plant gene for geminiviruses, putatively priming viral ss-DNA replication

09:25-09:40 **Shallu Thakur**
Genome editing strategies for improved powdery mildew resistance in cucurbits

09:40-09:55 **Ana Montserrat Martín-Hernández**
Niemann-Pick C1 protein - A new player in Cucumber Mosaic virus infection in melon

09:55-10:10 **Kevin Crosby**
Assessment of fruit quality and disease resistance in cantaloupe (Cucumis melo L.) hybrids developed at Texas A&M

10:10-10:25 **William M. Wintermantel**
Emergence of watermelon chlorotic stunt virus and its impact on virus population structure and infection dynamics in southwestern U.S. melon and watermelon production

10:30-11:00 **Coffee Break and Poster viewing (Session3)**

11:00-11:15 **Božena Sedláková**
Application of a new differential set for virulence study on Czech cucurbit downy and powdery mildew populations

11:15-11:30	**Onofrio Davide Palmitessa** *NFT with supplementary light as a technique to extend the production period of 'Scopatizzo' (Cucumis melo L.), even through the use of brackish water*
11:45-11:45	**Amnon Levi** *Genomic Prediction of Resistance to Fusarium Wilt (Fusarium oxysporum f. sp. niveum race 2) in Watermelon Using Parametric and Non-Parametric Approaches*
11:45-12:00	**Alejandro Flores-León** *Evaluation of cucumber (Cucumis sativus L.) for Drought Tolerance in Growth Chamber and Field Conditions*

12:00-13:30	**Lunch Break**
13:30	Departure for Excursion by Bus
19:00	Return to Hotel
20:30	Social Dinner

WEDNESDAY, NOVEMBER 6th

Session 4:
QUALITY TRAITS IMPROVEMENT
Chairpersons: Antonio Monforte Manuel, Jamilena Quesada

09:00-09:40	**Bhimanagouda S. Patil (Invited Speaker)** *Evaluating Sensory Attributes and Health-Promoting Compounds in Hybrid Melon Varieties Across Different Cultivation Regions of the United States of America*
09:40- 09:55	**Rebecca Grumet** *Mining the cucumber core collection for genetic control of fruit quality traits*
09:55-10:10	**Jie Zhang** *Identification of flesh color controlling genes in watermelon*
10:10-10:25	**Xiaoxi Liu** *Fine mapping of McTu4.1 controlling fruit wart in bitter gourd*
10:25-10:40	**Cecilia Martínez** *GWAS and BSA-seq approaches reveal several genomic regions and candidate genes regulating carotenoid content in Cucurbita pepo fruit*

10:40-11:10 Coffee Break and Poster viewing (Session4)

Session 5:

INNOVATIVE TECHNIQUES FOR BREEDING

Chairpersons: **Sara Sestili, Shuxia Chen**

11:10-11:50	**Abdelhafid Bendahmane (Invited Speaker)** *Leveraging Translational Biology to Enhance Plant Breeding*
11:50-12:05	**Hiroshi Ezura** *In planta Particle Bombardment (iPB): A novel gene editing technology for efficient breeding of cucurbit crops*
12:05-12:20	**Yong Xu** *Application of Molecular Breeding in Watermelon*
12:20-12:35	**Geoffrey Meru** *Genetics and breeding of the hull-less seed pumpkin in Cucurbita*
12:35-13:00	**Closing Remarks**

ORAL COMMUNICATION

OPENING LECTURE

OP.0.1

Melon genetic research in italy

Nadia Ficcadenti and Sara Sestili

Council for Agricultural Research and Economics (CREA) - Research Centre for Vegetable and Ornamental Crops, Via Salaria 1, 63077 Monsampolo del Tronto (AP), Italy

Cucurbitaceae are cultivated in almost all Italian regions as spring-forcing crops, semi-late, open field or under tunnel and also as winter crops under greenhouses. It includes about 900 species representing a precious reservoir of genetic material for genomics studies and a source of nutraceutical compounds being commonly used in diets . Melon made its first appearance in Italy during the period of the Roman Empire. In Italy, 25.500 ha of melon surface are cultivated with an average yield of 30.8 t/ha for a total production of 786 000 t (ISTAT data, 2023). Three important botanical varieties of melon are cultivated: the summer types (var. *reticulatus* and *cantalupensis*) and winter types (var. *inodorus*) mostly widespread in the southern regions. Italian breeding program aims at (i) improving several pure lines of all botanical varieties different for morphological and organolectical characters (ii) introducing resistance genes to the main disease. Research in Italy is carried on by seed companies interested in F1 hybrids having economically important traits and public research institutes. At CREA, the research activity started almost 25 years ago, by focusing on the resistance mechanism toward *Fusarium oxysporum* f.sp. *melonis* race 1,2 (FOM1,2) by using conventional and biotechnological breeding strategies. In 1995 through the parthenogenesis *in situ* technique, we developed a DH line named NAD e which is highly resistant to all races of FOM. This was the starting point for understanding the FOM-melon interaction mechanism and to discover the genes involved in the resistance. Furthermore NAD, is protected by plant rights CPVO/TQ-104/2-Rev and is suitable to be used as rootstock. . The results obtained over the years represent an important goal in the FOM resistance study and open new possibilities to use the CRISPR/Cas9 system to disclose which genes are involved in the resistance

OP.0.2

Introgression lines in melon genetics research.

Cristina Esteras[1], Maria José Gonzalo[2], Belén Picó[1], Juan Pablo Fernandez-Trujillo[3,4], Montserrat Martín[5,6], Marta Pujol[5,6], Jordi Garcia-Mas[5,6], Antonio J. Monforte[2]

[1] Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València (UPV), Valencia, Spain

[2] IBMCP Instituto de Biología Molecular y Celular de Plantas, CSIC/Universidad Politécnica de València, Ciudad Politécnica de la Innovación—Edificio 8E Ingeniero Fausto Elio, s/n, E-46022 València, Spain

[3] Department of Agronomical Engineering, Technical University of Cartagena (UPCT), Paseo Alfonso XIII, 48. ETSIA, E-30203 Cartagena, Spain

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[5] Center for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Edifici CRAG, Campus Universitat Autònoma de Barcelona (UAB), Bellaterra, E-08193 Barcelona, Spain

[6] Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Edifici CRAG, Campus UAB, Bellaterra, E-08193 Barcelona, Spain

Genomic libraries of introgression lines (ILs) consist of genetic lines containing different chromosomal introgressions from a donor genotype, within the same genetic background. Ideally, these introgressions represent the genome of the donor genotype. Typically, the donor genotype is an "exotic germplasm," such as a wild relative, a landrace, or an old traditional variety. The advantages of this strategy include the "Mendelization" of quantitative genetics and the incorporation of exotic variability into breeders' pools. The first IL library was constructed using the Korean cultivar “Songwan Charmi” (SC, PI 161375) as the donor and “Piel de Sapo” (PS) as the genetic background. Since then, through a collaborative effort involving IRTA-CRAG, UPV-COMAV, and IBMCP (CSIC-UPV) groups, five additional IL libraries have been developed: three with the PS background and two with the “Vedrantais” background. These IL libraries have been extensively phenotyped over recent years, primarily for fruit quality traits such as fruit morphology, color, ripening and postharvest behavior, sugar accumulation, and volatile composition. Other traits, including flowering earliness, root morphology, and disease resistance, have also been evaluated. Numerous QTLs have been identified, with effects ranging from low to very high, and some QTLs exhibiting opposite effects. The interaction between QTLs and genetic backgrounds has also been studied. In some cases, QTLs have been finely mapped, and the underlying genes identified, such as a NAC domain transcription factor, a negative regulator of ripening CTR1-like, and a putative DNA demethylase ROS1 involved in climacteric ripening, the vacuolar protein sorting 41 inducing recessive resistance to Cucumber Mosaic Virus, and a member of the OVATE gene family that modifies fruit shape. These IL libraries demonstrate the power of this approach in dissecting the genetic control of complex traits in melon and understanding the high phenotypic diversity among its varieties.

ORAL SESSION N. 1
K.1.1

Plant genetic resources of cucurbitaceae – maintenance, taxonomy and availability

Ulrike Lohwasser & Andreas Börner

Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben (IPK), Genebank Department, Corrensstrasse 3, D-06466 Stadt Seeland, Germany

Globally germplasm collections contain more than 5.8 million accessions of plant genetic resources. The crops with the largest number of accessions maintained ex situ are wheat, rice and barley, with a combined total of over 1.6 million accessions. Figures on the entire Cucurbitaceae family, which comprises 101 genera and 965 species, are difficult to find. For the main genera *Cucurbita* and *Cucumis* 70,232 accessions are maintained worldwide. The largest collections are in Europe with 18,674 accessions (https://www.fao.org/wiews/data/ex-situ-sdg-251/overview/en/). All the collections comprise wild and primitive forms, landraces as well as old and more recent cultivars of cultivated plants. The German *ex situ* genebank is one of the ten largest global collections worldwide with 151,000 accessions. 2,668 accessions of different cucurbit species are available. In general, one of the big problems is the identification and the taxonomy of the samples.

The genepool of some *Cucurbita* species is very well known but if the accessions are not taxonomically described the use for breeding is very difficult. And many of the species are not available in genebanks or only with a very few numbers of accessions. A gap analysis would be helpful in order to start collecting missions. Another topic is the availability. Due to international regulations like International Treaty of Plant Genetic Resources for Food and Agriculture (ITPGRFA) and Nagoya Protocol the access to material is getting more and more complicated. But nevertheless, screening of such collections shows a wide range of variability and gives the opportunity to find new breeding material.

O.1.1

The selection of new valuable alleles during the melon domestication process in sudan

Arthur Wojcik[1,2], Josefina Wohlfeiler[1,2], Stella Huynh[3], Nebahat Sari[1], Vincent Rittener-Ruff[4], Chelle Carlos[1,4], Aimeric Agaoua[1], Sonia Elbelt[1], Jacques Lagnel[1], Lucie Tamisier[1], Cécile Desbiez[4], Adnane Boualem[5], Abdelhafid Bendahmane[5], Sylvain Glémin[6], Yves Vigouroux[3], Maud I. Tenaillon[2], Catherine Dogimont[1]

1 INRAE Génétique et Amélioration des Fruits et Légumes GAFL, Montfavet, France

2 Univ Paris Saclay, INRAE, CNRS, AgroParisTech, Génétique Quantitative et Evolutive GQE, Gif Sur Yvette, France

3 Univ Montpellier, IRD, CIRAD, UMR DIADE, Montpellier, France

4 INRAE Pathologie Végétale PV, Avignon, France

5 Univ Paris Saclay, Univ Evry, Inst Plant Sci Paris Saclay IPS2, CNRS, INRAE, Gif Sur Yvette, France

6 ECOBIO UMR 6553 UR1 CNRS, Rennes, France

Cucumis melo genetic resources have been collected from all over the world. However, in Africa, melon genetic resources are still poorly known and their value underestimated. Our aim was to identify new traits and alleles in the gene pool of East Africa, known as a center of melon domestication. To this end, 20 wild populations and 20 domestic landraces collected in Sudan and maintained at INRAE Vegetable Germplasm Center were phenotyped for vegetative, flower and fruit traits and for virus resistance. They were resequenced using pair-end Illumina reads and mapped onto the Charmono reference genome. We have shown that wild and domestic melons have diverged on several vegetative and reproductive traits. They occupy distinct phenotypic spaces. Wild melons are branched vines with small leaves that produce numerous small bitter fruits. They are generally monoecious (male and female flowers). Domestic Tibish melons are vigorous, late flowering plants that produce medium-sized fruits with a low sugar content. They are generally andromonoecious (male and hermaphrodite). While a single mutation in the *CmACS7* gene, responsible for the sexual transition from monoecious to andromonoecious, has spread to most cultivated groups worldwide, the andromonoecious landraces of Sudan carry a functional monoecious allele of *CmACS7*. Using a positional cloning strategy, we isolated the *A2* gene responsible for this new andromonoecy and found that the sexual transition was associated with a deletion in the promoter of the *CmHB40* transcription factor. After inoculation with watermelon mosaic virus (WMV), some Sudanese accessions showed very mild symptoms. We showed that they carried a rare allele of the *CmVPS4* gene, responsible for WMV resistance in melon. Our results highlight that melon domestication in Sudan has led to the selection of specific traits and alleles, which are of great interest for melon breeding.

O.1.2

Phenotypic analysis of a *cucumis* f_2 interspecific population segregating for reproductive barriers.

David Bernabeu[1], Carme J. Mansanet[1], Belén Picó[2], Antonio J. Monforte[1], Carlos Romero[1] and María Ferriol[3]

[1] Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-Universitat Politècnica de València, Valencia, Spain,[2] Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València,Valencia, Spain [3] Instituto Agroforestal Mediterráneo, Valencia, Universitat Politècnica de València, Valencia, Spain

Interspecific reproductive barriers (IRBs) contribute to reproductive isolation in *Cucumis* L. by a complex network that may include incompatibility and incongruity overlapping processes (1). Thus, IRBs in *Cucumis* prevent genetic exchange between wild species and, particularly, with the cultivated ones (e.g. melon and cucumber). Understanding of the genetic basis controlling IRBs in *Cucumis* could be of great help in overcoming them and using wild germplasm for breeding. To this end, we have obtained an F_2 segregating population (90 individuals) from the self-fertilization of an interspecific hybrid derived from a cross between *C. prophetarum* and *C. anguria* species that show unilateral cross-incompatibility (UCI). Phenotyping of pre- and post-zygotic IRB is being performed by recording pollen tube growth using fluorescence microscopy and by evaluating fruit and seed set, respectively. Preliminary results confirm that UCI behaves as a dominant trait in this cross and show significant variability among the individuals comprised within this population regarding the rejection/acceptance of *C. prophetarum* pollen. Notwithstanding, further research will be necessary to use these data in order to map QTLs associated with IRBs in *Cucumis*.

O.1.3

Museomics-based analyses reveal new crop wild relatives in the genus *Cucumis*

Gentaro Shigita, Edgardo M. Ortiz, Alina Höwener, Hanno Schaefer

Technical University of Munich, Dept. Life Science Systems, Germany

Recent advances in plant genome editing technologies, represented by the CRISPR-Cas system, have enabled more precise, efficient, and flexible mutagenesis, even in non-model species. This progress brings to reality the long-held hope of introducing unique and innovative traits from wild species into crops without crossbreeding. At the same time, classical taxonomic and phylogenomic research is required to identify overlooked crop wild relatives as genetic resources for research and breeding.

The genus *Cucumis* currently comprises about 60 accepted species, including two major crops of global importance: melon (*C. melo*) and cucumber (*C. sativus*). According to the WFO Plant List (https://wfoplantlist.org), more than 300 species have been described in the genus to date, of which more than 200 are valid names.

In this study, we obtained type specimens of most of these validly described names from European herbaria and sequenced them using a combination of genome skimming and target capture sequencing of approximately 1,000 nuclear genes, including some related to agricultural traits. The targeted gene sequences were *de novo* assembled and aligned across the samples using the Captus pipeline (https://github.com/edgardomortiz/Captus) to infer phylogenetic relationships. We find that all currently accepted species are indeed distinct genetic entities, but several of them represent polyphyletic groups that comprise overlooked species. Among the names currently treated as synonyms, we discovered several genetically and geographically distinct taxa. Divergence times between species, ancestral states inferred from the trait-related genes, and potential polyploidization and interspecific hybridization events will also be discussed.

This study is conducted as part of the Taxon-OMICS project (SPP 1991) funded by the German Research Foundation (DFG).

O.1.4

Management and valorization of germplasm of Apulian typical unripen melon

C.Lotti[1], L. Ricciardi[2], F. Arcieri[2], M. Cannarella[2], C. Delvento[2], A. Giancaspro[1], A.G. Giudice[2], M .Guerriero[2], S. Pavan[2]

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[2]Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari, Via Amendola 165/A, 70126 Bari, Italy

The Apulia region (Southern Italy) is a secondary diversity center for many horticultural crops, where local populations and landraces and landraces are still propagated by farmers. Although they may have a significant economic impact for the area, they usually provide niche productions and are at risk of genetic erosion. A typical Apulian vegetable production is represented by unripen forms of melon (*Cucumis melo* L.), known as "carosello", "barattiere", or other folk names ("scopatizzo", "meloncella", "spuredda"). Their fruit are consumed fresh and raw, in salads or without dressings, and appreciated for their refreshing and organoleptic properties, their digestibility and nutritional value.

In the last decade, in the framework of several Regional Programs, a germplasm collection of 19 populations of "carosello" and "barattiere" was established at the GeneBank of the University of Bari Aldo Moro, which was characterized at the genetic and phenotypic levels. Here, we present molecular and morpho-agronomic data on these populations. Digital files were obtained for each population, with description of morpho-agronomic traits based on descriptors proposed by the International Union for the Protection of New Varieties of Plants (UPOV); molecular variation was assessed by genotyping by sequencing (GBS) and used to carry out parametric and non-parametric analysis of population structure. This revealed clear-cut differentiation among accessions named as "carosello and "barattiere". Accessions classified as "scopatizzo", "meloncella", "spuredda" were clustered together the group of "carosello". Overall, the results of this study provide elements for the taxonomical classification of within *C. melo*, and highlight superior genotypes which might be valorised as such or in breeding programs.

Currently, we are performing a fine-scale characterization of a wider germplasm, including 30 populations, using a whole-genome resequencing approach.

O.1.5

Southern Italy: Nativity of the Cocozelle Squash (*Cucurbita pepo* L. subsp. *pepo*, Cocozelle Group)

Harry S. Paris

Agricultural Research Organization, Newe Ya'ar Research Center (retired), present address: P. O. Box 6114, Yoqne'am 2065626 Israel

Cocozelle squash are the most elongate fruits of *Cucurbita pepo*. Their length-to-broadest-width ratio is at least 3.5, often much more, and they can attain a length of 75 cm at maturity. Besides being longer, cocozelle squash differ from the more cosmopolitan zucchini by not having a more-or-less uniform diameter along the entire length of the fruit. Instead, cocozelles are bulbous toward the distal end and obviously narrower at the mid-portion or stem end. Moreover, most cocozelle cultivars bear pistillate flowers that are much larger than those of any zucchini, and these are often sold freshly picked on the day of flowering. The word *cocozelle* is derived from a southern Italian dialect, being a diminutive of *cocuzze*, the bottle gourd. As the word indicates, cocozelle squash are mostly derived from southern Italy, though there are cocozelle cultivars and landraces that are native to other parts of Italy as well as other countries on the north shore of the Mediterranean Sea. The earliest-known illustrations of cocozelle squash are from the late-16th century and resemble the fruits of the strongly ribbed 'Romanesco'. The first description was by the Swiss botanist Johann Bauhin, in the 17th century. Several cocozelle fruits were illustrated in the 1770s by the father of *Cucurbita* taxonomy, the French scientist Antoine-Nicolas Duchesne, the most stunning illustration showing a section from a plant bearing a nearly fully grown, extremely long, bulbous striped fruit resembling those of 'Striato d'Italia' and 'San Pasquale'. 'San Pasquale' was mentioned by name by the Italian horticultural scientist Filippo Re in 1811. The cultivar-group Cocozelle is represented by dozens of open-pollinated cultivars of wide phenotypic and genetic diversity. Landraces from Italy, Spain, France, Turkey, and North Macedonia are preserved in genebanks in Europe and North America. Among the numerous commercially available cocozelle hybrids are 'Ortano' and 'Aquilone'.

ORAL SESSION N. 2

K0.2

Genetic Analysis of Nutrition, Texture and Flavor of Watermelon Fruits

Liu Wenge, *Zhu Hongju, He Nan, Lu Xuqiang, Yuan Pingli, Anees Muhammad, Gong Chengsheng, Zhao Yong, Yang Dongdong*

Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou, Henan 450009, China

As one of the world's top five fruits, watermelon fruit is rich in sugar, vitamins, amino acids, carotenoids and other functional nutrients beneficial to the human body, and it is a good product to eliminate the summer heat. The study of the genetic basis of watermelon fruit quality traits is important for meeting the increasing consumer demand and breeding new watermelon varieties with high nutritional and fruit qualities. Using natural and genetic populations as experimental materials, our team constructed the first metabolome database of watermelon fruits through a wildly targeted metabolomics approach. Based on watermelon genome resequencing data, combined with multiple omics analyses (HPLC, SPEM-GC-MS, BSA-Seq, GWAS, RNA-Seq), the stepwise selection and regulation mechanisms of major taste and flavor quality traits during the evolution of watermelon was revealed, the accumulation pattern of volatile organic compounds in watermelon fruits and the molecular basis of genetic variation were systematically investigated. The accumulation pattern and transcriptional regulation dynamics of carotenoids in watermelon fruits were explored. The key candidate genes that regulate the flesh hardness, flesh sourness, flesh bitterness, and amino acid content of watermelon fruit were excavated and verified, and a series of new watermelon varieties with nutritional flavors were selected and bred. This study lays the foundation for the improvement of watermelon fruit quality and the breeding of new high-quality watermelon varieties.

O.2.1

Development of a TILLING platform as a reverse genetic approach for functional genomics and plant breeding in *Cucurbita pepo*

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A TILLING platform has been developed in *Cucurbita pepo* as a reverse genetic approach to elucidate the function of genes, but also as a source of genetic resources for plant breeding. A total of 1,200 M2 lines of an EMS mutant zucchini squash collection, which is made up of 3,751 lines, were subjected to whole genome resequencing (WGS). The DNA of each 12 lines was combined into 100 pools and then sequenced in PE150 reads, generating 40 Gb per pool, which represents an average depth of 160 reads per genomic position. Bioinformatic variant calling analysis has identified a total of 52,000 high-impact and 730,000 moderate-impact mutations on the exome, the most frequent being G>A and C>T transitions. This massive sequencing project allowed the construction of a database containing 8-10 impactful mutations for most *C. pepo* genes, including mutations conferring resistance to critical cucurbit diseases, tolerance to abiotic stress, sex determination and parthenocarpy, and increasing fruit quality parameters such as postharvest chilling tolerance, and accumulation of nutraceutical compounds such as carotenoids and cucurbitacin. To validate the platform, several *in silico* mutations detected in genes of ABA biosynthesis, virus resistance, abiotic stresses, and carotenoid content were validated by genotyping M2 and M3 populations using KASP technology. The utility of the interesting agronomic traits conferred by these mutations in squash breeding is discussed.

0.2.2

Luffa Genome Sequencing, Germplasm Innovation, and Functional Gene Cloning

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Luffa is an important cultivated vegetable and medicinal plant in the family Cucurbitaceae. There are nine different species of the Luffa genus, of which only *Luffa acutangula (L.)* Roxb. and *L.cylindrica (L.)* Roem. are domesticated. In this study, the draft genome sequences of the *L.cylindrica* inbred line P93075 and *L.acutangula* inbred line S1174 were analyzed. Using Illumina, PacBio, and 10× Genomics sequencing techniques as well as new assembly techniques such as FALCON and chromatin interaction mapping (Hi-C), two chromosome-scale genomes were generated. The assembled 656.19 Mb genome of P93075 was obtained, and 25,508 protein-coding gene loci were identified. The 776.49 Mb genome of S1174 was assembled and has 27,405 genes. In terms of genome and protein-coding genes, S1174 (*L. acutangula*) has 120.3 Mb and 1897 more protein-coding genes than P93075 (*L. cylindrica*). Subsequently, we completed resequencing of 325 important luffa germplasm resources. In terms of germplasm innovation, we have created a new batch of important germplasm materials by constructing the chromosome segment substitution lines (CSSLs), EMS-induced mutations, and r-ray induced mutations. Based on the above research, we cloned important agronomic trait genes such as dwarf phenotype, daily flowering time, short vine, fruit length, Pericarp color, and resistance to powdery mildew. These results will greatly promote the development of molecular markers assisted breeding for luffa.

O.2.3

Expression of the melon NLR gene complement in response to multiple pathogens

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Resistance genes of the Nucleotide Binding and Leucine-Rich Repeats (NLR) family are organized across the genome in clusters, pairs, or single genes. In our research, we focused on the melon TIR-*NLR* genes *Fom-1* and *Prv*, situated in a head-to-head orientation at a single genetic locus. These genes confer resistance against *Fusarium oxysporum f. sp. melonis* (FOM) races 0 and 2, and to papaya ring spot virus (PRSV), respectively. We studied their expression and observed correlated spatial pattern of expression from a bidirectional promoter. Using CRISPR/Cas9 mutagenesis in melon we successfully demonstrated the function of *Prv* and *Fom-1*. Additional mutagenesis experiments are under way to probe the possible interaction between the two neighbor genes. Protein-interaction essays suggest that the two might differ in specificity. While certain NLR family members act as specific sensors, others were proposed as more general "helper NLR", and we asked whether this could be reflected also by their expression patterns. We thus turned to the complete NLR family repertoire of melon, and annotated 43 full-length NLRs, comprising 25 TNLs (TIR-NB-LRR), 15 CNLs (CC-NB-LRR), and 3 RNLs (RPW8-NB-LRR), the majority of which (65%) reside in clusters. Using an automated qRT-PCR microfluidic device, we quantified *NLR* transcripts in two multi-pathogen resistant melon genotypes, PI414723 and MR-1, and susceptible Charéntais-T, in response to six common melon pathogens. These included two potyviruses (PRSV, and Zucchini Yellow Mosaic Virus, ZYMV), three fungal pathogens (FOM races 1 and 2, and *Podosphaera xanthii* race 1, which causes powdery mildew), and an oomycete, *Pseudoperonospora cubensis*, which causes downy mildew. We observed varied *NLR* expression patterns, uncovering possible links to SA and JA defense pathways, responsiveness to different pathogens, and apparent degree of specificity.

O.2.4

Molecular mechanism analysis of aldehyde aroma in cucumber fruit

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Cucumber (Cucumis sativus L.) is cultivated and consumed worldwide, and China accounts for more than 80% of global production. Cucumber is popular with consumers for its fresh and distinct flavor. LOX oxidized linoleic and linolenic acids at C13 or C9 positions to form 13-hydroperoxylinolenic acid (13-HPOT) or 9-hydroperoxylinolenic acid (9-HPOT). Subsequently, 13-HPOT and 9-HPOT were cleaved by hydroperoxide lyase (HPL) to form C6 and C9 aldehydes. In this abstract, the aroma profile and sensory perception of cucumber fruit were analyzed, and the changes in aroma and the differential expression of genes related to the lipoxygenase metabolism pathway in Q16 and Q24 fruit during the development process of Q16 and Q24 fruit were analyzed. It was found that the CsLOX09 is a key gene involved in the synthesis of C9 aldehyde aroma in cucumber. The significant decrease in aldehyde and alcohol aroma content in CsLOX09 knockout strain fruit, and the aldehyde and alcohol aroma content significantly decreased in CsLOX09 knockout strain fruit. The transcription factors CsMYC2 and CsDof1.8 were selected using the promotor of CsLOX09, and the expression level of CsDof1.8 might be the reseason of C9 aroma in fruit of Q16 and Q24.

O.2.5

QTL stacking in *Cucumis sativus* to optimize resistance to ToLCNDV

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Tomato leaf curl New Dehli virus (ToLCNDV) is an emerging *Begomovirus* spreading in the Mediterranean basin since the last decade and is currently causing major economic losses to cucumber growers in Spain. By performing a biparental population mapping using a wild resistant cucumber source, we identified several QTLs for ToLCNDV resistance on chromosomes 1, 2 and 3. By determining the relative virus titer of ToLCNDV in plants containing different QTL combinations, we showed that only by stacking all QTLs we can reach low viral load and thereby high resistance to ToLCNDV compared to susceptible plants. This study showed the effective use of ToLCNDV virus quantification in determining which combination of QTLs is the best to reach high resistance to ToLCNDV in cucumber.

O.2.6

Pan-genome and multi-parental framework for high-resolution trait dissection in melon (*Cucumis melo*)

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Linking genotype with phenotype is a fundamental goal in biology and requires robust data for both. Recent advances in plant-genome sequencing have expedited comparisons among multiple-related individuals. The abundance of structural genomic within-species variation that has been discovered indicates that a single reference genome cannot represent the complete sequence diversity of a species, leading to the expansion of the pan-genome concept. For high-resolution forward genetics, this unprecedented access to genomic variation should be paralleled and integrated with phenotypic characterization of genetic diversity. We developed a multi-parental framework for trait dissection in melon (*Cucumis melo*), leveraging a novel pan-genome constructed for this highly variable cucurbit crop. A core subset of 25 diverse founders (*MelonCore25*), consisting of 24 accessions from the two widely cultivated subspecies of *C. melo*, encompassing 12 horticultural groups, and 1 feral accession was sequenced using a combination of short- and long-read technologies, and their genomes were assembled *de novo*. The construction of this melon pan-genome exposed substantial variation in genome size and structure, including detection of ~300,000 structural variants and ~9 million SNPs. A half-diallel derived set of 300 F_2 populations, representing all possible *MelonCore25* parental combinations, was constructed as a framework for trait dissection through integration with the pan-genome. We demonstrate the potential of this unified framework for genetic analysis of various melon traits, including rind color intensity and pattern, fruit sugar content, and resistance to fungal diseases. We anticipate that utilization of this integrated resource will enhance genetic dissection of important traits and accelerate melon breeding.

O.2.7

ETHQV8.1, encoded by *ethylene-responsive transcription factor ERF024*, regulates chromatin associated proteins before the onset of fruit ripening in melon

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Melon (*Cucumis melo* L.) is a good model to elucidate the genetic mechanism of ripening behaviour, because both climacteric and non-climacteric cultivars are available. In this work we have fine mapped *ETHQV8.1*, which was previously identified as a major QTL regulating climacteric ripening. The QTL was narrowed down to a genomic region which contains only one annotated gene, MELO3C024520.2, and encodes the ethylene responsive transcription factor ERF024 (*CmERF024*). Functional validation through CRISPR/Cas9 knock-out plants confirmed *CmERF024* as the causal gene regulating *ETHQV8.1*. The *erf024* mutants did not produce the expected ethylene peak and showed mild symptoms of climacteric ripening comparing to the wild type, delaying from 10 to 15 days ripening. To further elucidate the gene function, a DAP-seq experiment was performed to identify genes regulated by *CmERF024*, together with a gene co-expression analysis based on RNA-seq data. High confidence targets (HCT) were obtained, and a Gene Ontology analysis of these HCTs revealed a significant enrichment of DNA structure-related terms, suggesting an important role of *CmERF024* in the control of DNA structural changes associated with the transcriptomic switch happening during climacteric fruit ripening in melon.

O.2.8

Exploration of Novel Genetic Resistance to Powdery Mildew in *Cucurbita pepo* Using Genome-Wide Association Studies

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Powdery mildew (PM), caused by *Podosphaera xanthii*, poses a significant challenge to summer squash production (*Cucurbita pepo*), an important crop in the United States that generates over $216 million annually. Although some cultivars have partial resistance (*Pm-0*) from wild gourd *C. okechobeensis*, managing the disease still relies heavily on fungicide applications. This study aimed to identify novel genetic resistance in *C. pepo* to expand resistance alleles available for breeding programs. *C. pepo* USDA core collection (n= 207) was evaluated for PM-resistance in Florida (greenhouse, 2023), New York (greenhouse, 2022), and Michigan (field, 2022). Experiments used an RCBD with fifteen plants per accession in three replications. 'Success PM' (carrying *Pm-0*) and 'Early Prolific Straightneck' served as resistant and susceptible checks. Disease severity was assessed at 6th true-leaf stage on a 0-100% scale, evaluating pathogen sporulation on various plant parts. Across locations, Success PM and Early Prolific Straightneck were consistently tolerant and susceptible, respectively. However, wide phenotypic variation was observed among 207 *C. pepo* accessions, with accession 189 showing resistance across datasets. Phenotypic and genotypic data with approximately 4 million SNPs were analyzed using GAPIT for GWAS. Three models (MLM, FarmCPU, and Blink) were used to identify genetic loci linked to resistance. GWAS analysis for FL revealed significant PM-resistance loci for top 4th leaf (Chr 11 and 20), stem above 4th leaf (Chr 4, 14, and 16), and whole plant (Chr 13, 15, 18, and 20), with co-located loci on Chr 20 suggesting linkage/pleiotropy. For NY, significant hits for top 4th leaf (FarmCPU: Chr 2, 4, 7, 13 and 19; Blink: Chr 3, 4, 5 and 19) and bottom 4th leaf (Chr 6, 14 and 19) were observed while no significant loci were found in the Michigan field data. This data will be used to test potential markers and candidate gene validation.

O.2.9

Comparative genome-wide analysis of repetitive DNA and its structural proximity to functional sequences in the genus *Cucurbita*

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Transposable elements (TEs) are autonomous DNA sequences that play many roles in the evolution of eukaryotic genomes. In plants, TEs play a key role in the plasticity of genomes in response to environmental stresses, inducing DNA sequence alterations that can affect the activity of functional genes and diversifying their regulatory pathway, with a possible selective advantage for the host genome. Despite their differences in transposition mechanism and genomic abundance, both DNA transposons and retrotransposons can introduce genetic variation, representing an endogenous force that provides a degree of evolvability that would not be available otherwise. Furthermore, some TE families may contain stress-responsive elements in their promoters, being themselves responsive to abiotic or biotic triggers, influencing, for instance, resistance gene architecture and diversification.

Here, in the framework of the CucurGene project, we present our analysis to characterize the repetitive component of 5 different cucurbits, representing the mesophytic *Cucurbita* complex, through a comparative study of the abundance and evolutionary dynamics of TEs. The assembly genomes of the selected taxa were analyzed using a pipeline combining the highest-performing tools in repeat discovery to identify Class I and II TEs.

A pool of full-length elements (DNA transposons, long terminal repeat (LTR)-retrotransposons, and others) was retrieved from all the species. The identified repetitive components represent, on average, 20-30% of the genomes and consist mainly of long terminal repeat retrotransposons. Overall, large variability of repeat abundance at class, superfamily, lineage, and sublineage levels was observed, showing that repeats within individual genomes followed different evolutionary and temporal dynamics and that different events of amplification or loss of these sequences may have occurred after species differentiation.

Finally, the insertion of TEs in proximity or within genes was also assessed at a genome-wide level to infer the possible functional implications of TE activity in cucurbits genetic diversity.

O.2.10

Global identification of fruit-related noncoding RNAs in pumpkin

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Pumpkin (*Cucurbita* spp.) fruits play important roles both economically and nutritionally. In addition, due to the large inferior ovary and variable shape and size of the fruit, pumpkin is an ideal model species for studying fruit development. Recent research has confirmed that noncoding RNAs (ncRNAs) play critical regulatory roles in plant growth, development, and stress responses. However, the regulatory roles of ncRNAs in pumpkin have not been extensively studied. Here, strand-specific transcriptome sequencing and small RNA sequencing on pumpkin (*Curcurbita maxima* 'Rimu') fruits at six developmental stages was performed for systematic identification of ncRNAs. In total, 5425 reliably expressed long ncRNAs (lncRNAs) and 234 microRNAs (miRNAs) were identified. To identify ncRNAs involved in fruit development, we analyzed the expression profiles of ncRNAs throughout fruit development. A total of 322 lncRNAs and 186 miRNAs showed differential expression during the pumpkin fruit development process. These differentially expressed ncRNAs are important candidates for further investigation of the regulatory mechanisms of pumpkin fruit development. Moreover, the core ncRNA-mediated regulatory networks for pumpkin fruit development were predicted and constructed. Based on the results, the important regulatory roles of some fruit-related ncRNAs were revealed. For example, we have identified a lncRNA that functions in pumpkin fruit development through S-adenosyl-L-methionine synthetase. In addition, cma-miR156 was found to play an important role in the regulation of size and weight of the pumpkin fruit by regulating the expression of *CmaSPL*. These results show that the ncRNA-mediated regulatory pathways are significant in the process of pumpkin fruit development. Overall, our findings provide a rich resource for further exploration of pumpkin ncRNAs and a theoretical basis for genetic breeding of pumpkin.

O.2.11

Mapping a Novel Resistance to Powdery Mildew in *Cucurbita moschata*: Development of Markers for Varietal Improvement

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Powdery mildew resistance (PMR) in cultivated squash and pumpkin (*Cucurbita spp*) is currently relying on one major dominant gene, *Pm-0,* introgressed from *Cucurbita okeechobeensis* subsp. *martinezii*. Single gene resistances are fragile and easily overcome by pests through spontaneous mutations. Toward creating a more robust resistance package in *Cucurbita*, we have sought out and identified a new source of PMR in *Cucurbita moschata* that functions independent of the *Pm-0* introgression. For fine mapping this novel resistance, an F2 population was produced from a cross between the PMR parent and the susceptible butternut variety 'Waltham' and screened for resistance. Possible genomic regions responsible for the trait were found by genotyping 21 resistant and 22 susceptible individuals from the F2 screen and running a bulk segregant analysis (BSA), by which a highly significant 2Mbp region on chromosome 13 was subsequently identified. PCR and later high-resolution melting markers were generated to further fine map the region on chromosome 13 and to facilitate the introgression of this novel PMR. These markers were then used to select a new set of F2 plants with crossovers in this region. The F3 families selected were screened the following year in same manner as the F2. Segregation ratios in the F3 made it apparent that the loci on chromosome 13 was necessary but insufficient for the PM leaf resistance phenotype. Markers were then developed in significant regions on other chromosomes indicated by the BSA and used to genotype the most resistant and susceptible F3 individuals. During the 2024 field season, data will be collected on F2:4 and F2:3 families confirming the new markers correspond appropriately to phenotype. These markers will be useful for selecting individuals with the PM leaf resistance in *C. moschata* breeding lines that will be more widely adapted and useful for squash breeders.

O.2.12

Unraveling powdery mildew resistance in *Cucurbita pepo*: a transcriptomic and genomic exploration of two contrasting cultivars

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Resistance to powdery mildew in cucurbits is a desirable trait that enhances crop yield and quality, minimizes fungicide use, and offers significant environmental and health advantages. Despite substantial progress in identifying resistance sources over the past decades, powdery mildew remains a critical phytopathological challenge globally. Therefore, it is increasingly crucial to identify and utilize a broad range of resistant genotypes worldwide. The aim of this study was to identify the genetic differences between two *Cucurbita pepo* cultivars: one tolerant (968Rb) and one susceptible (True France, TF) to the host-specific fungal pathogen *Podosphaera xanthii*. A differential gene expression (DEG) analysis was performed on the transcriptome of 986Rb and TF plants comparing infested (i) and non-infested (ni) conditions. The comparison between 968i vs 968ni allowed the identification of 398 DEGs whilst the comparison between TFi vs TFni revealed 1129 DEGs. Among differentially expressed genes, 260 resulted up-regulated specifically in the 968Rb genotype, while 554 in TF. Conversely, 138 genes were downregulated in 968Rb and 575 in TF. Functional annotation revealed different response strategies in 968Rb and TF to pathogen attack. Pathways associated with signaling, cell wall, proteolysis, transcription factor, hormone signaling, and secondary metabolites emerged as the highly represented. Both genotypes showed enriched genes in cell wall biogenesis, suggesting a common defense strategy. However, 968Rb displayed stronger responses in stress responses and cell wall reinforcement (e.g., *FBA* upregulation), while TF focused on chitin catabolism (chitinase genes). In addition, a substantial number of genetic variants was revealed by the means of the single-nucleotide polymorphism (SNP) analysis in expressed transcripts. A total of 17,045 and 15,067 variants were detected in the 968Rb and TF genotypes, respectively. Notably, 9,232 SNPs were identified specifically in 968Rb, and 8,451 were identified specifically in TF. Genome SNP distribution analysis highlighted contrasting patterns in specific chromosomal regions. This work provided valuable insights for understanding zucchini-*Podosphaera xanthii* interaction. Moreover, the SNP contrasting pattern discovered in specific regions could be employed for studying the genetic basis of powdery mildew-resistance in cucurbits. This study paves the way for developing sustainable and effective powdery mildew control strategies, thereby ensuring the continued production of high-quality cucurbit crops.

ORAL SESSION N. 3

K0.3

Impairing plant susceptibility genes: what did/can we gain in cucurbits for resistance breeding

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In the past 10 years, our understanding of plant susceptibility (S) genes has been evolved rapidly. Plant S-genes are manipulated by adapted pathogens to cause diseases. Since S-genes are evolutionary retained across species, the same S-gene can be identified in many plant species. In this talk, I will give an overview on the progress of the S-gene concept in the last 10 years. By presenting a few novel S-genes identified in tomato, melon and cucumber, the future perspectives on editing plant S-genes to achieve disease resistance in cucurbits will be discussed. Further, I will show new evidence of the involvement of plant S-genes in responses to both biotic and abiotic stresses.

Although application of mutant S-genes in the breeding of resistant crops may be limited because of potential pleiotropy, new genomic techniques (NGTs) open up possibilities for proper modifications of plant S-genes. I will illustrate how the S-gene concept in combination with breeding tools and NGTs has been accepted as a novel breeding strategy to produce crop cultivars with improved resistance to different pathogens as well as abiotic stresses.

O.3.1

DNA primase large subunit is an essential plant gene for geminiviruses, putatively priming viral ss-DNA replication

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The family of *Geminiviridae* consists of more than 500 circular single-stranded (ss) DNA viral species that can infect numerous dicot and monocot plants. Geminiviruses replicate their genome in the nucleus of a plant cell, taking advantage of the host's DNA replication machinery. For converting their DNA into double-stranded DNA, and subsequent replication, these viruses rely on host DNA polymerases. In this study, sequencing of melon (*Cucumis melo*) accession K18 carrying the Tomato leaf curl New Delhi virus (ToLCNDV) recessive resistance quantitative trait locus (QTL) in chromosome 11, and analyses of DNA sequence data from 100 melon genomes, showed a conservation of a shared mutation in the *DNA Primase Large subunit* (*PRiL*) of all accessions that exhibited resistance upon a challenge with ToLCNDV. Silencing of (native) *Nicotiana benthamiana PriL* and subsequent challenging with three different geminiviruses showed a severe reduction in titers of all three viruses, altogether emphasizing an important role of *PRiL* in geminiviral replication. A model is presented explaining the role of *PriL* during initiation of geminiviral DNA replication, i.e. as a regulatory subunit of primase that generates an RNA primer at the onset of DNA replication in analogy to *DNA Primase*-mediated initiation of DNA replication in all living organisms.

O.3.2

Genome editing strategies for improved powdery mildew resistance in cucurbits

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Cucurbitaceae family has a wide range of vegetables and fruits contributing greatly to human diet and nutritional security. Various biotic and abiotic stresses are common impediment to the productivity of cucurbits. Among biotic stresses, powdery mildew (*Podosphaera xanthii*) (PM) is devastating. Development of disease resistance varieties, manipulation of metabolic pathways for tolerance/resilience and harnessing the host-pathogen crosstalk can be utilized for amelioration of the losses. Till date, the resistant source against the PM is not known in commercial cultivars except from wild species. Alternate approach for resistance breeding, is to disrupt susceptibility genes. The genome editing techniques especially CRISPR/Cas9 system has potential to knock-down susceptible (S) gene(s) and successfully employed in crops. We have optimized the protocol for genome editing in *C. pepo* (cv. Black Beauty) by targeting phytoene desaturase (PDS) gene and applying the same to create durable and broad-spectrum resistance to PM. The sequence of the MLO genes (dicots and monocots) was retrieved from the database and phylogenetic analysis was done. The 3 and 5 MLO genes of *C. lanatus* and *C. pepo* respectively has been sub-clustered (sub-cluster 1(Group1); 1 *C. lanatus* & 2 *C. pepo*, sub-cluster2 (Group2); 2 *C. lanatus* & 3 *C. pepo*) in clade V (genes associated with PM interaction). Conserved sgRNAs were designed for group 1 and group 2 genes using CRISPOR software with minimum off targets. CRISPR/Cas9 vector carrying sgRNAs targeting group 1 gene has been constructed and confirmed by sequencing and restriction digestion. The group 1 construct has been immobilized into agrobacterium strain (LBA4404) and used for transformation in cucurbits in addition to particle bombardment method. The positive transformants will be screened based on PCR and sequencing. This study will provide baseline information about the role of *S*-gene in PM interaction and aid in developing varieties with non-race specific and durable resistance.

O.3.3

Niemann-Pick C1 protein - A new player in *Cucumber Mosaic virus* infection in melon

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Plant viruses, as obligate parasites, rely on host cellular machinery to complete their life cycle and establish infection. To develop resistant plants against specific viruses, it is crucial to understand the infection to identify host proteins used by viruses for their life cycle. In our laboratory, we have recently uncovered a key player in the context of *Cucumber Mosaic Virus* (CMV) infection in melon: a Niemann-Pick C1 protein (NPC1). NPC1 is a transmembrane sterol transporter protein known for its associations with viral infections in mammals like those produced by SARS-CoV-2 and Ebola and Zika viruses but had never been related to plant viruses. Our research has demonstrated that CmNPC1 can interact with the CMV movement protein (MP), whose role is to facilitate virus movement between plant cells, enabling systemic infection. Our findings suggest that CmNPC1 may play a significant role in mediating intra- or intercellular viral movement within the plant, being crucial for viral dynamics. In melon, there are two genes encoding NPC1, one located in chr7 and the other in chr11. These genes exhibit differential expression patterns, especially in response to CMV infection, as elucidated using qRT-PCR. Through Yeast Two-Hybrid assays, we pinpointed the specific domain of CmNPC1 responsible for interacting with CMV-MP. Furthermore, utilizing the Bimolecular Fluorescence Complementation technique, we have precisely determined the subcellular localization of the interaction. Finally, we have demonstrated the functionality of CmNPC1 during viral movement by silencing. Our investigation suggests that both CmNPC1 proteins are used for the viral movement, not only by CMV but by a battery of viruses, suggesting that CmNPC1 could be a general hub protein used by several plant viruses. In summary, our research contributes valuable knowledge to the understanding of plant-virus interactions, contributing to the development of strategies to protect crops from viral infections.

O.3.4

Assessment of fruit quality and disease resistance in cantaloupe (*Cucumis melo* L.) hybrids developed at Texas A&M

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The hot, humid production regions of southern Texas create significant challenges for cantaloupe melon growers and threaten the sustainability of this iconic crop. High night temperatures, strong winds and numerous pests and diseases are omnipresent. The Texas A&M melon breeding program has been developing improved cultivars with abiotic and biotic stress resistance for 80 years. Currently, the program is assessing combining abilities for important quality and yield related traits in multi-disease resistant parent lines. A factorial mating design (partial Diallel) was utilized to create hybrids among 22 inbred parent lines. These were planted in 3 replications at two locations, Weslaco and Uvalde, Texas during the spring of 2024. Vines were rated on a 0-5 scale for resistance to powdery mildew (PM) and downy mildew (DM), while Monosporascus root rot and vine decline (MRRVD) was rated as resistant or susceptible. Additionally, the occurrence of gummy stem blight was sporadic but severe in some hybrids and commercial check cultivars. Resistance ratings for PM ranged from 0 to 4, while ratings for DM ranged from 1-5, with more than 50% of all entries losing all the leaves. Fruits were harvested during May and June. Data for total soluble solids (TSS), flesh firmness, color, peduncle scar size, cavity space, rind netting and size were recorded. Heterosis for fruit size, TSS and flesh firmness was observed in several hybrids, particularly with parents 3, 40, and 70. These parents were considered good general combiners for these traits. Resistance to PM and MRRVD demonstrated strong dominance in most hybrids, while resistance to DM was likely polygenic as both parents needed to exhibit resistance to produce a resistant hybrid, and there was a continuous distribution of resistance expression among all entries. The presence of slight PM on numerous parents and hybrids with known race 1,2 resistance suggested the presence of a multi-race population for this pathogen in the 2024 Weslaco field. At Uvalde, the DM pathogen originated in a nearby watermelon field, where it caused total defoliation, but did not overcome the resistance in TAM Perlita and TAM Dew cultivars, which were planted as checks. That resistance was derived from Smith's Perfect and is not effective in all environments or across all strains of the pathogen.

O.3.5

Emergence of watermelon chlorotic stunt virus and its impact on virus population structure and infection dynamics in southwestern U.S. melon and watermelon production

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Since the late 1990s, U.S. cucurbit production has experienced the emergence of five whitefly-transmitted viruses, all associated with higher abundance of the whitefly *Bemisia tabaci* MEAM1. These viruses often occur as mixed infections and have become important production constraints for US cucurbit crops including melon and watermelon. Recent multi-state surveys conducted in the southwestern U.S. demonstrated that mixed virus infections often include the criniviruses, cucurbit yellow stunting disorder virus (CYSDV) and cucurbit chlorotic yellows virus (CCYV) and the ipomovirus, squash vein yellowing virus (SqVYV). CYSDV is typically the dominant virus during the fall melon season, whereas CCYV is dominant in the spring melon season. Transmission studies revealed that infection timing influences dominance of CCYV or CYSDV in mixed infections of these two viruses in melon (*Cucumis melo*). Studies further demonstrated that CCYV must establish early to become the dominant virus. During the fall of 2023, the begomovirus, watermelon chlorotic stunt virus (WmCSV) was identified infecting melon and watermelon in the southwestern U.S. This was the first report of WmCSV in cucurbits in the United States. In areas where WmCSV was present, CYSDV was frequently identified in co-infections with WmCSV. Interestingly, CCYV was rarely found in mixed infections with WmCSV, and CCYV incidence was reduced compared to earlier sampling years. This suggests that the emergence of WmCSV may be altering the competitive dynamics of virus infections and influencing virus population structure in cucurbits. Current research is examining the regional overwintering hosts of both CCYV and WmCSV as well as the competitiveness of CCYV in mixed infections with CYSDV and WmCSV.

O.3.6

Application of a new differential set for virulence study on Czech cucurbit downy and powdery mildew populations

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Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev (1903), is highly destructive pathogen causing economically important problems of cucurbit vegetables production worldwide. Host parasite interactions between Cucurbitaceae and *P. cubensis* exhibit significant variation on both the individual and population level (Lebeda et al., 2013). Cucurbit powdery mildew (CPM) is mainly caused by two obligate ectoparasites, *Golovinomyces orontii* s.l. and *Podosphaera xanthii*, that are highly variable in virulence (Lebeda et al. 2018, 2021). There were screened twelve *P. xanthii* (Px) isolates and twelve *P. cubensis* isolates (PC) on a new differential set for virulence study developed by Lebeda et al. (2016, 2019, 2021, 2024). This new differential set for virulence study is composed of 22 *Cucumis melo* differentials. There were detected nine different PC races among twelve screened PC isolates. Races 127.127.127.64, 127.123.62.0, 127.127.127.0 were occurred twice, the other six races were unique. The most virulent race was 127.127.127.64 when all 22 *C. melo* differentials were sensitive to this race. All screened PC isolates showed the high level of pathogenicity. Among screened Px isolates, twelve different races were detected. All Px races were unique. The majority of screened Px isolates showed a medium level of pathogenicity. None of detected PM races was virulent to all 22 *C. melo* differentials. Significant differentiation of virulence patterns between PC and PM pathogen population was revealed. The research was supported by the Ministry of Agriculture CR, NAZV, projects no. QK21010064 and NPGZ-M/03-023 and IGA - PrF - 2024-001.

O.3.7

NFT with supplementary light as a technique to extend the production period of 'Scopatizzo' (*Cucumis melo* L.), even through the use of brackish water

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'Scopatizzo', belonging to the *Cucumis melo* L., is a local variety grown in Apulia region, which is consumed as unripe melon. This local variety is characterized by its relatively small-sized fruits, which have a light and sparse fuzziness. At the greenhouse of the experimental farm 'La Noria' of the National Research Council, Institute of Sciences of Food Production (Mola di Bari, Bari) two experimental trials were conducted between September and November 2023 and between March and June 2024. In these trials, 'Scopatizzo' plants were cultivated in the presence/absence of supplemental radiation provided by light-emitting diode (LED) technology and with the addition of NaCl in the nutrient solution (NS). In both trials, the cultivation technique used was closed-cycle NFT, aimed at increasing the efficiency of water (WUE) and nutrient use and proposing a cultivation system that reduces the production of waste from agricultural activities (substrate-free). In the first experimental trial the plants grown under LEDs showed an increase in productivity of about 300 g of fruits·plant^{-1} and a WUE approximately 4 L of NS·kg of fruit^{-1} better than the control. Instead, in the second experimental trial, the addition of 15 mM of NaCl in the NS resulted in a significant reduction in productivity compared to the control (more than 50%) as well as a reduction in WUE (almost 2.5 L of NS· kg of fruit^{-1}). Even in this second cycle, the application of LEDs led to an increase of about 700 g of fruits·plant^{-1} in productivity and an increase in WUE of about 3 L of NS· kg of fruit^{-1}. The research activity demonstrated that NFT is a valid cultivation technique for enhancing 'Scopatizzo' and that the application of LEDs provides advantages for the out of season production. Additionally, it was shown that the use of saline waters allows for acceptable fruit production, but with high NS consumption, as periodic renewals are necessary.

O.3.8

Genomic Prediction of Resistance to Fusarium Wilt (*Fusarium oxysporum* f. sp. *niveum* race 2) in Watermelon Using Parametric and Non-Parametric Approaches

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Breeding for complex traits that are influenced by many genes can be challenging, especially when using marker-assisted selection (MAS). Genomic selection (GS) offers a promising alternative by focusing on combining beneficial gene variations to create improved cultivars. In this study, we explored using GS to improve resistance to *Fusarium oxysporum* f. sp. *niveum* (*Fon*) race 2 in watermelon, a trait that is complex, controlled by multiple genes, and moderately heritable. Our goals were to: 1) evaluate how accurately genomic prediction (GP) models can predict Fon race 2 resistance in two watermelon populations (F_2 and recombinant inbred lines, RILs), 2) rank and choose the best families from these populations based on their genomic estimated breeding values (GEBVs) for developing new test groups, and 3) check if major genes linked to Fon race 2 resistance are present in the top-ranking families with the highest GEBVs. Resistance was measured by disease severity (1-5; 1 = healthy, 2 = stunted/chlorotic, or 3 =one or two cotyledon is wilted, 4= completely wilted, and 5=dead) 28 days after planting in soil infected with *Fon* race 2. We used genotyping-by-sequencing (GBS) data from 205 $F_{2:3}$ and 204 RIL families, with reference genomes from parental lines. We tested six different GS models, including both parametric (G-BLUP, BayesB, Bayes_LASSO) and non-parametric (Random Forest, SVM Linear, SVM Radial) methods. G-BLUP and Random Forest performed best, showing correlation values of 0.48 in the $F_{2:3}$ population and 0.68 in the RIL population, demonstrating the effectiveness of GP for improving *Fon* race 2 resistance in watermelon breeding.

O.3.9

Evaluation of cucumber (*Cucumis sativus* L.) for Drought Tolerance in Growth Chamber and Field Conditions

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Cucumber (*Cucumis sativus* L.) is an important crop belonging to the Cucurbitaceae family. Spain is a main producer and exporter in the European Union. Production of this crop and other cucurbits is hampered by climate change, especially in Spain where drought and water deficit affect both fruit yield and quality. Development of new varieties adapted to drought conditions requires a selection of resistant or tolerant plant material. In the context of the research project TED-2021-132130B-I00, a total of 36 cucumber cultivars from the Universitat Politècnica de València Genebank, were firstly evaluated under growth chamber conditions. A score-based drought screening method adapted to cucumber was employed, alongside indirect measurements with the leafclip sensor Dualex (METOS®), which measures the content of chlorophyll, flavonoids, anthocyanins and NBI (Nitrogen Balance index). Measurements were taken 4 and 7 days (T4 and T7) after an irrigation by immersion. Subsequently, a field assay was performed to evaluate tolerance to drought conditions with control treatment (all water necessities covered) and water stress treatment (35% of water necessities covered). An augmented design analysis was conducted with three different control varieties (high, medium and low tolerance). Plant relative water content (RWC) determination as well as measurements with the Dualex were performed. Finally, total production, fruit flesh firmness (FFF) and Soluble Solids Content (SSC) were measured. Our results revealed the correlation between parameters such as RWC and Flavonoid content with drought resistance. Performance under water deficit stress allowed for the identification of drought tolerant cucumber cultivars. The selected varieties from this study will contribute to the development of drought-resistant cucumber plant material.

K0.4

Evaluating Sensory Attributes and Health-Promoting Compounds in Hybrid Melon Varieties Across Different Cultivation Regions of the United States of America

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Muskmelon (*Cucumis melo* L.), a phenotypically and genetically diverse member of the Cucurbitaceae family, is a well-known source of health-promoting phytonutrients (including carotenoids, amino acids, vitamins, and neurotransmitters such as gamma-aminobutyric acid, GABA), and has numerous appealing sensory attributes. Recent crop improvement efforts have focused on enhancing these quality and consumer preference attributes. However, these attributes often vary significantly as a function of cultivation environment and management practices, and this often influences consumer preference patterns. Therefore, understanding the interactions between genetics, environment, and management practices is essential in identifying cultivars that can perform well in specific environments. This will also provide valuable information for continued crop improvement.

In a series of studies, we evaluated the variation in phytonutrient contents among different commercial melon varieties and newly developed hybrids at diverse locations across the US. Six new muskmelon hybrid cultivars (TH-1, TH2, TH3, TH4, TH5, and TH-6) and two commercial varieties ('Da Vinci', a Tuscan type and 'Chujuc,' a Western-Shipper type) were grown in five locations (Texas, Georgia, Arizona, California, and North Carolina) using standard commercial practices. Key phytonutrients including ascorbic acid, carotenoids, and sugars varied significantly across locations. β-carotene, ζ-carotene and β-cryptoxanthin also varied as a function of cultivar and location, with the highest β-carotene contents in Da Vinci (from Arizona and California), TH-1 (from Arizona), followed by TH-5 (from Arizona and North Carolina).

In another study to assess profiles of volatile organic compounds (VOCs; flavor aromatic compounds) we observed significant (ten-fold) variation in VOCs among nine cantaloupe cultivars. Similarly, in another location-based study, we evaluated novel muskmelon genotypes (TH-1 to TH-13) cultivated in different locations for phytochemicals (total soluble solids [TSS], sugars, carotenoids, and amino acids). The results revealed considerable variability across genotypes and location. Except for melons grown in Indiana, all had a TSS of more than 10 °Brix. The principal sugars were glucose, fructose, and sucrose. β-carotene levels were high, except in Indiana-grown melons and glutamine and GABA were at high concentrations.

In another time-based study, we looked at the sensory and phytonutrient profiles of commercial (Chujuc, DaVinci/TT-DV, Infinite Gold/HT-IG) and newly developed muskmelon cultivars (TH-5, TH-6) cultivated in Weslaco, Texas, from
2019 to 2020. In these cultivars, TSS varied from 7.3 to 11.1 °Brix, with Chujuc having the lowest TSS in 2020. Total sugars were greater in TH-5 and TH-6, particularly in 2020, than in the commercial cultivars. β-Carotene levels were comparable throughout TT-DV, HT-IG, and TH-5 in both years, but lower in Chujuc in 2020 compared to 2019.

Our exploration of these genotype–location interactions, with multiple varieties, multiple locations, and over multiple years, provides valuable information for choosing genotypes that will produce high-quality fruit at a specific location and for breeding improved varieties for these locations. Understanding these parameters is critical for enhancing melon sensory attributes, quality, and phytonutrient contents, and for directing breeding programs without compromising customer acceptance of melons.

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O.4.1

Mining the cucumber core collection for genetic control of fruit quality traits.

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Germplasm collections are a tremendous resource for important traits for crop improvement including resistances to biotic and abiotic stresses. However, valuable traits are often found in landraces or wild accessions with undesirable fruit quality. As improved cultivars must also meet market standards for fruit quality, identification of QTL or genes associated with desirable fruit quality traits can facilitate breeding efforts to introgress novel resistance or production traits. To this end we performed extensive phenotypic characterization and GWAS analysis for an array of cucumber fruit morphological traits (e.g., skin color, flesh color, length, diameter, shape, tapering, curvature, spine density, cuticle thickness, netting, seed cavity size, flesh thickness, hollowness) for the CucCAP cucumber core collection, a set of 388 accessions representing >96% of the genetic diversity present in the U.S. National Plant Germplasm System. The availability of resequencing data (40-50x) and identified SNPs for the full collection enabled the identification of significantly associated SNPs for each trait. In several cases, QTL for highly correlated traits were closely clustered. In other cases, accessions with extreme phenotypes provided evidence of genome-wide selection for multiple quantitative alleles. Many of the GWAS-identified SNPs were in close vicinity to QTL or candidate genes previously identified from bi-parental populations, providing confidence in the analyses and further support for QTL and candidate genes in the literature. In addition, several novel genes potentially important for these traits were also identified. The collected fruit quality phenotypic data, multi-year photographic documentation deposited to the Cucurbit Genomics Database (GuGenDB,), and associated genetic analyses provide valuable resources for breeding efforts and increased understanding of fruit development.

O.4.2

Identification of flesh color controlling genes in watermelon

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Watermelon (*Citrullus lanatus*) is an important cucurbit crop worldwide. Cultivated watermelons exhibit a wide range of flesh color: white, pale-yellow, canary yellow, orange and red. To unravel the flesh color controlling genes in watermelon, we first measured the inheritance of red, orange and yellow flesh color phenotypes and verified two flesh color controlling genes. The homozygous recessive *rf* (red flesh) locus is epistasis to *OF* (orange flesh) and *YF* (yellow flesh). The rf gene, encoding lycopene b-cyclase (*LCYB*) gene, was believed to determinate the red flesh formation. Meanwhile, the *OF* controlling gene was identified as a phytoene synthase (*PSY*) gene located in chromosome 1. The different genotypes of *PSY* decided yellow or orange flesh color.

Then, we identified the flesh color intensity controlling gene through constructing yellow/pale-yellow and scarlet-red/red flesh color segregate populations. A single dominant flesh color intensity (*ClFCI*) locus was identified in chromosome 6 in these two populations. A structural variation (SV) in the upstream region of the candidate *ClFCI* gene was discovered, contributing to heightened gene expression in dark-colored materials. The functional analysis of *ClFCI* indicated that it likely play an important role during chromoplast development.

What's more, we created a new photosensitive flesh color line by EMS mutagenesis in a red flesh material. This mutant exhibits an initial yellow hue that undergoes rapid photobleaching within 10 minutes under intense sunlight. A long-term light-emitting diode (LED) light treatment turned flesh color from yellow to pink. An EMS-induced G-A transversion which leads to a premature stop codon in 15-cis-ζ-carotene isomerase (*ZISO*) gene leaded to this phenotype.

In conclusion, our research reveals the genes located in carotenoid metabolic pathway governed different watermelon flesh colors formation. Additionally, we reveal that key genes governing chromoplast development may also exert a profound influence on flesh color formation.

O.4.3

Fine mapping of *McTu4.1* controlling fruit wart in bitter gourd

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Bitter gourd (*Momordica charantia*) is a vegetable and medicinal plant of the family Cucurbitaceae, cultivated in tropical and subtropical Asia. The skin pattern is an important appearance quality for bitter gourd, which affects consumers' purchasing intention and determines the range of variety promotion. The fruit wart locus *McTu4.1* is one of the major loci responsible for fruit skin pattern in bitter gourd. Identification of the underlying gene will improve our understanding of the molecular mechanism of fruit skin development. Genetic analysis showed that tubercules were controlled by a single gene and was partially dominant over the stripe. We employed the bulk segregant approach coupled to whole genome sequencing (QTL-seq) which led to the identification of a highly significant locus. To confirm the location of the gene underlying *McTu4.1*, we developed additional markers and conducted progeny tests. We fine mapped *McTu4.1* to a 39.9 kb region on chromosome 4 comprised of 8 predicted genes. This work provides a solid foundation not only for understanding the molecular mechanism of melon tubercule formation, but also for molecular breeding in bitter gourd.

O.4.4

GWAS and BSA-seq approaches reveal several genomic regions and candidate genes regulating carotenoid content in *Cucurbita pepo* fruit

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Carotenoids are a group of liposoluble pigments that have functional properties that reduce the incidence of certain human diseases. For this reason, increasing the carotenoid content in fruits and vegetables is a priority trait in many vegetable breeding programs. In the present work we have used two approaches to study the genetic control of color formation and carotenoid accumulation in mature *C. pepo* fruit. First, we combined the evaluation of lutein, zeaxanthin, α-carotene and β-carotene contents and genotyping by sequencing (GBS) data in 257 accessions of *C. pepo*. The genome-wide association study (GWAS) allowed the identification of genomic regions associated with carotenoid content. Lutein accumulation was associated with a region on chromosome 11 (S11_3084639) that carries a *carotenoid cleavage dioxygenase* (*CCD*) gene (Cp4.1LG11g05170) that participates in the formation of apocarotenoids. The study has also identified up to 9 regions associated with α-carotene and β-carotene content. Among them, a region on chromosome 13 includes variants of the gene Cp4.*1LG13g00040*, coding for the chaperone Hsp70 that regulates the accumulation of carotenoid in tomato fruit. Alternatively, the cross of accessions with orange and white fruits has led to the development of an F2 population that segregates 1:2:1 (white:yellow:orange) for fruit color and low, intermediate and high β-carotene and lutein content. BSA sequencing of contrasting bulks in generation F2 allowed the detection of a major QTL on chromosome 14 and a minor QTL on chromosome 5 that regulates the content of lutein and β-carotene. The role of candidate genes within these QTLs that may control carotenoid content and fruit quality in squash is discussed.

ORAL SESSION N. 5

K0.5

Leveraging Translational Biology to Enhance Plant Breeding

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Agriculture is currently confronting a multitude of challenges, ranging from climate change and disease resistance to the growing demand for higher yields. In this talk, I will explore how fundamental research and translational biology can play a crucial role in addressing these issues by helping to develop the resilient and high-performing crop varieties. I will introduce cutting-edge tools for characterizing gene functions and engineering key alleles, essential for advancing plant breeding. As example, I will share insights from our research on sex determination, and how we can use the finding to optimize the production of F1 hybrid seeds, a critical strategy for enhancing crop performance. Additionally, I will discuss our efforts to characterize complex traits, including plant interactions with pollinators, which are vital for improving both productivity and sustainability in agriculture.

O.5.1

In planta Particle Bombardment (iPB): A novel gene editing technology for efficient breeding of cucurbit crops

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Genetic and genomic research in Cucurbit crops have identified causal genes for important traits and revealed that these traits are often caused by small genetic mutations within the genes. Gene editing technologies can rapidly modify target genes to change important traits. Gene-edited tomato with enriched GABA has been developed and commercialized in Japan. By contrast, gene editing in Cucurbitaceae lags behind as it lacks efficient gene editing systems. Many causal genes for important breeding traits have yet to be be functional validated. Recently, one of the speakers' lab has developed *in planta* Particle Bombardment (iPB), a non-culture and DNA-free gene editing technology in wheat. The iPB method has been applied to different crop species including barley, maize, and soybean. In this presentation, we report application of this technology to melons to improve the shelf-life of melon fruit. We utilized CRISPR/Cas9 ribonucleoprotein (RNP) targeting *CmAOC1*, a key gene for ethylene biosynthesis in fruit, and directly delivered it to shoot apical meristems (SAMs) via particle bombardment. Under optimized conditions, we were able to detect mutations with high efficiency in 'Harukei-3', one of the popular melon lines in Japan and confirmed that the mutation was stably inherited in subsequent generations. The mutant lines showed an improved fruit shelf-life. Conventional gene editing using tissue culture is still difficult for melons, but the iPB method was verified to be effective in modifying target genes. There are few reports on gene editing in other cucurbits. Therefore, the iPB can be a breakthrough technology for gene editing in cucurbit crops.

O.5.2

Application of Molecular Breeding in Watermelon

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Watermelon ranks the world's top ten fruit crop, with China being the largest producer and consumer. As demand increases for high-quality from consumers, high diseases-resistant from grower and extend shelf-life and transportation durability from retailers, traditional breeding methods for integrating multiple elite traits are inadequate, labour-intensive and inefficient. This highlights an urgent need for improved breeding systems.

Since 2013, we have led advancements in watermelon genomics, developed the fine mapping of the East Asian watermelon "97103" genome and its variation map by 2019. These have established a solid foundation for identifying genes and SNPs linked to crucial traits such as sugar content, flesh color, firmness, resistance, bitterness, and sexual type, and facilitated the development of marker-assisted selection (MAS) strategies. Through MAS, we have successfully bred excellent varieties like “Jingmei 10K”, and “Jingcai”, which have achieved significant market success in China.

We have also established genome selection, genome editing and inducing double haploid (ID) breeding methodologies and integrated into watermelon breeding programs. These innovations promise to enhance MAS effectiveness by enabling high throughput pyramiding of polygenes, precisely creating new traits and rapidly producing homozygous lines. This journey from genomic mapping to the application of advanced breeding techniques is shaping the next generation of watermelon varieties to meet the evolving needs of producers and consumers.

O.5.3

Genetics and breeding of the hull-less seed pumpkin in Cucurbita

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Hull-less pumpkin seeds provide a significant source of income globally and are preferred for snacking and seed-oil processing as they eliminate the need for de-hulling prior to use. The hull-less seed trait in *Cucurbita* is controlled by a single major gene (designated *n*) and other modifier loci. Breeding for the hull-less pumpkin targets traits such as seed yield, seed nutrition, seed size, disease resistance, and more recently, marketable flesh quality. Availability of dual-purpose pumpkin for seed production and marketable flesh would be valuable to growers, with the added benefit of reduced food-waste. To facilitate the development of a dual-purpose pumpkin using marker-assisted selection (MAS), we recently mapped the genomic position (*Qtlhull-less-C12*) of the hull-less seed trait on chromosome 12 of *C. pepo* and identified *Cp4.1LG12g04350* (NAC- domain protein) as a likely a candidate gene. We sequenced *Cp4.1LG12g04350* gene across diverse genotypes varying in degree of seed-coat lignification, including segregating F_3 families, cultivars and accessions, and uncovered seven segregating sites and two haplotypes. Two SNP markers were significantly linked to *Qtlhull-less-C12* and are currently in use for MAS. Using a combination of MAS and embryo-rescue/ bridge crossing, we have introduced the hull-less trait from *C. pepo* into the genetic background of *C. moschata* and *C. maxima* with varying degree of flesh quality.

POSTER COMMUNICATION

POSTER SESSION N. 1

P.1.1

Characterization & Preservation of Bottle Gourd Collections

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Lagenaria siceraria, commonly known as bottle gourd, is a crop of immense historical significance, being one of the first domesticated crops, with its use dating back over 10,000 years. Its journey across almost every continent, adapting to a diverse range of climates and overcoming abiotic and biotic stressors, is a testament to its remarkable adaptability and diversity. The early interest in bottle gourd may have stemmed from its versatility: the immature fruits, leaves, stems, and flowers are edible, while the mature fruit's hard rind can be used for storage vessels, tools, musical instruments, amulets, or art. Despite this adaptability and importance for early humans, there is a pressing need for more research to better understand the adaptability and diversity of bottle gourds. Our study employs a three-part approach to provide resources for further discovery, investigate global diversity, and preserve bottle gourds. Objective 1 focuses on understanding the cultural importance of bottle gourds. In collaboration with Cornell University's Southeast Asia Program and Kasetsart University in Thailand, we are exploring the cultural significance of bottle gourds in Southeast Asian countries. Objective 2 aims to improve the sharing of scientific knowledge of bottle gourd by creating a crop ontology to help standardize data collection. This collaborative work includes researchers from various regions working on bottle gourds. Lastly, Objective 3 centers on enhancing our understanding of the phenotypic and genotypic diversity of bottle gourds globally. The United States Department of Agriculture National Plant Germplasm System and Tropical Vegetable Research Center at Kasetsart University bottle gourd collections are being characterized to explore this diversity.

P.1.2

Genetic diversity of the *Cucurbita maxima* accessions held at the Polish genebank

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Cucurbita maxima Duchesne is one of the world-wide cultivated and economically significant cucurbit species. Originating from the New World, it has a long tradition of cultivation in European countries. Several genebank germplasm collection of this species exist, but only a few of them have been characterized using DNA technologies. The Polish genebank collection of *Cucurbita maxima* comprises almost 200 accessions, mostly representing Central and Eastern European cultivars, landraces and local populations. This study aimed to characterize this collection using genome-wide SNP markers to understand the genetic variation among accessions. In total, 175 genebank accessions of *Cucurbita maxima* were genotyped using DArTSeq technology. Additionally, eight cultivars representing basic horticultural groups of *Cucurbita maxima* and two accessions, representing *Cucurbita pepo* and *Cucurbita moschata,* were included in the study. High-quality SNPs, that met the quality criteria (CallRate > 60% and MAF > 2%) and represented 20 chromosomes of *Cucurbita maxima*, were selected and used to access genetic diversity. SNP analysis confirmed that accessions of *Cucurbita pepo* and *Cucurbita moschata* were clearly separated from those of *Cucurbita maxima* and comprehensive analysis of the *Cucurbita maxima* genotyping data revealed the population structure. A core collection representing 99% of the genetic variability of Polish *Cucurbita maxima* germplasm collection was defined. Our study provides new insights into the genetic diversity of *Cucurbita maxima* to advance the use of genebank resources in breeding programs.

P.1.3

Development of core collections for melon and cucumber in the NARO Genebank, Japan

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Efficient use and management of genetic resources play a crucial role in advancing agricultural research and breeding. Core collections, representative subsets of diverse genetic resources, contribute to this by providing researchers and breeders with a common and compact diversity panel.

The melon (*Cucumis melo*) and cucumber (*Cucumis sativus*) genetic resources deposited in the National Agriculture and Food Research Organization (NARO) Genebank, a Japanese genetic resource center, are unique in that they include accessions from Southeast Asian countries, which are rarely available in other major genetic resource centers. In this study, we genetically characterized nearly 1,500 melon and cucumber accessions deposited in the NARO Genebank using the genotyping-by-sequencing (GBS) approach with the aim of developing core collections for these two important crops.

Analyses based on the genome-wide SNPs identified by GBS revealed phylogenetic relationships and population structures that are highly consistent with geographic distribution and reflect the contrasting domestication histories of the two crops. With a holistic approach that considers genetic diversity, historical significance, geographic origin, and unique agricultural traits, we have developed the World Melon Core Collection and the World Cucumber Core Collection. Each core collection comprises 100 accessions, capturing most of the genetic diversity within the species and representing diverse fruit morphologies. Compared to previous core collections from other genetic resource centers, our core collections are characterized by smaller collection sizes, in addition to the inclusion of Southeast Asian accessions. This compactness will allow them to fit into a wider range of research and breeding projects and will facilitate the efficient use and management of genetic resources. The core collections will be made publicly available from the NARO Genebank to researchers and breeders worldwide upon request.

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P.1.4

Screening of cucurbits for resistance to *Neocosmospora falciformis* and genetic variation of *N. falciformis* isolates associated with Fusarium wilt disease in cucurbits.

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Cucurbits, especially watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Naka) and melon (*Cucumis melo* L.), are of great economic importance in Spain. Pests and diseases are one of the main limiting factors for these crops. Diseases caused by soilborne phytopathogenic fungi are particularly important, highlighting the severity of symptoms caused by Fusarium wilt, such as vascular wilt and root rot. In Spain, *Neocosmospora falciformis* (*Fusarium falciforme*), a member of the Fusarium Solani Species Complex (FSSC), has been one of the predominant species in Fusarium wilt infection of cucurbits in recent years. Preliminary results have shown that this pathogen causes lesions in the genus *Cucumis*, *Citrullus*, and *Cucurbita*. In order to search for natural sources of resistance for the development of resistant varieties to help control this disease, a collection of 37 accessions belonging to different cucurbit species was screened. For the evaluation, artificial inoculations were carried out by root dipping of seedlings. Response to inoculation was assessed using a visual symptom scale from 0 (no symptoms) to 4 (dead plant). Most of the accessions evaluated were susceptible and only three accessions showed very few symptoms. On the other hand, given the limited information currently available on *N. falciformis*, a study of the genetic diversity of a collection of 38 cucurbit-associated isolates obtained in previous sampling surveys in 10 Spanish locations was carried out. Twelve ISSR (Inter Simple Sequence Repeats) primers were used for this study, generating a total of 247 amplification products, 97% of which were polymorphic. A high genetic variability was found among the isolates, but the dendrogram obtained did not show a clear grouping according to the geographical origin of the isolates or to host species.

P.1.5

Quality parameters of 'nugget' type *cucurbita maxima* fruits under high-temperature stress

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Among various *Cucurbita maxima* cultivars, the 'nugget' type has recently gained popularity among both producers and consumers. The semi-bush plants yield small to medium-sized fruits that are bright orange colored, with a distinctive brown pattern. The fruits are transverse to broadly elliptic in shape. The 'nugget' fruits are particularly noted for their pleasant nutty flavor and relatively high carotenoid content. The study aimed to assess variability in fruit quality parameters (weight, number per plant, dry weight content, flesh juice pH, fresh weight sugar and carotenoid content) within a breeding collection of 30 'nugget' type *C. maxima* accessions. Additionally, the study sought to assess the effect of high-temperature stress during the reproductive phase of plant development on these parameters. For that purpose, a comparative analysis of field-grown plants across two temperate and two high-temperature stress growing seasons was conducted. The accessions exhibited significant variation across all analyzed parameters, establishing a strong foundation for breeding high-quality varieties. High-temperature stress predominantly impacted carotenoid content, which was reduced by an average of 27.1% compared to temperate seasons. Additionally, sugar content and fruit weight were decreased by 15.2% and 5.7%, respectively. Conversely, flesh dry weight content was increased by 28.6%, while the number of fruits per plant and flesh juice pH remained largely unchanged. The differential response among accessions suggests variability in stress tolerance, which may be crucial for maintaining fruit quality under increasingly frequent high-temperature conditions.

P.1.6

Modulating the fruit morphology of traditional melon varieties through the introduction of genes identified in various introgression line libraries.

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Throughout the history of melon cultivation on the Iberian Peninsula, a large number of traditional varieties have been developed, many of which are highly valued in local markets. However, their cultivation is declining, being replaced by modern varieties. To promote their consumption, four traditional varieties have been selected ('Piel de Sapo', 'Amarillo', 'Rochet', and 'Blanco') for their extraordinary organoleptic quality. In parallel, several introgression line (IL) libraries have been developed in a 'Piel de Sapo' genetic background, containing introgressions from accessions: 'Vedrantais' (cantaloupe, France), PI 273438 (Dudaim, India), Ames 24297 (wild melon, Pakistan), and PI 124112 (acidulus, India). From these collections, ILs containing genes that induce round fruits (CALC8-2, DUD4-2, VED11-2), large fruits (TRI05-02), and small fruits (TRI08-2, DUD1-2, DUD4-2) were selected. These ILs were crossed with traditional varieties to verify if the effects of these genes persist when introduced into traditional genetic backgrounds. The results of this research will allow the selection of which genes have the greatest effects on the fruit morphology of traditional varieties, to introduce them into these varieties and implement a breeding-by-design program to expand the morphological diversity of traditional varieties with high organoleptic quality.

P.1.7

Exploitation of traditional snake melon (*Cucumis melo var. flexuosus* L.) landraces cultivated in the Mediterranean basin.

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Snake melon (*Cucumis melo subsp. melo* L. *var. flexuosus* (L.) Naudin) is a well-known and traditional vegetable in the Mediterranean region. It is a neglected crop has not attracted great interest for assessing its diversity by the accessions stored by some genebanks, and consequently its genetic resources have not been widely surveyed before.

In this study, we assessed the bio-morphometric and genetic diversity of twenty-four snake melon landraces previously collected from three different Mediterranean countries: Italy (Europe), Lebanon (Asia) and Tunisia (Africa), by using thirty-eight morphometric descriptors of the plant and 12 SSR markers. The data acquired shown the great polymorphism of the landraces analysed detecting an important phenotypic variability. PCA of the morphological characterization showed a diversity of 66% within the first three components and divided the collection into 3 groups independently of their origin. An important positive correlation was showed between root weight and fruit weight. Another important correlation, in this case negative, was ascertained among the interval of days from the transplanting to the first flower fruit setting and the plant length and weight, and for fruit length and diameter.

Based on the SSR molecular characterization, the hierarchical clustering grouped the accession in 3 main groups and expresses the diversity present of this crop although it showed some similarities among varieties coming from different part of the Mediterranean.

Findings of this study indicate a significant diversity for the Mediterranean snake melon germplasm that must be further conserved and investigated in order to improve the use of this ancient crop for diversifying vegetable farming and production.

POSTER SESSION N. 2

P.2.1

Genetic mapping reveals candidate genes controlling plant architecture in cucumber

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Plant architecture is one of the most important determinants of nutrient distribution and photosynthesis and has a major impact on crop management and yield efficiency. Identification of key genes and molecular mechanisms that control plant architecture, including plant height and branching is critical for modern breeding programs. In cucumber, only several genes determining altered growth habit at the molecular level have been identified, and there are attempts to develop novel compact cultivars. Here, we report research progress on three cucumber lines that exhibit altered growth habit controlled by single recessive genes. These lines were developed at the Department of Plant Genetics, Breeding and Biotechnology (WULS) and had not been studied at the molecular level so far. The segregating F_2 populations of 180 individuals each were developed from the crosses between line L500 characterized by the normal growth and lines characterized by altered growth L504, L505 and L511. For each population, plant phenotyping and DArTseq genotyping were performed, and next, identified SNPs were used for genetic mapping. Comprehensive analysis of genotyping data allowed to determine genomic regions associated with plant growth habit in all three populations. Then, PCR-based molecular markers were designed and tested in the segregating population to identify candidate genes controlling plant growth. In addition, for each line transcriptomic analysis was performed. Our studies provide new insights underlying the altered growth habit in cucumber.

P.2.2

Analysis of the cucumber chloroplast genome and expression levels of plastid-encoded genes

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Chloroplasts are organelles that are essential for the functioning of plant cells, not only because of photosynthesis, but also because they are involved in a number of processes that are important for maintaining homeostasis and responding to stress stimuli. Chloroplasts have their own genome that encodes the most important elements, including proteins, rRNA and tRNA required for gene expression, and proteins of the photosynthetic apparatus. Therefore, understanding the genome structure and plastid-encoded genes is important in expanding knowledge about the functioning of the entire plant organism and its improvement using breeding or biotechnological methods. The cucumber chloroplast genome has been sequenced and annotated, but not all identified open reading frames have been functionally annotated. Our aim is to discover the functions of undescribed ORFs and hypothetical open reading frames (YCFs) by plastid transformation using the biolistic method. In this study, we compared the chloroplast genome of the cucumber line B10 with chloroplast genome sequences from other plants. Whole genomes were compared, but also comparisons of individual genes were made, in particular with well-described tobacco sequences, which show a high degree of similarity. We also carried out expression analysis of plastid-encoded genes using qPCR. The analysis included known genes as well as ORFs and YCFs that have not yet been functionally described in cucumber. The obtained results will allow the selection of not well characterized plastid-encoded genes that are expressed for further analysis and understanding of their functions through biolistic transformation of the chloroplast genome using expression silencing vectors.

P.2.3

Multi-omics characterization of cucumber line B10 in the context of male flower development

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North-European cucumber (*Cucumis sativus* L.) Borszczagowski line, named also as B10, is a line often used as a reference in various studies. So far, the B10 line has the longest known genome sequence among cucumbers and is organized into chromosomes.. This comprehensive genomic knowledge, along with its derivation of various somaclonal, chemical, growth, and sexual mutants, underscores its significance as a reference standard. In this study, we explore the mechanism of sex determination and male flower development using multi-omics approach. This includes the analysis of transcriptome, miRNA, miRNA's target genes and metabolite identification. Utilizing high-throughput sequencing technologies such as RNA-seq, coupled with bioinformatics and laboratory validation, we identified differentially expressed genes (DEGs) and miRNAs (DE miRNAs) and their targets at various stages of male flower bud development. GO and KEGG pathway analyses elucidated the biological processes and metabolic pathways involved.. Our findings provide novel insights into flower morphogenesis and enhance the understanding of the B10 cucumber line's genetic framework, contributing significantly to plant developmental biology.

P.2.4

Interspecific hybridization in *Cucurbita* for improved disease resistance and novel traits

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Interspecific hybridization is an important tool for creating variation and introducing specific traits from one plant species to another. In the genus of *Cucurbita*, *C. pepo*, *C. moschata*, and *C. maxima* are the most economically important cultivated species. However, each of these species is superior in some desirable traits, but devoid of others. For example, *C. pepo* is the most diverse in fruit shape, size, and color, but is generally susceptible to many diseases. *C. moschata* carries resistance to many diseases but is low-yielding and late flowering. *C. maxima* is valued for its superior flesh quality but is fairly susceptible to diseases. The transfer of beneficial traits across *Cucurbita* species is necessary, but often hindered by hybridization barriers. The Cucurbit Breeding program at the University of Florida has developed methodologies for interspecific hybridization across *Cucurbita* species through embryo rescue and bridge crosses. Here, we highlight and discuss the success of the breeding program in interspecific transfer of disease resistance traits from *C. moschata* into *C. pepo* and *C. maxima*, including potyviruses (ZYMV and PRSV)), Phytophthora crown rot and powdery mildew. Furthermore, we report our progress on interspecific transfer of the hull-less seed trait (*h*) from *C. pepo* into *C. moschata* and *C. maxima* using a combination of marker-assisted selection targeting the *h* allele and bridge lines. The overall goal is to breed hull-less seed pumpkin with superior flesh quality across the three major *Cucurbita* species.

P.2.5

Functional validation of the melon *Fom-1* gene by CRISPR-Cas9 mutagenesis

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Fusarium oxysporum f. sp. *melonis* (FOM) causes Fusarium wilt in melons, and the *Fom-1* gene confers dominant resistance to races 0 and 2 of the pathogen. The gene was mapped to chromosome IX, close to another R-gene, *Prv*, that confers resistance to papaya ring spot virus (PRSV). Our laboratory identified a pair of TIR-NBS-LRR genes in the *Fom-1-Prv* locus by map-based cloning (Brotman et al. 2013). The function of *Prv* was validated recently by CRISPR-Cas9 mutagenesis (Nizan et al. 2023). To validate *Fom-1*, we transformed a FOM race 0 and 2 resistant melon genotype, Védrantais, with *Agrobacterium* carrying Cas-9 nuclease with guide RNAs directed against this gene. Four different plants were confirmed as transgenic, and carried several mutated alleles of the gene, *e.g.*, a 5 bp deletion, a 1 bp insertion and a large 6500 bp deletion. The T1 progeny of all four families became susceptible to FOM races 0 and 2, showing strong disease symptoms. This proves the role of Fom-1 in conferring Fusarium resistance and paves the way for further molecular research on melon-Fusarium interaction.

P.2.6

Candidate genes in the melon *Zym* resistance locus: expression in transgenic cucumber and CRISPR-Cas9 mutagenesis

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Zucchini yellow mosaic virus (ZYMV; potyviridae) represents a major pathogen of *Cucurbitaceae* crops. ZYMV resistance in melon PI 414723 is conditioned by a dominant allele at the *Zym* locus. In previous studies, *Zym* has been mapped to linkage group II of the melon genetic map. We utilized five mapping populations and two BAC libraries to construct a high-resolution genetic map of the *Zym* locus. We delimited the region to 17.6 kb sequence that harbors a NAC-like transcription factor and two R-gene homologs with a CC-NBS-LRR structure, named *NBL1* and *NBL2* (Adler-Berke et al. 2021). To prove which of the two homologs is the functional *R* gene, we generated transgenic cucumber plants that carry either *NBL1* or *NBL2* under the control of the 35S CaMV promoter. The cucumber line that we used is susceptible to ZYMV, and we ask whether the transgenic lines will become resistant to the virus by expressing the melon candidates. In addition, we apply CRISPR-Cas9 mutagenesis to inactivate the candidate genes (*NBL1*, *NBL2*, both of the above, and *NAC*) in a ZYMV-resistant melon genotype, and see whether resistance has been abolished.

P.2.7

Developing Tm-shift markers for selected traits in *Cucurbita spp.*

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Pumpkins of the genus Cucurbita are cultivated for a number of economically important uses including edible fruit, seed, and seed oil. Breeding of cucurbits frequently utilizes interspecific crosses, seen in the successful transfer of powdery mildew resistance from C. okeechobeensis to C. moschata and C. pepo, among other successful hybridizations. Due to its large size and vining architecture, squash is both space and labor-intensive to grow and conduct genetic work on. In an effort to accelerate selection, marker assays compatible with Tm-shift (thermal shift) genotyping technology were developed as a high-throughput, gel-free tool for genotyping at the seedling stage. Using polymorphisms within or near causal mutations, allele-specific marker assays were developed for powdery mildew resistance (PMR) conferred by the Pm-0 gene (C. moschata and C. pepo) and the hull-less seed phenotype (C. pepo). The marker assay for PMR was designed using a SNP in the candidate gene for Pm-0 and was found to be highly predictive, accurately identifying every genotype in a segregating F2 population of 51 lines of C. moschata. The marker assay for the hull-less seed phenotype was designed downstream of the causal gene and demonstrated a prediction accuracy of 97.5% in a population of 122 segregating lines of C. pepo. As the major gene controlling the hull-less phenotype is recessive, the marker assay is particularly useful for identifying heterozygous carriers of the mutation. Work is ongoing to develop additional marker assays for simple genes linked to desirable phenotypes such as the bush growth habit and genes controlling rind and flesh pigmentation (B, D, L1, L2). These assays can be used as tools for efficient selection in existing lines as well as to facilitate the introgression of desirable traits into other species of Cucurbita, serving to accelerate the rate of breeding and improvement of an economically important crop.

P.2.8

The development of long shelf-life melon by using in planta Particle Bombardment (iPB), a genome editing technique

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Melon production in Japan has been stagnant due to a decline in domestic consumption resulting from the increasing trend toward nuclear families. On the other hand, according to a survey by the Ministry of Agriculture, Forestry and Fisheries, exports to Southeast Asian countries, including Hong Kong and Singapore, are on the rise. Therefore, export expansion has become an important strategy in revitalizing domestic melon production. Extending the shelf life of fruits is expected to reduce food loss during transportation. We have been working on improving the shelf-life of melons by in planta Particle Bombardment (iPB), a genome editing technique. So far, we have succeeded in improving fruit shelf-life by introducing targeted mutations in the *CmACO1* gene, which encodes 1-aminocyclopropane-1-carboxylate oxidase (ACO), an ethylene biosynthetic enzyme, in a muskmelon line called Harukei No. 3. In this study, we tested whether this technology can be applied to other muskmelon lines. In this technology, gold particles coated with CRISPR/Cas9 ribonucleoprotein (RNP) were introduced into seed embryos immediately after germination. Sequence analysis or electrophoresis of plants grown from the treated embryos confirmed a mutation in *CmACO1* with an efficiency of about 10%. The mutation was also transferred on to the next generation with an efficiency of about 1%. The results of this study indicate that the iPB-based genome editing technique is applicable to many lines of melons.

POSTER SESSION N.3
P.3.1

The Emerging Viruses in Cucurbits Working Group: expanding stakeholder knowledge of cucurbit viruses in the United States

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Numerous viruses that infect cucurbits are established or emerging in cucurbit-producing regions of the United States (U.S.). These viruses impact cucurbit production by reducing marketable yields and increasing production costs. Knowledge of virus symptomology, mode of transmission, and effective management methods is critical for successful virus disease management. The Emerging Viruses in Cucurbits Working Group (EVCWG) was established in 2022 "to improve communication and knowledge about viruses across the cucurbit industry and develop strategies to successfully identify and mitigate virus threats to cucurbit production in the U.S." Since its establishment, the EVCWG developed seven fact sheets, nine educational postcards (in both English and Spanish), and two videos on cucurbit virus topics; these resources have been distributed at numerous stakeholder events and are freely available from the EVCWG website, eCucurbitviruses.org. EVCWG members also delivered over 15 presentations on emerging cucurbit viruses and the EVCWG to stakeholders. These efforts, along with electronic and personal communications and website visits, have reached over 5,000 stakeholders, increasing stakeholder knowledge of existing and emerging virus threats, virus spread, and virus management as well as their ability to recognize symptoms of potential virus threats in cucurbits. To increase opportunities for stakeholder participation and education, strengthen communication within the cucurbit industry, and build networks among cucurbit stakeholders, the EVCWG began holding open working group meetings and hosting webinars on key virus identification and mitigation topics. Through these efforts and the development of additional resources and opportunities, currently in progress, cucurbit stakeholders will not only continue to increase their knowledge of cucurbit viruses but also benefit from improved communication and strengthened networks in the U.S. and internationally. Increased awareness and knowledge, particularly of new cucurbit virus threats and available diagnostic protocols, are also critical for breeders and breeding programs striving to advance virus resistance.

P.3.2

Screening cucurbit germplasm for resistance to *Macrophomina phaseolina*

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The *Cucurbitaceae* family, which includes melons, watermelons, cucumbers and pumpkins, plays a critical role in global agriculture. These crops are vulnerable to the soil-borne fungus *Macrophomina phaseolina*, causal agent of charcoal rot, which produces significant economic losses. The fungus affects over 500 plant species. In cucurbits, frequent symptoms are stem lesions and partial wilting. Management of this pathogen is challenging due to its complex interactions with hosts and its survival through the formation of microsclerotia. Therefore, the identification of resistant germplasm is essential. The plant material used in this study in the screening for resistance to *M. phaseolina* included, *Cucumis melo* commercial cultivars and hybrids, wild *Cucumis* species and hybrids among them, *Cucurbita* spp. hybrids, and *Lagenaria siceraria* and *Citrullus lanatus* accessions. The inoculum was prepared using wheat seeds colonized by *M. phaseolina*, mixed with substrate, and distributed into pots where germinated seedlings were transplanted. A completely randomized design was used with six plants per genotype and a non-inoculated control. Two assays were performed, one in growing chamber and one in open-field. Plants were evaluated using a visual scale from 1 to 4, and roots were measured for various development and structure parameters using WinRHIZO software. Among the tested accessions, a *Fusarium oxysporum* resistant watermelon, the wild *Cucumis* species *C. sagittatus* and *C. ficifolius*, and the two hybrids derived from *C. ficifolius* and C. myriocarpus and C.anguria `Fimy´ and `Fian´, respectivelly showed the highest resistance. These accessions have been considered of interest for future studies about the genetic control and could help to breeding commercial varieties with *M.phaseolina* resistance.

P.3.3

Study of essential oils efficacy against pathogens occurred on cucurbit plants

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Efficacy of eleven essential oils (EO) were screened on two powdery mildew /PM/ isolates (causal agent: Podosphaera xanthii /Px/, 7/23 Px, 24/22 Px) from Czech Republic (CR). EOs originated from: Zingiber officinale, Valeriana officinalis, Ormenis multicaulis, Satureia hortensis, Acorus calamus, Illicium verum, Coriandrum sativum, Artemisia dracunculus, Artemisia absinthum, Pogostemon cablin, Citrus reticulata. There were also tested efficacy of other eight EOs at two cucurbit downy mildew isolates (causal agent: Pseudoperonospora cubensis /PC/: OL PC 1/23 3, PC 22/23 2) from CR. These EOs originated from: Picea abies, Abies alba, Abies alba cones, Larix decidua, Pinus mugo var. pumilio, Pinus sylvestris, Thuja orientalis, Pseudotsuga menziesi, Cupressus sempervirens, Citrus sinensis. A modified leaf discs bioassay by Sedláková et al. (2024) was used for screening EOs at both pathogens (PM, PC). A highly susceptible Cucumis sativus cv. Perzeus F1 served for preparation of leaf discs. There were screened these seven concentrations (0,025%, 0,04%, 0,05%, 0,06%, 0,075%, 0,085%, 0,090%) at all EOs for PM and PC. Efficacy of screened EOs towards PM and PC isolates varied significantly in relation to tested EOs and as well in comparison to both studied biotrophic groups of pathogens (PM, DM). Tested EOs also differed in fytotoxicity level.

P.3.4

Screening a watermelon *(Citrullus lanatus)* germplasm collection for resistance to *Alternaria cucumerina*

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Alternaria cucumerina, the causal agent of Alternaria leaf spot or blight of cucurbits, poses a significant threat to watermelon production under periods of wet weather and temperatures between 20° to 32°C in Europe and Asia. Cucurbits heavily infected by *A. cucumerina* defoliate resulting in reduction of the fruit yield and ultimately leading to economic losses for growers. Therefore, developing watermelon varieties more tolerant or resistant to *A. cucumerina* is crucial for sustainable disease management. Plant introductions (PIs) offer a valuable source of genetic diversity for breeding programs on resistance that add efforts to reduce the reliance on fungicides, as well as improve fruit quality and yield to increase profitability.

In our experiments, two cultivars and sixty watermelon PIs, originating from twelve countries were tested for resistance/susceptibility to *A. cumerina* using leaf disk assay. The source of the inoculum was *C. lanatus* leaves showing characteristic symptoms of the disease under open field conditions in Hungary. Disease Severity (DS) was scored on a five-point rating system and Disease Index (DI) was calculated from DS data to test statistical differences.

Our data on the watermelon germplasm collection tested for resistance to *A. cucumerina* might be used for breeding purposes. PIs exhibiting high levels of resistance are valuable resources that can be further characterized to develop more resistant watermelon cultivars or to incorporate resistance genes into commercial varieties.

P.3.5

Characterization and determination of aggressiveness of isolates of the fungus *Macrophomina phaseolina* identified in cucurbits

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Macrophomina phaseolina (Tassi) Goid. (Mp) is the causal agent responsible for charcoal rot, leading to substantial economic losses in various horticultural crops, particularly cucurbits such as melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsumara & Nakai), and squashes (*Cucurbita* spp). This pathogenic fungus is prevalent in tropical and temperate regions globally. In this study, a total of 115 Mp isolates were identified, collected from different cultivation areas of the Iberian Peninsula, specifically from the roots and stems of melon, watermelon, and various rootstocks (hybrids of *Cucurbita* and hybrids between different *Cucumis* species). These isolates were characterized morphologically and by comparison of their ribosomal ITS sequences. This characterization facilitated the selection of a set of isolates based on their morphotype for pathogenicity evaluation. The evaluation was conducted over two years using toothpick inoculation on various resistant and susceptible genotypes of melon, watermelon and squash. As a control, an isolate from a melon field in La Punta (Quatre Carreres, Valencia), previously used by the group in screenings, was employed. This study revealed variability in the aggressiveness of Mp strains species, depending on the isolate used. Notably, isolates capable of inflicting greater damage than the La Punta isolate were identified in every species. Three of the isolates that had greater damage than La Punta on the first year were repeated on the second year. The results obtained will guide the identification of resistance to Mp in different species, using isolates with different aggressiveness in screenings.

P.3.6

Occurrence of yellowing viruses infecting melons in Korea and molecular characterization of CCYV isolates

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In Korea, melon crops are significantly affected by yellowing viruses, including cucurbit Aphid-Borne Yellow Virus (CABYV), and cucurbit Chlorotic Yellows Virus (CCYV). This study examines the prevalence of these viruses and provides a detailed molecular characterization of CCYV isolates. The complete genome sequences of four CCYV isolates collected from melons were compared with previously reported isolates from GenBank. The analysis revealed very low genetic diversity among the CCYV isolates, offering insights into virus detection, host responses, and the development of resistant melon varieties, which are crucial for improving melon production in Korea.

P.3.7

Breeding program for the introgression of resistance to viral and fungal pathogens in melon traditional backgrounds

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Viral and fungal diseases are among the most challenging threatens for cucurbits cultivation worldwide. The best strategy to control these diseases in the long term is the use of genetic resistance. The breeding program presented here was aimed at the introgression of resistance to viral diseases and to powdery mildew in melon (*Cucumis melo* L.) traditional backgrounds. The resistance sources used were: the African accession TGR-1551 (acidulus group), with resistance to Watermelon mosaic virus (WMV), Cucurbit yellow stunting disorder virus (CYSDV) and powdery mildew (*Podosphaera xanthii* (Castagne) Braun); the Indian momordica melon group accession PI 414723, with resistance to WMV, zucchini yellow mosaic virus (ZYMV), Tomato leaf curl New Delhi virus (ToLCNDV) and powdery mildew; the Indian accession WM-7 (kachri group), with resistance to ToLCNDV; and the Korean accession PI 161375, 'Songwan Charmi' (chinensis group), as the source of resistance to Cucumber mosaic virus (CMV). The melon traditional varieties used as recurrent parents included the most appreciated Spanish types, 'Piel de Sapo', 'Blanco', 'Amarillo' and 'Rochet', as well as a snake melon, 'Alficoz' (*Cucumis melo* var. *flexuosus*). Marker assisted selection was used in the introgression program. The most advanced generations available were cultivated in open field in two different locations. The resistance to the viruses identified in the fields and to powdery mildew was confirmed in the resistant lines. Moreover, these lines had also recovered the internal and external morphological characteristics of the recurrent parent, as well as its sugars and acids profile related to sensory perception.

P.3.8

NAD: a case study of breeding for resistance to FOM in melon

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Fusarium oxysporum f.sp. *melonis* (FOM) is the most devastating and difficult-to-control melon disease, capable of crop losses of up to 100% of the product. The chemical does not guarantee adequate containment and agronomic and physical tools such as crop rotation, soil disinfection, solarization and the use of resistant rootstocks ensure only limited control. Therefore, resistant varieties represent the most effective defence method. Among the 4 races of the fungus identified (0, 1, 2, 1.2), race 1.2 ("yellow" and "wilt" pathotypes) is the most virulent and yield-limiting melon pathogen. Resistance to race 1,2 is controlled by multiple recessive genes, strongly affected by the environment. The genotypes available are characterized by intermediate resistance levels towards race 1,2. In 2002, the doubled-haploid (DH) line NAD, resistant to all four races of FOM, was developed by parthenogenesis *in situ* technique at the CREA-OF of Monsampolo del Tronto. The importance of NAD lies in its state of homozygosity which allows the full expression of all genes, conferring a high level of resistance to the FOM1,2 race. The NAD line was used to study the genetic basis of the heritability of resistance by crossing and backcrossing with the susceptible Charentais-T genotype and for transcriptomic analysis to identify resistance genes involved in the melon-FOM1.2 pathosystem. NAD is protected by plant rights confirmed by the CPVO/TQ-104/2- Rev which ascertained its high level of resistance to FOM 1,2 that makes NAD readily for use as a rootstock. NAD represents an important source of resistance to develop new breeding lines or F1 hybrids resistant to all 4 races of *Fusarium oxysporum* f.sp. *melonis* and suitable vegetal material for applying the New Breeding Techniques, such as the CRISPR/Cas9 system, to step forward in functional studies of the FOM1,2 resistance in melon.

P.3.9

High-throughput screening for salt tolerance in an EMS mutant collection of *Cucurbita pepo* and QTL-seq analysis of salt-tolerant mutants

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Salinity limits crop growth and yield in many arid and semi-arid regions worldwide. Identification of salt-tolerant genotypes is a challenge for breeding programs in many plant species. In this study, we used a germination-based method to assess salt tolerance in an EMS collection of *Cucurbita pepo* composed of 3,751 M2 lines. The screening identified different M2 lines that were salt tolerant at germination. Six of these lines were shown to transmit their phenotype to generations M3 and M4. In addition to improved germination under salt, these mutants showed better growth compared to WT in both seedling and plant stages, which was associated with increased production of osmoprotectants, especially in roots. The two best salt-tolerant lines, 1378 and 2075, were also tested as rootstocks, showing a positive effect on vegetative growth of the scions under control and saline conditions. To identify the causal mutations of salt-tolerant phenotypes in lines 1378 and 2075, salt-tolerant M2 plants were backcrossed with the MUCU16 genetic background but also with a different inbred line (L13B), obtaining two BC1S1 segregating populations. BC1S1 plants were phenotyped during germination and seedling etiolation, separating two salinity-tolerant and two salinity-susceptible bulks that were resequenced by WGS. In line 1378, the BSA- and QTL-seq at germination stage resulted in the identification of three QTLs on chromosomes 3, 4 and 20, but the analysis at seedling stage detected putative QTLs on chromosomes 2, 3 and 7. In line 2075, the BSA-seq at germination resulted in two putative QTLs on chromosomes 3 and 17, but at the seedling stage, only one candidate QTL on chromosome 3 was identified. This research not only provides an efficient protocol for high-throughput screenings for salt tolerance in *C. pepo*, but also new mutants useful for studying the molecular mechanisms behind salt tolerance, and valuable genetic resources for squash breeding programs.

P.3.10

RNA-Seq analysis of salt-tolerant mutants reveals potential mechanisms responsible for salt tolerance in *Cucurbita pepo*

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Salinity stress significantly impacts crop productivity, affecting >1 billion hectares worldwide and causing substantial economic losses. The purpose of the study was to analyze the transcriptomic changes that occur in two salt-tolerant zucchini mutants *(sal-1 and sal-2*) against wild-type under salt stress and control conditions to understand the molecular mechanisms of salt tolerance. The phenotypic traits, including leaf area and root biomass, showed that WT plants were severely affected compared to salt-tolerant mutants. RNA-Seq analysis revealed 154 and 1068 salt-specific differentially expressed genes (DEGs) in *sal-1* and *sal-2*, respectively. Key plant hormones such as ABA, auxin, cytokinin, brassinosteroid, jasmonate, and ethylene biosynthesis and signaling-related genes were up-regulated in mutants, suggesting a role in stress tolerance. Furthermore, the MAPK and Ca^{2+} signaling networks were also differentially expressed in the mutants, indicating their contributions to salt tolerance. The genes coding for antioxidant enzymes (PODs, CAT, PRXs, GSTs, and GRXs) and regulation of the cell wall components also showed increased expression in the mutant compared to WT, which improves ROS regulation and structural integrity under stress. DEGs coding for ion transporters (potassium, ammonium, nitrate, phosphate, magnesium) and ion channels (cation/proton exchanger 3, two-pore potassium channel, cation/carnitine transporter, cation/calcium exchanger) were significantly upregulated in *sal-2* with possible involvement in salinity tolerance. In the mutant, transcription factors from different families were found that were enhancing tolerance of the plant to salt stress. In general, the findings emphasize the complexity of molecular responses involved in the salinity tolerance of Zucchini mutants and prioritize further exploration of specific genes that contribute to resilience in crops under saline conditions.

P.3.11

Deciphering the biosynthesis, regulation and distribution of cucurbitacins in *Cucurbita pepo*

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Cucurbitacins are a group of tetracyclic triterpenes with a cucurbitane skeleton, produced by species of the Cucurbitaceae family, primarily known for contributing to the bitter taste of certain crops. Beyond this property, cucurbitacins play a crucial role in enhancing plant defense mechanisms against herbivores and preventing plant diseases. Additionally, these secondary metabolites are important for their pharmacological properties, including antioxidant and antitumoral effects.

Given the importance of *C. pepo* in the food industry, understanding the genetic regulation of cucurbitacin biosynthesis and their tissue-specific distribution is crucial. For this purpose, in this study, the quantification of five types of cucurbitacins (A, B, D, E, and I) by UHPLC/QTOF-HRMS was performed in the inbred line MUCU16. The analysis was conducted across different seed stages (dry, soaked and germinated), as well as in roots, cotyledons, leaves, ovaries, and fruits. The highest accumulation of cucurbitacins in MUCU16 was observed in roots after two weeks of plant development (21.5 µg), followed by germinated seeds (4.4 µg) and cotyledons (2.8 µg). Cucurbitacin I was the predominant found in *C. pepo* roots, followed by cucurbitacins B, E, and D, whereas cucurbitacin B dominated in cotyledons. Furthermore, transcriptional profiling of putative genes involved in cucurbitacin biosynthesis and signaling was conducted. Notably, germinated seeds with a radicle length of 2 mm exhibited the highest expression levels of biosynthesis and signaling genes.

To further explore genetic variability, cucurbitacin profiling was performed across a diverse collection of *C. pepo* accessions conserved in germplasm banks. The observed variability in cucurbitacin content among the accessions is discussed. These findings provide valuable genomic resources and highlight potential sources of genetic varieties with enhanced cucurbitacin production.

POSTER SESSION N.4
P.4.1

Specialty Pumpkin Cultivars for Organic and Conventional Resilient Cropping Systems in Southern Puerto Rico

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Specialty pumpkins, such as the tropical pumpkin or "calabaza" (*Cucurbita moschata* Duchesne), are widely grown and consumed in Puerto Rico, ranking second among the most important vegetables on the island. Yield, fruit quality, and disease resistance of 21 genotypes were evaluated under conventional and certified organic management conditions in Puerto Rico at the University of Puerto Rico Lajas Research Station from January to May 2022, and from November 2022 to March 2023. The genotypes were evaluated using a randomized complete block design with three replications. The number of marketable fruits, total fruits per plot, yield (kg ha^{-1}), and incidence of whiteflies, among other variables, were recorded. Overall, the number of fruits per plot and marketable fruits ranged from 2 to 12, while the yield reached 9,229 kg ha-1 to 65,707 kg ha-1. Under organic certified management, the highest yield was obtained by UFTP42 with 46,567 kg ha^{-1}, while UFTP4 obtained the lowest yield with 5,110 kg ha^{-1}. Under conventional conditions, the UFTP34, UFTP80, and 'Soler' lines obtained the highest performance, with 66,677; 63,974, and 75,971 kg ha^{-1}, respectively. On the other hand, the genotypes UFTP4, UFTP10, UFTP22, and UFTP46 obtained lower yields with 9,229; 10,881; 17,635, and 15,408 kg ha^{-1}, respectively. Verde Luz, showed the lowest incidence of whiteflies (< 10%), under both management conditions, while the remaining genotypes showed no significant differences between them. In summary, UFTP34, UFTP44, UFTP45, and UFTP80 could be released as prospective cultivars for the southern area of Puerto Rico, and they could be used as parents for breeding purposes under organic and conventional cropping systems.

P.4.2

Unraveling the Interplay between Ethylene Synthesis, Aroma Volatiles and Respiration in Melon Fruit Ripening

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The process of fruit ripening has a very big impact in fruit quality, post-harvest durability, and shelf life, making it a crucial aspect of plant development and breeding goals. Fleshy fruits are broadly classified into climacteric, when a peak of ethylene and a respiratory burst occur at the onset of ripening, and non-climacteric, when neither the ethylene peak nor the respiratory burst occur. Nonetheless, the genetic control of ripening is not fully understood yet. Melon (*Cucumis melo L.*) has garnered attention as a new model for studying fruit ripening due to the coexistence of both climacteric and non-climacteric varieties within the same species, which makes it an ideal crop to study this complex process. Recently, our group has identified a major QTL on chromosome 8, *ETHQV8.1*, governing climacteric ripening. This QTL was fine mapped using two recently developed reciprocal introgression lines funded by the climacteric variety 'Védrantais' and the non-climacteric 'Piel de Sapo'. Introducing the non-climacteric allele into a climacteric background delayed and reduced ethylene production whereas the presence of the climacteric allele in a non-climacteric background led to a mitigated climacteric response characterized by low ethylene production and limited expression of ethylene-associated traits, including aroma production. However, the underlying molecular mechanisms of the interrelationships between volatiles biosynthesis, ethylene and respiration are yet poorly understood.

To elucidate these links and shed light into the regulation mechanisms of this complex process we measured ethylene production in both the parental lines and the ILs containing the *ETHQV8.1* QTL and characterized their respiration, primary metabolite and aromatic profiles at different fruit development stages. Preliminary results are presented and discussed in the context of the possible roles of mitochondrial respiration and ethylene biosynthesis for aroma production during melon fruit ripening.

P.4.3

CRISPR/Cas9 mutation of *CmOFP13*, a gene controlling fruit shape in *Cucumis melo* L.

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Melon fruit shape is an important trait that influences both storage and consumers' preference. Fruit shape is known to be regulated by several factors, such as Ovate Family Proteins (OFPs), Tonneau Recruiting Motif proteins (TRMs), IQ67 domain proteins (IQDs) or brassinosteroids (BRs). Recently, *CmOFP13* was identified as a controller of melon organ shape by a map-based cloning approach and validated by overexpression in *Arabidopsis*, generating rounder leaves and shorter siliques. Nevertheless, the functional validation on melon was pending. CRISPR-Cas9 was applied to melon in order to confirm the function of *CmOFP13*. 'Védrantais' (VED), a round fruit accession, was selected for transformation. Seed cotyledons were transformed with Cas9 obtaining T0 tetraploid edited plants carrying a +1 mutation. After self-pollination and two rounds of crosses with wild type VED, heterozygous diploid plants were obtained. Finally, self-pollination led to the obtention of edited homozygous diploid plants showing more elongated fruits. This phenotype was associated to the loss of the OVATE domain, matching expected results and previous studies in Arabidopsis. The mutation of OFPs offers the possibility to obtain new melon fruit shapes, adapting them for a safer storage or to consumers' preferences.

P.4.4

First results on the occurrence of cucurbitacins in an Apulian landrace of unripe melon (*Cucumis melo* L.)

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Apulia region (Southern Italy) is an important secondary center of diversity for *Cucumis melo* L. Several landraces of this species are still grown here including the so-called unripe melons, such as 'Scopatizzo'. Although this landrace is taxonomically *C. melo*, its fruits are harvested at the immature stage to be consumed fresh and raw, in salads or without dressings and are appreciated as an alternative to cucumber (*C. sativus* L.) due to their better quality profile. Cucumber may occasionally have a bitter taste due to the presence of cucurbitacins, while, as far as we know, evidence regarding the presence of cucurbitacins in Apulian landraces of unripe melons is lacking in the literature. Cucurbitacins are tetracyclic triterpenes synthesized by some Cucurbitaceae species, known to confer an unpleasant taste to fruits and cause gastrointestinal distress and other health issues. Following the discovery of 'Scopatizzo' fruits with bitter taste, cucurbitacins were searched for in the ethanolic extract. Flow injection analysis with detection performed by atmospheric pressure chemical ionization-high resolution mass spectrometry provided evidence for the presence of at least four cucurbitacins (D, B, R, and C and/or their isomers), which were absent in typical unbitter fruits. The discovery of 'Scopatizzo' fruits with a bitter taste represents a negative point even in the case of a low percentage of fruits with this characteristic, because consumers could associate this sporadic feature with a distinctive trait of the 'Scopatizzo', translating in a setback of its market rise. Further insight into this discovery will be required in the near future to assess if the detection of cucurbitacins may mark the appearance of genotypes whose fruits have features not compatible with commercialization. Future research should also be conducted to identify genes associated with cucurbitacin production in 'Scopatizzo' fruits and develop markers for selective breeding, considering the possibility of back-mutations as well as cross-pollination between different genotypes of the Cucurbitaceae family.

P.4.5

The genetic mapping and candidate gene analysis of the major QTL controlling fruit length in Luffa

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The fruit length is a critical trait for both yield and aesthetic quality in cucurbit vegetables, and understanding its regulatory mechanisms is a key biological question. Luffa is one of the most diverse species in terms of fruit length within the Cucurbitaceae family, making it an excellent experimental material for studying fruit elongation. In this study, we obtained a Luffa germplasm named WJ55, which produces exceptionally long fruits reaching up to 1.6 meters. Cytological observations indicated that an increased cell number is the primary cause of the elongated fruit phenotype. To clone genes associated with fruit length, we crossed the extremely long-fruit inbred line WJ55 with the short-fruit inbred line P93075 (commercial fruit length 0.32 meters) to generate a segregating population. Using Quantitative Trait Genes-Seq (QTG-Seq) analysis, three loci controlling fruit length were identified on chromosomes 4, 9, and 13 with a confidence level of 95%, explaining 23.5%, 8.9%, and 13.8% of the phenotypic variation, respectively. Focusing on the major locus on chromosome 4 (designated *LacFL4*), we narrowed down the region to a 133-kb interval containing 15 candidate genes through high-density marker mapping. Integrating gene expression analysis, sequence variations, cytological observations, and auxin (IAA) content measurements, we hypothesize that the auxin response gene *Lcy04g015060* (*GH3.1*) is the most likely candidate gene for *LacFL4*. This research contributes significantly to our understanding of the molecular mechanisms underlying fruit elongation in cucurbits and provides a foundation for future breeding efforts aimed at improving fruit length in Luffa cultivars.

P.4.6

Recurrent excision of a hAT-like transposable element in *CmAPRR2* leads to the 'Shooting Star' melon phenotype

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The external appearance of fruit commodities is an essential trait that has profound effects on consumer preferences. A natural melon variety exists that is characterized by an uneven and patchy arrangement of dark green streaks and spots in the white-skinned rind, which looks like shooting stars streaking across the sky; thus, this variety is called 'Shooting Star' (SS). To investigate the mechanism underlying the SS melon rind pattern, we initially discovered that the variegated dark green color results from chlorophyll accumulation on the white skin. We then constructed a segregation population by crossing a SS inbred line with a white rind (WR) inbred line and used bulk segregant analysis (BSA) to reveal that the SS phenotype is controlled by a single dominant gene, *CmAPRR2*, which has been previously confirmed to determine dark green coloration. Further genomic analysis revealed a hAT-like transposable element inserted in *CmAPRR2*. The transposable element in *CmAPRR2* is excised recurrently from rind tissues. The excision of this transposon activates the expression of *CmAPRR2*, thereby promoting the accumulation of chlorophyll and resulting in the formation of variegated dark green color on the rind, ultimately resulting in the SS rind phenotype. Therefore, we propose that the SS phenotype results from the recurrent excision of the hAT-like transposable element in *CmAPRR2*.

P.4.7

Fruit qualitative evaluation of Chinese watermelon cultivar adapted for the mediterranean cold greenhouse conditions for the early production

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Watermelon (*Citrullus lanatus* L.), is one of the most appreciated fruits in Europe especially during the hot summer. Nowadays, the main criteria for varieties selection is the earliness of the production, the uniformity of maturation and the fruit size.

In this study we evaluate under greenhouse conditions five varieties of small watermelon (baby type) developed in China for the European market in comparison with the commercial cultivar "Perla nera", grown and commercialized in Italy. Plant vigor and leaves coverage, days from transplant to first female flower apparition and fruit harvest (maturity, tendril drying) were recorded. Peduncle diameter on the harvest day was registered Fruit dimensions, rind thickness and coloration, flesh coloration and crispiness were registered for the fruits in addition to the degree Brix and the pH of the juice.

A high diversity was registered among the different cultivar compared, especially in the first female flower apparition and plant vigor. Regarding fruits characteristics "Sugar lycopene" showed to have the thinnest rind and a lighter green color with dark green stripes. "Ice flower" Showed to have a medium rind with the orange to red flesh with the highest sugar content with a brix of13.42 compared to the commercial variety with12.31of Brix.

The Pearson correlation showed a positive relation between fruit weight and rind thickness. An interesting negative correlation also was registered between peduncle diameter and days to first female flower apparition

This study showed some of the important varieties developed in China for European interest and highlighted the competitiveness of some of the lines to the regular and standard varieties in use. Thus, have to be more exploited in order to increase the quality of the varieties grown in Europe.

P.4.8

QTL Analysis of Major Effective Locus Related to Melon Seed Size

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Seed size is an important agronomic trait in melon, but its genetic basis and candidate gene were still unclear. In wild type melon, the seed size was quite small while the cultivated melon accessions with a larger seed size and exhibited abundant variations among different germplasm resources. In this study, the F_2 population (including 650 plants planted in spring and 153 plants planted in autumn) derived from M1-15 (cultivar, ssp. *agrestis*) and PI 614174 (wild type, ssp. *agrestis*) with significant differences in seed size were used for genetic inheritance verification and QTL analysis. Totally 16,060 seeds were evaluated for seed length and diameter, statistical analysis indicated that seed size was quantitative trait with a high significant positive correlation between seed length and diameter. A 3.75 Mb segment on chromosome 2 related with seed size was detected through BSA-seq (Bulked Segregant Analysis by Sequencing) strategy with two seed size gene pools. 10 polymorphic InDel markers were designed and used for genetic linkage analysis. Finally, a stable major effective QTL both related with seed length and diameter was detected in the BSA-seq region explained phenotype variation from 18.51% to 27.32% for seed length and diameter in spring and autumn. Our research provides data basis for further fine mapping and the precise localization of key genes in melon seed size and the cloning of candidate genes in the future.

P.4.9

Identification of genomic regions and candidate genes controlling postharvest cold tolerance in *Cucurbita pepo*

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b

The storage of fruits at low temperatures during transport and commercialization period is commonly used to prevent physiological and nutritional losses in fruits and vegetables. However, due to the subtropical origin of *Cucurbita pepo*, a physiological disorder known as chilling injury (CI) occurs when zucchini fruits are stored at low temperatures during this period. Postharvest cold tolerance is variety-dependent, with Natura and Sinatra as contrasting cultivars for this trait, being tolerant and sensitive, respectively. Physiological studies comparing these two varieties have revealed that the phytohormone abscisic acid (ABA) plays a key role in conferring cold tolerance. However, the complete regulatory and molecular mechanisms controlling this trait remain unknown. Accordingly, the aim of this work was to define putative genomic regions and genetic variants linked to the chilling tolerance phenotype in zucchini fruit. For this purpose, offspring plants from the cross between Natura and Sinatra were characterized for postharvest quality parameters, specifically weight loss percentage and CI, across two different seasons. The obtained results were used to create two DNA bulks with contrasting phenotypes to perform a Bulk Segregant Analysis (BSA), coupled with whole genomic sequencing (WGS). The BSAseq approach allowed the identification of two major QTLs located on chromosomes 5 and 18, which are responsible for the cold tolerance observed in the Natura cultivar. Within these regions, several candidate genes related to phytohormones, cell wall composition, membrane stability, and antioxidant defense were identified, along with several transcription factors. These genes are discussed as potential candidates of controlling postharvest chilling tolerance and the associated genetic variants could be used for future selection of this trait. These findings will support the development of cultivars with enhanced postharvest quality, potentially reducing or eliminating the need for environmentally unfriendly postharvest technologies

P.4.10

A mutation leads to the production of stenospermocarpy melon fruit "has been successfully completed and your data have been recordedproperly.

Maria Florencia Cocaliadis

basf

Seedless is a desirable trait to have in a fruit breeding program. It is a trait appreciated by consumers for flesh consumption, as well as for the fresh-cut industry. Seedless fruit could occur naturally or be induced by hormone treatment, crossbreeding, or breeding through the ploidy. Parthenocarpy and stenospermocarpy are the two described mechanisms underlying seedless fruits. The first one is known as true seedlessness due to the fruit setting occurring independent of pollination and fertilization processes. However, in the case of stenospermocarpy, pollination, and fertilization regularly occur, and embryo abortion leads to seedless fruits. In melon, parthenocarpy fruits have been reported in Makuwa cultivars (Ahmad Zaelani et al., 2015) and stenospermocarpy melon EMS mutants were patented in Charentais type (Tadmor, Yaakov., et al 2015). However, to date there is no seedless melon in the market. The present project aims to evaluate the genetic architecture of a novel seedless trait as well as its pleiotropic effects on other commercial breeding segments.

P.4.11

Agronomic Performance of DH Winter Type Melons (*Cucumis melo* L. var. *inodorus*)

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Melon is an important vegetable species that is grown economically in the world and in Türkiye. Winter type melons melons (*Cucumis melo* var. *inodorus*) are the primary melon groups in most regions of Türkiye. The study was carried out in the open field in the research and application area of Çukurova University, Faculty of Agriculture, Department of Horticulture during the 2024 spring-summer growing season. The agronomic performance of 46 double haploid lines developed by irradiated pollen technique were evaluated. Within the scope of the research, plant length, main stem diameter, number of nodes, total yield (kg/m^2), fruit weight, fruit height and diameter, seed cavidity height and diameter, fruit flesh and rind thickness and TSS parameters were examined. According to the measurements, all plant characters were statistically different among the DH lines. Highest yield (3.6 kg/m^2) was obtained from Si-6-200S while the heaviest fruits were obtained from SR-14 (3620.0 g). In terms of TSS, the highest values were determined in SR31 line (13.36%). In general, it can be concluded that there is no specific line superior for all the examined parameters. The performance of the selected lines should be evaluated in the hybridization combinations in the future breeding programs.

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