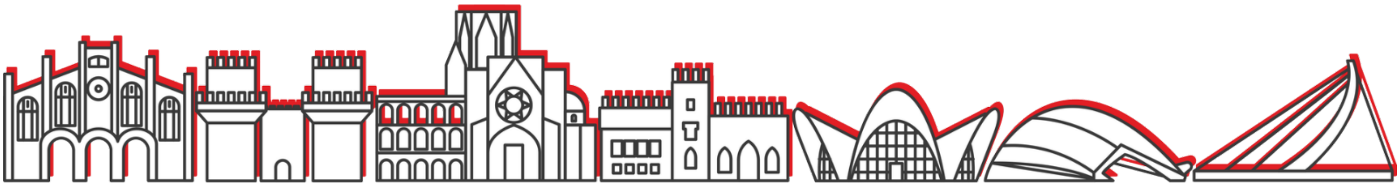

XX Eucarpia Meeting of the Tomato Working Group

Valencia, May 31 - June 3, 2022

XX^th EUCARPIA Meeting of the Tomato Working Group

Valencia, Spain
May 31 – June 3, 2022

Programme &Abstracts

EUCARPIA

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XXth EUCARPIA Meeting of the Tomato Working Group

31 May – 3 June 2022
Valencia, Spain

Conveners:
Antonio Granell and Jaime Prohens

International Scientific Committee
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Amalia Barone (Univ. Naples, Italy)
Mathilde Causse (INRAE, France)
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María José Díez (Univ. Politècnica de València, Spain)
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Mariola Plazas (Univ. Politècnica de València, Spain)
Clara Pons (Univ. Politècnica de València, Spain)
Jaime Prohens (Univ. Politècnica de València, Spain)

Congress Secretariat:
Scientific issues:
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Universitat Politècnica de València
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Registration and invoicing issues:
Centro de Formación de Posgrado
Universitat Politècnica de València
e-mail: congresos@cfp.upv.es

Meeting venue:
Paraninfo Hall (3A building)
Universidad Politécnica de Valencia
Carrer Jean Dausset
46022 Valencia, Spain
(see map at https://eucarpia2022.webs.upv.es/venue/ or type “Paraninfo UPV” at Google Maps: https://tinyurl.com/yckjs8md)

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Important and Useful Information about the Meeting

Badges: All delegates will be provided with a name badge to be worn throughout the Congress.

Reaching the Botanical Garden for inauguration conference and reception: Inaugural conference and reception at Botanical Garden of Valencia (http://www.jardibotanic.org/). Location: Carrer Quart 80, 46008 Valencia. See location map at Google Maps typing “Jardín Botánico de la Universidad de Valencia” (https://tinyurl.com/3r4454t6).

Reaching the Meeting venue: The Meeting venue (1-3 June 2022) is situated in the building 3A of the campus of the Universidad Politécnica de Valencia (Carrer Jean Dausset, 46022 Valencia, Spain). See location at: https://eucarpia2022.webs.upv.es/venue/ or at Google Maps (https://tinyurl.com/yckjs8md)

Several hotels are at walking distance from the venue (see https://eucarpia2022.webs.upv.es/nearby-hotels-accomodation/). Tram and bus stops are close to the venue.

Metro and bus for reaching the venue: There are metro and bus stops near the venue. The metro stop is “Universidad Politécnica”. Maps, timetables and other info on the Valencia metro is available from https://www.metrovalencia.es/. For bus (you have to choose as destination “Universidad Politécnica de Valencia”), a route planner is available from https://www.emtvalencia.es/.

Registration / Information desk: It will be located at the entrance hall of the Conference venue and will operate from Wednesday 1st of June, 8:30 h until the closure of the Meeting. Registration will also be possible at the inaugural conference and reception on Tuesday 31st of May at the Botanical Garden of Valencia between 18:00 and 19:00 h.

Certificate of attendance and invoice: A certifícate of attendance and invoice of registration fees will be provided to participants in the Meeting.

Speakers: Speakers should hand in their Power Point Presentations to the organization as early as possible after their arrival to the Congress venue or submitted in advance to eucarpiatomato2022@upv.es. The time schedule of oral presentations can be consulted in this programme.

Poster presentations: All posters will be displayed in the Hall of the congress venue. Numbers allocated to each poster can be consulted in this programme.
The maximum poster size recommended is 90 cm (horizontal) x 120 cm (vertical). Adhesive tape and drawing pins will be provided.

Internet connection: Participants will receive at arrival with a password and instructions for wifi connection. For participants belonging to an institution affiliated to Eduroam (see http://www.eduroam.org) wi-fi connection will be possible directly without any passwords.

Language: The official Meeting language is English.

Medical service: The University has a medical service, situated in the campus at 10 m walking from the Meeting venue (https://tinyurl.com/nm3ny8eb). Please contact a member of the organization in case of need. For emergencies call 112.

Covid: Masks are advised but are not mandatory. If you have covid symptoms you should require medical advice (see above) and not participate in the Meeting activities. Updated information will be provided in case of changes of policy.

Insurances: The organizing committee accepts no liability for personal injuries sustained, for any loss or damage to property belonging to Meeting participants, either during or as a result of the Meeting.

Scientific Programme, XX EUCARPIA Meeting of the Tomato Working Group, Valencia, Spain

Tuesday 31st of May 2022

Inaugural conference and reception at Botanical Garden of Valencia (http://www.jardibotanic.org/). Google Maps location: https://tinyurl.com/3r4454t6.

18:00-19:00	Registration	
19:00-19:45	**MJ Díez, R. Fernández-Muñoz**	*Inaugural conference:* Tomato genetic resources in Spain: conservation and use
19:45	Visit to tomato materials garden exhibition in the Botanical Garden and welcome cocktail dinner	

Wednesday 1st of June 2022

Meeting venue: Paraninfo UPV.
Carrer Jean Dausset, 46022 Valencia (https://eucarpia2022.webs.upv.es/venue/). Google Maps location: https://tinyurl.com/yckjs8md

8:30 – 9:30	Registration and poster set up
9:30 – 9:45	Welcome and Opening of the Meeting

Session 1: Genetic resources

Chairpersons: Roger Chetelat and *María José Díez*

9:45-10:15	**R. Chetelat**	*Keynote talk:* The molecular basis of pollen rejection in tomato wide crosses
10:15-10:35	**J.Blanca et al.**	American and European Tomato History unveiled using haplotype and GBS analyses
10:35-10:55	**C. Pons et al.**	Unlocking the European traditional tomato genetic resources
10:55-11:15	**Aprea et al. (*presenter*: M.J. Díez)**	SPET genotyping of over 15,000 accessions of tomato reveals the genetic structure of the tomato germplasm at world level

11:15-11:45	Coffee break + Poster session

Session 2: Breeding for morphological and quality traits

Chairpersons: Mondher Bouzayen and *Antonio Monforte*

11:45-12:15	**J.Benejam**	*Keynote talk:* Genetic control of tomato fruit quality: from GWAS to breeding

12:15-12:45	**M. Bouzayen**	*Keynote talk:* Uncoupling fruit softening from fruit ripening or *"Give to Caesar what is Caesar's"*
12:45-13:05	**E. Bineau et al.**	Improving tomato quality in hybrid varieties: inheritance and gene expression of key compounds
13:05-13:25	**D. Alonso et al.**	Phenotypic and metabolomic characterization of the G2P-SOL tomato core collection
13:25-13:45	**Mellidou et al (*presenter*: A. Kanellis)**	Transcriptome profiles of tomato fruit enriched in vitamin C through overexpressing PG-GDP-L-galactose phosphorylase1 (GGP1)

13:45-15:00	Lunch at venue hall or garden + Poster session

Session 3: Breeding for abiotic stresses

Chairpersons: Amalia Barone and *María José Asins*

15:00-15:30	**A. Barone**	*Keynote talk:* Enhancing tolerance to abiotic stresses: a multi-combined approach to face high temperatures in tomato
15:30-15:50	**Bashary et al. (*presenter*: M. Lieberman-Lazarovich)**	Characterization of *Solanum pimpinellifolium* Backcross Inbred Lines as a resource for heat stress tolerance in tomato
15:50-16:10	**Ruggiero et al.**	Tomato responses to combined water and nutrient stress: a view from transcriptomic and splicing analyses
16:10-16:30	**Fang et al. (*presenter*: K. Chen)**	Genetic characterization of tomato pollen viability and pollen number under heat stress

16:30-17:00	Coffee break + Poster session

Session 4: EU-funded projects on tomato

Chairpersons: Antonio Granell

17:00-17:10	**A. Granell**	TRADITOM
17:10-17:20	**A. Granell**	HARNESSTOM
17:20-17:30	**G. Giuliano**	G2P-SOL
17:30-17:40	**T. Cardi**	BRESOV
17:40-17:50	**M. Bouzayen**	TOMGEM
17:50-18:00	**A. Schubert**	TOMRES
18:00-18:10	**F. Sunseri**	SOLNUE
18:10-18:20	**M. Lapidot**	VIRTIGATION
18:20-19:00	General discussion on synergies	

Thursday 2nd of June 2022

Meeting venue: Paraninfo UPV.
Carrer Jean Dausset, 46022 Valencia (https://eucarpia2022.webs.upv.es/venue/). Google Maps location: https://tinyurl.com/yckjs8md

Session 5: Breeding for biotic stresses

Chairpersons: Moshe Lapidot and *Yuling Bai*

9:00-9:30	**M. Lapidot**	Tolerance and resistance to tomato brown rugose fruit virus (ToBRFV)
9:30-9:50	**Schol et al.**	Predicting *R* gene durability through virulence studies with CRISPR-Cas9-generated effector mutants of the tomato leaf mold pathogen *Cladosporium fulvum*
9:50-10:10	**D'Esposito et al. (*presenter*: M. Ercolano)**	Integrated omic approaches for dissecting the tolerance to *Tuta absoluta* in tomato
10:10-10:30	**Fonseca et al.**	Tomato hairplus mutation induces changes in the epigenome and increases glandular trichome density

10:30-11:00	Coffee break + Poster session

Session 6: Tools and materials for breeding and genomics

Chairpersons: Lukas Mueller and *Aureliano Bombarely*

11:00-11:30	**L. Mueller**	Breedbase, a digital ecosystem for plant breeding
11:30-11:50	**Ziarsolo et al (*presenter*: J. Cañizares)**	K-seq, a reliable and low-cost genotyping technology
11:50-12:10	**Vidavski et al (*presenter* : A. Eybishitz)**	Old Battle and New War - Resistance Sources and Markers against TOBRFV
12:10-12:30	**Nieuwenhuis et al (*presenter*: S. Diaz-Trivino)**	Tandem gene breeding in tomato
12:30-12:40	**Guayson et al (*presenter*: M. Zouine)**	High-quality de novo genome assembly of the Solanum pimpinellifolium TO-937 genome using PacBio HiFI Long Read technology

12:40-12:50	Group picture outside the Meeting hall
12:50-16:00	Departure by bus for El Palmar (Albufera Lake Natural Park) and lunch

Technical visit

Fundación Ruralcaja Experimental Farm (Paiporta, Valencia)

16:00-19:00	Technical visit: Demonstration trial of tomato diversity and modern cultivars

Meeting social dinner

Restaurant “La Hípica de Valencia”, Calle Jaca 23, Valencia. Google Maps location: https://tinyurl.com/2p98as24

21:00	Meeting social dinner

Friday 3rd of June 2022

Meeting venue: Paraninfo UPV.
Carrer Jean Dausset, 46022 Valencia (https://eucarpia2022.webs.upv.es/venue/). Google Maps location: https://tinyurl.com/yckjs8md

Session 7: New Plant Breeding Technologies

Chairpersons: Hiroshi Ezura and *Diego Orzáez*

9:30-10:00	**H. Ezura**	The world’s first CRISPR tomato launched to a Japanese market: its impact on the implementation of genome editing technology
10:00-10:30	**D. Orzáez**	From intragenesis to genome editing: new breeding techniques and their use for improving fruit composition
10:30-10:50	**Sevi et al.**	Editing Tomato for Low Allergenicity and Improved Nutritional Composition
10:50-11:10	**Nicolia et al (*Presenter*: T. Cardi)**	CRISPR/Cas9-mediated mutagenesis in tomato: application to parasitic plant resistance, technical developments, and future perspectives
11:10-12:00	Coffee break + Posters	

Session 8: Round Table on challenges in tomato breeding

Chairperson: Jaime Prohens

12:00-13:00	**J. Prohens**	Round Table with seed companies
13:00-13:15	Closure and next meeting announcement	
13:15-15:30	Lunch and removal of posters	

List of Posters XXth EUCARPIA Meeting of the Tomato Working Group

Session 1. Genetic resources

Poster No.	Title	Authors
P1.01	Phenotypic diversity and molecular characterization of the tomato germplasm from the Lazio region in Central Italy, with a focus on landrace distinctiveness	**Farinon et al.**
P1.02	Exploiting natural epigenetic variation for tomato improvement	**Ibañez et al.**
P1.03	Preliminary agronomic and quality evaluation of four new tomato (*Solanum lycopersicum* L.) hybrids of the Muchamiel, De la pera and Rosa types with virus resistance genes	**Carbonell et al.**
P1.04	n-Tomatomics: The profile of Greek tomato landraces	**Iakovidis et al.**
P1.05	Caracterization of the Valencian collection of the “De Penjar” tomato in greenhouse and open field on organic conditions	**Casanova et al.**

Session 2. Breeding for morphological and quality traits

Poster No.	Title	Authors
P2.01	Molecular characterization of the *Aubergine* mutation and its relevance in the anthocyanin-enrichment of tomato fruits	**Menconi et al.**
P2.02	Tomato domestication and breeding resulted in a large variety of fruit volatile profiles in modern and traditional tomato varieties away from those of their ancestors	**Rambla et al.**
P2.03	Identification of QTLs for volatile organic compounds, sweetness, and consumer perception from introgression lines between *S. lycopersicum* cv. Moneymaker and *S. pimpinellifolium*	**Petit et al.**
P2.04	Response of tomato traditional varieties to microbial biostimulants	**Sánchez-Sánchez et al.**
P2.05	Use of CRISPR/Cas9 to study ascorbic acid metabolism in a *S. pennellii* introgression subline	**Principio et al.**
P2.06	Agronomic performance of four types of traditional tomato grown under different fertigation regimes	**Sánchez-Sánchez et al.**
P2.07	The HARNESSTOM participatory plant breeding platform: unlocking the results of EU tomato breeding research projects	**Casals et al.**
P2.08	Assessment of fruit quality traits of tomato F1 hybrids	**Tringovska et al.**
P2.09	Comparative evaluation of tomato hybrids for agronomic traits	**Grozeva et al.**
P2.10	Yield and fruit quality traits of F1 hybrids obtained from Bulgarian heirloom varieties	**Ganeva et al.**
P2.11	Evaluation of fruit quality in F1 tomato hybrids derived from traditional landraces with particular focus on their bioactive composition	**Flores et al.**
P2.12	Genetic variability and characterization of fruit quality traits related to functional and organoleptic quality of tomato landraces	**Hellín et al.**
P2.13	The loss of tomato POD2 function impairs meiosis and tapetum degradation, resulting in male-sterility and parthenocarpy	**Micol-Ponce et al.**

P2.14	Tomato *CRABS CLAW* paralogues regulate carpel development and ensure floral determinacy by interacting with chromatin remodelling factors responsible for *WUSCHEL* repression	**Castañeda et al.**
P2.15	“Malacara” tomato nutritional characterization in organic agriculture	**Raigón et al.**
P2.16	Classical reverse breeding approach and combined selection under low input conditions for the production of tomato recombinant lines suitable for organic culture	**Avdikos et al.**
P2.17	BIOSUVEG: an innovative biostimulant for tomato	**Gresta et al.**
P2.18	Strigolactones promote flowering through the miR319-*LANCEOLATE-SINGLE FLOWER TRUSS* module in tomato	**Visentin et al.**
P2.19	Preliminary study of the aromatic profile of Muchamiel and De la pera tomato breeding lines with different genotypes for *Ty-1* and *ty-5* genes	**Cabrera et al.**
P2.20	Morpho-physiological determinants of the long shelf-life (LSL) fruit phenotype in Mediterranean tomato landraces	**Conesa et al.**
P2.21	The tapetal tissue is essential for the control of REDOX homeostasis during microgametogenesis in tomato	**Salazar-Sarasua et al.**
P2.22	CLASS-II KNOX genes redundantly coordinate spatial and temporal patterns of the tomato ripening	**Keren-Keiserman et al.**
P2.23	Superior fruit total soluble solid content of red x pink F1 hybrids over nearly-isogenic parental lines	**Mateos del Amo et al.**

Session 3. Breeding for abiotic stresses

Poster No.	**Title**	**Authors**
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P3.02	Using heat-coupling proteomics with phenotypic classification to identify suitable germplasm for breeding of heat stress tolerant tomato	**Biermann et al.**
P3.03	Strategies of Na and Cl accumulation in *Solanum lycopersicum* and its wild halophyte relative *Solanum chilense* under salt stress	**Bigot et al.**
P3.04	Genetic control of reproductive traits under different temperature regimens in segregating tomato populations	**Gonzalo et al.**
P3.05	Effect of indole-3-acetic acid methyltransferase mutations in tomato high-temperature responses	**Lobato-Gómez et al.**
P3.06	Evaluation of tomato accessions for nitrogen use efficiency in plantlet stage	**Flores et al.**
P3.07	Gene editing of key effectors improves tomato abiotic stress tolerance	**Punzo et al.**
P3.08	Loss of function of *SlSSI2* encoding a stearoyl-ACP-desaturase negatively affects plant growth and development in tomato	**Quevedo-Colmena et al.**
P3.09	Searching for new tomato salt stress tolerance candidate genes, using Introgression Lines (ILs) of *Solanum pennellii* x *Solanum lycopersicum* grown *in vitro*	**Americo et al.**
P3.10	Low production of the ethylene-precursor ACC in roots improves adaptation to salinity stress in tomato	**Martín-Rodríguez et al.**

P3.11	Heat stress during the progamic phase: Impact on male gametophytes of *Solanum lycopersicum* at transcriptome and proteome level	**Flores-Tornero et al.**
P3.12	Dissection of QTLs involved in the control of stigma position in tomato	**Riccini et al.**
P3.13	Agronomic potential of new tomato hybrids as rootstocks for organic agriculture	**Fonseca et al.**
P3.14	Screening of a selected set of tomato accessions for tolerance to nitrogen and water stresses	**Tripodi et al.**
P3.15	Association of stress resilience phenotyping with a wide SNPs screening in Mediterranean tomato landraces revealed by GWAS	**Juan-Cabot et al.**
P3.16	Hormone spraying treatments are effective in modulating stomatal behaviour and osmotic rearrangements in response to moderate drought in tomato plants	**Morabito et al.**
P3.17	Inhibition of deoxyhypusine synthase by GC7 induces modification of polyamine catabolism in tomatoes during salt stress	**Szepesi et al.**
P3.18	Exploring the natural diversity in tomato germplasm for phosphate starvation responses	**Mishev et al.**
P3.19	Short-term transcriptome changes in NUE-contrasting tomato genotypes in response to low nitrate availability	**Aci et al.**
P3.20	Grafting alters plant vigor, yield, fruit chemical contents and resistance to tomato yellow leaf curl virus in tomato under heat stress	**Rakha et al.**
P3.21	Microorganisms and climatic conditions effects on *S. pimpinellifolium* performance	**Ruiz-González et al.**
P3.22	Effects of heat wave on reproductive organ development in tomato	**Bollier et al.**
P3.23	Development and evaluation of interspecific hybrids of eggplant as rootstocks for grafting tomato for cultivation under heat stress	**Hashem et al.**

Session 5. Breeding for biotic stresses

Poster No.	**Title**	**Authors**
P5.01	Identification of an efficient source of resistance to pepino mosaic virus in tomato	**Ruiz et al.**
P5.02	Denomination of five *Cf-9* breaking isolates of *Passalora fulva* on tomato	**Sangster et al.**
P5.03	Screening of wild tomato germplasm for resistance to Tomato brown rugose fruit virus (ToBRFV)	**Figàs et al.**
P5.04	ORLY - another source for resistance to *Sw-5* breaking strain of TSWV	**Stamova et al.**
P5.05	Role of ethylene in viroid symptomatology of tomato plants	**Vázquez-Prol et al.**
P5.06	Transformation of CAPS markers linked to the *Ty-2* gene for use by HRM in tomato (*Solanum lycopersicum* L.)	**García-Martínez et al.**
P5.07	Screening of tomato germplasm for resistance to ToBRFV	**Pasev et al.**

P5.08	Identification of genomic regions associated with the resistance to black leaf mold based on the genotyping-by-sequencing of *Solanum habrochaites* (LA1777) introgression lines	**Choi et al.**

Session 6. Tools and materials for breeding and genomics

Poster No.	Title	Authors
P6.01	Elucidating the role of microProteins in modulation of tomato plant development	**Vitozzi et al.**
P6.02	Morphoagronomic characterization and whole-genome resequencing of the eight founders of the first interspecific tomato MAGIC population	**Arrones et al.**
P6.03	Agronomic techniques for speed breeding in tomato	**Gimeno-Páez et al.**
P6.04	Phenotypic and genetic characterization of a tomato mutant altered in adventitious organogenesis and root development	**Jáquez-Gutiérrez et al.**
P6.05	HarnesstomDB, a database for facilitating the visualization and mining of tomato omics resources	**Pierre et al.**
P6.06	A 3D imaging approach for evaluating tomato fruit shape	**Blazakis et al.**
P6.07	Live plant monitoring using electrical impedance spectroscopy	**Bar-On et al.**
P6.08	Genome-wide dissection of epistatic QTLs in tomato BILs population	**Torgeman and Zamir**
P6.09	Multi-trait genomic prediction to improve tomato aroma contents	**Bénéjam et al.**
P6.10	Tandem gene breeding in tomato	**Nieuwenhuis et al.**

Session 7. New Plant Breeding Technologies

Poster No.	Title	Authors
P7.01	Generation time reduction in tomato using TEV virus-mediated overexpression of flowering positive regulator Flowering Locus T (AtFT)	**Petit et al.**
P7.02	Increased GABA accumulation in tomato fruits by promoter region genome editing	**Gramazio et al.**
P7.03	Enhancing cold stress tolerance in tomato by engineering lipid composition in fruit and plant	**Vittani et al.**
P7.04	Characterization of *green flesh* and *tangerine* CRISPR/Cas9 edited tomato lines in San Marzano variety	**Dono et al.**
P7.05	An efficient workflow for obtaining tomato elite-edited cultivars using the CRISPR/Cas9 system	**Amoroso et al.**
P7.06	In-depth characterization of *greenflesh* tomato mutants obtained by CRISPR/Cas9 editing: a case study with implications for breeding and regulation	**Gianoglio et al.**
P7.07	Down regulation and loss of Auxin Response Factor 4 function using CRISPR/Cas9 alters plant growth, stomatal function and improves tomato tolerance to salinity and osmotic stress	**Bouzroud et al.**

We gratefully acknowledge the sponsors of the XX EUCARPIA Meeting of the Tomato Working Group:

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ABSTRACTS

Inaugural Invited Conference
IC.01

Title: IC.01 Tomato genetic resources in Spain: conservation and use

Authors: Díez, M.J.*# [1], Fernández-Muñoz, R.*# [2]

Affiliations: 1- Instituto de Conservación y Mejora de la Agrodiversidad Valenciana – Universitat Politècnica de València (COMAV-UPV), Valencia, Spain; 2- Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" CSIC-UMA, Algarrobo-Costa, Málaga, Spain

Presenting author: mdiezni@btc.upv.es; rafael.fernandez@ihsm.uma-csic.es
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Abstract:

The usefulness of New World plants gave rise to an intense traffic of vegetable products between America and Europe, centralized in its beginnings in Seville and the Canary Islands. Spain played a strategic role in the acclimatization of these crops in the Old World, that were subsequently expanded to the rest of countries. The extraordinary wealth of climates and environments in Spain led to the diversification of the varieties introduced from America, being an outstanding example the tomato. Today, Spain is considered a secondary center of diversity of this crop. Tomato breeding activities started in Spain in the second half of the 20th century. The need of variation led to the first tomato breeders to organize collecting expeditions to the center of origin of tomato and to the Spanish geographical areas, constituting the beginning of the Spanish tomato germplasm collection. The growing needs of the tomato production in Almeria, the most important tomato production area in Europe, and the processing production of tomato in other areas of Spain, have driven the breeding objectives of the Spanish breeders for the last decades. Breeding for resistance to diseases, adaptation to salinity, and quality have been major breeding objectives. The valorization of the outstanding richness of traditional Spanish varieties is an important current breeding objective. The development of high throughput genotyping and sequencing technologies have facilitated the study of the Spanish germplasm in a world context contributing to the wide utilization of the Spanish tomato germplasm.

Session 1: Genetic Resources

Keynote invited presentation
K1.01

Title: K1.01 The molecular genetic basis of pollen rejection in tomato wide crosses

Authors: Qin, X. [1], Chetelat, R.T.*# [1]

Affiliations: 1- Dept. of Plant Sciences, UC Davis, Davis, CA, U.S.A.

Presenting author: trchetelat@ucdavis.edu
Corresponding author: trchetelat@ucdavis.edu

Abstract:

Reproductive barriers can limit the utilization of traits in wild tomato germplasm. Crosses between cultivated tomato and its green-fruited wild relatives are compatible only if tomato is the female parent; in the reciprocal crosses, *S. lycopersicum* pollen tubes are rejected on pistils of the wild species. This unilateral incompatibility (UI) involves multiple pistil-side barrier factors and corresponding pollen-side resistance/susceptibility proteins. One form of UI occurs when pistils express active S-RNases, the specificity determinants of self-incompatibility (SI); pollen of cultivated tomato lack essential S-RNase resistance factors, including Cullin1 and S-locus F-box proteins. Other types of UI act independently of pistil S-RNases. We previously showed that low expression of a farnesyl pyrophosphate synthase gene (*FPS2*) in cultivated tomato pollen leads to its rejection on *S. pennellii* LA0716 pistils, which lack S-RNase. We used pollen of *fps2* knockouts in *S. pennellii* to map a pistil factor conferring FPS2-dependent pollen rejection to a region within IL3-3. A single genetic locus, comprised of four tightly linked ornithine decarboxylase genes (*ODC2s*) from *S. pennellii*, are required and sufficient for rejection of *fps2* pollen. We combined the *ODC2*-containing introgression of IL3-3 with IL12-3, which contains the SI genes *HT-A* and *HT-B*. *S. lycopersicum* pollen express low levels of FPS2 and are compatible on either single IL, but are rejected on the IL3-3 x IL12-3 double IL. While the biochemical mechanism of FPS2/ODC2-based UI is unknown, our results show it does not require S-RNase but may interact with other SI factors such as the HT proteins.

Oral presentation. Session 1: Genetic Resources
O1.01

Title: O1.01 American and European Tomato history unveiled using haplotype and GBS analyses

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Abstract:

Not many historical or archeological records remain from the tomato journey from Solanum pimpinellifolium (SP) to the vintage varieties, however, its domestication, migrations and diversification in Europe can be unveiled using genetic analyses. The study of 628 SP, Solanum lycopersicum var. cerasiforme (SLC), and Solanum lycopersicum var. lycopersicum (SLL) revealed: 1) SP evolved into SLC during a migration from Peru and Ecuador, 2) there is a wild SLC Mesoamerican population, 3) there are no wild SLC populations in Ecuador and Peru, 4) Peruvian and Ecuadorian SLC are an admixture of Mesoamerican SLC and SP, 5) SP introgressions in SLC harbor flowering control and light response genes, 6) at least some Mesoamerican SLL derives from domesticated Peruvian and Ecuadorian SLC. A GBS analysis of 1,254 accessions, that included mainly European vintage materials, was used to analyze the European tomato history. The main conclusions were: 1) the vintage materials PCA was organized along a Spanish-Italian axis with mixed origin accessions between these two groups. 2) Spanish and Italian vintage materials were more diverse than the rest. 3) vintage materials have a very low genetic diversity, out of 64,943 only 298 were polymorphic (95% threshold), 4) many of these polymorphic markers are associated with morphological traits like fruit size and color, 5) 25% of the vintage accessions carry introgressions from the wild species. Thus, Spain and Italy became secondary diversity centers and its vintage accessions carry some loci that maintain its high diversity due to balancing artificial selection.

Oral presentation. Session 1: Genetic Resources
O1.02

Title: O1.02 Unlocking the European traditional tomato genetic resources

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Abstract:

The Mediterranean basin countries are considered secondary centres of tomato diversification. However, information on phenotypic and allelic variation of local tomato materials is still limited. To unveil the molecular basis of Southern European tomato phenotypic diversity, we carried out the most comprehensive phenotypic and genomic variability analysis on traditional European tomato by using 1) a collection of 1,499 traditional European tomatoes (TRADITOM collection) and 2) a multipurpose core collection (TCC), comprising 227 European traditional tomato accessions from the original TRADITOM collection, that captured most of genotypic and phenotypic variation and geographical origin present in traditional tomato. The TRADITOM collection was used to gain insights into the phenotypic and genetic architecture of plant and fruit morphological and quality traits. The TTC was used to understand how traditional varieties respond to environmental variation and to investigate the genetic architecture of trait sensitivity to the environment The results presented here revealed a broad range of phenotypic variability in traditional European tomato and indicated that despite the low polymorphism reported for cultivated and traditional tomato germplasm, as compared to the wild relatives, traditional European tomato is a rich repository of crop genetic diversity, as revealed by both old and new, previously unreported loci.

Oral presentation. Session 1: Genetic Resources
O1.03

Title: O1.03 SPET genotyping of over 15,000 accessions of tomato reveals the genetic structure of the tomato germplasm at world level

Authors: Aprea, G. [1], Fain, V.V. [2], Alonso, D. [3], Barchi, L[4], Ferrante, P. [1], Schafleitner, R. [5], Börner, A. [6], Salinier, J. [7], Bovy, A. [8], Boyaci, F. [9], Tringovska, I. [10], Lefebvre, V. [7], Stein, N. [6], Prohens, J. [3], Díez, M.J.*# [3], Giuliano, G. [1]

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Abstract:

Tens of thousands tomato accessions are conserved in genebanks all over the world, constituting a valuable source of genes of interest for tomato breeding. However, the lack of accurate passport data and the scarce phenotypic and genotypic data of these collections represents a drawback for its exploitation. Genetic characterization of such large collections can improve their management in genebanks through the identification of duplicates and misclassifications, contribute to decipher the distribution of the worlwide genetic diversity, the domestication and diffusion processes and the identification of valuable genotypes. The G2P-SOL project has carried out the largest-scale genotyping to date, by assembling a 5-k probes Single Primer Enrichment Technology (SPET) tomato platform, used to genotype a collection of over 15,000 accessions from worldwide genebanks. Genomic data allowed to identify about 2,000 duplicates within and between genebanks and 174 misclassifications, mainly between the fully cross-compatible *Solanum lycopersicum* and *S. pimpinellifolium*. The PCA and STRUCTURE analyses revealed several bottlenecks occurred in the tomato domestication and diffusion processes, which lead to a marked loss of diversity from the Peruvian and Ecuadorian *S. lycopersicum* var. *cerasiforme* to the ones of Central and rest of South America and from *S. lycopersicum* var. *cerasiforme* to the tomato landraces. On the other side, a higher genetic diversity in modern cultivars, more evident within the most recently developed breeding materials was observed at worldwide level.

Poster presentation. Session 1: Genetic Resources
P1.01

Title: P1.01 Phenotypic diversity and molecular characterization of the tomato germplasm from the Lazio region in Central Italy, with a focus on landrace distinctiveness

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Abstract:

Italy is notoriously a secondary center of diversification for cultivated tomato (*Solanum lycopersicum* L.). The study of phenotypic and genetic diversity in landrace collections is important for germplasm conservation and local biodiversity protection. Here, we set up to study the germplasm collected in the region of Lazio in Central Italy with a focus on the distinctiveness among landraces and the attribution of membership to unnamed accessions. Our regional collection included 30 accessions belonging to 6 different locally recognized landraces and 21 unnamed accessions. All accessions were gathered in Lazio and belonged to the collection held at the Regional Agency for the Development and Innovation of Agriculture in Lazio (ARSIAL) and at the University of Tuscia. Control genotypes (n=13) comprising both landraces from neighbouring regions and cultivars, were included as reference. The collection showed wide phenotypic variability for several vegetative and fruits traits, such as fruit weight (range 14-277 g), locule number (2-12), shape index (0.54-2.65), yield (0.24-3.08 kg/plant), and soluble solids (3.4-7.5 °B). A few landraces showed uncommon phenotypes, such as potato leaf, colourless fruit skin, or delayed ripening. Multivariate analysis of 25 cardinal phenotypic variables grouped the named varieties and allowed to assign some of the unnamed to recognized clusters. Genotyping of the 7,720 SNPs from the SolCAP tomato array allowed to better identify the membership group of unnamed accessions and was useful to perform a study for molecular distinctiveness of the flattened-ribbed types, that presented overlapping distribution according to the phenotypic data.

Poster presentation. Session 1: Genetic Resources
P1.02

Title: P1.02 Exploiting natural epigenetic variation for tomato improvement

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Abstract:

DNA methylation is a hallmark of repressive heterochromatin occurring mostly over transposable element (TE) sequences. Changes in the methylation level of TEs (known as epialleles) can potentially affect the expression of nearby genes and, in some cases, underlie heritable trait variations. Most crop’s genomes, including that of tomato, are largely populated by TEs, hence offering a rich source of potential epialleles that may contribute to phenotypic diversity. Nonetheless, very few comprehensive population epigenomic surveys have been performed in crops, which precludes the use of heritable epigenetic changes for breeding programs. Here, we present our efforts to characterise natural epigenomic diversity in tomatoes by generating high-quality single cytosine methylomes for circa 100 tomato accessions ranging from wild species to modern cultivars. Bioinformatic analysis uncovered more than 200,000 differentially methylated regions (DMRs) genome-wide, most of them located within or nearby genes, including loci controlling important agronomic traits. Our work provides a first comprehensive view of natural epigenetic variation in tomato and sheds light on its contribution to tomato diversity.

Poster presentation. Session 1: Genetic Resources
P1.03

Title: P1.03 Preliminary agronomic and quality evaluation of four new tomato (Solanum lycopersicum L.) hybrids of the Muchamiel, De la pera and Rosa types with virus resistance genes

Authors: Carbonell, P.* [1], Cabrera, J.A. [1], Grau, A. [1], Salinas, J.F. [1], Alonso, A. [1], Amoros, A. [1], Sanchez, E. [2], Lopez, N. [2], Gomariz, J. [2], Ruiz, J.J. [1], Garcia-Martinez, S.# [1]

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Abstract:

The tomato breeding program from CIAGRO-UMH has a collection of pure lines and hybrids of the Muchamiel, De la pera and Rosa types, which are popular tomato landraces in province of Alicante (Spain). These improved cultivars have different combinations of Tm-2a, Ty-1 and Sw-5 genes, which confer resistance to ToMV, TYLCV and TWSV, respectively. Recently, we have developed new hybrids together with IMIDA, using Muchamiel, De la pera and Rosa lines from CIAGRO-UMH and traditional accessions Muchamiel, Pera and Flor de Baladre selected from BAGERIM, the germplasm bank belonging to IMIDA. The aim is to obtain new material of interest to the farmer, with traditional characteristics and the added value provided by the genetic resistance to three diseases of great impact in tomato. In this preliminary trial, three yield factors and two fruit quality parameters of the four hybrids with the greatest potential have been evaluated. Regarding the total yield and number of fruits, the Pera hybrid stands out with 4,8 Kg per plant and almost 60 fruits per plant on average. Instead, the Muchamiel hybrid UMH1200 x IM3 shows the highest average fruit weight, exceeding 220 g. In fruit quality, the Rosa-Flor de Baladre hybrid achieves interesting values in both soluble solid content (5,56 °Brix) and acidity (0,56%), being the best hybrid by far in this aspect. This initial evaluation is a part of a series of trials that will be carried out in the coming years.

Poster presentation. Session 1: Genetic Resources
P1.04

Title: P1.04 n-Tomatomics: The profile of Greek tomato landraces

Authors: Iakovidis, M. [1], Avdikos, E. [2], Kalyvas. A. [3], Kyriakoudi, A. [4], Mourtzinos, I. [4], Tagiakas, R. [2], Gossaine, N. [1], Mavromatis, A.G., [2], Mellidou, I. [3], Krommydas, K. [3], Sampathianaki, M. [1], Biliaderis, C. [5], Kanellis, A. [6], Kalaitzis, P.# [1]

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Abstract:

Tomato production in Greece has been dominated by hybrid seeds, developed mainly by multinational companies, without taking into consideration the extensive variation in desirable traits of local landraces related to physicochemical properties, sensory, resistance to abiotic stress, which have been acclimated to local conditions since the 19th century. n-Tomatomics is a multidisciplinary project aimed at assessing, characterizing, and improving with useful traits the main collection of Greek local tomato varieties that exist in the Greek Seed Bank and in national plant breeding laboratories and departments. Local varieties like Santorini, Makedonia, Oxheart, Pantarosa, Lotos, and others, are grown in commercial greenhouse and field conditions to improve yield potential, disease resistance, fruit quality traits, appearance, and postharvest behavior. Utilizing Omics approaches, genomic, transcriptomic, and metabolomic information is being extracted for each variety and assessed for breeding potential alongside agronomic and marketing performance indicators. Interesting traits have been identified in these varieties controlling hybrid vigor, ethylene signaling, fruit shape, fruit color, carotenoid, and lycopene content, which are being investigated. Complex breeding strategies like classical reverse breeding using recurrent selection under honeycomb experimental design or pure line selection with moving average method, are being implemented to generate hybrid vigor and transgressive segregants, in order to incorporate novel traits, which will propel these local varieties to a commercially competitive level for the Greek farmers and reverse the decline in tomato production of the last 20 years.

Poster presentation. Session 1: Genetic Resources
P1.05

Title: P1.05 Caracterization of the Valencian collection of the "De Penjar" tomato in greenhouse and open field on organic conditions

Authors: Casanova, C.* [1], Figàs, M.R. [1], Burguet, R. [1], Prohens, J. [1], Soler, S.# [1]

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Abstract:

The 'De Penjar' tomato (*Solanum lycopersicum* L.) is a group of resilient local varieties from the Spanish Mediterranean region carrying the alc mutation, which provides long shelf-life. We have evaluated under organic cultivation a collection of 66 accessions of 'De Penjar' tomato varieties from the Valencian region (Spain) in two sites (Alcalà de Xivert, open field; Borbotó, greenhouse). Plants were characterized with 24 morphological and 11 agronomic descriptors and five quality traits. We found a great diversity in the collection. For example, there were accessions with red, pink, orange-red and orange-pink external color of the fruit, as well as for the shape of the fruit, with seven morphologies (flatten, slightly flattened, rounded, rectangular, ellipsoid, heart-shaped, pear-shaped). For quantitative characters, significant differences were observed between varieties for all the characters evaluated (40) in the two cultivation conditions. We observed a significant effect of the site for all the evaluated characters except for plant inflorescence number, fruit disease incidence, green fruits stripes, blossom end-rot incidence, fruit weight and color parameter b. Particularly, the differences in vigor of the plants, the number of fruits per bunch, the content of soluble solids and the flavor index were remarkable. In the latter the highest value was observed in Alcalà de Xivert, where the 'De Penjar' type has a long tradition of cultivation. The results obtained highlight the value of the 'De Penjar' Valencian tomato collection.

Session 2: Breeding for Morphological and Quality Traits

Keynote invited presentation. Session 2: Breeding for Morphological and Quality Traits

K2.01

Title: K2.01 Genetic control of tomato fruit quality: from GWAS to breeding

Authors: Causse, M.*# [1], Bineau, E. [1], Bénéjam, J. [1], Zhao, J. [1], Brault, M. [1], Carretero, Y. [1], Pelpoir, E. [1], Pellegrino, P. [1]
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Abstract:

Tomato flavor has changed over the course of domestication and later during breeding although it was not a target for breeders until recently. Consumers then complained about the taste of modern varieties. Although tomato taste is influenced by environmental and post-harvest conditions, the varieties show a large diversity for fruit composition and texture. We will present our recent work intended to improve tomato fruit quality. Since the availability of large numbers of SNP, genome-wide association studies (GWAS) were performed on several panels of tomato lines. We thus performed a meta-analysis of GWAS for 18 traits, combining results on 775 tomato accessions and 2,316,117 SNPs from three GWAS panels. We discovered 305 significant associations for the contents of sugars, acids, amino acids and flavor-related volatiles. We showed for instance that fruit citrate and malate contents have been impacted by selection during domestication and improvement, while sugar content has undergone less stringent selection. Volatile organic compound contents also evolved and some trends for improvement were identified. More recently we checked the inheritance of volatiles at the hybrid level in order to help the production of F1 hybrids with good quality. We are now assessing how to use these data to improve tomato quality.

Keynote invited presentation. Session 2: Breeding for Morphological and Quality Traits

K2.02

Title: K2.02 Uncoupling fruit softening from fruit ripening or *"Give to Caesar what is Caesar's"*

Authors: Bouzayen, M.*# [1]
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Abstract:

Softening has been classically considered as a ripening-associated process, and so far, the main way to control softening has been the slowdown of ripening which has detrimental impact on sensory qualities. Also, addressing the mechanisms underlying fruit softening mainly focused on factors/events occurring at the ripening phase in pericarp tissues. Our study shows that the MADS-box transcription factor SlMBP3 is a master regulator acting at very early pre-ripening stages. Sufficient expression level of SlMBP is required for locular gel formation and *KO* mutation of this transcription factor results in All-flesh phenotype with a total absence of gel formation giving rise to an *"aubergine-like"* inner tissue. Interestingly, *SlMBP3-KO* lines exhibit enhanced fruit firmness starting at early pre-ripening stages and maintained throughout advanced stages of ripening. Consistently, overexpression of SlMBP3 results in extreme softness that manifests already at very early pre-ripening stages. Combined RNA-seq and ChIP-seq revealed that misexpressing *SlMBP3* impairs locular gel formation through massive transcriptomic reprogramming at initial phases of fruit development. *SlMBP3* influences locule gel formation via the control of cell cycle and cell expansion genes at early pre-ripening stages. Our work also shows that dual KO mutation of MBP3 and AGL11, two members of the same class D clade, results in severe detrimental phenotypes affecting plant growth, fruit size and seed formation. Such deleterious features preclude any potential use in breeding strategies and tomato breeders have inadvertently selected specific alleles of *SlAGL11* to avoid major detrimental effects of dual *SlMBP3/SlAGL11* mutants. Our findings provide leads for novel breeding strategies that are anticipated to circumvent losses of sensory quality associated with the incomplete ripening in tomato and possibly other fleshy fruits.

Oral presentation. Session 2: Breeding for Morphological and Quality Traits O2.01

Title: O2.01 Improving tomato quality in hybrid varieties: inheritance and gene expression of key compounds

Authors: Bineau, E.* [1,2], Rambla, J.L. [3,4], Duboscq R. [1], Corre, M.N. [1], Bitton, F. [1], Lugan, R. [5], Granell, A. [3], Plissonneau, C. [2], Causse, M.# [1]
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Abstract:

Flavour and nutritional quality are important goals for tomato breeders. This study aimed at shedding light upon transgressive behaviors breeders may exploit to improve both flavour and nutritional quality composition. We studied the metabolic contents of 44 volatile organic compounds (VOCs) and 20 phenolic compounds, together with transcriptome profiles in a factorial design. The experimental design comprised six parental lines and their 14 F1 hybrids (HF1) among which five pairs of reciprocal HF1. After cluster analyses of the metabolome dataset, and co-expression network construction of the transcriptome dataset, we characterized the mode of inheritance of each component. Both overall and per-cross mode of inheritance analyses revealed as many additive and non-additive modes of inheritance. Up to 66% of metabolites displayed transgressions relative to parental values in at least one cross. Analysis of the modes of inheritance of metabolites revealed that: i) transgressions for a metabolite were mostly of a single type whichever the cross; ii) modes of inheritance for a metabolite were scarcely consistent between the 14 crosses, except for terpenoid and benzenoid VOCs; iii) metabolites belonging to the same cluster displayed similar modes of inheritance for a given cross and iv) transgressions for metabolite content or gene expression were not correlated to the genetic distance between parental lines. We identified few reciprocal effects in any of the dataset. Integrating metabolome, transcriptome and modes of inheritance analyses suggested a few candidate genes that may drive important changes in fruit VOC contents.

Oral presentation. Session 2: Breeding for Morphological and Quality Traits O2.02

Title: O2.02 Phenotypic and metabolomic characterization of the G2P-SOL tomato core collection

Authors: Alonso D.*# [1], Demurtas, O. [2], Costanzo, M. [2], Casanova C. [1], Grozeva, S. [3], Ganeva, D. [3], Pasev, G. [3], Figàs M.R. [1], Soler S. [1], Tringovska, I. [3], Alseekh, S. [4] Fernie, A. [4], Rambla, J.L. [5], Granell, A. [5], Prohens J. [1], Diez-Niclos, M.J. [1], Giuliano, G. [2]

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Abstract:

The phenotypic and metabolomic diversity present in tomato (*S. lycopersicum* L.) cultivars, landraces and wild relatives has been previously studied on a limited number of accessions and species. In the frame of the EU-funded project G2P-SOL, about 15,000 accessions of cultivated tomato and wild relatives have been inventoried and most of them have been genotyped with the 5k probes SPET tomato platform. The gathered information has been used for the establishment of a core collection based on passport and genotypic data. The core collection consisted of 383 accessions of the cultivated species and 49 wild relatives, and was phenotyped in two different locations, in Plovdiv (Bulgaria) and in Alboraya (Valencia, Spain). Ten qualitative morphological traits and 12 quantitative agronomic traits were recorded. In addition, fruits were harvested and sampled to perform metabolomic profiling for semi-polar metabolites, lipids, and volatile compounds. Overall, a wide phenotypic diversity related to plant and fruit characteristics, such as fruit size, shape, firmness, colour and °Brix was found in the core collection. Regarding metabolomic profile, 162 semi-polar compounds, 195 lipids and 55 volatile compounds were identified. Significant variation in several metabolites between genotypes has been observed. These results are being used for GWAS analysis aimed at identifying quantitative trait loci (QTLs) underlying the control of the main traits.

Oral presentation. Session 2: Breeding for Morphological and Quality Traits
O2.03

Title: O2.03 Transcriptome profiles of tomato fruit enriched in vitamin C through overexpressing PG-GDP-L-galactose phosphorylase1 (GGP1)

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Abstract:
Although several ascorbic acid (AsA) biosynthetic pathways have been proposed to be functional in different species/cultivars, the consensus is that the L-galactose pathway is the dominant biosynthetic route in most plant species, including tomato. Previous studies indicated that GDP-L-galactose phosphorylase (GGP) represents a key target for AsA manipulation in tomato fruit. Œ§he present study aims to unravel the transcriptional responses of GGP1 overexpression in tomato fruit, under the PG ripening fruit specific promoter, and to identify other metabolic pathways that were affected by high vitamin C contents by using a transcriptomic approach. Our analysis demonstrated that 164 and 13 DEGs were up- or down-regulated, respectively, in transgenic (PG-GGP1) compared to WT fruit, highlighting series of changes in their gene expression profiles. A broad number of up-regulated transcripts in PG-GGP1 fruit were related to cell wall modification, ethylene biosynthesis and signaling, pollen fertility and carotenoid metabolism. Further, this higher AsA accumulation caused an up-regulation of transcripts related to the AsA main and alternative biosynthetic pathways suggesting that the entire metabolic path has been influenced suggesting a master regulation. Our results revealed that AsA enrichment in tomato ripe fruit via PG promoter driving GGP1 overexpression has the potential to affect various aspects of fruit development and ripening and provide a robust database to further investigate the function of AsA induced genes.

Acknowledgment: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH “CREATE” INNOVATE (project code: Œ§2EDŒö-01332-n-Tomatomics).

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.01

Title: P2.01 Molecular characterization of the *Aubergine* mutation and its relevance in the anthocyanin-enrichment of tomato fruits

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Abstract:

Anthocyanins are secondary metabolites belonging to flavonoids conferring the typical red, purple and orange colours in the plant kingdom. They serve many functions in plants: to attract pollinators and seed dispersers and as defence against biotic and abiotic stresses. Being part of the human diet, they have been proven as health-beneficial, antinflammatory and antitumoral compounds. Common tomato plants can synthetize anthocyanins in their vegetative parts, but not in the fruits. The anthocyanin pathway is well characterized and conserved in higher plants but its regulation is not fully understood. The nutraceutical value of tomato makes it a good starting point for the anthocyanin enrichment of its fruits and mutants able to accumulate high quantities of anthocyanins have been exploited to obtain anthocyanin-enriched tomato fruits and to study the pathway. The mutations *Anthocyanin* fruit (*Aft*), introgressed from *S. chilense*, and *atroviolacea* (*atv*), introgressed from *S. cheesmaniae*, led to the development of novel anthocyanin rich tomato varieties and to the discovery of key regulators of the biosynthetic way. These are the genes AN2like and MYB-ATV, acting, respectively, as a positive and a negative regulator of the pathway in the fruits and in the vegetative parts of the plant. Another mutation, called *Aubergine* (*Abg*), introgressed from *S. lycopersicoides*, has long been known but never fully characterized: it confers the ability to accumulate anthocyanins in the fruit peel in a light-dependent manner. Our present study focuses on the *Abg* mutation to identify its molecular basis and characterize its potential in tomato breeding.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.02

Title: P2.02 Tomato domestication and breeding resulted in a large variety of fruit volatile profiles in modern and traditional tomato varieties away from those of their ancestors

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Abstract:

Domestication of the wild species *Solanum pimpinellifolium* L. in the Andean region and in Mesoamerica resulted in the cultivated tomato species (*Solanum lycopersicum* L.), in a process which included selection of alleles involved in fruit weight and shape but several other characters were also either on purpose or unintentionally altered. Tomato fruit flavour is the result of our combined perception of a few sugars, organic acids and over thirty different volatile compounds, which provide the tomato fruit its characteristic flavour and aroma notes. A large set of 496 genotypes from *S. lycopersicum* and *S. pimpinellifolium* were evaluated for their volatile compound composition in the ripe fruit. Results revealed a shift in the volatile profile associated to the domestication process, from *S. pimpinellifoium* until *S. lycopersicum* var. lycopersicum, with *S. lycopersicum* var. cerasiforme (an intermediate step in the domestication process) also showing intermediate volatile profiles. Volatile compounds were also determined in a second set of varieties, including 180 different European traditional tomato varieties and 31 modern tomato hybrids or breeding lines, revealing characteristic volatile profiles for different groups of varieties and ample variability within traditional tomato varieties and, in a lesser extent, also within modern ones. These results are being used to understand the molecular genetic basis of volatile composition and as a basis for tomato flavour breeding.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.03

Title: P2.03 Identification of QTLs for volatile organic compounds, sweetness, and consumer perception from introgression lines between *S. lycopersicum* cv. Moneymaker and *S. pimpinellifolium*

Authors: Petit, J.* [1], Mirabel, S. [1], Rambla, J.L. [1], Casals, J. [2], Romero del Castillo, R. [2], Monforte, A.J. [1], Granell, A.# [1]

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Abstract:

Throughout decades, tomato breeding interest focused on improving producers' interests such as yield, and little attention was given to consumers,Äô interests. Consequently, tomato flavour was strongly affected. Currently, breeding interest shifted towards improving consumer-driven traits, mainly flavour-related compound composition, while maintaining characteristics of interest for producers. Major Volatile Organic Compounds implicated in consumer liking have been defined and good progress is made to understand the underlying genetics. The main goal of this study is to identify QTLs associated to consumer liking-VOC and sweetness and to develop pre-breeding lines with improved tomato flavour. Wild relative tomatoes are known to be good sources of genetic variants lost in modern cultivars. An IL collection derived from the S. pimpinellifolium x S. lycopersicum cv. Moneymaker has been extensively characterized. In the present study, six IL lines with outstanding VOC composition were selected. F2 populations of 70 recombinant plants were created for each line backcrossed with Moneymaker and QTL mapping performed. In addition, sensory analyses were performed on 5 selected IL lines using an untrained taster panel. 32 QTLs for different VOCs were identified. The panel detected significant differences between the selected lines and Moneymaker. Particularly, SP9-2 line was significantly tastier than Moneymaker. QTLs for Brix, benzaldehyde and benzylalcohol associated to higher content with the S. pimpinellifolium alleles were identified in SP9-2 line. QTLs for benzaldehyde and 3-methylbutanol, positively associated to consumer-liking, were identified in SP11-2 line. These two lines were selected for further QTL fine mapping and sensory descriptive analyses using a trained panel.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.04

Title: P2.04 Response of tomato traditional varieties to microbial biostimulants

Authors: Sánchez-Sánchez, A.* [1], Hernández, V. [1], Hellín, P. [1], Fernández, I. [1], Garrido, I. [1], Sánchez, E. [1], García-Martínez, S. [2], Fenoll, J. [1], Flores, P.# [1]

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Abstract:

In the light of the loss of genetic diversity caused by the intensification of agriculture and modern plant breeding, access to traditional and locally adapted varieties plays a fundamental social, ecological and agronomic role in agro-ecosystems. In addition to their cultural and culinary value, these varieties are a source of resistance to different abiotic and biotic stresses and constitute an alternative to dependency on modern varieties. One of the aspects in which traditional varieties seem to differ from commercial varieties is their ability to respond to beneficial soil micro-organisms to promote plant growth. This work examines the response of five traditional tomato varieties (Lyco-168, Lyco-285, Lyco-236, Lyco-212, Lyco-H7 and Lyco-H10) from IMIDA Germplasm Bank (BAGERIM) and one commercial tomato variety (Mongo) to microbial biostimulants (N-fixing + K and P-solubilizing bacteria + mycorrhizal endomycorrhizae fungi). Plant growth and yield of the commercial variety were not significantly affected by the application of microbial biostimulants and their effect on the traditional varieties depended on the cultivar type. While biostimulants increased plant growth in varieties Lyco-168 and Lyco-212 (stem thickness), Lyco-H10 and Lyco-H7 (plant height) and Lyco-285 (both parameters), and yield in Lyco-285, Lyco-H10 and Lyco-H7, such enhancing effects of plant growth promoting microorganisms were not observed in Lyco-236. No effect of biostimulants on the concentration of carotenoids, vitamin C and phenolic compounds was observed except in variety Lyco-168 in which the increase in vegetative growth of the plant was accompanied by a decrease in the concentration of carotenoids and vitamin C.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.05

Title: P2.05 Use of CRISPR/Cas9 to study ascorbic acid metabolism in a *S. pennellii* introgression subline

Authors: Principio, L.* [1], Barone, A. [1], D'Ambrosio, C. [2], Stigliani, A.L. [2], Giorio, G. [2], Rigano, M.M.# [1]

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Abstract:

The antioxidant ascorbic acid (AsA) cannot be synthesized in the human body, and it is necessary to get AsA in the daily diet; therefore, the possibility of increasing AsA content in the fruit constitutes one of the major objectives of tomato breeding. In recent years, *Solanum pennellii* introgression lines (IL) have been exploited to improve several quality traits in tomato. These lines are characterized by the presence in homozygosity of a portion of the genome of the wild species *S. pennellii* introduced into the cultivated species *S. lycopersicum* cv.M82. In previous studies, one subline (R182) of chromosome 7 showed better performance in terms of yield and AsA content respect to M82. We investigated the function of two genes, coding for the Nucleobase Ascorbate Transporter (NAT) and for the Major Facilitator Superfamily Protein (MFSP), which could be involved in the biosynthesis and accumulation of AsA in R182. The gene MFSP is present in the genomic region introgressed from *S. pennellii*, while the gene NAT is located in the *S. lycopersicum* region flanking the insertion. The role of these genes in AsA accumulation has been investigated by editing through the CRISPR/Cas9 technology. Both R182 and M82 were transformed with sgRNA/Cas9 cassettes targeted to the MFSP or NAT genes. The T0 plants were characterized by molecular analysis and DNA sequencing to confirm gene editing. We are currently analyzing the progeny of some T0 biallelic plants to study the stability of the mutant phenotypes and the inheritance of the edited alleles.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.06

Title: P2.06 Agronomic performance of four types of traditional tomato grown under different fertigation regimes

Authors: Sánchez-Sánchez, A. [1], Flores, P. [1], Hellín, P. [1], Molina, E. [1], Lozano, J.L. [1], Sánchez, E. [1], García-Martínez, S. [2], Fenoll, J. [1], Hernandez, V*# [1]

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Abstract:

The loss of genetic diversity, the scarcity of water resources and the excessive use of fertilizers are having serious repercussions that threaten the sustainability of production. The objective was to evaluate the potential of traditional tomato varieties to promote the adaptation of production systems to low-input conditions and the preservation of biodiversity. Four traditional tomato types, Moruno, Muchamiel, Flor de Baladre and Pera, and a commercial variety (Mongo) as control were grown under two fertilization (F) regimes (100% and 50%) combined with two irrigation (I) doses (100% and 75%). While the total yield of the commercial variety was not affected by reduced fertilization or irrigation rates, its effect on the traditional varieties depended on the type of tomato. Yield of Moruno type tomato decreased as a result of lower fruit number when fertilization was reduced from 100% to 50% at the highest irrigation rate (100% I), suggesting a higher nutrient demand of this variety with respect to the control. In Muchamiel and Flor de Baladre types, the 100F+75I treatment decreased mean fruit weight, probably due to a greater sensitivity of both tomato types to salinity. Finally, in the Pera type variety, the treatments did not affect any of the production parameters. The effect of the treatments on the organoleptic quality and physical parameters of the fruit was also very diverse and depended on the type of tomato. These results highlight the importance of adapting fertigation to each type of tomato in order to optimize yield and quality while reducing inputs.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits
P2.07

Title: P2.07 The HARNESSTOM participatory plant breeding platform: unlocking the results of EU tomato breeding research projects

Authors: Casals, J.* [1], Mazzucato, A. [2], Tringovska, I. [3], Barone, A. [4], Ercolano, M. [4], Soler, S. [5], Pons, C. [5,6], Fumelli, L. [2], Grozeva, S. [3], Ganeva, D. [3], Pasev, G. [3], Romero del Castillo, R. [1], Picarella, M. [2], Asins, M.J. [7]; Prohens, J. [5]; Díez, M.J.# [5]; Granell, A.# [6]

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Abstract:

Tomato genetic diversity stored in seedbanks represents a tool for farmers to diversify their productions. The scant phenotypic data available, and the difficulty for farmers to navigate among thousands of accessions, renders this diversity underutilized. In the last years different EU-funded projects have characterized more than 3,000 accessions (TRADITOM, 1,700 accessions; TOMGEM, 658; G2PSOL, 435; ROOTOPOWER, 150; and BRESOV, 404), generating essential information that allows to "fish" in these germplasm collections. This information is being published in scientific journals, but still difficult to access for farmers, consumers or chefs. With the aim of transferring this knowledge, within the HARNESSTOM project different participatory strategies are being implemented. As a first step, an on-line survey to study consumer preferences has been conducted, with the participation of more than 3,000 consumers. Information on consumption habits, and preferences for sensory profile and cooking properties have been collected. In parallel, researchers have screened the aforementioned collections in order to select promising genotypes. The use of five selection criteria (breeder- annotations; phenotypic data; renowned landraces; resistance to biotic/abiotic factors; use as rootstock) has enabled to select 241 promising accessions. These genotypes are being re-phenotyped and genotyped to unravel the diversity of this panel. A sub-collection of 25 varieties is being evaluated by farmers and citizens by means of Participatory Variety Selection (more than 70 farmers) and Citizen Science (more than 500 citizens) trials. Overall this project is evaluating different participatory methodologies to speed-up the transfer of results from European research projects to the farmer fields.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.08

Title: P2.08 Assessment of fruit quality traits of tomato F1 hybrids

Authors: Tringovska, I.*# [1], Grozeva, S. [1], Ganeva, D. [1], Stoeva, V. [1], Nankar, A.N. [2], Pasev, G. [1], Kostova, D. [2]

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Abstract:

The tomato fruit is a good source of micronutrients and antioxidants with protective effect against degenerative and infectious diseases for humans. Breeding targets in tomatoes are changing over the years - from yield at the beginning through shelf-life and to enhanced fruit nutritional quality in recent years. Most modern cultivars are F1 hybrids due to their benefits from heterosis phenomenon. The aim of the current study is to assess fruit quality traits of 152 tomato F1 hybrids resulted from hybridization program for yield and quality improvement. The evaluated traits are Dry matter (DM) content, Total Soluble Solids (TSS), Vitamin C, Ferric reducing antioxidant power (FRAP) and Total polyphenols (TP) as well as fruit color (CIELab) and fruit firmness. Within the whole set of genotypes, a wide variability is observed for all traits. The most variable ones are Vitamin C and FRAP, with coefficient of variation 47.9% and 45.8%. The studied fruit quality traits are also assessed depending on fruit size. The group of small (cherry type) tomato is distinguished by the highest values of all traits, except for fruit firmness. A tendency of decreasing the mean group values of DM and TSS content with increasing the fruit weight is observed. Interestingly, the group of very big tomatoes (fruit weight above 300 g) shows relatively higher mean values of Vitamin C (23.7 mg 100 g-1) and TP (31.3 mg GAE 100 g-1). F1 hybrids possessing combinations of best fruit quality traits are selected.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.09

Title: P2.09 Comparative evaluation of tomato hybrids for agronomic traits

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Abstract:

Heterosis is a popular breeding method used to improve the yield and fruit quality traits of tomato to generate the perspective hybrids. A total of 152 tomato hybrids were grown in an open field experiment and assessed for agronomic traits. The F1 hybrids along with their respective parents were characterized by 30 conventional morphological and agronomic descriptors including plant, flower, and fruit traits. According to fruit weight, evaluated hybrids were divided into four main groups: small (cherry type), medium (31 g-90 g), big (91 g-200 g) and very big (over 201 g) as each group was sub-divided based on their suitability for fresh consumption or processing. Based on a single trait the best-parent heterosis was established in 41%, 19%, and 38% of the hybrids for productivity per plant, average fruit weight, and number of fruits per plant, respectively. In the group of very big tomato over 50% of hybrids expressed high heterosis in productivity and number of fruits. In addition, 30% of the hybrids in this group exceeded the better parent by average fruit weight. Among the 152 hybrids only 10 F1 were the best performing ones exhibited heterosis for the productivity and productivity-related traits. The promising hybrid combinations were F1 (LYC-52 x LYC-49) - determinate, medium size tomato for processing and F1 (LYC-6 x LYC-3) - very big, indeterminate tomato for fresh consumption.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.10

Title: P2.10 Yield and fruit quality traits of F1 hybrids obtained from Bulgarian heirloom varieties

Authors: Ganeva, D.*# [1], Grozeva, S. [1], Stoeva, V. [1], Tringovska, I. [1]

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Abstract:

The current challenge of tomato breeding is development of elite varieties, combining productivity, disease resistance, with high nutritional value. Due to their good taste and quality as well as their adaptability to local climatic conditions, heirloom varieties are recognized as valuable genetic material and may provide solutions for enhancing the crop. Breeding potential of such germplasm in Bulgaria is still unexploited. Aiming at crop improvement by these valuable genetic resources, a total of 20 F1 hybrids were developed from the crossing between big fruited lines, obtained as a result of individual plant selection in local population. The current study was designed to evaluate the F1 hybrids, grown in a greenhouse, in terms of yield, yield-related traits and fruit quality. In the hybrid combinations total yield varied from 4.4 to 8.6 t/da, while yield-related traits as average fruit weight and number of fruits per plant were 250.1 - 435.4 g, and 4.4 - 13.9, respectively. The yield obtained from several hybrids was close to or exceeded those obtained by the commercial open pollinated tomato variety “Rozovo sartse” (6.5 t/da) originated from a landrace and used as a reference. Two F1 hybrids (27/20 and 37/20) were distinguished with high yield potential, good taste and flavor. All studied F1 hybrids had relatively high values for total soluble solids (5.5-6.5%) and Vitamin C content (16.4-20.6 mg/100g FW) and firm enough for this tomato type. The results indicated that local germplasm can be successfully used in breeding new and improved tomato varieties.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.11

Title: P2.11 Evaluation of fruit quality in F1 tomato hybrids derived from traditional landraces with particular focus on their bioactive composition

Authors: Flores, P.* [1], Fernández, I. [1], Colomer, C. [1], Molina E. [1], Garrido, I. [1], Molina M.V. [1], Cava, J. [1], Gomariz, J [1]., Hellín, P.# [1]

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Abstract:

In recent years, tomatoes have been identified as a fruit of great interest due to their high content of health-related compounds. Unfortunately, modern agriculture focuses its efforts on prioritising characteristics such as high yield, long life and visual impact, neglecting organoleptic and nutritional quality. In this sense, landraces cover a wide genetic diversity that can help mitigate the current genetic erosion within agricultural diversity, enhancing nutritional and organoleptic traits compared to commercial F1. This work aims to characterise and evaluate the bioactive composition (vitamin C, phenolic compounds and carotenoids) of hybrids obtained from local tomato varieties selected for their added value, with the objective of selecting superior quality genotypes, which may be candidates for new cultivars or potential parents to be used in new crosses. Nineteen traditional tomato varieties were used as parents and twenty-seven F1 hybrids were obtained. Both parents and hybrids were grown under glass together with two commercial controls. The bioactive composition in the landraces was strongly influenced by genotypic factors. Thus, vitamin C content ranged from 12,0-34,8 and 11,7-33,9 mg 100 g^{-1} for parents and hybrids, respectively, and β-carotene content ranged from 0,3-11,6 to 0,8 and 13,6 µg g^{-1} and lycopene from 0-53,4 and 0-41,9 µg g^{-1}, for parents and hybrids, respectively. In general, compared to the parental varieties, the content of phenolic compounds decreased in the hybrids, with mean values of 172,8 and 141,7 µg g^{-1}, for parents and hybrids, respectively.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits
P2.12

Title: P2.12 Genetic variability and characterization of fruit quality traits related to functional and organoleptic quality of tomato landraces

Authors: Hellín, P.*# [1], Hernández, V. [1], Sánchez, E. [1], Molina, M.V. [1], López, N. [1], Colomer, C. [1], Cava, J. [1], Fenoll, J. [1], Flores, P. [1]

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Abstract:

Among the different vegetable crops, tomato is one of the most popular and widely cultivated in the world due to its socio-economic and nutritional importance. There are more than 25,000 tomato varieties, although only 20% are marketed, which is causing a loss of valuable genetic diversity through the loss of cultivars and the disappearance of traditional and local varieties. In this sense, landraces cover a wide genetic diversity that can help mitigate the current genetic erosion within agricultural diversity, improving nutritional and organoleptic traits compared to commercial F1. The objective of this study was to determine the genetic diversity of fruit quality parameters related to nutritional and bioactive compounds such as sugars, organic acids, vitamin C, lycopene and Œ≤-carotene of 48 tomato landraces belonging to nine different cultivar groups: 'Flor de Baladre', 'Corazv≥n', 'de la Sierra', 'Muchamiel', 'de la Pera', 'Pimiento', 'Kumato', 'Mesa Murciano' and 'Cherry'. Fruits with different shapes (oblate, slightly flattened, rounded, heart-shaped, long oblong and pyriform), colours (yellow, pink and red) and sizes (from very small to very large) were included in this study. The highest values in compounds related to the organoleptic quality of the fruits were found in the orange and yellow 'Cherry' varieties. While the varieties belonging to the red 'Cherry' and 'Pimiento' types showed a high content of compounds related to the functional quality (vitamin C and carotenoids). The results obtained show an important intravarietal and intervarietal variability, which would allow the selection of varieties to be used in breeding lines focused on obtaining fruits of high organoleptic and nutritional quality.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.13

Title: P2.13 The loss of tomato POD2 function impairs meiosis and tapetum degradation, resulting in male-sterility and parthenocarpy

Authors: Micol-Ponce, R. [1,4], García-Alcázar, M .[1], Lebrín, R. [1], Capel, C.[1], Pineda, B.[2], García-Sogo, B. [2], Alche, J.D. [3], Ortiz-Atienza, A. [1], Yuste-Lisbona, F.J. [1], Moreno, V. [2], Capel, J. [1], Lozano R.*# [1]

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Abstract:

Pollen development takes place within the anthers and entails a plethora of biological processes that require coordinated activity of both sporophytic and gametophytic cells, leading to the release of functional pollen and plant fertilization. Little is known about the molecular mechanisms regulating pollen development in crop species, including tomato. We have identified a novel male-sterile tomato mutant, *pollen deficient 2* (*pod2*), bearing abnormal pollen grains, which causes the formation of small parthenocarpic fruits. Using a combined strategy of mapping-by-sequencing and RNA interference-mediated gene silencing, we proved that *POD2* encodes the *Solanum lycopersicum* G-type lectin receptor kinase II.9 (SlG-LecRK-II.9). Histological observations of *pod2* anther development revealed abnormalities in meiosis and tapetum programmed cell death, which result in the formation of four dysfunctional microspores, leading to aberrant microgametogenesis. To further understand the role of *POD2* in pollen development, we carried out a transcriptomic analysis of RNA extracted from wild-type and *pod2* floral buds. Among the most de-regulated genes, we found putative orthologs of Arabidopsis genes that are essential for male meiosis and cytokinesis. Therefore, the essential role of POD2/SlG-LecRK-II.9 for the formation of functional pollen grains is first revealed, providing valuable insight into the functional role of receptor kinases in regulating tomato pollen development.

Acknowledgements. This work was partially supported by grants PID2019-110833RB-C31, PID2019-110833RB-C32, and PID2020-113324GB-100 funded by the Spanish Ministry of Science and Innovation, and the Research and Innovation Programme of the European Union Horizon 2020 (BRESOV Project, ID 774244). The authors would also thank CeiA3 for providing research facilities.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.14

Title: P2.14 Tomato *CRABS CLAW* paralogues regulate carpel development and ensure floral determinacy by interacting with chromatin remodelling factors responsible for *WUSCHEL* repression

Authors: Castañeda, L. [1], Giménez, E. [1], Pineda, B. [2], García-Sogo, B. [2], Ortiz-Atienza, A. [1], Micol-Ponce, R. [1,3], Angosto, T. [1], Capel, J. [1], Moreno, V. [2], Yuste-Lisbona, F.J. [1], Lozano R.*# [1]

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Abstract:

Flowers consist of sepals, petals, stamens and carpels that are sequentially generated from a pool of stem cells located in the floral meristems (FM). Once a set number of floral organs has been initiated, stem cell activity is arrested, and the FM is thereby determined to form the gynoecium. The precise timing of this event, also known as floral determinacy, is crucial for crop species since alterations in FM termination led to a reiterative carpel formation pattern resulting in indeterminate fruits, thus affecting fruit shape and size, two quality attributes that influence consumer's acceptance and postharvest handling. Here we investigate the molecular mechanisms underlying floral determinacy through the functional characterization of tomato paralogous *CRABS CLAW* (*CRC*) genes. We revealed that the incomplete penetrance and variable expressivity of the indeterminate carpel-inside-carpel phenotype observed in *fruit iterative growth* (*fig*) mutant plants is due to the lack of function of the *Solanum lycopersicum CRC* homologue *SlCRCa*. Furthermore, a comprehensive functional analysis of *SlCRCa* and *SlCRCb* paralogues allowed us to propose that they operate as positive regulators of FM determinacy by acting in a compensatory and partially redundant manner to safeguard the proper formation of flowers and fruits. Likewise, our results provide the first evidence of the physical interaction of putative *CRC* orthologues with members of the chromatin remodelling complex that epigenetically terminates floral stem cell activity by repressing *WUSCHEL* expression.

Acknowledgments. This work was supported by the PID2019-110833RB-C31 and PID2019-110833RB-C32 grants (MICI/AEI/FEDER, UE) and the BRESOV project (Horizon 2020, No. 774244).

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.15

Title: P2.15 “Malacara” tomato nutritional characterization in organic agriculture

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Abstract:

Plant genetic resources for food and specifically traditional varieties, play an important role in sustainability and security of global food system. Traditional varieties are an essential component of agricultural biodiversity, adapted to territory as livelihood of people who depend on agriculture. Traditionally small farmers and in particular, the important role of organic farmers, have carried out the work of conservation of the genetic material adapted to local conditions. Tomato is an excellent source of nutrients and bioactive antioxidant compounds that are important for human health. Chemical composition of tomato fruit depends on factors such as cultivar, maturity, environmental conditions and cultivation method. The identification of cultivars with high nutritive value, represent a useful approach to select tomato cultivars with better quality and health-promoting properties. This study mainly aims to characterize, physical and nutritional parameters, of two Malacara “for hanging” tomato, differentiated by exocarp color (red and yellow), grown under organic farming conditions. Tomato cultivation was carried out in "La Verde" cooperative, involved in organic agriculture certificated for 33 years and promotes re-seeding and seed exchange, as a model of in situ conservation. Malacara Tomato produced during summer, with long-life characteristic allows it to be consumed fresh, in winter, being a high source of bioactive components such as vitamin C, carotenoids and polyphenols, and an important source mineral, especially potassium, without detracting from its aromatic characteristics. Results can aid in selection of tomato varieties for a sustainable production system and to produce tomatoes with high nutritional value and rich in aroma.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.16

Title: P2.16 Classical reverse breeding approach and combined selection under low input conditions for the production of tomato recombinant lines suitable for organic culture

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Abstract:

Nowadays, there are limited cultivars specifically bred for organic and low-input systems. Most selections have been made through conventional breeding programs, which did not include specific traits required for organic and low-input systems. The majority of commercial tomato varieties are hybrids, to take advantage of heterosis. In tomato, continuous selfing enabled homozygosity to accumulate favorable traits of additive nature, resulting in the so-called inbred vigor. This experiment showed potential in exhibiting inbred vigor at a level equal to or greater than the hybrid vigor of commercial hybrid lines when cultivated under organic and low input conditions. The recombinant lines produced through classical reverse breeding from four F1 single cross hybrids was conducted at low- and high-input farming systems. Our results show that, following the appropriate breeding process in early generation selection (Pedigree and Reccurent selection with Honeycomb design scheme) and under low-input conditions, it is possible to produce recombinant lines with inbred vigor in yield potential and fruit quality. Further improvement is underway to introduce multiple disease resistance and quality traits via a multiparental backcrossing scheme that converts each variety into near-isogenic hybrids, taking advantage of the simple or monogenic nature of the underlying genes controlling them, like NLRs, sft, sp, and rin. These genetic materials can stand out as novel elite cultivars for cultivation in organic, low-input, or high-input conditions, depending on their performance in a given environment.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.17

Title: P2.17 BIOSUVEG: an innovative biostimulant for tomato

Authors: Gresta, F.# [1,2], Campo, E. [1], Visentin, I.#*[1,2], Cardinale, F. [1], Schubert, A [1].

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Abstract:

The research for new potential biostimulant components and their characterization and usage optimization represent a challenging topic of research and has been subjected to ever intensive investigation in recent years. In this context, the EIT Food innovation and research project "Innovative biostimulants for sustainable fruit production from vegetable crops (BIOSUVEG)" is being implemented to achieve an innovative biostimulant to launch onto the market. The BIOSUVEG project involves a spin-off company from the University of Turin (Italy), StrigoLab Srl, which produces an innovative raw biostimulant ingredient obtained from roots exudates of tomato plants following a proprietary process. Also, we aimed to formulate it to optimize its activity with a sticking agent provided by Koppert Biological Systems B.V. (The Netherlands). A series of foliar sprays were performed with two dilutions of the Strigolab "raw powder mixed with the Koppert" sticky agent. The trial was performed in tomato variety KOKORO F1 (Unigen Seeds, Italy), in an open field under a plastic tunnel. To validate the two different dilutions, different traits are being evaluated such as flowering induction and flowering acceleration, yield, and fruit quality. In the end, all this information from the field trial would allow choosing the best solution dosage to meet farmers' expectations and needs and further develop strict claims for the biostimulant.

This project has received funding from EIT-FOOD Innovation programme 2021-22, 21014 - BIOSUVEG

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.18

Title: P2.18 Strigolactones promote flowering through the miR319-*LANCEOLATE-SINGLE FLOWER TRUSS* module in tomato

Authors: Visentin, I. [1], Russo, G. [1], Frizzo Ferigolo, L. [2], Gresta, F. [1], Deva, E. [1], Korwin Krukowski, P. [1], Tarkowski, D. [3], Tebaldi Silveira Nogueira, F. [2], Schubert, A. [1], Cardinale, F.*# [1]

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Abstract:

Strigolactones are a class of phytohormones with various functions in plant development, stress responses, and in the interaction with (micro)organisms in the rhizosphere. As developmental regulators, they control above and below-ground morphology, but while their effects on vegetative development are rather well studied, little is known about their role in reproduction. We investigated the mechanisms underlying reported defects by strigolactone-related mutants in solanaceous plants, using tomato as a model. The results showed that strigolactone levels in the shoot, whether endogenous or exogenous, inversely correlate with the time from germination to flowering, with the number of flowers and with the transcript levels of the florigen-encoding gene SINGLE FLOWER TRUSS (SFT) in the leaves. Genome-wide and targeted transcript quantifications coupled to metabolite analyses demonstrated for the first time that strigolactones induce the activation of the miR319-LA (LANCEOLATE) module in tomato leaves and affect the gibberellin flowering pathway in tomato. The induction of SFT by exogenous strigolactones can occur both before and after floral transition and is blocked in plants expressing a miR319-resistant version of LA under the control of the LA native promoter. Our study represents the first positioning of strigolactones in the context of the flowering regulation network in any plant species.

This work was partly funded by the project VEG- ADAPT ("Adapting Mediterranean vegetable crops to climate change-induced multiple stress"), supported by PRIMA - Partnership for Research and Innovation in the Mediterranean Area.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits
P2.19

Title: P2.19 Preliminary study of the aromatic profile of Muchamiel and De la pera tomato breeding lines with different genotypes for *Ty-1* and *ty-5* genes

Authors: Cabrera, J.A.* [1], Carbonell, P. [1], Salinas, J.F. [1], Grau, A. [1], Alonso, A [1], Noguera-Artiaga, L. [1], Ruiz, J.J. [1], Garcia-Martinez, S.# [1]

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Abstract:

Traditional varieties retain more organoleptic characteristics than modern varieties, which are selected for their handling, uniformity, size, yield or disease resistance. This decrease in compounds related to taste and smell is also caused by the consequent decrease in alleles that control the accumulation of compounds related to taste. The recovery of flavour involves obtaining cultivars that do not present many difficulties in their management, such as, for example, traditional varieties with resistance to the main viruses affecting tomato cultivation and which maintain the characteristics of these native varieties. Consequently, the CIAGRO-UMH plant breeding group has interesting materials with added value, improved tomato lines of the Muchamiel and De la pera types, with resistance to ToMV (Tomato mosaic virus), TYLCV (Tomato yellow leaf curl virus) and TSWV (Tomato spotted wilt virus), as a result of the breeding programme of traditional varieties that began in 1998.

In this preliminary study, and with the aim of studying the effects of TYLCV resistance introgressions on the profile of volatile compounds, several Muchamiel and De la pera breeding lines have been analysed, with the same genotypes for the Tm-22 (ToMV) and Sw-5 (TSWV) alleles but in different combinations for the alleles responsible for TYLCV resistance, the Ty-1 allele and the ty-5 allele. The analysis was performed using a gas chromatograph coupled to a single quadrupole mass detector. Absorption of the compounds was carried out by headspace solid phase micro-absorption (HS-SPME) technique. Thirty-eight volatile compounds were quantified and statistically significant differences were found for some of them.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.20

Title: P2.20 Morpho-physiological determinants of the long shelf-life (LSL) fruit phenotype in Mediterranean tomato landraces

Authors: Conesa, M.À.*# [1], Castanyer, X. [1], Fullana-Pericàs, M. [1], Ribas-Company M.À.J. [1], Domínguez, E. [2], Heredia, A. [2], Galmés, J. [1]

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Abstract:

The long shelf-life (LSL) fruit phenotype characterizes diverse drought-tolerant Mediterranean tomato landraces. This phenotype allows fruits to maintain integrity up to 6-12 months after harvest, harvesting fruits ripen on the vine. The genetic basis for the LSL phenotype has been related to mutations in the NAC-NOR region, although literature evidences raise some concerns to this assumption. Studies on the Balearic “de Ramellet” tomato landrace show heterogeneity in the LSL phenotype, and contrasting impairment of it in some accessions, but not others, when cultivated without water shortage. The morpho-physiological determinants of the LSL phenotype may be related to fruit cuticle traits, which could also explain different interactive effect with irrigation during cultivation. Fruit integrity maintenance for such extended periods should prevent internal water loss and limit fruit respiration to a minimum baseline. Cuticle (im)permeability to H2O, CO2 and/or O2 may be key determining factors to explain those behaviours. Using a customized fruit respiration chamber connected to an infra-red gas-exchange analyser, and a customized hi-sensitivity permeameter we measured, along 8 months, in-vivo fruit respiration and isolated cuticle permeability of diverse Mediterranean tomato landraces cultivated both under severe water shortage and well-watered conditions. We observed contrasting shelf-life behaviours that can be attributed to cuticle and fruit traits. Ongoing research will deepen in the molecular determinants underlying those behaviours.

Funding: VEG-ADAPT (PCI2019-103706; PRIMA-European Union, and the Spanish Ministerio de Ciencia, Innovación y Universidades) and SEQ-LIFE (PDR2020/59; Direcció General de Política Universitaria i Recerca, Govern de les Illes Balears).

Poster presentation. Session 2: Breeding for Morphological and Quality Traits
P2.21

Title: P2.21 The tapetal tissue is essential for the control of REDOX homeostasis during microgametogenesis in tomato

Authors: Salazar-Sarasua, B.* [1], López-Martín, M.J. [1], Roque, E. [1], Hamza, R. [1], Cañas, L.A. [1], Beltrán, J.P. [1], Gómez-Mena, C.# [1]

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Abstract:

The tapetum is a specialized layer of cells within the anther adjacent to the sporogenous tissue. During its short life, it provides nutrients, molecules and materials to the pollen mother cells and microsporocytes being essential during callose degradation and pollen wall formation. The interaction between the tapetum and sporogenous cells in tomato plants, despite its importance for breeding purposes, is poorly understood. To investigate this process, gene editing was used to generate loss-of-function mutants that showed complete and specific absence of tapetal cells. These plants were obtained targeting the previously uncharacterized Solyc03g097530 (SlTPD1) gene, essential for tapetum specification in tomato plants. In the absence of tapetum, sporogenous cells developed and callose deposition was observed. However, sporocytes failed to undergo the process of meiosis and finally degenerated, leading to male sterility. Transcriptomic analysis conducted in mutant anthers lacking tapetum revealed the downregulation of a set of genes related to redox homeostasis. Indeed, mutant anthers showed a reduction of reactive oxygen species (ROS) accumulation at early stages and altered activity of ROS scavenging enzymes. The obtained results highlight the importance of the tapetal tissue in maintaining redox homeostasis during male gametogenesis in tomato plants.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.22

Title: P2.22 CLASS-II KNOX genes redundantly coordinate spatial and temporal patterns of the tomato ripening

Authors: Keren-Keiserman, A. [1], Shtern, A [1], Levy, M. [2] , Chalupowicz, D. [3], Furumizu, C. [4,6], Alvarez, J.P. [4], Amsalem, Z. [5], Arazi, T. [1], Alkalai-Tuvia, S. [3], Efroni, I. [2], Ori, N. [2], Fallik, E. [3], Goldshmidt, A.*# [1]

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Abstract:

Ripening is a complex developmental change of a mature organ, the fruit. In plants like *Solanum lycopersicum* (tomato), it involves softening, pigmentation, and biosynthesis of metabolites beneficial for the human diet. Examination of the transcriptional changes towards ripening suggests that redundant uncharacterized factors may be involved in the coordination of the ripening switch. Previous studies have demonstrated that *Arabidopsis CLASS-II KNOX* genes play a significant role in controlling the maturation of siliques and their transition to senescence. Here we examined the roles of four tomato *CLASS-II KNOX* genes in the maturation and ripening of fleshy fruits using CRISPR-CAS9 (cr) derived knockout alleles of the individual genes and an artificial microRNA line simultaneously targeting all four genes. The mutant and knockdown plants (*35S::amiR-TKN-CL-II*) had leaves with increased complexity, reminiscent of the tomato leaves overexpressing *CLASS- I KNOX* genes, which antagonize *CLASS-II KNOX* gene functions. The fruits of *cr-slknat3*, *cr-slknat5*, and *cr-slknat7* mutants were smaller than the control. The *cr-slknat3* fruits had delayed accumulation of the red pigmentation and yellow stripes around the pericarp veins. The internal *cr-slknat3 cr-slknat7/+* and *35S::amiR-TKN-CL-II* fruit tissues softened and turned red. However, the pericarp color break of these fruits took place ten days later than control, turning yellow instead of red. Strikingly, the *35S::amiR-TKN-CL-II* fruits showed an early ethylene release peak; however, their pericarps remained significantly firmer than control even after three weeks of shelf storage. These findings suggest that *CLASS-II KNOX* genes redundantly coordinate the spatial and temporal patterns of tomato fruit ripening.

Poster presentation. Session 2: Breeding for Morphological and Quality Traits P2.23

Title: P2.23 Superior fruit total soluble solid content of red x pink F1 hybrids over nearly-isogenic parental lines

Authors: Mateos del Amo, J.* [1], Domínguez Carmona, E. [1], Gallardo Alba, F. [2], Fernández-Muñoz, R.# [1]

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Abstract:

Pink-coloured tomatoes are reputed as good flavoured and are very popular in local markets. Pink tomatoes show a transparent, colourless fruit cuticle in contrast to the naringenin-chalcone flavonoid-rich, orange-yellow cuticle of normal, red tomatoes due to the y (colourless epidermis) recessive mutation in MYB12 gene located in chromosome 1. In order to investigate the basis of the alleged higher quality of pink vs. red tomatoes and, especially, our previous observations of good flavour in hybrids between red and pink tomato varieties, two red x pink F1 crosses between pairs of tomato near-isogenic lines were obtained, namely ‘Ailsa Craig’ wt/wt x ‘Ailsa Craig’ y/y and ‘Ponderosa Red’ wt/wt x ‘Ponderosa Red’ y/y, being the latter a pink tomato line in which y allele was introgressed from its closely related ‘Ponderosa Pink’ cultivar. The parental lines and the two F1 hybrids were grown to harvest in a greenhouse and total soluble solids (°Brix) and titratable acidity (TA) were measured in red ripe fruits. While no differences for °Brix were observed between the parents of each cross, significantly higher °Brix was measured in the two red x pink F1’s compared to their corresponding parental lines. No clear differences between parental lines and F1 crosses were shown for TA. The experiment was repeated but including also the pink x red, reciprocal F1 crosses. The overdominance for °Brix but not for TA was confirmed in the red x pink hybrids while the pink x red hybrids produced °Brix values similar to those of their parents.

Session 3: Breeding for Abiotic Stresses

Keynote invited presentation. Session 3: Breeding for Abiotic Stresses
K3.01

Title: K3.01 Enhancing tolerance to abiotic stresses: a multi-combined approach to face high temperatures in tomato

Authors: Barone, A.*# [1], Francesca, S. [1], Graci, S. [1], Olivieri, F. [1], Rigano, M.M. [1]
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Abstract:

Plants are highly sensitive to climate changes that modify the environmental conditions in which they grow and produce biomass, fruit, tubers, or other commercial products. In the last few years, increasing temperatures and water scarcity have been the most limiting environmental factors causing yield reduction. In our laboratory, we focused on studying the responses of tomato crop to the increase in average temperatures. Following an initial phenotypic characterization of around 100 genotypes grown under high temperatures in open fields, we selected a group of tolerant genotypes, which were evaluated for two years under plastic tunnel and in open fields. The selection of the best performing genotypes was carried out on yield-related traits. As a result of these trials, we selected one superior genotype, whose response to high temperatures was investigated at physiological levels and evaluating fruit quality traits. Growth parameters and leaf gas exchange measurements revealed that this genotype used an efficient physiological strategy and had an effective capacity to activate antioxidant defence mechanisms to counteract abiotic stresses. In addition, it also exhibited good fruit quality traits even when grown under elevated temperatures. Finally, it has been genotyped using high-throughput platforms. A GBS analysis evidenced the high genetic variability of this genotype, and the presence in its genome of wild introgressions from *S. pimpinellifolium*. We are now investigating its whole genome with the aim of finding allelic variants in candidate genes potentially involved in the thermotolerance exhibited by this genotype.

Oral presentation. Session 3: Breeding for Abiotic Stresses
O3.01

Title: O3.01 Characterization of *Solanum pimpinellifolium* Backcross Inbred Lines as a resource for heat stress tolerance in tomato

Authors: Bashary, N. [1], Miller, G. [1], Lieberman-Lazarovich, M.*# [1]

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Abstract:

The occurring temperature increment in crop production areas worldwide is generating conditions of heat stress that negatively affects crop productivity. Tomato (*Solanum lycopersicum*), a major vegetable crop, is highly susceptible to elevated temperatures. Under such conditions, fruit-set is dramatically reduced, leading to significant yield losses. *Solanum pimpinellifolium*, a wild species closely related to the cultivated tomato, was shown to have beneficial attributes under various abiotic stress growth conditions. We have utilized a population of backcross inbred lines (BILs) originated from a cross between *Solanum pimpinellifolium* and *Solanum lycopersicum*, in order to evaluate its potential as a new genetic resource for the improvement of reproductive performance under heat stress conditions. This population was screened for numerous heat stress-related traits, under controlled heat stress and non-stress conditions. Our results show that significant variation exists in all of the heat stress related traits that were examined and point out individual lines with better reproductive performance under heat stress. Thus, our results place this population as a valuable new resource for the discovery of heat stress related genetic loci for the future development of heat stress tolerant tomato cultivars.

Oral presentation. Session 3: Breeding for Abiotic Stresses
O3.02

Title: O3.02 Tomato responses to combined water and nutrient stress: a view from transcriptomic and splicing analyses

Authors: Ruggiero, A.*[1], Punzo, P. [1,2], Van Oosten, M. J. [2], Cirillo, V. [2], Costa, A.[1], Maggio, A. [2], Grillo, S. [1], Batelli, G.# [1]

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Abstract:
Cultivation of tomato is often subjected to limited water and nutrient availability, causing severe yield reductions. To identify genotypes with contrasting stress responses, we subjected 10 tomato varieties to drought, low nitrate and a combination of both. By analyzing physiological (chlorophyll content, stomatal conductance and relative water content) and biometric parameters (plant height, shoot fresh/dry weight, leaf area, root length and dry weight) we selected genotype T270 as the most severely affected, and T250 as tolerant to limited input availability. To identify common and genotype-specific molecular responses, transcriptomic and differential splicing analyses were performed for T250 and T270 subjected to combined stress, in root and leaf samples. In both tissues, combined stress elicited a lower number of differentially expressed genes (DEGs) in T250 compared to T270. Interestingly, in roots the number of DEGs was higher than leaves in both genotypes. Enriched GO categories similar in the two genotypes included “nitrate assimilation” and “nitrate transport”, up-regulated in leaves, and “response to water deprivation” and “abscisic acid-activated signaling pathway” up-regulated in roots. The analysis of differential alternative splicing (DAS) events showed a higher number of DAS genes in roots compared to leaves. The low percentage of common DAS genes detected, highlighted the different behavior of the two genotypes, especially in leaves. Taken together, these results reveal important insights into the transcriptional and post-transcriptional mechanisms regulating tomato adaptation to growth under reduced water and nitrogen inputs.

This work was supported by the European Commission H2020 program, TOMRES project under grant agreement no. 727929.

Oral presentation. Session 3: Breeding for Abiotic Stresses
O3.03

Title: O3.03 Genetic characterization of tomato pollen viability and pollen number under heat stress

Authors: Fang, Y.F. [1], Lin, Y.C. [1], Gonzalo, M.J. [2], Lee, Y.C. [1], Najera, I. [3], Montero, T. [4], Gil, D. [4], Baixauli, C. [3], Granell, A. [2], Monforte, A.J. [2], Hanson, P. [5], Chen, K.Y.*# [1]

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Abstract:

High temperature reduces the fruit set rate in tomatoes. The pollen viability and pollen number can be the main factors involved in the fruit set. In the current study, an F8 recombinant inbred line population derived from a cross of CA4 and the heat-tolerant cultivar CLN1621L was used to investigate the genetic factors responsible for maintaining pollen viability and pollen number under heat stress. The results showed that the correlation coefficient between pollen number and pollen viability ranged between 0.39 ~ 0.72 in 7 trials. Over 17K SNPs were discovered from the transcriptome data but highly clustered within two introgression regions on chromosomes 6 & 9. SNPs from transcripts outside the introgression regions are approximately 0.2 SNP per kb and were used to construct the high-density genetic map. The QTL analysis identified two QTLs for maintaining pollen viability under heat stress on chromosomes 3 and 10. However, we found no same QTL for maintaining pollen number under heat stress in repeated trials.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.01

Title: P3.01 The tomato SHW1 homolog, SlSHW1, is involved in the regulation of plant development and stress responses

Authors: Gupta, N.*# [1], Nath, U. [1]

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Abstract:

Light is an important factor for plant survival. Although many genes showing light signaling functions have been reported in Arabidopsis, similar information is not available in crop plants. SHW1 (Short hypocotyl in white light) encodes a serine-arginine-aspartate rich protein that acts as a negative regulator of photomorphogenesis, however plays a positive regulatory role in light-regulated gene expression and root development in *A. thaliana*. In this study, Arabidopsis SHW1 homologue (Solyc04g005120.2) was isolated from tomato (*Solanum lycopersicum* var. Pusa Ruby) mature leaf cDNA and named it SlSHW1. We determined the effect of light on the expression of SHW1 during early seedling development in tomato, it was highly expressed in white light (WL) also upregulation was found in blue light (BL). Spatial-temporal expression analysis of SlSHW1 revealed a clear preferential expression in flower and fruit, especially at early stages of fruit development, and a weak expression in vegetative organs. In addition, we found that SlSHW1 was differentially expressed under various abiotic stresses such as high salinity, oxidative, and drought. Moreover, we assessed the functional orthology of SlSHW1 by their ability to complement Arabidopsis loss-of-function mutants and results indicate that tomato protein is a true functional homolog of its Arabidopsis counterpart. This study provides evidence that SHW1 is a regulatory protein and it plays an important role in overall plant growth and development.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.02

Title: P3.02 Using heat-coupling proteomics with phenotypic classification to identify suitable germplasm for breeding of heat stress tolerant tomato

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Abstract:

Crops will be exposed to distressing abiotic conditions more often in the future due to climate change, out of which heat stress is used as an example for this study. To support the identification of tolerant germplasm, advance screening techniques and identify heat stress tolerance markers, a diversity panel of 14 tomato genotypes, comprising Mediterranean landraces of *Solanum lycopersicum*, the cultivar Moneymaker and *Solanum pennellii* LA0716, was assessed towards their tolerance against long-term heat stress. For this, young tomato plants were exposed to either control (22/18 ºC) or heat stress (35/25 ºC) conditions for two weeks. Within this period, phenotypical traits were recorded. Subsequently, leaf samples were taken and used for untargeted proteomics. To test the hypothesis, that more tolerant genotypes have less affected phenotypes upon stress adaptation, a cluster-analysis based approach was developed, which allowed for the classification of tolerance levels among the diversity panel. The resulting classification of the internal references as "tolerant" highlights the applicability of our proposed tolerance assessment model. Using the assigned tolerance attributes as supplemental information for the interpretation of proteomes, existing markers for heat stress as well as putative candidates that might confer heat stress tolerance have been identified. Hence, the combined evaluation of phenotypic plasticity and proteomes are useful tools to support the identification of novel genetic resources amongst existing germplasms and support breeding for stress adapted cultivars.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.03

Title: P3.03 Strategies of Na and Cl accumulation in *Solanum lycopersicum* and its wild halophyte relative *Solanum chilense* under salt stress

Authors: Bigot, S.*# [1], Martínez, J.P. [2], Lutts, S. [1], Quinet, M.# [1]

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Abstract:
Salinity is a growing global concern that affects the yield of crop species, including tomato (*Solanum lycopersicum*). In contrast to *S. lycopersicum*, its wild relative *Solanum chilense* was reported to have halophyte properties. We compared salt resistance of both species during vegetative and reproductive phases and investigated Na and Cl accumulation in the different organs. Plants were exposed to NaCl (0, 60, 120 mM) from the seedling stage up to flowering (85 days after stress). Overall, *S. chilense* accumulated more Na than *S. lycopersicum* while Cl concentration was similar in both species under salt stress. Sodium accumulated mainly in roots in *S. lycopersicum* and in stems in *S. chilense*, showing that *S. lycopersicum* had an excluder behavior and *S. chilense* an includer behavior regarding Na accumulation while such a difference was not observed regarding Cl accumulation. Both species limited the accumulation of Na and Cl in the inflorescences. The concentration of Na and Cl in the inflorescences were respectively lower and higher in *S. lycopersicum* compared to *S. chilense* under salt stress. Na localization in the flowers was analyzed by laser ablation ICP-MS. Na was mainly located in male floral organs of *S. chilense* but in non-reproductive floral organs *S. lycopersicum* under salinity. Na and Cl accumulation and repartition could be partly explained by gene expression of transporters involved in Na and Cl distribution. Overall, our results indicated that *S. chilense* was more salt-resistant than *S. lycopersicum* and that both species differed towards strategies of Na and Cl accumulation.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.04

Title: P3.04 Genetic control of reproductive traits under different temperature regimens in segregating tomato populations

Authors: Gonzalo, M.J.* [1], Maia, L. [1.2], Nájera, I. [3], Baixauli, C. [3], Gil, D. [4], Montoro, T. [4], Guiliano, G. [5], Ferrante, P. [5], Chen, K.-Y. [6], Asins, M.J. [7], Granell, A. [1], Monforte, A.J.# [1]

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Abstract:

The global temperature increase due to climate change is jeopardizing the yield in most crops. Tomato is one of the affected crops, therefore, in the current work, we study the genetic control of reproductive traits under different heat stress conditions in several populations of inbred lines derived from crosses between S. pimpinellifolium accessions and tomato cultivars (MoneyMaker x TO-937, E9xL5 and E6203xLA1589) and between heat tolerant and heat sensitive tomato cultivars. The temperature increase affected reproductive traits, especially at extremely high temperature, where only few lines were able to set fruits. Even though a relative modest number of QTLs were identified, two clusters of QTLs involved in the response of reproductive traits to heat stress were detected on chromosomes 1 and 2 for most of the populations. Interestingly, several epistatic interactions were detected in the E9xL5 population, that were classified in three classes based on the allelic interaction: dominant (one locus suppressed the allelic effects of a second locus), co-adaptive (the double homozygous for the same parent alleles showed a higher phenotypic value than the combination of homozygous for alternative alleles) and transgressive (combination of double homozygous for alleles from different parents showed better performance than double homozygous for the same parent alleles) epistasis. These results reinforce the important role of non-additive genetic variance for response to heat stress and the potential of the new allelic combinations arisen after wide-crosses.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.05

Title: P3.05 Effect of indole-3-acetic acid methyltransferase mutations in tomato high-temperature responses

Authors: Lobato-Gómez, M.* [1], Minguet, E. [1], Gonzalo, M.J. [1], Sánchez del Coso, E. [2], Monforte, A.J. [1], Granell, A.# [1]

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Abstract:

Tomato fruit development is sensitive to high temperature, and phytohormones play an important role in plant environmental responses. Auxins regulate high temperature responses, and indole-3-acetic acid (IAA) is the most studied one. IAA inactivation pathways include IAA methylation by IAA carboxyl methyltransferases. In tomato, two IAA methyltransferases (IAA-MET) have been identified, located at chromosomes 7 (IAA-MET7) and 12 (IAA-MET12). We hypothesize that mutations in tomato IAA-METs could let to higher free IAA concentrations and therefore to higher resistance to high-temperature. TILLING and CRISPR-Cas9 were used to mutate IAA-MET12 in tomato cv. Micro Tom (MT) and cv. MoneyMaker (MM), respectively. The mutated and wild type (WT) plants were grown at normal temperature (NT, 24¬∫C day/20¬∫C night) and once the first fruit was set, half of the plants continued at NT and half were transferred to constant HT (28¬∫C day and night). At NT, the MT mutated line showed an earlier appearance of the first inflorescence (after 4.35 ¬± 0.61 leaves) comparing with the WT (after 5.25 ¬± 0.58 leaves) and an acceleration of 10 days in the first anthesis comparing to the WT. A MM mutated line showed a significant higher number of seeds than the WT at HT. In HT greenhouse conditions these mutant lines also showed good heat tolerance as assessed by fruit sets. A backcross strategy has been initiated to introduce the TILLING mutation in different backgrounds These preliminary results can be used to develop tomato lines with a higher tolerance to HT due to the climate change.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.06

Title: P3.06 Evaluation of tomato accessions for nitrogen use efficiency in plantlet stage

Authors: Flores, M. [1], Villanueva, G .[1], Prohens, M. [1], Plazas, M.*# [1]

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Abstract:

Identification of tomato materials with high nitrogen use efficiency (NUE) is a first step to develop new varieties for more sustainable and resource-efficient horticulture. In this work, a collection of 30 tomato accessions were grown in a substrate with low N content (coconut fiber and perlite) and irrigated with a low N (0.5 mM) or high N) solution. Plantlets were grown up to 40 d (HN) and 65 d (LN) when most of the accessions had reached a similar development stage (first flower buds). For each of the accessions and treatments, growth and biomass parameters, chlorophyll, anthocyanins, and flavonoid content, as well as the N balance index, C, N contents, and NUE and its components NUpE and NUtE were evaluated. Many differences were observed among accessions for the traits evaluated. Even though plants of both treatments were at the same stage of development, plants from the HN treatment had a higher fresh and dry weight of the different plant parts, due to an increase in stem, leaf, and root, as the availability of nitrogen increases. Plant pigments were affected by treatments with a decrease of chlorophyll, increase of flavonoids and anthocyanins, and reduction of NBI under LN conditions. N content was much lower under LN conditions. NUE and NUtE were higher under LN, while NUpE was lower. Significant interactions between accession and N treatment were detected, which allowed identifying accessions with high NUE in each of the conditions. Evaluation of these selected materials in adult plant stage is the next step for identifying materials with higher NUE that can be used in breeding programs.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.07

Title: P3.07 Gene editing of key effectors improves tomato abiotic stress tolerance

Authors: Punzo, P.* [1,2], Ruggiero, A. [1], Cirillo, V. [3], Maggio, A. [3], D'Agostino, N. [3], Cardi, T. [1,2], Nicolia, A. [2], Grillo, S. [1], Batelli, G. #[1]

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Abstract:

Breeding for crops tolerant to abiotic stress is a priority in plant research, given the ongoing climate change and population increase dynamics. Towards this goal, we applied new breeding technologies to edit the sequence of key salt and water stress determinants within the tomato genome. In detail, we targeted i) SALT OVERLY SENSITIVE1 (SOS1), encoding a plasma-membrane located Na+/H+ antiporter responsible for Na+ extrusion from the cytosol, to obtain a constitutively active form through deletion of an autoinhibitory C-terminal domain; ii) PYRROLINE-5-CARBOXYLATE DEHYDROGENASE (P5CDH), involved in the catabolism of proline, to generate loss-of-function mutants capable of accumulating higher levels of this compatible osmolyte. Thus, we generated constructs containing the CRISPR/CAS9 enzyme coding sequence and two single guide RNAs for each target gene. After sgRNAs validation through the hairy roots system, stable transgenic plants were obtained and genotyped through PCR and DNA sequencing to verify the presence and nature of the induced mutations. Three independent p5cdh knock-out mutants and three sos lines with large deletions of the SOS1 C-term domain were obtained. In vitro seed germination and root growth analysis confirmed a greater tolerance to salt stress of sos and p5cdh edited lines. In addition, leaf proline content was higher (up to 10-fold) in p5cdh lines compared to wild-type, and resulted in a lower water loss in detached leaf assays. Phenotyping of edited plants subjected to stress in soil is underway to verify the impact of mutations in vivo. This work was funded by the Italian Ministry of Agriculture, BIOTECH-Cisget project.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.08

Title: P3.08 Loss of function of *SlSSI2* encoding a stearoyl-ACP-desaturase negatively affects plant growth and development in tomato

Authors: Quevedo-Colmena, A.S.* [1], Yuste-Lisbona, F.J. [1], Ortiz-Atienza, A. [1], Jáquez-Gutiérrez, M. [2], Quinet, M. [3], Atarés, A. [2], Moreno, V. [2], Angosto, T. [1], Lozano R.# [1]

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Abstract:

ACP desaturase (SACPD) is a key enzyme catalyzing 18:0-ACP conversion to oleic acid (18:1)-ACP in the chloroplast stroma. Thus, SACPD activity regulates the ratios of saturated to unsaturated fatty acids, playing a crucial role in regulating membrane stability and fluidity, as well as photosynthesis efficiency. Here, we report the molecular cloning and characterization of a new tomato (*Solanum lycopersicum*) T-DNA recessive mutant, named as *pale dwarf* (*pad*), which exhibits a dwarf and chlorotic phenotype promoted by a severe reduction of chlorophyll content. Cloning of flanking sequences at T-DNA integration site, and co-segregation analysis performed using allele-specific primers, denoted that *pad* phenotype was caused by a single T-DNA insertion disrupting the tomato homologue of the Arabidopsis *SUPPRESSOR OF SALICYLIC ACID INSENSITIVITY 2* (*SlSSI2*), encoding a plastid localized isoform of SACPD. The phenotype of CRISPR/Cas9 *SlSSI2* knockout lines confirmed that the morphological abnormalities shown by *pad* plants was due to the *SlSSI2* lack of function. Through functional, metabolomic and expression analyses we prove that *SlSSI2* gene activity is crucial in regulating 18:1 levels, as well as show that *SlSSI2* disruption alters the basal defense response against pathogenic microorganisms through the JA- and SA-mediated signaling pathways.

Acknowledgments: This work was supported by the PID2019-110833RB-C31 and PID2019-110833RB-C32 grants (MICI/AEI/FEDER, UE) and the BRESOV project (Horizon 2020, No. 774244).

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.09

Title: P3.09 Searching for new tomato salt stress tolerance candidate genes, using Introgression Lines (ILs) of *Solanum pennellii* x *Solanum lycopersicum* grown *in vitro*

Authors: Americo, S.* [1], Ferrari, G. [2], Beretta, M.* [2], Desiderio, F. [3], Lo Piero, A.R. [1], Morelli, G. [4], Cattivelli, L. [3], D'Orso, F. [4]

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Abstract:

Global warming is reducing the availability of fresh water, making the use of saline water frequent for agricultural purposes, causing a sharp increase in soil salinity (Flowers, 2004; Foolad, 2004). This condition is unsuitable for cultivated tomato plants (*Solanum lycopersicum*), that display a reduction in root and shoot development, yield loss and death (Xiong, Schumaker and Zhu, 2002), when grown in saline soils. Previous studies analysed some Introgression Lines (ILs) of *Solanum pennellii* (LA0716) x *Solanum lycopersicum* (UC82). *S. pennellii* is a wild non-commercial tomato specie, highly tolerant to drought and salt stress. The results of these studies focused on chromosome 7 of *S. pennellii*, particularly on the introgressed region of accession LA4067 (IL 7-4), considered a potential source of candidate genes. This study aims to find candidate genes for salt stress tolerance, so that they can be used in genetic improvement for *S. lycopersicum*. For this purpose, ILs and sub-ILs of chromosome 7 were germinated and phenotyped in vitro using a MS medium with a concentration of 40mM NaCl and a control MS medium without salt. After 30 days, root fresh and dry weight (FW, DW) data were scored and statistically analysed. LA4068 (IL 7-4-1) and LA4070 (IL 7-5-5) resulted to be the most tolerant accessions (Ferrari et al., 2022; Submitted by ACTA Horticulturae). RNA extracted from roots of LA4068, LA4070 and UC82 grown in stress and control conditions will be sequenced (RNA-seq). In the next months, sequencing output will be analysed by bioinformatics procedures, to obtain the DEGs.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.10

Title: P3.10 Low production of the ethylene-precursor ACC in roots improves adaptation to salinity stress in tomato

Authors: Martín-Rodríguez, J.A.* [1], Martínez-Melgarejo, P.A. [1], Prudencio, A. [1], Albacete, A. [1], Pereira Peres, L. [2], Martínez-Andújar, C. [1], Pérez-Alfocea F. [1]

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Abstract:

Ethylene is a plant hormone considered as a growth inhibitor and senescing promoter in response to abiotic stresses as salinity. Although it is produced in different plant tissues, an important role has been proposed for this hormone in the root as stress sensor and root-to-shoot signalling molecule. In this study, the role of root-sourced ethylene in response to salinity has been assessed in tomato by using rootstocks altered in ethylene production. A commercial tomato cultivar was grafted onto the ethylene overproducing mutant (epinastic, Epi); a root-specific ethylene deficient transgenic line (ACCD) overexpressing an ACC-deaminase (that degrades the ethylene precursor aminocyclopropane-1-carboxylic acid, ACC); and onto their wild type genetic background the cv. Micro-Tom (MT-WT). The grafted plants were cultivated in a greenhouse under control and saline stress (75 mM NaCl) conditions for 3 months. Plant yield and yield components, biomass, photosynthetic traits, changes in major plant hormones (UHPLC-MS) and ionome (ICP-MS), and expression of ethylene-related genes were analysed. Root-specific alteration of ethylene production modified growth, physiology and yield of grafted plants. Expression of ethylene biosynthetic genes were similarly induced by salinity in the leaves of the three graft combinations, suggesting that the precursor ACC produced in the roots and transported to the shoot is influencing the putative ethylene evolution in both organs and the response to stress. Hence, ACCD rootstocks reduced the toxic accumulation of Na+ in leaves and flowers, and improved mineral nutrients and photosynthetic status in the scion, while the opposite was found in the high-ethylene producer Epi grafts.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.11

Title: P3.11 Heat stress during the progamic phase: Impact on male gametophytes of *Solanum lycopersicum* at transcriptome and proteome level

Authors: Flores-Tornero, M.*# [1], Abreu I.A. [1], Becker, J.D.# [1]

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Abstract:

Climate change has a strong impact on seed yield because sexual reproduction in flowering plants is particularly sensitive to elevated temperatures. Heat stress during pollen development results in a reduction of viable pollen in several species, including tomato and Arabidopsis. The subsequent progamic phase, during which a pollen tube grows through the transmitting tissue of the pistil to deliver two sperm cells to the embryo sac for double fertilization, can be equally affected by heat stress. This phase is characterized by pollen-pistil and gamete-gamete interactions, but the effect that acute heat stress has on the male gametophyte during the progamic phase is unknown. Our hypothesis is that acute heat stress during the progamic phase causes deregulation of transcript and/or protein levels in tomato male gametophyte that ultimately leads to reduced fertilization efficiency and reduced seed set. Therefore, we aim to determine the threshold temperatures at which the progamic phase starts being affected, and to obtain transcriptional profiles for FACS isolated vegetative nuclei, generative cell, and sperm cell from tomato pollen grains and semi in vivo grown pollen tubes under normal and heat stress conditions. Additionally, we will also generate proteome data for tomato pollen grains and whole pollen tubes under normal and heat stress conditions to identify candidate genes with the potential to alleviate heat stress responses of male gametophytes during the progamic phase.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.12

Title: P3.12 Dissection of QTLs involved in the control of stigma position in tomato

Authors: Riccini, A. [1], Farinon B. [1], Pons, C. [2], Causse M. [3], Granell A. [2], Mazzucato A.*# [1]

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Abstract:

A change in stigma position with respect to stamens is a domestication trait in tomato, contributing to the transition from the allogamy of ancestors to the autogamy of cultivated counterparts. Although most modern varieties exhibit a stigma stably positioned inside the antheridial cone, in specific genotypes, vintage or traditional varieties, an exserted position is maintained. This phenotype may be magnified under heat stress. Shifts in stigma position increase cross-pollination and generate fertility problems. Here, the genetic control of stigma position in tomato has been studied in three different populations, the core collection selected within the EU project Traditom (n=224) essentially composed by landraces, a collection of wild or semi-feral genotypes (n=136) and MAGIC population developed at INRAE from both wild and cultivated parents (n=255). Deep phenotyping showed that exserted stigma is more frequent in populations listing wild genotypes or segregating wild alleles and, in the cultivated germplasm, in landraces with pear, oxheart and flat fruit types. A genome wide association study yielded 13, 11 and 12 unlinked QTL positions in the three populations respectively, including both shared and population-specific loci. Such QTLs both confirmed previous studies (eg. the cloned Style2.1 locus on Chr2) or represented novel positions (eg. on Chr1 and Chr11). Biparental populations built to validate the evidenced QTLs suggested that different genes, whit different allelic interactions, underpin the phenotype in different genotypes and populations. First validation data confirmed the QTLs on Chr1, Chr8 and Chr11.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.13

Title: P3.13 Agronomic potential of new tomato hybrids as rootstocks for organic agriculture

Authors: Fonseca, R.* [1], Ozuna, C. [1], Figàs, M.R. [2], Soler, S. [2], Prohens, J. [2], Lozano, R.# [1]

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Abstract:

Global climate change is the uppermost challenge for agriculture nowadays. The increase of temperatures, most of the time accompanied by a reduction of water availability, has a negative impact in plant growth and ultimately in crop production. Moreover, the growing population rate poses a huge concern on food availability and safety in this context of climate change. Tomato (*Solanum lycopersicum* L.) is a major vegetable crop highly appreciated by its nutritional qualities. Over the past decades, huge efforts have been made to genetically improve this species through breeding activities mainly directed to the development of varieties adapted to high input traditional systems instead of organic low input farming. Thus, the combined action of climate change and the consumers demand on greener and safer agricultural practices, makes necessary the development of varieties suitable for organic production under the climate change scenario. Funded by the European Union-Horizon 2020, the BRESOV project aims to the development of new tomato varieties better adapted to climatic adverse conditions and to organic agriculture. The present work includes the implementation of a successful breeding program which has allowed to obtain new inter- and intra-specific tomato hybrids. Their agronomic potential as rootstocks was tested under organic conditions, and BT02530 x BT00120, BT08300 x BT00250 and BT02220 x BT00230 hybrids were found to show higher fruit yield when compared to commercial rootstock hybrids. These hybrids also showed better agronomic performance under stress conditions, providing a suitable material for increasing resilience of organic tomato farming to climate adverse conditions.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.14

Title: P3.14 Screening of a selected set of tomato accessions for tolerance to nitrogen and water stresses

Authors: Tripodi, P. [1]*, Figàs, M.R. [2], Leteo, F. [3], Soler, S. [2], Díez, M.J. [2], Campanelli, G. [3], Cardi, T. [1,4], Prohens, J.*# [2]

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Abstract:

Tomato varieties able to grow with reduced inputs of fertilizers and water are required for a more sustainable horticulture. In this study, we evaluated 42 tomato varieties selected in the H2020 BRESOV project in replicated trials in two organic farms (Italy and Spain) under three treatments in each location: standard (control), no nitrogen fertilization, and reduced (30% of control) water supply. Plants were phenotyped for 32 traits related to plant, fruit, and root characteristics. A broad range of variation was found for all traits, with significant differences between the applied treatments and the cultivation sites. Genotypic (G) variation was generally the most important, although treatment (T) and location (environment; E) and GxE interactions were also significant for many traits. No N fertilization led to an average decrease yield of 17.9% (Italy) and 11.3% (Spain) while reduction of water supply resulted in average yield reductions of 8.9% (Italy) and 19.0% (Spain). However, in general the reduction in yield from the reduced irrigation treatment was compensated by higher soluble solids content under these conditions (7.9% in Italy and 16.8% in Spain). Wide variation was observed in the responses of individual varieties to both stresses, with some varieties with similar or better performance under the low input treatments than under the control. Differences were observed among varietal groups for some traits, with breeding lines and cultivars having in general higher yield and heirlooms higher soluble solids content. The results suggest that selection and breeding of resilient tomato materials with improved performance under N and water stresses under organic farming conditions is feasible.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.15

Title: P3.15 Association of stress resilience phenotyping with a wide SNPs screening in Mediterranean tomato landraces revealed by GWAS

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Abstract:

Cultivation practices in last decades, especially for horticultural crops like tomato (*Solanum lycopersicum* L.), have been based in excessive irrigation and nutrition to maximize yield. Climate change conditions envisage aridity increases that may force more sustainable cropping systems to ensure profitability and optimized use of resources in horticulture. One of the cornerstones in the transition is to adapt crops to higher degrees of abiotic stress, especially to drought. In this regard, Mediterranean tomato landraces are a notorious genetic resource to breed for stress resistance, since they have been selected for centuries to produce under the stressful conditions existing in the Mediterranean during summer. In this study, with the aim of identifying the molecular determinants behind drought tolerance in a wide collection of Mediterranean tomato landraces, we combine phenotyping and genotyping datasets resulting from two H2020 projects (TOMRES and TRADITOM, respectively). Genome-wide association studies in a set of 149 accessions and 1.631 informative (MAF ≥ 5%) SNPs were performed in a wide set of phenotypic traits measured in both a control well-watered (WW) and a water-stress (WS) treatment. Phenotypic traits include plant morpho-physiological traits and gas-exchange, field production, and fruit quality parameters. The results from this study show significant SNP associations in chromosomes 4, 5 and 12, with multiple key traits that offer essential information for improving drought resistant high-quality varieties via marker-assisted selection in tomato breeding programs.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.16

Title: P3.16 Hormone spraying treatments are effective in modulating stomatal behaviour and osmotic rearrangements in response to moderate drought in tomato plants

Authors: Morabito, C. [1], D'Angeli, A. [1], Gualtieri, C. [1], Secchi, F. [1], Cardinale, F. [1], Visentin, I. [1], Schubert, A. [1]

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Abstract:

Solanum lycopersicum is one of the economically most important crop in the Mediterranean basin. During the last decades, increasing drought events severely affect final yields in terms of quantity and quality. The VEG-ADAPT project aims to individuate new strategies to help plants coping with abiotic stresses, such as drought. In order to investigate hormone-based mechanisms involved in stress responses, we sprayed the canopy of tomato plants with four different hormones and hormone-like compounds, respectively abscisic acid (ABA), gibberellins (GA3), strigolactones (the artificial homologous GR24) and karrekins. Each group of ten treated plants was divided into two subgroups, five plants were exposed to moderate water stress (Ψw= -0.9 Mpa), while the remaining five were irrigated up to field capacity for the whole experimental trial. When plants reached the stress level, we re-watered them up to field capacity to fulfill recovery. Physiological parameters (water potential, stomatal conductance) were monitored during whole experiment. Treatments affected stomatal behavior under stress condition and during the following recovery. Samples collected in the final stage of the experiment (after recovery fulfillment) showed significant changes in the osmolarity values, total soluble sugar and proline contents in both leaves and roots. During our project, we used plants belonging to the wild genotype M82 and to the mutant line ccd7, strigolactones deficient. Mutant plants responded differently to drought and to the treatments. Our study will provide useful results and insights for unrevealing the role of hormone cross-talk in conferring an increased tolerance towards water stress in tomato.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.17

Title: P3.17 Inhibition of deoxyhypusine synthase by GC7 induces modification of polyamine catabolism in tomatoes during salt stress

Authors: Szepesi, Á.*# [1], Bakacsy, L. [1], Sípos, L .[1], Szőllősi, R. [1], Zsigmond, L .[2], Molnár, Á. [1], Pálfi, P .[1]

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Abstract:

Salinity is one of the most threatening abiotic stress factors affecting not only agriculture but also our food safety. Tomato is usually exposed to salt stress using brackish water for irrigation or in some ground water contaminated with salty water. Hypusination is essential metabolic posttranslational modification of eIF5A translation factor which is dependent from spermidine content. To decipher the connection between polyamine catabolism and hypusination in fruit development, we used *Solanum lycopersicum* cv. Mano and exogenously applied GC7, a DHS inhibitor for investigating the importance of hypusination in salt stress. Our results demonstrate the important role of hypusination and DHS in reproductive stages of tomato plants during salt stress. Polyamine catabolism was also reduced after GC7 treatment in control and salt stress treated fruits. We could conclude that reduced DHS activities result in higher polyamines contributing improved growth and development in tomatoes not only in control but also in salt stressed conditions. Our results could enhance the knowledge about hypusination during salt stress in plants.

This research was funded by the grant of NRDI (National Research, Development and Innovation) Office (FK129061) and NTP-NFTÖ-21-B-0238 by Hungarian Ministry.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.18

Title: P3.18 Exploring the natural diversity in tomato germplasm for phosphate starvation responses

Authors: Mishev, K.*# [1], Zehirov, G. [1], Srivastava, R. [2], Dobrev, P.I. [3], Velikova, V. [1], Kerchev, P. [4], Kumar, R. [2]

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Abstract:

Phosphorus is an essential macronutrient that ensures plant growth and development, and its mobilization is critical for crop yield. A major challenge in modern agriculture is the limited phosphate (Pi) availability, especially in acid soils where Pi form insoluble complexes. The overuse of Pi fertilizers as a way to compensate for the low Pi availability is inefficient and poses severe risks for the environment. An alternative strategy is to better exploit the plasticity in conditions of Pi starvation that plants have acquired in the course of their evolution. In the present study, we screened Bulgarian tomato germplasm comprising 17 cultivars and 18 F1 hybrids for their response to Pi deficiency. Transfer of hydroponically grown tomato seedlings from a growth medium with optimal Pi concentration to a medium with 250-fold lower Pi induced substantial changes in the growth phenotype and the photosynthetic performance that allowed us to identify genotypes with contrasting sensitivity to Pi starvation. The tolerant varieties displayed differences in the root system architecture, the Photosystem II efficiency, the levels of leaf pigments, and the expression of plastid-encoded photosynthetic genes. Further on, we explored the hormone of root tissues and root exudates under low Pi stress. The LC-MS/MS analysis revealed differential accumulation of several classes of plant hormones and their metabolites that could be used as promising molecular markers for assessment of low Pi tolerance in tomato.

Acknowledgments: This work was funded by the National Science Fund of Bulgaria (Grant КП-06-India-9) and the Department of Science and Technology of India.

Title: P3.19 Short-term transcriptome changes in NUE-contrasting tomato genotypes in response to low nitrate availability

Authors: Aci, M.M. [1], Lupini, A. [1], Mauceri, A. [1], Puccio, G. [2], Caldiero, C. [1], Mercati, F. [3], Abenavoli, M.R. [1], Sunseri, F. [1]*#

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Abstract:

Understanding the regulatory networks underlying plant N responses as well as to identify N-responsive genes, associated with high Nitrogen Use Efficiency (NUE) is one of the main challenges for sustainable cropping systems. Nitrate (NO3-), the main nitrogen form in agricultural soils, provokes a plant transcriptome reprogramming which changes in transcripts accumulation, cell- and tissue-specific, take quickly place under NO3- limited N supply. Here, two NUE contrasting tomato genotypes, Regina Ostuni (RO, high-NUE) and UC82 (low-NUE), were adopted for evaluating transcriptomic changes to different N dosages, in both root and shoot, after short-term (24h) resupply. Differential expressed genes for G (genotype), G×N (N supply) and G×N×T (sampling Time) were identified by ANOVA using a multivariate linear model. According to their expression profiles across the time-course, N-responsive genes were selected. A Weighted Gene Co-expression Network Analysis (WGCNA) allowed us to detect modules significantly correlated to LN in RO. In both tissues, a co-expressed module was highly correlated to LN resupply in RO after 24h. In root, phenylpropanoid and flavonoid biosynthesis pathways are more enriched in high NUE genotype that agree with the higher anthocyanin accumulation observed in plants under N stress, while in shoot hormones and protein kinases signaling are involved in high NUE. Finally, WGCNA decoded the dynamic regulatory network related to LN, facing the key role played by cytokinins and ROS balancing in early LN-tolerance mechanisms adopted by N-use efficient genotype RO. These results should provide new insights into developing N use efficient tomato genotypes useful for breeding.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.20

Title: P3.20 Grafting alters plant vigor, yield, fruit chemical contents and resistance to tomato yellow leaf curl virus in tomato under heat stress

Authors: Rakha, M.*# [1], Hashem, A. [1], Bayoumi, Y. [1], Soltan, M. [2]

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Abstract:

Tomato (*Solanum lycopersicum* L.) is one of the most widely produced vegetable crops in the world, but production in tropics and subtropics is highly affected by both biotic and abiotic stresses. Grafting aims to improve plants' root traits, which enhances access to water and nutrients and resulting in higher crop yield and tolerance to biotic and abiotic stresses such as such as drought, extreme temperature, salinity, tomato yellow leaf curl virus (tylcv), wilt diseases, and others. Here, tylcv-resistant tomato hybrid 023 plants were grafted onto six tomato rootstocks under heat stress in late summer season (May to October 2020) in open field. Interestingly, all tomato rootstocks, except Edkawi, were conferred higher plant vigor, chlorophyll contents, fruit set, and total marketable yield to the scion compared to non-grafted tomato plants. Tomato plants grafted onto T1 and T4 rootstocks had the highest total marketable yield followed by Maxifort. In addition, tomato rootstock T4 provided the highest vitamin C and total soluble solids in the tomato scion fruits. The most abundant antioxidants, lycopene and beta carotene were found in fruits of tomato plants grafted onto T1, T6, Edkawi and Maxifort. However, plants grafted onto Edkawi rootstock exhibited tylcv symptoms and decreased tylcv resistance levels to the tomato scion 023 compared to non-grafted plants and other rootstocks. This study suggests that the grafting of tomato plants onto suitable rootstocks may improve plant vigor, yield, fruit chemical contents, and manage tylcv resistance under heat stresses in open field.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.21

Title: P3.21 Microorganisms and climatic conditions effects on *S. pimpinellifolium* performance

Authors: Ruiz-González, M.X.*# [1,2], Acosta-Quezada, P.G. [1], Tandazo-Yunga, J.V. [1], Neira-Briceño, A. [1], Prohens, J. [2], Loján, P. [1]

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Abstract:

The XXIst century has imposed severe emerging challenges to agriculture. On the one hand, there exists a pressure to boost sustainable agriculture. On the other hand, plants and crops face the effects of climate change; thus, there exists the need to find tolerant varieties. The characterisation of both plant biodiversity and the biological response to abiotic stresses, plays a pivotal role in developing sustainable solutions; because it reveals phytogenetic materials with fine-tuned performance under specific climatic conditions. Moreover, up to date research has remarked the important role of soil microorganisms in sustainable agriculture, but we lack knowledge on the ecological effects of the interaction of microbial diversity and their plant partners. We have investigated the effects of microorganisms and climatic background of two populations of *Solanum pimpinellifolium* under two climatic conditions. First, we isolated microbial species present in two types of soils (Podocarpus: cold and humid; and Paltas: warm and dry) that where characterised through a metagenomics approach. Then, we randomly allocated 15 *S. pimpinellifolium* seedlings from either a cold (Azuay) or warm (Manabi) background populations to each a control, inoculum from Podocarpus, or inoculum from Paltas treatments. The set up was replicated in a Loja greenhouse (cold to mild conditions) and in a Paltas field (warm conditions). Plants were grown into pots and we gathered 33 agromorphometric and phenological characters. All treatment, condition and accession, as well as their interactions produced significant overall differences, highlighting the role of microorganisms and climatic effects above the genetic background.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.22

Title: P3.22 Effects of heat wave on reproductive organ development in tomato

Authors: Bollier, N. [1], Micol-Ponce, R .[1], Jarry, L. [1], Chevalier, C. [1], Maza, E. [2], Zouine, M. [2], Djari, A. [2], Bouzayen, M. [2], Hernould, M.* [1], Delmas, F.# [1] and Gonzalez, N.# [1]

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Abstract:

While we are entering a period of increasingly rapid climate change, there are strong requests to design new strategies to maintain high yields of fruits and vegetables produced in harsh temperature conditions. By using Tomato as a reference fleshy fruit crop, we propose to address these requests by studying reproductive development under elevated ambient temperatures in commercial tomato varieties. Within the TomGEM project (Genetics, Environment Management, European Union, Horizon 2020 research and innovation program, grant agreement N679796, started in march 2016) our objectives are to (1) study the cellular and molecular bases of reproductive organ development under heat wave, (2) identify the key regulators conferring improved ability for reproductive organ development under elevated ambient temperatures. To date, 1) a morphological and cytological definition of developmental stages at high resolution from initiation of floral meristems to floral maturity has been produced by analyzing flower buds of different sizes from various genotypes submitted to high ambient elevated temperatures, 2) five commercial tomato varieties have been grown under heat wave and genome-wide expression analysis has been performed on inflorescence meristems and pollen tetrad stage buds. Differentially Expressed Genes (DEGs) related to abiotic and biotic stress responses in plants have been identified and their functional analyses are in progress.

Poster presentation. Session 3: Breeding for Abiotic Stresses
P3.23

Title: P3.23 Development and evaluation of interspecific hybrids of eggplant as rootstocks for grafting tomato for cultivation under heat stress

Authors: Hashem, A. [1], Bayoumi, Y. [1], Plazas, M. [2], Prohens, J. [2], Rakha, M.*# [1],

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Abstract:

Grafting is a common horticultural practice for tomato (*Solanum lycopersicum* L.) production, particularly in intensive cultivation systems. Grafting is usually performed to manage soil pathogens, and to improve plant vigour, earliness and yield of the scion. Eggplant (*Solanum melongena*) and its wild relatives have good grafting compatibility with tomato. Interspecific hybrids of eggplant with wild species are known to be highly vigorous. Hybridizations between cultivated eggplant and its two closest wild relatives (*S. insanum* and *S. incanum*) had a high success rate and many hybrid seeds per fruit were obtained. Four interspecific hybrids of eggplant with *S. insanum* and one with *S. incanum* were tested as rootstocks for grafting two fresh market F_1 cultivars ($023F_1$ and Alisa F_1) in the late summer (May to October 2020) in Kafrelsheikh region in Egypt, where heat stress is a limiting factor of the potential yield. Interestingly, all the rootstocks had 100% graft compatibility with the scion $023F_1$ and ≥80% with Alisa F_1. All vegetative growth parameters including plant height, stem diameter, number of leaves, number of branches and chlorophyll content were generally higher in grafted tomato plants compared to non-grafted tomatoes. Similarly, eggplant rootstocks improved the number of flowers and marketable fruit number in grafted tomato plants compared to non-grafted ones. In particular the hybrid between eggplant and *S. insanum* MEL4xINS2 displayed the highest number of fruits per plant in both scions. These are very promising results for using interspecific hybrids of eggplant as rootstocks in commercial production of tomatoes to alleviate heat stress in tomato cultivation.

Session 5: Breeding for Biotic Stresses

Keynote invited presentation.
K5.01

Title: K5.01 Tolerance and resistance to tomato brown rugose fruit virus (ToBRFV)

Authors: Lapidot M.*# [1]

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Abstract:

An outbreak of a new disease infecting tomatoes occurred in southern Israel during the autumn of 2014. Symptomatic plants showed a mosaic pattern on leaves accompanied by narrowing of leaves and yellow-spotted fruit. It was found that the causal agent is a new tobamovirus that was identified earlier in Jordan and named tomato brown rugose fruit virus (ToBRFV). ToBRFV induces severe losses due to reduction in plant vigor and fruit quality and is fast spreading worldwide. The virus has become a major limiting factor for the production of fresh-market tomatoes because plants carrying the tobamovirus resistance genes Tm-1, Tm-2, and Tm-22 were found to be susceptible to the virus.

We assayed the effect ToBRFV has on tomato yield and yield components of four different tomato genotypes, which included two near-isogenic lines, one with and one without the Tm-22 gene, a tolerant genotype and a resistant genotype. Two yield experiments were carried out at two different geographical locations. In each experiment, the yield performances of ToBRFV-inoculated plants were compared to those of control non-inoculated plants of the same genotype.

To identify and characterize ToBRFV-resistant tomato genotypes, different tomato genotypes which included commercial hybrids, wild-type solanum species and genotypes polymorphic in Tm-1 and Tm-2 loci were screened for viral resistance. The screen resulted in the identification of an unexpectedly high number of tolerant genotypes and a single resistant genotype. A selected tolerant genotype and the resistant genotype were further analyzed, and the loci controlling tolerance and resistance were mapped.

Oral presentation.
Session 5: Breeding for Biotic Stresses
O5.01

Title: O5.01 Predicting *R* gene durability through virulence studies with CRISPR-Cas9-generated effector mutants of the tomato leaf mold pathogen *Cladosporium fulvum*

Authors: Schol, C.R.*# [1], Hilgers, A.M. [1], Bai, Y. [1], Joosten, M.H.A.J. [1]
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Abstract:

We have successfully established and applied a marker-free CRISPR/Cas9-mediated gene editing system in the biotrophic fungal pathogen of tomato leaf mold, *Cladosporium fulvum*. This was done by first producing *C. fulvum* protoplasts by means of enzymatic digestion, subsequently applying PEG-mediated transformation with a plasmid encoding for Cas9 and a single short guide RNA and, finally, selection and genotyping of hygromycin resistant transformants. After successful genotyping of CRISPR/Cas9 causal mutations, the selection pressure is removed which leads to loss of the plasmid, resulting in a marker free CRISPR/Cas9 editing strategy. Using this system, several novel Extracellular protein (Ecp) effector genes in *C. fulvum* have been successfully edited, leading to frameshift mutations which are highly likely to result in gene knockouts. Subsequently, the effect of loss of these Ecp effectors on fitness, pathogenicity and virulence will be assessed by means of inoculations with the knockout strains on susceptible tomato plants. This will provide valuable data for making predictions on the potential durability of novel Cf-Ecp resistance genes that have recently been identified to recognize these Ecp effectors in wild tomato germplasm. *C. fulvum* is well-known for mutating or completely losing effector genes under selective pressure of corresponding resistance genes in the field, resulting in evasion of host immunity and breaking of resistance genes. By investigating the impact of loss or mutation of an effectors in a pathogen, we can actively predict the durability of corresponding resistance genes on the host side, which allows us to more durable resistance genes in the future.

Oral presentation. Session 5: Breeding for Biotic Stresses
O5.02

Title: O5.02 Integrated omic approaches for dissecting the tolerance to *Tuta absoluta* in tomato

Authors: D'Esposito D. [1], Guadagno A. [1], Amoroso C. [1], Di Natale A. [2], Cascone P. [3], Guerrieri E. [3], Garonna A. [1], Pennacchio F. [1], Ercolano M.R. *# [1]

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Abstract:

Tuta absoluta is one of the most devastating insect pests both in tomato open field and greenhouse production. The development of tolerant varieties to *T. absoluta* represents a crucial breeding effort, but, to date, very limited information on traits related to this leaf miner tolerance are available. In this framework, a comparative transcriptome analysis between two tomato cultivars, one tolerant and one susceptible to *T. absoluta* led us to identify several genes, related to the recognition of the insect, jasmonic acid metabolism, trichome development and terpenes synthesis, that play a key role. The two analyzed genotypes also revealed clear differences in leaf trichome density, in the degree of infestation and at genomic level. The distribution of variants was not uniform along the chromosomes and the tolerant genotype showed more chromosome regions with SNP and InDel hotspots. The chromosome regions with variant dissimilar pattern were investigated highlighting potential traits contributing to the different response to *T. absoluta*. We identified numerous genes affected by variants involved in the defense response to herbivory and participating in the production of secondary metabolites. These genes that represent good candidates to further investigate for enhancing our understanding of tolerance to insect pests in tomato. Finally, a metabolic analysis for volatile organic compounds released from the two cultivars, constitutively or upon attack by *T. absoluta*, showed a significant difference in several classes of chemicals. Overall, all these findings are a valuable for tomato breeding programs aiming to develop tolerant plants to *T. absoluta*.

Oral presentation. Session 5: Breeding for Biotic Stresses
O5.03

Title: O5.03 Tomato *hairplus* mutation induces changes in the epigenome and increases glandular trichome density

Authors: Fonseca, R.* [1], Capel, C. [1], Suárez-Alcaraz, A. [1], Yuste-Lisbona, F.J. [1], Gómez-Martín, C. [2,3], Lebrón, R. [2,3], Hackenberg, M. [2,3], Oliver, J.L. [2,3], Angosto, T. [1], Lozano, R. [1], Capel, J.# [1]

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Abstract:

Trichomes are specialized epidermal cells developed in the aerial surface of most terrestrial plants. They have been related to abiotic stress responses like avoidance of excessive radiation or water loss, but their implication in biotic stress responses have been far demonstrated. Thus, glandular trichomes are natural biological factories that synthetize complex compounds which have a toxic or dissuasive effect on pests. However, non-glandular trichomes form a physical barrier that prevents plague dissemination. This becomes particularly relevant for agriculture under the climate change scenario, where the increase of temperatures has resulted in an increase of plague pressure. In this work, the tomato *hairplus* (*hap*) mutant is characterized and we demonstrate that the increase of glandular type I trichome of the mutant plants increase their resistance to pest. Map based cloning combined with whole geno me sequencing strategies allowed the identification of the HAP gene. The HAP protein belongs to a group of histone tail modifying proteins, although some of them also bind to differentially methylated DNA regions. Loss of function of HAP results in epigenomic modifications expressed as a large number of differentially methylated cytosines throughout the entire genome of mutant plants, which ultimately produces transcriptomic changes never before observed in tomato trichome mutants previously described. All these results demonstrate that HAP is a master regulator of processes linked to epigenome remodeling, as well as a valuable tool for tomato breeding for pest resistance.

Poster presentation. Session 5: Breeding for Biotic Stresses
P5.01

Title: P5.01 Identification of an efficient source of resistance to pepino mosaic virus in tomato

Authors: Ruiz, F. [1,2], Rodriguez-Sepulveda, P. [2], Bretó, P.*# [2], Donaire, L. [1], Hernando, Y. [2], Aranda, M.A.# [2]

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Abstract:

To date, no sources of resistance against pepino mosaic virus (PepMV) have been found in tomato, and those identified in relative species have not proven useful for breeding resistant commercial varieties. Therefore, we undertook a reverse genetics approach to identify new sources of resistance, under the assumption that the knockout of a key host factor for virus infection would limit or impede viral spread inside the tomato plant, causing a loss-of-susceptibility phenotype. To that end, we screened a collection of 1,000 M2 tomato EMS mutagenized families by inoculating them with an aggressive PepMV isolate, and after two rounds of selection, the progeny of a symptomless plant showed stable absence of symptoms and reduced accumulation of four different viral strains. Analysis of mapping populations derived from this line confirmed the monogenic and recessive nature of the resistance; besides, the loss-of-susceptibility was maintained after 5 passages of the virus, suggesting that the resistance caused by this mutation could be durable. A bulk segregant analysis coupled to next generation sequencing identified a genetic interval which encompasses five candidate genes carrying single nucleotide mutations. Later studies pointed to one of these genes as the main candidate for several reasons: a mapping analysis of recombinants narrowing the interval, the observed SNP in the candidate is predicted to cause a truncation of the protein, and its edition through CRISPR/Cas9 reproduced the loss-of susceptibility in a different cultivar. In both mutants, originated by EMS and edition, there are no other noticeable phenotypic alterations. Altogether, the mutation and associated markers open the possibility to breed tomato varieties with stable broad-spectrum resistance to PepMV.

Poster presentation. Session 5: Breeding for Biotic Stresses
P5.02

Title: P5.02 Denomination of five *Cf-9* breaking isolates of *Passalora fulva* on tomato

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Abstract:

Background: Hubbeling (1978) has introduced groups, some equivalent to races, called A, B, C, D and E, for the tomato leaf mould pathogen *Passalora fulva*, as a practical replacement for complex race numbers. No type isolates were defined by Hubbeling, and none of the races were virulent on *Cf-9*. Nowadays, *Cf-9* breaking races are increasingly important and new tomato varieties with resistance to *Cf-9* breaking isolates are reaching the market. To clarify the situation, the Expert Group on Disease Resistance Terminology (EG-DRT) of the International Seed Federation (ISF) carried out a ringtest project from 2019 till 2021.

Test plan: Ten *Cf-9* breaking isolates collected in Asia and Europe were evaluated for their virulence spectrum on a differential set of eight differentials and two candidate differentials resistant to *Cf-9* breaking isolates, side-by-side with an evaluation of candidate Ff: 0 and A-E type isolates.

Results: Five new virulence patterns were described; four isolates had a unique pattern, 6 isolates had the same pattern. Five *Cf-9* breaking races were denominated as Pf: F, G, H, I and J. The virulence patterns of Ff: 0, A, B, C, D and E were confirmed and type isolates of these races are now available. *Cf-6* provided resistance to all *Cf-9* breaking isolates except race J. The new set contains *Cf-2*, *Cf-4*, *Cf-5*, *Cf-6*, and *Cf-9*.

Conclusion: an up-to-date differential table with 6 differentials and 11 races is published at www.worldseed.org/.../disease-resistance/differential-hosts

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Poster presentation. Session 5: Breeding for Biotic Stresses
P5.03

Title: P5.03 Screening of wild tomato germplasm for resistance to Tomato brown rugose fruit virus (ToBRFV)

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Abstract:

Tomato brown rugose fruit virus (ToBRFV) is an emerging viral disease causing a lot of concern in tomato production. Search for sources of resistance is required in order to develop of new varieties with durable resistance. In this respect, tomato wild relatives have proved to be an excellent source of resistance genes for tomato breeding. As a first step, we devised a protocol for screening and we tested mechanical inoculation of several materials at three stages of development (expanded cotyledon, two true leaves and four true leaves) and found that ToBRFV inoculation was successful in all cases. We screened a germplasm collection of 199 accessions belonging to 13 species of tomato wild relatives. Most represented species were *S. chilense* (n=63), *S. pimpinellifolium* (n=45) and *S. peruvianum* (n=30). Plants were mechanically inoculated at the expanded cotyledon stage and infection evaluated by symptoms and DAS-ELISA as diagnostic technique. By 30 DPI, sixty-one out of the 199 accessions showed symptoms associated with ToBRFV systemic infection such as mosaics, deformation and filimorfism of leaves. These accessions were discarded unless a plant without symptoms was identified. Subsequently (60 DPI), 18 additional accessions were discarded with apparent symptoms. Finally, plants displaying no infection symptoms and low ELISA values were found among 11 accessions of *S. chilense* (1), *S. habrochaites* (7), *S. pennellii* (1), *S. peruvianum* (1) and *S. pimpinellifolium* (1), although some plants were still infected in several of these accessions. Interestingly, only three of the resistant accessions of *S. habrochaites* are carriers of the Tm1 gene. Putatively resistant plants of these accessions are currently being crossed with cultivated tomato to study the genetic control of the resistance and to introgressing resistance to ToBRFV into tomato.

Poster presentation. Session 5: Breeding for Biotic Stresses
P5.04

Title: P5.04 ORLY - another source for resistance to *Sw-5* breaking strain of TSWV

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Abstract:

Tomato Spotted Wilt Virus (TSWV) is a causal agent of Tomato spotted wilt disease - one of the most destructive diseases in many countries. Using resistant varieties is the best strategy for controlling TSWV. Resistance has been found in several wild species and gene *Sw-5* from *S. peruvianum* has been used for developing resistant tomato cultivars. Some years ago *Sw-5* resistance breaking (RB) isolates were detected in many countries. Last year, after testing lines coming from interspecific crosses we reported high level of resistance in line Bogdana having three species in its genetic background. Recently, we directed our investigation on segregating group of lines, G55. Twenty lines were screened with California RB isolate of TSWV. We used Moneymaker (susceptible), line Bogdana and LA 2667 (*Sw-5*) as controls. The plants were evaluated based on scale 1 (susceptible) to 5 (resistant). Five lines segregated and the rest of them showed good levels of resistance (rating 4 and 5). The best line, named ORLY, showed the highest level of resistance. F1crosses with Moneymaker were also resistant, suggesting a dominant inheritance of the factor controlling resistance. The segregation in F2 (ORLY x Bogdana) resulted in a ratio of 9 resistant and 7 susceptible plants showing differences between the genes responsible for resistance in both lines. ORLY is a cultivated type tomato line with vigorous indeterminate plants and midsize fruits.

Poster presentation. Session 5: Breeding for Biotic Stresses
P5.05

Title: P5.05 Role of ethylene in viroid symptomatology of tomato plants

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Abstract:

The volatile hormone ethylene plays an important role in viroid pathogenesis and symptom development. Previous work has shown a possible relation between ethylene, symptom development and ribosomal stress of tomato plants upon Citrus Exocortis Viroid (CEVd) infection. To understand the role of this hormone in viroid pathogenicity, treatments with an ethylene precursor (etephon) and an inhibitor of ethylene signaling (1-Methylcyclopropene; 1-MCP) were used to analyze the response of tomato plants to CEVd. Tomato cultivar VFN8 wild-type plants and epinastic (epi) mutants, which show ethylene-related phenotypes resembling viroid symptoms, were either treated with etephon or infected with CEVd and ethylene levels and ribosomal stress were analyzed. Whilst ethylene levels increased in etephon-treated plants but not in CEVd-inoculated plants, ribosomal stress was observed in both. The highest levels of ribosomal stress were observed in epi mutants infected with CEVd, suggesting that ribosomal stress observed in plants with severe symptoms could be a combination of the stress produced by ethylene and CEVd. To further characterize the role of ethylene in disease progression, plants of the Rutgers cultivar, which is known to be sensitive to CEVd-infection, were inoculated with CEVd and treated with 1-MCP to block ethylene signaling. CEVd-inoculated plants displayed higher levels of ethylen e and more severe symptoms when compared with non-infected Rutgers plants. However, these symptoms were reverted in 1-MCP treated plants. Our results indicate that ethylene is involved in the development of ribosomal stress in viroid-inoculated plants, since ethylene signaling appears necessary for symptom development upon an infection by CEVd in tomato.

Poster presentation. Session 5: Breeding for Biotic Stresses
P5.06

Title: P5.06 Transformation of CAPS markers linked to the *Ty-2* gene for use by HRM in tomato (*Solanum lycopersicum* L.)

Authors: García-Martínez, S.*# [1], Cabrera, J.A. [1], Carbonell, P. [1], Grau, A. [1], Alonso, A. [1], Scavuzzo, A. [2], Lo Piero, A.R. [2], Ruiz, J.J. [1]

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Abstract:

The traditional tomato cultivars breeding program of CIAGRO-UMH (Spain) has introduced the *Ty-1* and *ty-5* genes, which confer a good level of resistance to TYLCV. In 2021, the introduction of the Ty-2 gene began in Muchamiel and De la pera tomato breeding lines carrying the *Tm-2*, *Sw-5*, *Ty-1* and *ty-5* genes, by backcrossing, using marker-assisted selection. A search for markers linked to the Ty-2 gene was performed, and the 3 CAPS markers TG36, T0386A and cLEN-11-F24, previously described by several authors, were selected. These markers are located in the interval 84 to 87 cM of chromosome 11, where the *Ty-2* gene is found. Ten pairs of primers were designed that flank the restriction enzyme targets of the CAPS markers, amplifying fragments between 73 and 173 bp. The amplification reaction was performed with 6 individuals of known genotype (2 RR, 2 Rs and 2 ss), and a subsequent agarose gel electrophoresis, to verify that the size of the fragments had the expected size. The amplification reactions were repeated using the HRM technique, and the 4 pairs that best distinguished the 3 genotypes were chosen. These 4 pairs were amplified 20 more individuals, and the one that best distinguished the plants of known genotype was selected. The Ty-2-5 primer pair was used in March 2022 to screen the BC1 generation, made up of 96 plants, and the expected proportions of each genotype were obtained.

Poster presentation. Session 5: Breeding for Biotic Stresses
P5.07

Title: P5.07 Screening of tomato germplasm for resistance to ToBRFV

Authors: Pasev, G.*# [1], Radeva-Ivanova, V. [1], Pashkoulova, V. [1], Grozeva, S. [1], Ganeva, D. [1]

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Abstract:

In recent years Tomato Brown Rugose Fruit Virus (ToBRFV) has become a serious treat for tomato production worldwide. The extensive use of Tm22 gene in commercial cultivars does not provide reliable resistance against this tobamovirus. For this purpose, 194 tomato (192 *S. lycopersicum* L. and two *S. peruvianum* L.) accessions from the collection of Maritsa Vegetable Crops Research Institute was subjected to screening for resistance to ToBRFV as part of the HARNESSTOM project. The germplasm consisted of breeding lines, varieties, wild accessions and landraces from Bulgaria and the rest of the world. The screenings were performed with an Israeli strain in growing chamber with controlled light and temperature regime. The phenotypic reaction against ToBRFV was scored up to 30-40 dpi. According to the results accessions were divided into extremely susceptible (73,7%), susceptible (20,1%), tolerant (2,6%), and heterogenic (2,6%). Eleven genotypes out of 194 accessions possessing Tm2 or Tm22 gene were found to be susceptible or extremely susceptible. Only one accession possessing Tm1 gene showed a potential for resistance. Systemic spread of the virus was not proved in this accession but in all other groups by ELISA. Additional analysis for resistance in later developmental stages as flowering and fruit maturation are needed of the resistant candidate accession.

Poster presentation. Session 5: Breeding for Biotic Stresses
P5.08

Title: P5.08 Identification of genomic regions associated with the resistance to black leaf mold based on the genotyping-by-sequencing of *Solanum habrochaites* (LA1777) introgression lines

Authors: Choi, Y.* [1], Kwon, S. [1], Park, J. [1], Park, S. [1], Jang, K. [1], Lee, S. [1], Park, G. [1], Park, Y.# [1]

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Abstract:

Black leaf mold (BLM) caused by *Pseudocercospora fuligena* is a destructive disease in tomato. In this study, BLM resistance to Korean isolate of P. fuligena was evaluated in *Solanum habrochaites* introgession lines (ILs) derived from a cross between the resistant donor LA1777 and the susceptible recurrent parent LA4024 (*S. lycopersicum*). Bioassay revealed 8 ILs including LA1777 with high levels of resistance (DSI =0.0~10.0) to BLM and the rest of the ILs including LA4024 (DSI =53.0) showed a wide range of intermediate resistance and susceptibility (DSI =10.3~53.0). These accessions were genotyped by the Genotyping-by-sequencing (GBS), which produced a total of 59,216 single nucleotide polymorphisms (SNPs). The SNPs were used to map introgressed regions (IRs) of LA1777 genome in each IL, and a total of 135 IRs were identified with an average of 1.5 regions per IL. Using 2,666 SNPs after filtering, a Genome-wide Association Study was performed on fixed and random model circulating probability unification mode, and a total of 10 significant SNPs (P-value <0.003) were identified from the IR of chromosome 1 (6 SNPs) and 7 (4 SNPs). Two SNPs located on genes Solyc07g045400.1.1 and Solyc07g045353.1.1 that encodes pentatricopeptide repeats and zinc finger BED domain, respectively were selected for CAPS marker conversion and genotyped on 404 tomato accessions evaluated for the BLM resistance. A high level of correlation between the marker genotype and BLM resistance was detected in these accessions, indicating strong association of these IRs with the BLM resistance.

Session 6: Tools and Materials for Breeding and Genomics

Keynote invited presentation
K6.01

Title: K6.01 Breedbase, a digital ecosystem for plant breeding

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Abstract:

Modern breeding methods integrate next-generation sequencing (NGS) and phenomics to identify plants with the best characteristics and greatest genetic merit for use as parents in subsequent breeding cycles to ultimately create improved cultivars able to sustain high adoption rates by farmers. This data-driven approach hinges on strong foundations in data management, quality control, and analytics. Of crucial importance is a central database able to 1) track breeding materials, 2) store experimental evaluations, 3) record phenotypic measurements using consistent ontologies, 4) store genotypic information, and 5) implement algorithms for analysis, prediction and selection decisions. Because of the complexity of the breeding process, breeding databases also tend to be complex, difficult, and expensive to implement and maintain. Here, we present a breeding database system, Breedbase (https://breedbase.org/). Originally initiated as Cassavabase (https://cassavabase.org/) with the NextGen Cassava project (https://www.nextgencassava.org/), and later developed into a crop-agnostic system, it is presently used by dozens of different crops and projects. The system is web-based and is available as open source software. It is available on GitHub (https://github.com/solgenomics/) and packaged in a Docker image for deployment (https://dockerhub.com/breedbase/). The Breedbase system enables breeding programs to better manage and leverage their data for decision making within a fully integrated digital ecosystem.

Oral presentation. Session 6: Tools and Materials for Breeding and Genomics O6.01

Title: O6.01 K-seq, a reliable and low-cost genotyping technology

Authors: Ziarsolo, P. [1], Hasing, T. [2], Hilario, R. [1], Garcia-Carpintero, V. [3], Blanca, J. [1], Bombarely, A. [3], Cañizares, J.*# [1]

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Abstract:

We have developed a new Genomic Reduced Representation methodology based on the amplification of a genomic fraction and Illumina sequencing, K-seq. The amplification is carried out using short oligonucleotides, but its reproducibility is excellent because it starts with the large DNA Pol I Klenow fragment polymerase. The short primers are selected for the different species by using a software application that favors the regions that can be mapped with high MAPQ values, increasing the number of specific markers on the samples. K-seq has been used in two crops, tomato and wheat, and in dog. The tomato primers have also been successfully used in other Solanaceae: potato, pepper, eggplant and petunia. When compared with GBS in the same tomato accessions, K-seq obtained more markers with the same number of reads, and with similar error and reproducibility rates. We have also shown that K-seq is useful on polyploid species, subgenome specific markers were created for tetraploid and hexaploid wheat samples. This methodology was also used to build a tomato genetic map of a F2 with 147,326 SNP markers in which several QTLs implicated on trichome density were identified. The principal advantages of K-seq are its low cost, flexibility, and its simple technological requirements; any laboratory capable of doing PCRs could carry out K-seq experiments.

Oral presentation. Session 6: Tools and Materials for Breeding and Genomics O6.02

Title: O6.02 Old battle and new war - resistance sources and markers against ToBRFV

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Abstract:

The appearance of Tomato brown rugose fruit virus (ToBRFV) in commercial tomato cultivation created a substantial threat for tomato growers around the globe, causing severe damage to commercial varieties, and represented a significant challenge for tomato breeders. We developed a controlled virus inoculation system that allowed, on the one hand, to infect our material with a uniform timing and amount of the virus and, on the other, to characterize the effect of the inoculation. Using this system, we were able to characterize and catalog our material according to the level of resistance of each line and source. After identifying sources of resistance, our research was focusing on two main goals: genetic research to try and find those genetic markers. The second, a breeding process to introduce the resistance source to all TomaTech segments. We performed whole genome sequencing (WGS x30). At the same time, we created segregating populations, those performing control inoculation, disease severity index (DSI) phenotyping, and molecular tests. The WGS provided us 28,798,244 variants (SNPs + indels). After filtration we reduce the number and reach 549,749 SNPs that were used for analysis. We identified differential variance spread over several chromosomes; some have the highest probability of containing the genes responsible for the resistance trait and some might be found as a minor gene. These SNPs are used to identify and characterize advanced lines from the source of resistance to create resistant hybrids.

Oral presentation. Session 6: Tools and Materials for Breeding and Genomics O6.03

Title: O6.03 Real-time electrical plant-based dehydration sensors

Authors: Bar-On, L. [1], Zeron, Y. [2], Sade, N. [2], Jog, A. [1], Shacham-Diamand, Y. [1], Avni, A.*# [2]

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Abstract:

We present a technology for real-time monitoring along with early detection of drought levels using in-vivo electrical impedance spectroscopy measurements. . The plant is monitored using the novel four-point electrical impedance spectroscopy. The suggested four-point probe connection is established galvanically to the plant vascular cambium, while readings are collected continuously across short time intervals. In this configuration we eliminate contact effects getting more reliable measurements compared to the conventional two-probe setup. In this manner, a trend of the plant's daily behavior, as well as its response to different externally induced changes can be monitored. Large data sets can be created and compared using this measurement approach. Effective electrical impedance spectroscopy was conducted to monitor plant hydration status. At the same time, an automated gravimetric weight-based system was connected. Data collected from both monitoring systems across time, and comparison of the impedance behavior to known plant parameters, show that the impedance measurement yields similar behavioral patterns to the well-established weight-based method. The method we present here can be distributed for field use offering a simple and accessible method for monitoring plant health and stress response. The method has been demonstrated on both tobacco and tomato plants, for periods ranging from a few days to a few months, showing response to water stress and a daily cycle trend.

Oral presentation. Session 6: Tools and Materials for Breeding and Genomics
O6.04

Title: O6.04 High-quality de novo genome assembly of the Solanum pimpinellifolium TO-937 genome using PacBio HiFI Long Read technology

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Abstract:

Solanum pimpinellifolium (SP), the wild species from which cultivated tomato was domesticated, has been successfully used by breeders to improve many valuable traits. In this study, we generated a high-quality chromosome-level genome sequence of SP TO-937 using PacBio HIFI technology followed by Hi-C scaffolding. The accession TO-937 is a true-breeding line obtained by repeated selfing from an ccession collected in origin at Lambayeque, Peru. It shows good-quality fruit trais and the presence of type-IV glandular trichomes producing acylsugars that confer resistance to a range of pests such as spider mites, whiteflies and aphids. TO-937 was utilized as the wild parent for development of interspecific RIL and IL mapping populations that served to localize QTLs associated to, e.g. fruit-quality related traits such as metabolite contents (including volatile aromes), mineral content, cracking resistance, composition and color of cuticle, and also to vegetative and reproductive heat-tolerance traits. The PacBio HIFI reads were first assembles into contigs using Hifiasm software. Interestingly, the highest contig length was greater than 75.8 Mb and correspond to one full chromosome (Chromosome 7) assembled without any gap. The contig N50 is 15 Mb assembled in only 19 contigs. Hi-C scaffolding increased the N50 to 50 Mb assembled into 7 scaffolds only. The BUSCO score is 98.3% that correspond to a good completeness of the assembled genome. When compared to two released S. pimpinellifolium genome assemblies (LA2093 and LA1670), several chromosome rearrangements have been identified. The genome annotation is in progress. This ressource will be released soon for further studies at the fundamental and applied research.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics

P6.01

Title: P6.01 Elucidating the role of microProteins in modulation of tomato plant development

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Abstract:

MicroProteins (miPs) are small proteins that contain typically only a single protein-protein interaction domain (PPI). MicroProteins are related to larger multidomain proteins. By interacting with the related protein, they can interfere with the formation of higher order protein complexes, thereby imposing a potential negative role on relevant biological mechanisms. We have recently shown in cereals, that the microProteins can be used as a post-transcriptional tool to modify biologically relevant traits. In my project I am using CRISPR-based gene editing approaches to create microProtein genes in tomato plants. The use of these microProteins can lead to improvement in plant breeding. However, microProteins have not been explored in crops yet, where they could have a high potential for agricultural purposes. In this study we are working on elucidating the possible application of microProteins in the crop species *Solanum lycopersicum* cv. MicroTom. Preliminary results suggest that modulation in the expression of a microProtein might affect the flowering time regulation.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics
P6.02

Title: P6.02 Morphoagronomic characterization and whole-genome resequencing of the eight founders of the first interspecific tomato MAGIC population

Authors: Arrones, A. [1], Gramazio, P. [2], Pereira-Dias, L. [1], Prohens, J. [1], Soler, S. [1], Esteras, J. [3], Garmendia, A. [4], Díez, M.J. [1], Vilanova,S*# [1]

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Abstract:

The tomato relatives *Solanum pimpinellifolium* (SP) and *S. lycopersicum* var. cerasiforme (SLC) are extremely valuable for tomato breeding. However, so far, their potential has been barely exploited. We have carried out a comprehensive morphological and genomic characterization of four accessions of the wild SP and four of the weedy SLC that have been selected from 1,008 accessions genotyped by 7,414 SNPs to maximize the range of variation of both taxa. These eight accessions are the founders of the first interspecific tomato MAGIC (Multiparent Advanced Generation InterCross) population. Morphoagronomic characterization of the parents was performed with 39 descriptors including plant, inflorescence, fruit and agronomic traits, revealing the wide range of diversity captured. The whole-genome resequencing (~20X) identified more than 12 million polymorphisms among the parents, ranging from 1.2 to 1.9 million variants in SLC and 3.1 to 4.8 million in SP, confirming the hypothesis of the emergence of a strong genetic bottleneck during the evolution of the former from the latter. The PCA analyses also confirmed the high diversity of SP and the complex evolutionary history of SLC. The analysis of variants distribution revealed large regions with similar patterns, which could be a footprint of common ancestral introgressions or introgression of genetic material from one accession into another. Functional characterization of the variants revealed a significant enrichment of GO terms related to cell wall changes that would have been negatively selected during domestication and breeding. Currently, the final generation of 383 S5 MAGIC lines are being genotyped by skim whole-genome resequencing. This MAGIC population will be of interest for tomato genetics and breeding purposes.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics P6.03

Title: P6.03 Agronomic techniques for speed breeding in tomato

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Abstract:

Shortening the reproductive cycle is important for speed breeding in tomato. Different genetic and cultivation approaches can be used for this purpose in this crop. The objective of this work is to compare five different agronomic treatments on flowering and ripening earliness in 'Moneymaker' (indeterminate) and 'M82' (determinate) tomato cultivars. In a first experiment, five different pot sizes (0.2 L, 0.45 L, 0.8 L, 1.3 L and 6 L) were tested. We found a negative correlation between pot size and time to flowering so that the earliest flowering was obtained with the largest pot size (6 L), requiring 43.8 d in 'Moneymaker' and 45.0 d in 'M82' from sowing to first flower opening. In a second experiment, four different treatments were evaluated and compared to the control: cold pre-treatment at 14ºC for 7 d in the cotyledonary stage, water stress (50% reduction of water supply), and high concentrations (10x) of potassium or phosphorus in the substrate. Preliminary analysis shows that compared to the control, keeping plantlets at the expanded cotyledon stage at 14°C for 7 days results in a shorter time from sowing to first flower in 'Moneymaker' (3.5 d). Reduction of watering resulted in a flowering delay in 'Moneymaker' (2.2 d) and in smaller plants with less fruit set in both varieties. The high potassium treatment accelerated fruit ripening (3.3 d in 'Moneymaker' and 4.6 d in 'M82') compared to the control. In subsequent experiments, the most efficient combination of treatments will be combined with embryo rescue to accelerate the reproductive cycle of tomato

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Poster presentation. Session 6: Tools and Materials for Breeding and Genomics
P6.04

Title: P6.04 Phenotypic and genetic characterization of a tomato mutant altered in adventitious organogenesis and root development

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Abstract:

Adventitious organogenesis and root development are bottlenecks when applying tissue culture techniques in micropropagation or plant breeding. In vitro plant regeneration is usually approached empirically by searching for a suitable explant and adjusting growth regulators and other components of the culture medium. Although this empirical approach has been very successful from a practical point of view, new insights into the genes that regulate adventitious organogenesis and root development in vitro are urgently needed. Alternatively, root development determines the plant's capacity to absorb water and nutrients from the soil, which indicates the importance of identifying the genetic determinants that regulate growth and root architecture. Here we report the characterization of the defective in organogenesis and rooting recessive mutant (*dor*) identified in our collection of tomato T-DNA lines. Explants of the mutant develop callus but are unable to differentiate adventitious shoot-buds. In vitro evaluation reveals an alteration in embryonic root development as well as a reduced capacity for adventitious root formation. The alteration in root development causes the mutant plants to collapse in vivo unless they are grown with a special supply of water and nutrients. However, mutant development can be normalized by grafting onto the wild-type root so that, under these conditions, it develops normal flowers and fruits. Functional analysis of the altered gene currently underway will provide new insights into adventitious organogenesis and root development in tomato.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics P6.05

Title: P6.05 HarnesstomDB, a database for facilitating the visualization and mining of tomato omics resources

Authors: Pierre, D.* [1], Pons, C.# [2,3], Darnige, E [1], Grossberger, A. [4], Semel, Y. [4], Granell, A. [3], Zouine, M. [1]

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Abstract:

For the last two decades, EU-researchers and EU-funded projects have made important contributions in characterizing tomato genetic resources of relevance for the EU economy/agriculture. However, this information is still untapped or is dispersed in the literature or in specific websites and databases from diverse projects. Information is not connected, have different architectures, or use different descriptors, units, etc. and therefore an effort is needed to collect all the data and information and make it easily available to the different stakeholders. Furthermore, information related to accessions in seedbanks/germplasm banks contain only passport data and a very basic phenotypic description of the accessions. However, what would make them more valuable would be to have information about the genetics and phenotypic traits associated to each accession. By collecting this information for a large number of accessions and making it accessible in a single repository together with tools will result in a boost in the utilization of accessions conserved in Germplasm Banks and facilitate breeding. We have built the HarnesstomDB v1.0, a comprehensive publicly available open-source multi-omics database for harnessing the potential of tomato germplasm collections for breeding. HarnesstomDB v1.0 enables to store, integrate and interconnect curated omics data and metadata from different projects, trials and experiments including images using a controlled vocabulary and accepted standards. HarnesstomDB v1.0 will store genomic, transcriptomic, phenotypic and metabolic data. To access, upload, manage, retrieve and search information, a web interface connected to HarnessTomDB v1.0 is under development. The high impact will then derive from the opportunity of unification of the data produced so far mainly in EU tomato projects, in a single-stop shop that will complement others like SGN or PHEN.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics
P6.06

Title: P6.06 A 3D imaging approach for evaluating tomato fruit shape

Authors: Blazakis, K. [1], Abou Zeid, M. [1], Iakovidis, M. [1], Avdikos, E. [2], Kalyvas, A. [3], Mavromatis, A.G. [2], Ralli, P. [3], Kalaitzis, P.* [1]

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Abstract:

In plant breeding programs, precise and quantitative phenotypic data is of vital importance for assessing genotype performance. Accurate measurements from phenotypic data together with molecular data are used for the determination of quantitative traits of agronomic importance. Traditional two-dimensional tomato fruit morphological analysis rely on manual, subjective measurements which are labor intensive and time-consuming to assess morphological characteristics. A methodology of detecting various morphological characteristics based on image analysis tools is presented. A three dimensional scanner was used to capture the fruit shape and then by using scientific computing techniques we were able to calculate quantitative and qualitative traits related to size, shape, symmetry and presence of additional structures such as the shape of blossom end and depression at peduncle end. We illustrate the applicability of our approach to assess the profile of the fruit shape of 22 Greek tomato landraces. Finally, the proposed methodology might be considered a useful, rapid and reliable image-based tool to identify agronomically important morphological attributes for tomato breeding programmes as a cost effective phenotyping technique.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics
P6.07

Title: P6.07 Live plant monitoring using electrical impedance spectroscopy

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Abstract:

We present a technology to measure a live plant in an ongoing manner. The plant is monitored using the novel four-point electrical impedance spectroscopy. The suggested four-point probe connection is established galvanically to the plant vascular cambium, while readings are collected continuously across short time intervals. In this configuration we eliminate contact effects getting more reliable measurements compared to the conventional two-probe setup. In this manner a trend of the plant daily behavior, as well as its response to different externally induced changes can be monitored. Large data sets can be created and compared using this measurement approach. The method we present here can be distributed for field use offering a simple and accessible method for monitoring of plant health and stress response. The method has been demonstrated on both tobacco and tomato plants, for periods ranging from a few days to a few months, showing response to water stress and a daily cycle trend.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics P6.08

Title: P6.08 Genome-wide dissection of epistatic QTLs in tomato BILs population

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Abstract:

The study of epistasis, the nonlinear interaction between genes, requires the analysis of very large populations, which generate multiple independent genotypes that carry specific genomic regions. To zoom-in on epistasis we will employ a specifically designed backcross-inbred line (BIL) population, of an unprecedented size, derived from a cross between a newly discovered self-compatible accession of the drought tolerant tomato species *Solanum pennellii* (LA5420) and two divergent modern cultivated inbred lines (*Solanum lycopersicum*). The homozygous BILs (BC2S6) include 1500 lines in a determinate processing background (LEA) and 500 BILs in an indeterminate, large-fruited greenhouse background (TOP). The 1500 BILs from the LEA background was sequence by SPET technology and the SNP genotypes were used to partition the genome of the populations into unique mapping bins. Preliminary estimation of the mapping resolution shows a lot of recombination events between adjacent open reading frames (ORFs). The BILs and their hybrids (BILHs) were repeatedly phenotype for yield and quality associated traits in irrigated and non-irrigated fields and in the greenhouse. The data was analyzed relative to the sequence derived mapping bins using multiple epistasis detection software. The analysis revealed a few single-QTLs that improved total yield in drought conditions. Importantly, we found cases of interaction between two regions in the genome, from two different chromosome that effect total yield*Brix in the BILH. To validate and fine-map presumptive interacting genes the following genetic screen will be implemented: We will test in the field the BILs that carry individual chromosome segments that gave rise to the mapped epistasis and their combined hybrids to evaluate the reproducibility of the epistatic phenotypes. Upon validation we will fine-map interacting pairs of genes (or more) by crossing the first BIL carrying a large genomic segment to the BILs with densely mapped recombinants in the second segment, and vice versa. This will identify the sub-region necessary for the epistasis and due to the high mapping resolution, it is likely that the responsible ORFs will be identified. Thus, we expect that in the timeframe of the project we will be able to genetically identify candidate genes involved in epistasis. The *S. pennellii* QTL/genes will be introduced into diverse tomato hybrids to maximize the benefits from favorable epistatic and heterotic interactions. These BILs and the associated data will be made available to the academic community for research of a wide range of morphological and biochemical traits. We believe that such a resource, which to the best of our knowledge is not available for any plant species, will uncover higher dimensions of the complex relationship of genotype to phenotype.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics
P6.09

Title: P6.09 Multi-trait genomic prediction to improve tomato aroma contents

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Abstract:

The increasing consumer demand for tastier tomatoes led to the development of several breeding projects for quality. The first ones are based on increasing the sugar content and improving the acid balance (malic and citric acids) as both traits have been widely described as the main contributors to tomato flavor. However, sugar content and fruit weight are primarily regulated by linked genetic regions with opposite effect, so increasing sugar content reduces fruit weight. In this context, other breeding levers have been considered: the most promising is the diversification of tomato aromas. Tomato aromas are combinations of large number of volatile compounds among which 30 have a major effect on taste. Each of these is controlled by a large number of QTLs. Genomic prediction is a statistical method to estimate the breeding value of genotypes based on their genotyping information. It is well suited for the improvement of low effect polygenic traits. Thus, we explored the efficiency of this method for aroma content prediction on GWAS data previously published by Bineau et al. (2021): 44 volatiles assessed by GS-MS in a collection of 121 cherry tomato lines. The ability and accuracy of prediction models adapted to multiple traits were tested by cross-validation in comparison with single trait models. The second challenge was to predict the breeding values of hybrids (derived from the line collection). Third, we explored some index based on combined volatiles content representing aroma to predict global tomato flavour. I will present the latest results on these three axes.

Poster presentation. Session 6: Tools and Materials for Breeding and Genomics P6.010

Title: P6.10 Tandem gene breeding in tomato

Authors: Nieuwenhuis, R. [1], Warris, S. [1], Rommel-Fuentes, R. [1], Hesselink, T. [1], Bakker, L. [1], Schijlen, S. [1], de Ridder, D. [1], Peters, S. [1], Diaz-Trivino, S.*# [1]

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Abstract:

Many genes related to agronomical and quality traits are present in tandem arrangements (metabolic, abiotic stress tolerance, resistance) in *Solanum* species. Those genes can locate in heterochromatic or repetitive zones of the genome, where the effects of their rapid evolution, rearrangements and transcription can be buffered from the rest of the genome. Here we study recombination by pooled pollen sequencing of interspecific crosses in tomato, specially focusing on the presence of hot and coldspots of recombination in tandem gene areas of breeding interest, looking for genomic features that explain how these key regions can be introgressed.

Session 7: New Plant Breeding Technologies

Keynote invited presentation
K7.01

Title: K7.01 The world’s first CRISPR tomato lunched to a Japanese market

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Abstract:

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats / CRISPR associated proteins 9) technology was awarded the Nobel Prize eight years after its initial publication, which represents unprecedented speed. Genome editing (GE) is featured as a technology for rapid plant breeding, and CRISPR/Cas9 technology is attracting attention due to its convenience and editing efficiency. On December 11, 2020, notification was given to the Japanese government of the first GE tomato containing a high level of GABA (ɣ-aminobutyric acid), which is well known for its human health-promoting benefits, developed using this technology. By May 12, 2021, a venture company launched from University of Tsukuba, Sanatech Seed Co. Ltd., had begun providing seedlings to 4,200 home gardeners free of charge to monitor cultivation. Subsequently, on September 15, 2021, online sales of GE tomato fruits began. This was the first agricultural product in the world to be launched using CRISPR/Cas9 technology. Here, I provide background into how this new development occurred, and how it prompted other governments around the world to re-evaluate GE technology for practical application.

Keynote invited presentation. Session 7: New Plant Breeding Technologies
K7.02

Title: K7.02 From intragenesis to genome editing: new breeding techniques and their use for improving fruit composition

Authors: M. Vázquez-Vilar [1], S. Gianoglio [1], A. Fernández-del-Carmen [1], V. García-Carpintero [1], M. Drapal [2], S. Presa [1], G. Diretto [1], J.L. Rambla [1], P. Fraser [2], C. Martin [4], C. Comino [5], A. Moglia [5], A. Acquadro [5], F. Sevi [3,6], G. Dono [7], Danila Valentino [5], E. Moreno-Giménez [1], M. Fullana-Pericàs [8], M.A. Conesa [8], J. Galmés [8], S. Lanteri [5], A. Mazzucato [7], A. Granell [1], D. Orzáez*# [1]

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Abstract:

New plant breeding techniques offer new possibilities for enriching tomato fruit composition without the interplay of transgenic DNA sequences, either by introducing natural genetic variants already available in breeder´s genetic toolbox via genome editing, or more sophisticatedly, by creating new rearrangements of tomato gene sequences using intragenesis. In this talk we will provide examples of these two approaches. In one of them, we will show the production of staygreen tomatoes using CRISPR/Cas9 genome editing and will describe how this affects fruit metabolic composition. In a second example, we will describe an intragenesis strategy to produce high flavonol tomatoes. In this second example we will also describe the successful use of an intragenic marker for tomato transformation and will discuss how this marker contributes, as an unexpected effect, to an enrichment in the composition of branched chain amino acids in the fruit.

Oral presentation. Session 7: New Plant Breeding Technologies
O7.01

Title: O7.01 Editing tomato for low allergenicity and improved nutritional composition

Authors: Sevi F. [1,2], Lico C. [2], Frusciante S. [2], Presa S. [3], Orzaez D. [3], Granell A. [3], Dellino M. [3,4], Barone A. [1], Rigano M. M. [1], Woo S. L. [5], Sinno M. [1], Pennacchio F. [1], Di Lelio I. [1], Diretto G. [2], Fiore A. [2]

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Abstract:

Tomato breeding has been mainly driven by increases in fruit size, productivity and only recently by consumer driven traits. Tomato is highly consumed vegetable and contributes positively to our health due to its high content of carotenoids, vitamins, flavonoids, folates, fibers and minerals, but still can elicit allergic reactions in a small number of consumers. Moreover, in tomato there are many "antinutritional" compounds, so called for interfering with the normal absorption of nutrients or showing toxic effects in human. Among those compounds several glycoalkaloids. The purpose of our work has been to eliminate and/or decrease, some of these antinutritional and/or allergenic molecules using the new breeding technology "genome editing". In our approach we designed specific gRNAs for the genes of interest: the GAME4 gene involved in a key step in the biosynthetic pathway of the glycoalkaloids and the Sola l 4 which is among the major allergens in tomato. We obtained genetically stable plants carrying loss of function mutations in both GAME4 and Sola l 4 genes. Moreover, the edited plants showed a reduction in the glycoalkaloids content of up to 90% compared to control plants and also a complete absence of allergenic protein. These results indicates that is possible to target two different types of antinutritional compounds (a protein and a small metabolite) by gene editing and to obtain plants with the desired phenotype. The effect of editing in the biotic stress responses towards fungi and insects will be also presented.

Oral presentation. Session 7: New Plant Breeding Technologies
O07.02

Title: O7.02 CRISPR/Cas9-mediated mutagenesis in tomato: application to parasitic plant resistance, technical developments, and future perspectives

Authors: Nicolia, A.# [1], Cuccurullo, A. [1], Andersson, M. [2], Yoneyama, K. [3], Contaldi, F. [1], Camerlengo, F. [1], D'Agostino, N. [3], Festa, G. [1], Hofvander, P. [2], Cardi, T.#* [1,5]

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Abstract:

Broomrapes (*Orobanche* spp and *Phelipanche* spp.) represent a serious threat for several crops cultivated in the Mediterranean basin, and some regions of Asia and Europe. Tomato (*S. lycopersicum*) cultivation can be significantly affected mainly by the diffusion of *Phelipanche ramosa*, but also of other species. Strigolactones (SLs) are the main stimulants that trigger the germination of *P. ramosa* seeds in infested soils. Knock-out mutants for the four main genes (SlD27, SlCCD7, SlCCD8, SlMAX1) responsible of the biosynthesis of SLs, have been generated by CRISPR/Cas9 mutagenesis in the same tomato genetic background. Phenotypic analysis of the mutant progenies has evidenced the expected phenotype of the SL- plants and a significant reduction of *P. ramosa* infection in soilless experiments. A multiplexing (SlCCD7 + SlCCD8) mutagenesis approach based on ribonucleoprotein (RNP) delivery into tomato protoplasts has been also explored, showing high frequency of mutagenesis in derived calli (up to 90 and 26% for single and both genes, respectively). Besides a high (multiplex) mutation frequency with reduced frequency of mosaicism and off-target effects, the RNP delivery in protoplasts has the advantage of producing DNA-free mutants. Plant regeneration from edited calli, however, remains a limiting step for applications to a wide number of genotypes. To apply mutagenesis procedures bypassing potential limitations of lengthy and complicated tissue culture protocols, their genotype-dependency, and the possibility to induce unwanted somaclonal variation, the development and implementation of alternative editing systems will be discussed.

Poster presentation. Session 7: New Plant Breeding Technologies
P7.01

Title: P7.01 Generation time reduction in tomato using TEV virus-mediated overexpression of flowering positive regulator Flowering Locus T (AtFT)

Authors: Petit, J.* [1], Mirabel, S. [1], Aragonés, V. [1], Amoroso, C.G. [2], Carere, N. [1], Rici, R. [1], Darós, J.A. [1], Granell, A.# [1]

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Abstract:

Tomato breeding is a long-term process often requiring many crosses and backcrosses to obtain the desired plant/fruit characteristics. The urgent need to develop new cultivars able to cope with climate change challenges or with the consumer demands requires new technologies to speed up breeding. Generation time can be reduced by accelerating flowering time. The transition from vegetative growth to floral meristems in tomato is regulated through a combination of genetic and environmental cues. In this study, virus-mediated overexpression of a positive flowering regulator (Flowering Locus T, FT gene) was tested to accelerate tomato flowering. A potyvirus vector (TEV) was used to transiently overexpress AtFT in three tomato genotypes: Moneymaker, M82 determinate and MicroTom. Plasmid pGTEV::AtFT was assembled and introduced into *A. tumefaciens*. Cotyledons of young seedlings were agroinfiltrated. The viral vector was able to infect all tomato plants. Moneymaker and M82 infected with TEV::AtFT flowered after 7.7 and 6 leaves stage, respectively, which can be compared to the controls that flowered at 11 and 8 leaves. Virus-based overexpression of AtFT in Moneymaker and in M82 reduced generation time 7 days; reduction in MicroTom was 5 days. As a side effect of infection, plants showed reduced plant vigour and lower number of trusses and fruits, but seed number per fruit was not affected. Overall, transient overexpression of AtFT in the three tomato genotypes results in a significant reduction in flowering and generation time without modifying the plant genetic background. Importantly the viral vector is not transmitted to the next generation.

Poster presentation. Session 7: New Plant Breeding Technologies

P7.02

Title: P7.02 Increased GABA accumulation in tomato fruits by promoter region genome editing

Authors: Gramazio, P.*# [1,2], Takayama, M. [3], Hunziker, J. [4], Lu, Y. [1], Shinozaki, Y. [5], Ezura, H. [1,3]

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Abstract:

Hypertension is a primary risk factor for cardiovascular disease. The ɣ-aminobutyric acid (GABA) is a non-proteinogenic amino acid that has shown effective in lowering the blood pressure of mild hypertensive patients. Intake of GABA through the daily diet may help reduce their symptoms and might be an effective way to prevent hypertension. Tomato contains high levels of GABA in mature tomato green fruits that rapidly decrease in ripe fruits due to two main mechanisms of the GABA pathway. In fact, GABA is synthesized from glutamate by glutamate decarboxylases (GAD) and reversibly converted to succinic semialdehyde by GABA transaminase (GABA-T). A high-GABA content Micro-Tom lines have been already developed by CRISPR/Cas9 deleting the C-terminal autoinhibitory domain of the *SlGAD2* and *SlGAD3* genes by introducing a premature stop codon (Nonaka et al., 2017). On the other hand, simply knocking out *SlGABA-T* would lead to severe dwarfism and affect vegetative and reproductive growth as already reported by RNAi approach. Thus, in this study, we aimed at further increasing the GABA content in tomato by applying an alternative CRISPR/Cas9 multiplexing strategy by targeting the promoter region of *SlGABA-T* in either Micro-Tom WT and SlGAD3ΔC37#3-12 genotypes. In that way, we produced novel cis-regulatory alleles that provided a continuum of variation in *SlGABA-T* gene expression and a better balance between GABA accumulation and plant development. Some of the edited lines presented higher GABA levels than their respective Micro-Tom and SlGAD3ΔC37#3-12 WT genotypes, without apparently severe morphological defects.

Poster presentation. Session 7: New Plant Breeding Technologies
P7.03

Title: P7.03 Enhancing cold stress tolerance in tomato by engineering lipid composition in fruit and plant

Authors: Vittani, L.*# [1,2], Busatto, N. [2], Granell, A. [3] , Costa, F. [1]

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Abstract:

After harvest, fruits are stored to guarantee an extended and continuous marketability. Low temperature can lead to a series of post-harvest related disorders due to the occurrence of chilling injuries. Apple, a climacteric fruit like tomato, is susceptible to a postharvest disorder known as superficial scald. To gain knowledge in the understanding of this phenomenon, we develop a biparental mapping population crossing a susceptible variety “Granny Smith” to superficial scald with a resistant cultivar “Pinova”. After harvest, apples were stored at 4ºC for six months and maintained at 24ºC shelf-life for 7 days, and a metabolite QTL mapping analysis identified two fatty acids are major compounds mainly involved in the cold stress response: Linolenic and Vaccenic acid. A genetical genomic approach carried out by RNA-Seq on the individuals of the bi-parental population allowed to select a list of DEGs involved in fatty acid metabolism, out of which three genes located within the interval of the QTL associated to Linolenic and Vaccenic acid, such as a member of the Cytochrome p450 family, an Esterase/Lipase and an Omega-3-desaturase emerged from the analysis, were further functionally validate in *Solanum lycopersicum*, the model species for fleshy fruit. To confirm their possible role in enhancing cold stress resistance we are investigating the role of these elements by generating constitutive expression and fruit specific expression line, together with orthologous gene silencing by CRISPR-Cas9 techniques. The investigation of three fatty acids related genes will elucidate the role of these specific fatty acids in promoting cold stress resistance.

Poster presentation. Session 7: New Plant Breeding Technologies
P7.04

Title: P7.04 Characterization of *green flesh* and *tangerine* CRISPR/Cas9 edited tomato lines in San Marzano variety

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Abstract:

Stay Green Protein (SGR) and Carotenoid Isomerase (CRTISO) were targeted using computationally designed gRNAs with the stable transformed CRISPR/Cas9 system, to reproduce the effects of these mutations into the Italian tomato landrace San Marzano (SM). Tomato fruits of green flesh (*gf*) ripen to a brownish colour for the simultaneous presence of lycopene and chlorophyll, whereas tangerine (*t*) abolishes all-trans-lycopene in fruits, which appear orange, due to pro-lycopene. These phenotypes represent an alternative to the red tomato also because of their different nutraceutical and organoleptic qualities. Both mutations had been introgressed into SM by backcrossing. Here, genotypic, phenotypic, and biochemical characterization was performed in *gf* and *t* introgressed and edited lines and compared to SM. The average gene editing efficiency was 67.8% for SGR and 71.4% for CRTISO. Interestingly, edited *gf* lines showed higher soluble solids and longer shelf-life than SM. To evaluate putative perturbations at the whole metabolome level, the polar, non-polar and volatile metabolite fraction were studied. The *gf* volatile profile did not differ from SM. For *t*, the dissimilarity of the introgressed line attributed to Benzenoids and Terpenoids was not observed in the edited lines. Lastly, the diverse content of lycopene in *t* had no effect in volatile apocarotenoid formation, responsible for tomato flavour acceptability. A targeted analysis has been carried out, by quantifying the levels of specific metabolites associated to nutritional activity like sugars, carotenoids, vitamins, and phenylpropanoids. These genotypes offer new opportunities to improve and diversify SM fruit quality by modifying its flavour and nutraceutical properties.

Poster presentation. Session 7: New Plant Breeding Technologies
P7.05

Title: P7.05 An efficient workflow for obtaining tomato elite-edited cultivars using the CRISPR/Cas9 system

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Abstract:

Given climate changes and decreasing cultivable areas, food security for a growing population represents a critical challenge in the coming decades. Tomato breeding could enhance resistance to different stress. However, this process is tedious and time-consuming. Therefore, novel strategies are required to obtain tomato cultivars with improved traits under multiple stress conditions. In this work, a protocol to evaluate the suitability of elite tomato lines to be employed in CRISPR/Cas9-mediated genome editing experiments was set up. Tomato cultivars were assessed for their phenotypical traits, the presence or absence of resistance loci, and the in vitro regeneration rates. The comparison of phenotypical and genetic analysis allowed the selection of SanMarzano2 as the most suitable line. The choice of target genes to mutate was carried out by analyzing massive transcriptomic data. Raw reads, retrieved from twelve RNA-seq studies of tomatoes exposed to biotic and abiotic stress, were *de novo* analyzed using a common pipeline. Comparison of differential expressed genes (DEGs) induced by each stress allowed the identification of 126 genes involved in response to different biotic and abiotic stresses. In particular, *SlWATI* (involved in IAA accumulation) and *SlHyPRPI* (participant in vascular immunity) were selected as optimal targets. Genes were knock-out separately and simultaneously in SanMarzano2 and MoneyMaker genotypes. Out of a total of 82 mutated plants, 25 plants carried bi-allelic mutations at target loci, suggesting a high transformation efficiency. In conclusion, our work was useful for testing a gene-editing workflow that can be employed routinely to produce improved elite-edited varieties.

Poster presentation. Session 7: New Plant Breeding Technologies
P7.06

Title: P7.06 In-depth characterization of *greenflesh* tomato mutants obtained by CRISPR/Cas9 editing: a case study with implications for breeding and regulation

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Abstract:

Gene editing has increased our ability to define the relationship between certain mutated loci and their associated phenotypes with greater depth and precision than traditional breeding techniques. We focused on the *greenflesh* (*gf*) gene in tomato, targeting this locus in three cultivars (MicroTom, MoneyMaker and San Marzano) and evaluating the efficiency, specificity and mutation patterns associated with CRISPR/Cas9 activity. *gf* encodes a Mg-dechelatase responsible for the initiation of chlorophyll degradation, and its mutation determines a clearly detectable phenotype, with ripe fruits developing a distinct colour due to the simultaneous presence of chloroplasts and chromoplasts. Mutations in gf orthologs have also been associated to pathogen resistance in various plant species. It is known from the characterization of spontaneous gf tomato mutants that different mutations (SNPs, small indels, or larger deletions) all result in the same phenotype. In our work, phenotypic evaluations were performed in the transgene-free T2 generation on two MoneyMaker *gf* lines, which carry two different mutant alleles (a small insertion of 1 nucleotide and a larger deletion of 123 bp). Fruit metabolic profiles were assessed, and the link between tomato CRISPR-induced *gf* mutations and pathogen infection was explored by infecting leaves and fruits with *Botrytis cinerea*, which resulted in a significant reduction in pathogen proliferation. Our data suggest that the CRISPR/Cas9-mediated mutation of the gf locus in tomato is robust and reproducible and mimics known features of *gf* genotypes obtained through traditional breeding, while also shedding light on functions so far undescribed in tomato, such as pathogen resistance.

Poster presentation. Session 7: New Plant Breeding Technologies
P7.07

Title: P7.07 Down regulation and loss of Auxin Response Factor 4 function using CRISPR/Cas9 alters plant growth, stomatal function and improves tomato tolerance to salinity and osmotic stress

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Abstract:

Auxin is a key hormone that regulates several aspects of plant growth and development. However, its role in stress responses remains poorly understood. Auxin acts on the transcriptional regulation of target genes, mainly through Auxin Response Factors (ARF). This study focuses on the involvement of SlARF4 in tomato tolerance to salinity and osmotic stress. Using CRISPR/Cas9 and antisense approaches, we found that down-regulation of SlARF4 promotes root development and density, increases soluble sugars content and maintains chlorophyll content at high levels under stress conditions. Furthermore, ARF4-as displayed higher tolerance to salt and osmotic stress through reduced stomatal conductance coupled with increased leaf relative water content and Abscisic acid (ABA) content under normal and stressful conditions. This increase in ABA content was correlated with the activation of ABA biosynthesis genes and the repression of ABA catabolism genes. Cu/ZnSOD and mdhar genes were up-regulated in ARF4-as plants which can result in a better tolerance to salt and osmotic stress. A CRISPR/Cas9 induced SlARF4 mutant showed similar growth and stomatal responses as ARF4-as. Our data support the involvement of ARF4 as a key factor in tomato tolerance to salt and osmotic stresses and confirm the use of CRISPR technology as an efficient tool for functional reverse genetics studies.

NOTEBOOK