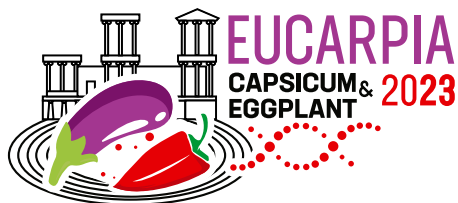




EUCARPIA

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Eucarpia 2023

18th EUCARPIA MEETING ON
Genetics and Breeding of
Capsicum and Eggplant



Session:

GENETIC RESOURCES





Keynote talk 1

TITLE: FROM WILD TO MILD AND BACK AGAIN: GENETIC DIVERSITY, CROP IMPROVEMENT STRATEGIES AND OUR SHARED CULTURAL HERITAGE WITH CHILE AND EGGPLANT

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Abstract: *Capsicum* fruits and eggplants are among the top ten most important vegetables consumed worldwide. They are vital contributors to human health and nutrition, and unique, diverse types are appreciated by many cultures around the world. It has been estimated that genetic improvements in our crops must increase to twice the present global rate to keep up with demand. Intense breeding of elite cultivars can result in the commodification of some varieties – for example, sweet peppers. Selection for broad adaptation, high yield, mechanical harvestability, shelf-life, and universal consumer appeal often results in the creation of modern cultivars that are mild, bland-tasting, or just plain boring. In some areas, the concomitant decline of locally adapted, and venerated, land race varieties resulted not only in the loss of genetic diversity, local income, and culinary quality, but also an element of our cultural heritage. As our appreciation of landraces has grown, so too has our realization of the need for enhanced preservation, collection, characterization, and utilization of crop wild relatives. Modern technologies, and the organization of multi-disciplinary teams, can open the gates of innovation to make more useful genes accessible for varietal improvement. We would also be well served to incorporate societal priorities into our plant breeding programs. Ensuring that all participants in the food chain have a voice will encourage policy makers and funders to support accelerated development of future crop varieties – varieties that will fulfill societal needs for productivity and value coupled with sustainability of our agroecosystems, both small and large. Listening to all stakeholders in the value chain will help assure that breeders release improved varieties that also help sustain our cultural heritage and pride of place for *Capsicum* and eggplant varieties.

Keynote talk 2

TITLE: THE POTENTIAL OF GENETIC RESOURCES AND NEW EXPERIMENTAL POPULATIONS FOR THE ENHANCEMENT OF EGGPLANT BREEDING

Authors: Gramazio, P.[1], Alonso, D.[1], Arrones, A.[1], Villanueva, G.[1], Plazas, M.[1], Toppino, L.[2], Barchi, L.[3], Portis, E.[3], Ferrante, P.[4], Lanteri, S.[3], Rotino, G.L.[2], Giuliano, G.[4], Vilanova, S.[1], Prohens, J.*[1]

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Abstract: Eggplant (*Solanum melongena*) is a globally important vegetable crop. However, the exploitation of wild/allied genetic resources, particularly from the secondary and tertiary genepool, for the development of new cultivars is rather limited and still lags behind other major vegetables. Here we review the potential of cultivated and wild genetic resources for eggplant breeding enhancement. Over 19,000 accessions of eggplant and relatives are stored in germplasm banks worldwide. Most of them correspond to *S. melongena*, while the other cultivated eggplants (*S. aethiopicum* and *S. macrocarpon*) and wild relatives are much less represented. Exploration of the *S. melongena* germplasm has revealed a wide diversity for morphological and agronomic traits, and the use of the intraspecific diversity allowed dramatic increases over the last decades in yield and quality of eggplant. However, the new challenges posed by climate change and the need of a more sustainable agriculture will require the use of the largely unexplored immense wealth of the genetic diversity of eggplant relatives for broadening the genetic base and introgressing adaptive traits, including tolerance to biotic and abiotic stresses. Despite its economic importance, few experimental populations, such as mutant libraries, core collections, immortal biparental and multiparent recombinant inbred populations, as well as introgression lines have been developed. Nevertheless, the results obtained so far

with these populations have shown that they represent a powerful tool for eggplant breeding. Overall, we foresee that the systematic and extended use of genetic resources will be needed for developing new eggplant cultivars with superior features and improved resilience to climate change.

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Oral presentations

1 Oral presentation

TITLE: THE MUTATION OF *CACK1* CAUSES SEEDLESS FRUITS IN CHILI PEPPER (*CAPSICUM ANNUUM*)

Authors: Maki, T.*[1], Kusaka, H.[1], Matsumoto, Y.[1], Yamazaki, A.[1,2], Yamaoka, S.[1], Ohno, S.[1], Doi, M.[1], Tanaka, Y.[1]

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Abstract: In fruit crops, seedlessness is a preferred trait because seed removal is cumbersome for consumers and seeds become industrial waste for the processing industry. However, knowledge for developing seedless peppers is limited. Our previous study isolated a pepper mutant, tn-1, that stably produces seedless fruits. In this study, we characterized tn-1 and identified the causative gene. Although pollen germination and pollen tube elongation was normal, observation by confocal laser microscopy revealed that embryo sac development was defective in tn-1. Marker analysis narrowed the tn-1 locus to a 313 kb region on chromosome 12. Further mapping-by-sequence analysis identified CA12g21620, encoding a histidine kinase, as a candidate gene. Phylogenetic analysis of histidine kinases revealed that CA12g21620 is a homolog of Arabidopsis CKI1 (Cytokinin Independent 1), which plays an important role in the development of female gametophytes, and CA12g21620 was named as CaCKI1. Sequence analysis revealed that tn-1 has a 3 bp insertion in the 6th exon which causes single lysine (K) residue insertion into the receiver domain of CaCKI1, and that the sequence around this site is widely conserved among CKI1 orthologs in various plants. This suggests that the insertion of one K residue may disturb the phosphorylation relay downstream of CaCKI1 and inhibit normal development of the female gametophyte, resulting in tn-1 seedless fruit. Furthermore, we found that virus-induced gene silencing of CaCKI1 increased the percentage of defect in development of female gametophyte in chili pepper. This study demonstrates the important role of CaCKI1 in seed development in chili pepper and the potential utilization of its mutation to develop seedless varieties.

2 Oral presentation

TITLE: CURRENT STATUS, CHALLENGES AND BREAKOUT OPPORTUNITIES IN PEPPER BREEDING

Authors: Szamosi, Cs.*[1], Palotás, G.[2], Tímár, Z.[2], Pápai, B.[3], Kovács, Zs.[3], Fári, M.[4], Ruskó, J.[5], Gatto, G.[6], Csilléry, G.[7]

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Abstract: In the last decades cultivation technologies and growing methods (e.g., soilless culture or intensive open field cultivation) have had more impact in the yield increase of F_1 pepper production than the genetic improvement through breeding approaches. Yield potential of pepper production seems to have reached its limits even within high-tech glass house production (25-30 kg/m²). Considering the achievable yield (80-120 kg/m²) of tomato, it is understandable why greenhouse pepper production is decreasing.

Breakthrough solutions are needed in order to significantly increase yield within commercial pepper production in a more sustainable way! We must boost pepper yields not with higher doses of synthetic fertilizers and other chemicals but by exploring and understanding how to utilize the genes responsible for productivity.

Some examples of potentially useful *Capsicum* mutants and wild capsicum collection of PepGen Ltd. will be demonstrated from a commercial point of view:

- novel solutions similar to the jointless tomato treat? Easy calix detachment or *Brp*-breaking peduncle traits could enhance the efficacy of open field mechanical harvest
- fruit quality traits (capsaicin, antioxidant capacity)

- usage of *tti*-tortuous internode and *Pcx*-Procumbent plant mutants enables laying down the stem similar to tomato production
- “multiflower/multiset” traits: could be the breakthrough in order to double the pepper yield by focused introgression from wild *Capsicum chinense*, *C. frutescens*, *C. baccatum*
- the capsicum mutant collection of PepGen having numerous “hidden gems” which may help either to increase pepper yield or expand growing technology possibilities: *Bil*-brilliant leaf may confer increased stress tolerance, more efficient production under low light conditions (winter months, vertical farming), *dwx*-dwarf/semi dwarf plants for vertical farming, *sfx*-super fastigiate plant to run extra short cycle crop even in Space.

3 Oral presentation

TITLE: GENETIC DIVERSITY, POPULATION STRUCTURE AND RELATIONSHIPS IN A COLLECTION OF PEPPER (*CAPSICUM* SPP.) INSULAR SPANISH LANDRACES OBTAINED WITH GENOTYPING-BY-SEQUENCING (GBS)

Authors: Ortega-Albero, N.*[1], Fita, A.[1], Martínez, F.[1], Luna-Prohens, J-M[2], Ruiz-Ramírez, F.[3], Mallach-Pérez, M.[3], Rodríguez-Burruazo, A.[1]

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Abstract: Pepper (*Capsicum* spp.) is one of the most important crops worldwide; hence, finding new sources of interesting genes would have major impacts on agriculture. Understanding the species' genetic background is key to finding new sources of variation that could be incorporated into future breeding programs. This work explores the genetic diversity of 66 Spanish Balearic accessions, through genotyping-by-sequencing (GBS) to find traits adapted to specific insular environments. Palma and Eivissa landraces were genotyped with SNP markers and population structure and patterns of diversity were obtained with bioinformatic analysis. The results show a moderate genetic diversity for the studied landraces with no clear clusters within each island population. Genetic divergence between Spanish peninsular and insular landraces was observed – which suggests specific adaptation to each environment. These findings may serve as the basis for study of the divergent evolutionary patterns associated with the respective populations.

These results can contribute to further elucidation of genetic basis of Balearic landraces, facilitating interesting gene identification through genome-wide association studies (GWAS), or developing new varieties with marker-assisted selection (MAS).

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Poster presentations

1 Poster presentation

TITLE: RECUPEVO: A MULTIDISCIPLINARY TASKFORCE FOR THE RECOVERY AND VALORIZATION OF THE “VOGHERA” PEPPER LANDRACE

Authors: Toppino, L.*[1], Sala, T.[1], Losa, A.[1], Tassone, M.R.[1], Fibiani, M.[2], LoScalzo, R.[2], Picchi, V.[2], Martufi, G.[2], Tripodi, P.[3], Gattolin, S.[4], Zefelippo, M.[5], Olezza, A.[6], Balestrazzi, A.[7], Mancovei, A.[7], Pagano, A.[7], Genone, A.[7], Ferrari, F.[8], Rossi, G.[8], Rotino, G.L[1].

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Abstract: Pepper (*Capsicum annum* L.) is a vegetable/spice crop originated from the New World and amongst the most cultivated within the *Solanaceae* family together with potato, tomato and eggplant. Around the genus *Capsicum* there is an increasing interest and fascination due to the considerable variation existing for several traits among landraces, ancient varieties as well as domesticated and wild species, which are a valuable resource of traits of interest for breeding and make this crop suitable and versatile for innumerable uses as food and non-food products. The “Voghera” landrace is a pale

green variety (Citrin) of sweet pepper grown within the Po flood plain area (Northern Italy) near Voghera town, usually harvested and consumed in its early (green) ripening stage in salade, but also ripe (yellow stage) in risotto, whose peculiarities are related to its sweetness, the thinness of its pulp and low water content, which allow traditional pickled preservation of fruits. Pollination phenomena, hybridization, and the presence of different varieties in the surrounding cultivation areas progressively caused the loss of the purity of "Voghera" landrace, with consequently occurrence of undesired high pungency of the berry. The RECUPÉVO project aimed at the recovery of the purity of "Voghera" pepper, with primary attention to sweetness and phenotypic features, aimed to return the "original" landrace to the seedbanks and growers. Conservative breeding activity enabled the reconstitution of capsaicin-free "Voghera" lines. A deep molecular and biochemical characterization of different lines of "Voghera" pepper recovered from the cultivation area allow to better position this landrace in the context of the genetic variability existing among citrin peppers, together with expression analyses of genes involved in the capsaicin biosynthetic pathway, aimed at identifying the genetic determinants for the loss of sweetness in this landrace.

2 Poster presentation

TITLE: GENETIC BACKGROUND OF LILAC COLOURATION IN PEPPER FRUITS (*CAPSICUM ANNUUM L.*)

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Abstract: Pepper is one of the most consumed vegetable and spice crops. Besides numerous characteristics that determine the consumer acceptance of the fruit, colour in particular ranks very high in the perceived palatability and flavour intensity of the berries.

For our study, 'Black Pearl' an extreme lilac genotype carrying a LINE-1 retrotransposon insertion in the CaMYBa gene was crossed with a breeding line that matures from pale green to red. F₁ hybrids were grouped and kept under high and low light conditions and tested for expression of both the enzyme encoding structural genes and the transcription factors associated with anthocyanin biosynthesis at two stages of ripening. Parallel measurements of the berry nutraceutical properties were performed. We could detect the effect of the LINE-1 retrotransposon insertion in terms of both gene expression and higher anthocyanin content measured in both hybrid groups. We could not identify the effect of elevated anthocyanin content on the antioxidant capacities of the berries at their economically ripe stage.

After self-fertilization of the F₁ plants, fourteen tissues of the resulting F₂ plants were phenotyped in two-week intervals. Out of the 196 plants, 103 were genotyped and the genotypic data was compared with the phenotypic data. We also monitored the correlation of the berry shape to the colouration of the berries. Ten of the tested F₂ plants showed genotypic data that did not match with the phenotypic data. In these cases, we hypothesized the presence of another R2R3-MYB transcription factor altering the colouration of the vegetative and generative organs.

3 Poster presentation

TITLE: ASSESSMENT OF BALKAN PEPPER (*CAPSICUM ANNUUM* L.) ACCESSIONS FOR PEST RESISTANCE, AGRONOMIC AND FRUIT QUALITY TRAITS

Authors: Todorova, V.*[1], Yankova, V. [1], Tringovska, I. [1], Markova, D. [2], Nankar A.N. [3]

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Abstract: Pepper is an economically significant crop due to the nutritional and health benefits of its fruits. They combine a high content of diverse vitamins, pigments, phenols, minerals and other bioactive substances which have proven antioxidant activity. Pepper plants are attacked by various pests throughout the vegetative period. Pests damage leaves, fruits and whole plants and some of them, like aphids and thrips, are vectors of pathogenic viruses. The diversity of pepper forms, the traditions in cultivation and numerous usages of the fruits, are characteristic for Bulgaria and other Balkan countries. During two consecutive years (2018-2019) 21 pepper accessions were evaluated for various morphological, economic and biochemical traits at Maritsa Vegetable Crops Research Institute, Plovdiv. Naturally occurring pest infestations under field conditions were also assessed. The examined genotypes originated from Albania, Greece, North Macedonia and Bulgaria and belonged to different varietal types - Corniforme, Blocky, Pumpkin (Ratundum) and Kapia. The accessions K1115 (type Pumpkin) and K1081 (type Kapia) showed the highest productivity (0.74 and 0.70 kg per plant, respectively). The accession K712 (type Pumpkin) had the highest amount of total polyphenols (203.44 mg GAE/100 g FW) in its fruits, while accessions K1103A and K1055 from Pumpkin type were the best in terms of their antioxidant activity (11.49 and 11.48 $\mu\text{mol Fe}^{2+}/\text{g FW}$, respectively). No infestation from aphids (*Myzus persicae* Sulz.) was reported in 38% of the studied accessions. The genotype K697 (Kapia type) was considered as the most tolerant to pest attack. This accession was not infested by aphids;

it had the lowest damaged plants (20 %) and infestation rate (0.75) by thrips (*Frankliniella occidentalis* Perg. and *Thrips tabaci* Lindeman) and comparatively low percent of damaged plants (6.7%) and fruits (8.3%) by cotton bollworm (*Helicoverpa armigera* Hubn.).

Acknowledgements: The research leading to these results has received funding from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

4 Poster presentation

TITLE: IMPROVING THE QUALITY OF CAPSICUM COLLECTION THROUGH MUTATION BREEDING AND SUPPORTING MOLECULAR TECHNOLOGY

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Abstract: Bulgaria was the secondary gene pool for pepper (*C. annuum* L.); however, during the political transformation and economic crises, the growing lands were reduced, and considerable genetic diversity was lost. Pepper is a major natural source of β -carotene which contributes to food quality. In pepper, integrated application of nuclear and advanced proteomics technologies, and molecular-marker techniques, is expected to provide new opportunities for modern pepper breeding. We studied the degree of polymorphisms in a collection of 73 pepper Bulgarian landraces and varieties, introductions, and mutants. For the first time a retroelement-based Inter-SINE Amplified Polymorphism (ISAP) method was used in *Capsicum*, initially developed for potatoes. From the seven Solanaceae SINE families as well as two sub-families studied, markers from SolS-II and SolS-V generated the most informative banding patterns.

Old and new mutant varieties (Oranzheva kapia, Albena, Desislava), and advanced mutant lines in M_8 generation were evaluated by different HPLC methods. Improved quality of mutant pepper fruits intended for fresh consumption was assessed for high β -carotene concentration and anthocyanin-free traits. Mutations associated with orange fruit colour in sweet pepper varieties and five carotenoid pigment levels were detected through comparison and they did not show an adverse affect on concentrations of essential mineral elements in pepper fruit.

Two hydroxylase enzymes, converting α - and β -carotene, were characterized through PCR amplification, cloning, sequencing and expression of the two hydroxylase genes. Proteomics characterization of the mutant variety through 2D-DIGE and Maldi Toff sequencing

suggested alteration in the regulation of proteins such as Capsanthin capsorubin synthase. Molecular marker was developed for performing marker assisted selection.

Acknowledgements: This research was funded by the International Atomic Energy Agency, grant number BUL/5/016 "Sustaining National effort in improving the productivity and quality of selected crops through nuclear techniques" and RER/5/024 "Enhancing productivity and resilience to climate change of major food crops in Europe and Central Asia". The author also acknowledges the financial support received by EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

5 Poster presentation

TITLE: SCREENING BALKAN PEPPER (*CAPSICUM ANNUUM L.*) COLLECTION FOR FRUIT QUALITY

Authors: Tringovska, I.*[1], Todorova, V.[1], Grozeva, S.[1], Stoeva, V.[1], Asenova, V.[1], Nankar, A.N.[2], Kostova, D.[2]

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Abstract: A total of 182 pepper accessions were phenotyped for fruit quality during two consecutive years. The traits of interest were Dry matter (DM) content, Total Soluble Solids (TSS), Vitamin C, Ferric reducing antioxidant power (FRAP) and Total polyphenols (TP) evaluated at fully ripened fruits. A wide variation is observed for all evaluated traits: Dry matter – 7.4-25.5%; Vitamin C – 62.8-299.2 mg/100g FW; TSS – 5.4-18.3 oBrix; FRAP – 3.7-24.9 $\mu\text{m Fe}^{2+}/\text{g FW}$ and TP – 112.9-480.2 mg GAE/100g FW. The genotypes were separated in five pre-defined groups in accordance with their usage: *Pungent*, *Sweet Green*, *Pumpkin*, *Kapia* and *Sweet Paprika Powder*. Sweet Paprika Powder group is distinguished with the highest average values of all traits, followed by Pungent group, while Sweet Green group has the lowest values of evaluated fruit quality traits. Relatively high Vitamin C content is observed in *Kapia* and *Pumpkin* groups. Based on the analyses of the five fruit quality traits valuable accessions could be identified in each group. For the whole collection, a selection of “the best quality accessions” is made. Accessions number CAPS-173 from *Kapia* varietal type and CAPS-109 from Pungent group are distinguished with high values of all studied traits.

Acknowledgements: The research leading to these results has received funding from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

6 Poster presentation

TITLE: ELEMENTAL PROFILING OF PEPPER FRUITS OF BALKAN CAPSICUM ANNUUM L. COLLECTION

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Abstract: Pepper fruits are widely consumed for their unique taste, aroma, pungency and colour. They are also rich source of various nutritional compounds possessing antioxidant, antibacterial and anticarcinogenic properties, which are beneficial for human health. As a part of larger scientific program on comprehensive phenotyping of Balkan pepper collection the current study was conducted (i) to determine the concentration of some macro, micro- and trace elements in pepper fruits and (ii) to identify genotypes enriched with some elements so that they could be further used in future breeding. A collection comprised by 181 *Capsicum annuum* L. accessions originated and/or collected across 62 geographical sites from six Balkan countries is used in this study. Examined genotypes were grown during two consecutive years in Bulgaria, split into five predefined groups based on their end-use: *Pungent*, *Sweet Green*, *Pumpkin*, *Kapia* and *Sweet Paprika Powder*. In the first two groups fruits were harvested before maturity as they are commonly used while rest three groups were harvested at fully ripened stage. Freeze dried samples were subjected to microwave digestion in closed PTFE vessels, and subsequently analyzed by ICP-OES with dual-view configuration. A total of fifteen different macro and microelements were quantified. Obtained results displayed wide range of variation for mineral composition profiles between the predefined groups and among accessions within each group and indicated significant genotype influence. In each group, accessions with high content of individual elements were distinguished. The observed variability is anticipated to enable the selection and breeding novel varieties with enhanced mineral composition.

Acknowledgements: The research leading to these results has received funding from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

7 Poster presentation

TITLE: FIELD TRIALS AND EVALUATION FOR EUROPEAN EVALUATION NETWORK FOR PEPPER ACCESSIONS IN THE ARMENIA

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Abstract: Pepper was selected as a fruit vegetable of significant interest to establish an EVA network. Activities in the pepper network include field evaluations for agronomic traits in various geographic locations in Armenia. The research was conducted within 2021 in the experimental household of Scientific Centre of Vegetable and Industrial Crops of Darakert community (the Ararat valley) of the Armenia. The experiment was carried out according to the "Methodological regulations of randomized block experimental design" of the World Vegetable Center. The characteristics of the genetic recourses are described according to the methods of VIR, AVRDC and IPGRI (Bioversity International). The content of biochemical tests in fruits was determined by an agrochemistry method and Morion OK2i nitrate tester. The field experiment and productivity were subjected by the method of ANOVA analysis. Evaluation environment- open field, sowing, transplanting, evaluating, for harvesting number of 83 accessions. Pepper accessions experiment of the testing, evaluation, characterization can be classified according of fruit type, plant habit, and variety type. The number of days to germination- ripening in the studied pepper accessions was 111-142 days. The results of the yield of the pepper accessions was 85,3 – 450,7 c/ha. The results of qualitative parameters. The content of dry matter in the pepper accessions was 3.2 - 5.3% 8.9%, content of sugars was 4.6 – 7.8%, the content of vitamin C - respectively – 78.55 – 156.95 mg%, acidity – 0.47 - 0.50% concentration of nitrates in the product – 37 - 45%. In the testing pepper accessions have good results *Capsicum annuum* - 006, 010, 012, 015, 018, 020, 021, 027, 028, 029, *Capsicum baccatum* var. *pendulum*-139, *Capsicum baccatum* var.

pendulum -140, *Capsicum chinense* - 160,166, 171, *Capsicum chacoense* - 181, *Capsicum frutescens* - 209, 210.

Acknowledgements: The research leading to these results has received funding from International Atomic Energy Agency Technical Cooperation project RER5024/ Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia.

8 Poster presentation

TITLE: ADAPTABILITY EVALUATION TRIAL OF BULGARIAN PEPPER IN SOUTH KOREA

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Abstract: Pepper is one of the most important vegetables that is consumed in variable forms across different cultures. Different classes of market varieties display unique flavor and taste characteristics making them economically significant for the pepper industry. The overall goal of this project was to test the regional adaptability of Bulgarian pepper accessions in continental climate of South Korea and contribute to the improved genetic diversity of WorldVeg germplasm. Those genetic resources are intended to address the global challenges that affect food security, sustainability, and adaptation to climate change. During 2022, a total of 11 pepper accessions representing different varietal groups (Sweet Green/Sweet Pepper, Kapia, Paprika, Pungent, and Pumpkin) with two Korean cultivar controls were evaluated for an array of horticultural traits including plant and fruit agromorphological traits, disease/insect resistance, and fruit quality. Evaluated accessions were divided into sweet and hot pepper groups and were evaluated in open field and greenhouses, respectively. Hot pepper did not show significant differences in vegetative growth in comparison to sweet pepper—except for thicker stem diameter, which likely varied due to water stress. Among evaluated accessions, CAPS-67 was the tallest and it displayed the thickest stem, the greatest plant width, and had the longest leaves and light fruit. CAPS-51 was shortest, with the thinnest stems, and lowest stem diameter and plant width. Based on the comparison between sweet pepper and hot pepper, hot pepper accession fruits had thin fruit walls and low fruit weight whereas sweet pepper fruits were heavier with thicker

walls. Molecular marker analyses for several diseases showed that all evaluated accessions were susceptible to CMV, powdery mildew, late blight, bacterial spot and TSWV. Overall, evaluated Bulgarian accessions appeared to be unadapted to the South Korean continental climate.

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Session:

PRE-BREEDING AND
BREEDING – AGRONOMIC
AND FRUIT QUALITY
TRAITS





Keynote talk 3

TITLE: GENETIC AND MOLECULAR BASIS OF PEPPER DOMESTICATION TRAITS

Authors: Paran, I.*[1], Borovsky, Y.[1]

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Abstract: Wild peppers are characterized by late flowering, small, round, red colored, pungent, erect, soft flesh and dehiscent fruit. Genetic and molecular studies using natural variation sources allowed to identify major QTLs and genes controlling the domestication syndrome of pepper. Modification of the activity of major genes involved with biosynthetic and developmental pathways associated with plant and fruit growth and morphology have been selected during domestication for improved adaptation to agricultural needs. Major findings of these processes will be reviewed.

Oral presentations

4 Oral presentation

TITLE: BULKED SEGREGANT ANALYSIS REVEALS CANDIDATE LOCI ON CHROMOSOME 3 FOR THE INHIBITOR OF *PHYTOPHTHORA CAPSICI* RESISTANCE (*IPCR*) GENE IN PEPPER

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Abstract: Phytophthora blight, caused by *Phytophthora capsici*, is one of the most devastating diseases facing pepper (*Capsicum annuum* L.) producers worldwide. Management strategies for phytophthora root-rot are limited, leaving host resistance as the best approach. However, breeding for host resistance is complex and confounded by numerous factors, including the existence of an inhibitor gene to host resistance, known as the Inhibitor of *Phytophthora capsici* resistance (*lpcr*) gene. Our aim was to identify loci associated with the *lpcr* gene to improve the accuracy and efficiency of breeding for host resistance in chili pepper. We developed an F₂ population consisting of 250 individuals derived from the resistant parent Criollo de Morelos 334 (CM334) and *lpcr* parent NMCA10399. The population, along with parental lines and checks were screened using a highly virulent isolation of *P. capsici* following standardized methodologies. Inheritance in the F₂ population did not significantly deviate from the expected ratio of 3:13, indicating dominant suppression epistasis. A total of 50 F₂ individuals, with the Phyto5NBS1 gene on chromosome 5, were selected, 25 of which were highly resistant and 25 were susceptible. QTL-seq was used to identify the significant trait-associated SNP/indel between two phenotype extreme pools in the F₂. In each pool, the proportion

of variants was calculated for a SNP-index, and $\Delta(\text{SNP-index})$ between two pools, representing a percentage of the alternative allele in total read depth and the difference. We identified 10 SNPs and a 45 bp indel in a large region on chromosome 3, which was significantly associated with susceptibility in our population and is a strong candidate for the *lpcr* gene in pepper. Further work, such as fine mapping, is required to confirm the location and possibly understand the function of the *lpcr* gene.

5 Oral presentation

TITLE: MICROSCOPIC AND METABOLIC INVESTIGATIONS DISCLOSE THE FACTORS THAT LEAD TO SKIN CRACKING IN CHILI-TYPE PEPPER FRUIT VARIETIES

Authors: Marinov, O.[1,2], Nomberg, G.[1,2], Arya, G.C.[1], Manasherova, E.[1], Cohen, H.*[1]

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Abstract: The hydrophobic cuticle encasing the fruit skin surface plays critical roles during fruit development and post-harvest. Skin failure often results in the fruit surface cracking and forming a wound-periderm tissue made of suberin and lignin. The factors that make the fruit skin susceptible to cracking have yet to be fully understood. Herein, we investigated two varieties of chili peppers (*Capsicum annum* L.), NumexGarnet, whose fruit has intact skin, and Vezena Slatka, whose fruit has cracked skin. Microscopical observations, gas chromatography-mass spectrometry, biochemical and gene expression assays revealed that Vezena Slatka fruit form a thicker cuticle with greater levels of cutin monomers and hydroxycinnamic acids, and highly express key cutin-related genes. The skin of these fruit also had a lower epidermal cell density due to cells with very large perimeters, and highly express genes involved in epidermal cell differentiation. We demonstrate that skin cracking in the Vezena Slatka fruit is accompanied by a spatial accumulation of lignin-like polyphenolic compounds, without the formation of a typical wound-periderm tissue made of suberized cells. Lastly, we establish that skin cracking in chili-type pepper significantly affects fruit quality during post-harvest storage in a temperature-dependent manner. In conclusion, our data highlight cuticle thickness and epidermal cell density as two critical factors determining fruit skin susceptibility to cracking in chili-type pepper fruit.

6 Oral presentation

TITLE: UTILIZATION OF THE *TTI* GENE ENABLES LYING DOWN THE PEPPER CROP DURING LONG CYCLE GREENHOUSE CULTIVATION

Authors: Ruskó, J.[1], Kovács, I.[1], Buleca, Cs.[2], Szamosi, Cs.[3], Csilléry, G.*[4]

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Abstract: Due to the expensive F_1 hybrids and labour problems in agriculture, long cycle crops are grown in active glasshouses. The interior height of modern growing facilities exceeds 5 meters. In the second part of the growing season the harvest is often carried out at a height 3-4 meters. In high-tech greenhouses the stem of the tomato is procumbent. During the 10-11 months of cultivation, the stem grows to 12-14 meters. However, the stem is laid down horizontally and the producing part is usually only 2 meters high. On the other hand, pepper cannot even tolerate laying down the plants at 45 degrees without fruit quantity and quality reduction. Our research objective was to create white-conical pepper varieties which can be laid down similar to tomatoes.

Based on years of co-breeding between Duna-R Kft. and PepGen Kft., the plants containing the *tti* gene (tortuous internode) found suitable for the novel pepper cultivation method. *Tm2/Tm3*, *TSWV*, *Lt* are considered as basic resistances for greenhouse pepper cultivation. Therefore, the first step of our collaboration was to introduce all of these into the *tti* mutant.

The pre-screened breeding lines were first tested under commercial growing practice in 2021 at the facility of Árpád Zrt. During the parent line selection, the focus was on the thickest stems while maintaining a softer stem structure. In the 2022-23 experiments, we compared the yield results of 7 new hybrids. Zentus F_1 of Duna-R Kft. was used as a control. The 11 months long experiment started on August, 2022. Harvest was done weekly from the beginning of December 2022. The yield result of the F_1 hybrids containing the *tti* gene, does not differ significantly from the results of traditionally grown control. The experiment will be continued till July, 2023.

7 Oral presentation

TITLE: CLARIFICATION OF GENETIC REGULATION MECHANISM REGARDING PUNGENT TRAITS IN JAPANESE CHILI PEPPER 'SHISHITO' *CAPSICUM ANNUUM*

Authors: Kondo, F.*[1,2], Nemoto, K.[1], Matsushima, K.[1]

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Abstract: The pungent tastes of chili peppers (*Capsicum* spp.) are derived from chemical constituents known as capsaicinoids. Their contents determine the pungent level that is one of the critical traits. However, capsaicinoid content is known as quantitative trait; thus, it's difficult to control pungent levels in the breeding, and they're liable to change depending on the cultivation environments. To control them, it's indispensable to understand the detailed mechanism regarding the genetic regulation of pungency in chili pepper fruits. To get the new insights, we investigated unique pungent traits observed in the Japanese chili pepper 'Shishito' (*Capsicum annuum*); the occurrence of pungency was established in the seedless fruits, even though its pungency is usually deficient (kept quite low). We initially focused on the seedless-dependent fluctuation of pungency, and performed transcriptome analysis via RNA sequencing in seeded/seedless 'Shishito' fruits. As the results, we clarified that pungent seedless fruits specifically exhibited high expressions of 20 genes, which were responsible for the metabolic pathway around capsaicinoid biosynthesis. Therefore, these transcriptional changes were considered to induce the fluctuation of pungency in 'Shishito' fruits. On the other hand, we also focused on the deficiency of pungency in 'Shishito', and implemented quantitative trait locus (QTL) analysis regarding capsaicinoid content in the F₂ population ('Shishito' × pungent cultivar). As the result, it was revealed that the deficiency of pungency in 'Shishito' was quantitative trait controlled by multiple loci. We identified the two major loci named Shq13 and Shq17 respectively, of which total genetic variance explained (GVE) was approximately 60%. Non-pungent trait was previously known as qualitative trait controlled by a single recessive gene, so that 'Shishito' appeared to lose its pungency due to the unique genetic mechanism. These new insights, clarified in 'Shishito', are useful information for breeding about genetic control of pungency levels in chili peppers.

8 Oral presentation

TITLE: STUDY OF THE ANTHOCYANIN RELATED TRAITS IN PHOTSENSIBLE (ASI-S-1) AND NON-PHOTSENSIBLE (IVIA-371) CULTIVATED EGGPLANT VARIETIES

Authors: Gomis-Cebolla, J.*[1], Arrones, A.[1], Manrique, S.[1], Plazas, M.[1], Prohens, J.[1], Gramazio, P.[1]; Vilanova, S.[1]

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Abstract: One of the unique characteristics of *Solanum melongena* is the accumulation of anthocyanin in the fruit skin. Anthocyanin accumulation is generally light-dependent, but some cultivars present light-independent accumulation. This is a desirable trait as it guarantees homogeneous colouring of the fruits regardless of light incidence. This character is easily visualized by the presence of anthocyanins under the calix (purple under calix-PUC), as this zone is protected from light. The research team identified four QTL along chromosome 10 that are involved in characteristics linked to anthocyanins (vegetative anthocyanin, fruit anthocyanin and PUC) through the generation of a MAGIC population. To reduce the number of potential QTL involved in the anthocyanin related traits, a bulk segregation analysis (BSA-seq) from the F₂ of the cross of IVIA-371 (light-independent) x ASI-S-1 (light dependent) had been carried out. *SmMYB113* (key positive regulator in the anthocyanin biosynthesis pathway) was identified in the highest peak of chromosome 10 in the GWAS analysis. To identify *SmMYB113*'s partners in IVIA-371 (light-independent) and ASI-S-1 (light dependent) eggplant varieties, we assessed the protein-protein interactions formed by *SmMYB113*.

9 Oral presentation

TITLE: IDENTIFICATION OF TWO *CAPSICUM* ACCESSIONS RESISTANT TO THE FOXGLOVE APHID *AULACORTHUM SOLANI*

Authors: T'Joen, G.A.*[1,2], Voorrips, R.E.[1], van Loon, J.J.A.[2], Caarls, L.[1]

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Abstract: The foxglove aphid *Aulacorthum solani* (Kaltenbach) is a major pest insect in pepper cultivation. *A. solani* secretes a salivary toxin that causes chlorosis and necrosis around feeding sites, potentially leading to leaf death or even whole plant defoliation and thus greatly decreasing fruit yield. This makes *A. solani* an especially aggressive species that is difficult to control. Moreover, like other aphids, *A. solani* can cause indirect damage by transmitting viruses. Breeding for insect-resistant pepper varieties is an alternative to relying on environmentally harmful insecticides to control aphids. However, to the best of our knowledge no pepper material resistant to *A. solani* had been identified until now. This study aims to identify a source of resistance to *A. solani*, to characterize its mechanism and study its genetics. We screened 119 *Capsicum* accessions from different geographical origins for resistance to *A. solani* in no-choice clip-cages assays with 1-day old nymphs. Survival (percentage of alive first generation aphids) and reproduction (number of second generation aphids) were measured as resistance parameters. Two accessions, one *Capsicum annuum* and one *C. baccatum* showed lower reproduction than the susceptible *C. annuum* and *C. baccatum* controls respectively. Callose deposition was also observed 24 hours upon aphid infestation in plants of both accessions but not in susceptible plants, hinting at a quick defense response in the plant to aphid feeding. The identified resistant accessions have been crossed to susceptible *C. annuum* and *C. baccatum* accessions respectively in order to generate an F_2 population for further QTL mapping. The identification of such a QTL will provide tools for pepper breeders to create novel varieties that are resistant to *A. solani*.

10 Oral presentation

TITLE: GENETICS UNDERLYING FLAVONOID VARIATION IN PEPPER FRUIT

Authors: Tikunov, Y.*[1], Wu, Y.[1], Popovsky-Sarid, Sigal.[2], Borovsky, Y.[2], Tumino, G.[1], Baruch, K.[3], Tripodi, P.[4], Lefebvre, V.[5], Visser, R.G.F.[1], Paran, I.[2], Bovy, A.[1]

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Abstract: Pepper fruit (*Capsicum* spp.) is a good source of specialized nutritional metabolites such as flavonoids, which play an important role in both plant physiology and ecology as well as human nutrition. The regulation of flavonoid biosynthesis has been extensively studied in *Arabidopsis*, tomato and petunia, but is only partially explored in pepper. In this study, a QTL on chromosome 5 of pepper was found to be highly associated with quantitative variation in flavonoids in an F₂ population and later verified in NILs developed from a cross between *C. annuum* accessions Long Sweet and AC2212. A transcription factor highly homologous to the master regulator of the flavonoid pathway in tomato – SIMYB12 – was identified as a candidate gene underlying the effect of the locus. VIGS of CaMYB12-like in the high-flavonoid accession cv. Long Sweet led to a significant decrease in the expression of flavonoid pathway genes and drastic decrease of flavonoid levels in silenced fruits. Overexpression of CaMYB12-like in the SIMYB12-deficient tomato *y* mutant led to a partial complementation of its low flavonoid phenotype. In a parallel study ripe fruit of a core collection of about 400 *C. annuum* accessions was developed and fruit metabolomics was carried out in the G2P-SOL project. A GWAS analysis revealed the most significant association for flavonoid variation

precisely in the CaMYB12-like locus, which suggests the general key role of this gene as a master regulator of flavonoid content in *C. annuum* and makes it a straightforward target for classical breeding. Introgression of the CaMYB12-like allele into two cultivated varieties confirmed the potential of CaMYB12-like to breed for high flavonoid content in different pepper backgrounds.

Acknowledgment: G2P-SOL project was funded by the European Union Horizon 2020 research and innovation program under Grant Agreement No. 677379. We are also grateful to all the partners involved in G2P-SOL project.

11 Oral presentation

TITLE: EVALUATION OF THREE SETS OF ADVANCED BACKCROSSES OF EGGPLANT AND THEIR RECURRENT PARENTS UNDER LOW N FERTILIZATION CONDITIONS

Authors: Villanueva, G.*[1], Plazas, M.[1], Gramazio, P.[1], Prohens, J.[1], Vilanova, S.[1]

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Abstract: Excessive use of nitrogen (N) fertilizers in crop production has negative impacts on the environment and human health. Eggplant wild relatives possess inherent adaptations to natural conditions and are a potential source for developing new varieties with improved nitrogen use efficiency (NUE) for more sustainable agriculture. In this study, we evaluated traits of three *S. melongena* under two different nitrogen fertirrigation conditions and three sets of advanced backcrosses (ABs) with introgressions from wild eggplant relatives *S. dasyphyllum*, *S. elaeagnifolium* and *S. insanum* under low N fertilization. The results show that different N treatments significantly affected plant and composition traits in *S. melongena* recurrent parents, with higher values observed in normal N conditions. Compared to the recurrent parents cultivated under low N conditions, the sets of advanced backcrosses showed wider distribution ranges for the evaluated traits. The PCA results for each set of ABs revealed specific trait correlations and distribution patterns among individuals and with the recurrent parent. Significant common correlations in some sets among traits were observed, while each set also exhibited specific correlations. A total of 16 significant QTLs were identified, of which eight QTLs detected in ABs of *S. dasyphyllum*, three in ABs of *S. elaeagnifolium*, and five in ABs of *S. insanum*. Different allelic effects were observed on the traits evaluated. The study highlights the potential of these three eggplant wild relatives in developing new varieties with improved nitrogen use efficiency for sustainable agriculture, particularly for adaptation to low N conditions.

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12 Oral presentation

TITLE: IDENTIFICATION OF *SMAPRR2* AND *SMGLK2* AS GENES UNDERLYING CHLOROPHYLL DISTRIBUTION IN THE EGGPLANT FRUIT PEEL

Authors: Arrones, A.*[1], Gramazio, P.[1], Alonso, D.[1], Baraja-Fonseca, V.[1], Plazas, M.[1], Prohens, J.[1], Mangino, G.[1], Portis, E.[2], Barchi, L.[2], Giuliano, G.[3], Vilanova, S.[1]

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Abstract: Chlorophyll distribution in eggplant fruit peel can be uniform or irregular, the latter referred to as green netting. These two traits are involved not only in fruit appearance but affect also fruit nutritional quality. Therefore, the identification of putative candidate genes controlling these traits is of great relevance for eggplant breeding. A total of 420 individuals from the first eggplant multi-parent advanced generation inter-cross (MAGIC) population were phenotyped for these traits and high-throughput genotyped by the eggplant single primer enrichment technology (SPET) platform. Through a genome-wide association study (GWAS), strong associations were identified for both traits of interest. For the uniform green fruit pigmentation trait, a major peak was identified on chromosome 8 while for the green netting fruit trait a significant peak was localized on chromosome 4. Under these peaks we identified *SmAPRR2* gene as the best candidate for the uniform green fruit pigmentation, and *SmGLK2* for the green netting, traits. Subsequently, 277 accessions from the G2P-SOL eggplant core collection were examined and several allelic

variants were identified as responsible for the disruption of *SmAPRR2* and *SmGLK2* genes, corresponding to phenotypes that exhibited the absence of uniform fruit chlorophyll distribution and netting pattern, respectively. Moreover, these mutations revealed strong geographical clustering, suggesting that these phenotypes may have arisen and been selected independently several times during domestication. This study identified the causative genes of two relevant breeding traits that will foster eggplant breeding programs focused on fruit color and nutritional quality.

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13 Oral presentation

TITLE: ROLE OF FRUIT CUTICLE IN *COLLETOTRICHUM* INFECTION

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Abstract: Chili anthracnose is a major fungal disease, causing fruit rots across tropical and subtropical regions worldwide. Among species of the *Capsicum* genus, *Capsicum annuum* emerges as the most pivotal species from an economic standpoint. Notably, certain genotypes have demonstrated variance in resistance to anthracnose through non-wound inoculation at distinct fruit maturation stages. Among 12 accessions distinguished by varying anthracnose responses during mature green and ripe stages, an exploration into their wax attributes was conducted. Interestingly, the observed variance in anthracnose resistance did not exhibit a correlation with cuticle thickness and wettability. The wax profiles of the highly resistant genotype, CA758, and the most susceptible genotype, CA1113, exhibited similar characteristics throughout both fruit developmental stages. Noteworthy findings revealed that spore germination levels on extracted cuticular wax from most genotypes exceeded 80%, with CA965, an intermediate susceptible genotype, displaying the lowest and slowest germination rates. Appressoria formation on all chili genotypes was above 67% higher than the control, indicating that the wax promoted the formation of appressoria. Thus, no correlation between host resistance reaction to infection by *Colletotrichum truncatum* and the composition of cuticular wax of the chili genotypes was observed. This implies the participation of alternate host mechanisms in modulating anthracnose resistance.

14 Oral presentation

TITLE: A NOVEL GLOBAL *CAPSICUM* CORE COLLECTION DELIVERS CANDIDATE GENES FOR ROBUST AGRONOMIC QTLs THROUGH A MULTI-ENVIRONMENT GWAS APPROACH

Authors: McLeod, L.[1,2], Barchi, L.[3], Tumino, G.[4], Tripodi, P.[5], Giuliano G.[6], Voorrips, R.[4], Paran, I.[7], Lefebvre, V.*[1]

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Abstract: Deciphering the genetic architecture of quantitative traits through genome-wide association mapping on core collections paves the way for breeding adapted crops. We constructed the G2P-SOL pepper world core collection, a set of 423 *Capsicum* spp. accessions of great genotypic and phenotypic variability, representing the diversity of more than 10,000 pepper accessions from 10 major genebanks. To tackle the modulation of complex quantitative traits by the environment and by the genotype-by-environment (GxE) interaction effects, a highly diverse subset of 350 *C. annuum* accessions was extensively phenotyped in a multi-location effort. Multi-environment genome-wide association studies (GWAS) were conducted for 23 agronomic traits pertaining to fruit flavor, color, size and shape, and to plant productivity, vigor and precocity. Robust QTLs and environment-specific QTLs were thus detected, and 97 well-known and new genes potentially underlying 53 of the most high-confidence and robust QTLs

were highlighted. These results assert the usefulness and universality of the G2P-SOL core collection, which will be available upon request for the pepper community, alongside the genotypic and phenotypic data collected as part of the G2P-SOL project. This unique resource will be a key asset in accelerating gene discovery, developing genetic markers for marker-assisted selection, and breeding pepper varieties adapted to various climatic environments.

Poster presentations

9 Poster presentation

TITLE: *IN VITRO* ANALYSIS AND MICROPROPAGATION OF THE *TTI* (*TORTUOUS INTERNODE*) *CAPSICUM ANNUUM* PLANTS

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Abstract: Vegetable production in greenhouses needs new ideas, plant materials, and cultivating methods to produce more. Involving mutant traits into plant breeding might make possible to create new cultivating styles, such as vertical farming. We involved the *tti* (*tortuous internode*) mutant in our experiments. *tti* plants have elongated hypocotyl with slight spiraling. The mutant trait is not easily observable in the early phenophase. In our experiment we germinated the progeny seeds of the self-fertilized mutants on MS medium. We dissected the hypocotyls and the cotyledons of the mutant plants, then we placed them on MS medium without any hormone supplementation. We observed and documented the plant explants one week later. All the cotyledons presented excessive rooting. The hypocotyl segments started to grow small calluses and extreme amounts of accessory roots. We only were able to regenerate a small percentage of plants from the calluses, however the shoot tips and offshoots were perfect explants for micropropagation due their increased rooting ability. Considering the elongated hypocotyls, internodes, and the great rooting finesse we suspect that the background of the mutant phenotype has an abnormal level of auxins or gibberellins in the plants. Our further experiments will focus on these phytohormones.

10 Poster presentation

TITLE: PEPPER FRUITS - CONSUMER PREFERENCES IN SERBIA

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Abstract: In Balkan cuisines, as well as in Serbia, pepper (*Capsicum annuum* L.) has a very diverse use. Knowledge about consumer preferences is of great importance for a breeding process as well as in market-orientated production. According to the authors' knowledge, there is little data in the literature about consumer preferences in terms of pepper fruit shape, colour and hotness, especially in the region of southeast Europe and Serbia. An online survey to collect data regarding consumer preferences was conducted via Google forms. Four hundred and two participants (52% females and 48% males), answered the survey questions. In the questionnaire, participants were asked about basic personal data: gender, education level, and age. Questions regarding their preferences were: Do you eat fresh peppers?; What type of pepper fruit do you usually eat?; What colour of pepper fruit do you usually eat?; Do you eat hot peppers?; Level of hotness?; Level of colour importance?; Level of fruit type importance?. According to our research, the most preferred pepper type in Serbia is kapia, while the bell pepper is the second chosen type. Also, it was revealed that the favourite colour of pepper fruit is red. There is a tendency for higher importance of fruit type rather than fruit colour. The highest percentage of hot pepper consumers prefer medium hot peppers. When we compared preferences about the level of hotness with age, participants between 18-25 and 45-65 like hot pepper fruits more than other age groups. The obtained trend shows that men in Serbia generally prefer more hot pepper fruits than women.

11 Poster presentation

TITLE: RESULTS DURING THE LAST 20 YEARS AND PRIORITIES IN THE FUTURE BREEDING AND RESEARCH WORK WITH PEPPER (*CAPSICUM ANNUUM* L.) AT MARITSA VEGETABLE CROPS RESEARCH INSTITUTE

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Abstract: The summarized results of the pepper breeding and research work at the Maritsa Vegetable Crops Research Institute, Plovdiv during the last 20 years (2003–2023) are presented. The main breeding directions in this period are indicated: earliness, productivity, improved morphological, organoleptic and biochemical properties, resistance to biotic and abiotic stress factors, suitability for organic production etc. Thanks to national and European projects and programs, the Institute's pepper collection has been enriched. Accessions originating from six Balkan countries were characterized by: agrobiological and biochemical traits, some mineral elements in the fruits, and their reaction to attack by viral (TMV, PMMoV, TSWV and CMV), and fungal (*Verticilium dahliae* Kleb and *Phytophthora capsici* Leon.) diseases under artificial infection conditions, and in natural background conditions to important insect pests (*Myzus persicae* Sulz., *Frankliniella occidentalis* Perg., *Thrips tabaci* Lindeman and *Helicoverpa armigera* Hubn.). Their androgenic response and genetic diversity were also assessed. Achievements of the pepper research programs are highlighted. A brief characterization of the original cultivars (Kurtovska kapia 1, IZK Delikatess, IZK Rubin, IZK Kalin, Milkana F₁, Yasen F₁ and Ivaylovska kapia) developed in this period is reported. New candidate cultivars are also mentioned: Baltovska kapia, Ruevit and Dan-Dan. In conclusion the main priorities in the future breeding and research work are presented.

12 Poster presentation

TITLE: EFFECTS OF DIRECT SEEDING AND TRANSPLANTING ON YIELD AND FRUIT CHARACTERISTICS OF HOT CHILI PAPRIKA (*CAPSICUM ANNUUM* L.)

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Abstract: In the last decade, hybrid chili peppers have been grown on an increasingly large surface area in Hungary. The price of hybrid pepper seeds is significantly higher than that of the OP varieties, so it was mainly grown with transplanting technology. In the last few years, however, the direct seeded cultivation of hybrids has become more and more widespread. Cultivation by direct seeding requires less manual work, so costs can remain lower compared to transplanting. We investigated the effect of direct seeding (DS) and transplanting (TP) in 2 hybrid hot varieties (Hetényi Parázs F1- HP, Uniring F1- UR).

The experiment was carried out on sandy soil at the border of Lakitelek. The direct seeding was done on April 20, 2021 by a belt seeder (200,000 seeds/ha), and transplanting was done by hand on May 11 (70,000 plants/ha). The level of nutrient supply and irrigation was determined for each technology based on the needs of the plants and the experience of previous years. At pepper maturity, mature fruits were harvested in 4 replicates per treatment from a 2 m² area. The yield was calculated from the weight of the harvested fruits and a sample of about 0.35-0.5 kg was taken per replicate for analytical tests. The yield was higher at the direct seeded technology (DS-HP: 44,56 t/ha, TP-HP: 23,32 t/ha, DS-UR: 44,75 t/ha TP-UR: 27,71 t/ha). The dry material, total capsaicinoid and dye content (ASTA) were better at transplanting technology (DS-HP: 18,60%, 1339 mg/kg, 71,5 ASTA, TP-HP: 20,93%, 2158 mg/kg, 87,5 ASTA, DS-UR: 14,11%, 1990 mg/kg, 48,5 ASTA, TP-UR: 14,51%, 2779 mg/kg, 61,5 ASTA).

Based on this experiment the direct seeded cultivation of hybrid paprika can be much more economical than the seedling one.

13 Poster presentation

TITLE: CAPSAICINOIDS AND PHYTOCHEMICAL CONTENT OF THE RECOVERING SWEET PEPPER “VOGHERA” LANDRACE

Authors: Martufi, G.[1], Lo Scalzo, R.[1], Picchi, V.[1], Tassone, M.R.[2], Toppino, L.[2], Fibiani, M.*[1]

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Abstract: “Voghera” landrace is a sweet pepper variety typically grown in Northern Italy, traditionally harvested and consumed at green stage. It has progressively lost its purity, with the occurrence of pungency, due to the presence of capsaicinoids, that are mainly synthesized in the placenta. Within a larger typifying project which aims to try to recover the native “Voghera” landrace in its phenotypical and sweetness peculiarity, this study concern with the biochemical characterization, focusing on the capsaicinoids content and the relationship between it and other phytochemicals, which are highly present in the pepper, giving it health-promoting characteristics. Berries from thirteen “Voghera” lines (1-4 plants per line) were harvested and separated in seeds, placenta, and pericarp tissues. Also, three local sweet landraces and one hot pepper were collected and analyzed as a reference for biochemical results. For the capsaicinoids content, “Voghera” lines showed wide variability, between and within lines, with plants of the same line showing fruits almost completely free of capsaicinoids, while others very spicy. As expected, higher values were found in the placenta, with an average content of 177 mg/kg dw (range 0.01-1073 mg/kg), comparing to the hot pepper variety (2734 mg/kg) and on the contrary to the sweet reference landraces (range 0-4.3 mg/kg). Occasionally, small quantities were also found in the “Voghera” seeds and pericarps (average content 12 and 14 mg/kg, respectively). Among the other phytochemicals, the “Voghera” landrace was characterized by an average content that did not deviate from the reference landraces. To support both the different selection steps and the genetic research, biochemical analyses were crucial in order to recover the original sweetness along with keeping variety’s interesting nutraceutical contents, as ascorbic acid.

Acknowledgements: This research was funded by Regione Lombardia, PSR 2014-2020 misura 10.2.01, project “RECUPEVO”.

14 Poster presentation

TITLE: IDENTIFYING CANDIDATE GENES FOR THE PRICKLINESS TRAIT IN EGGPLANT USING AN INTROGRESSION BREEDING APPROACH

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Abstract: Eggplant exhibits a broad range of useful traits for breeding but is largely limited by narrow genetic variation. To overcome this limitation, introgression breeding approach has been used to introduce natural variation and introgress traits of interest. The use of introgression lines (ILs) in eggplant holds great potential for developing improved varieties with desirable traits, such as adaptation to climate change, biotic and abiotic stress tolerance, and enhanced nutritional value. Additionally, this material is an interesting resource for genetic studies.

To dissect the genetic control of the prickliness trait in eggplant, a set of ILs has been used developed between *S. insanum*, a prickly common eggplant ancestor, and the recurrent parent *S. melongena*. Prickles act as a defense against herbivores and a major dominant QTL (*PI*) for the prickliness in eggplant was previously located on chromosome 6. To fine map the *PI* locus, advanced backcrosses (ABs) lines with introgressions from chromosome 6 were used to narrow down the *PI* locus to a genomic region of 96 kb. Introgressions of the *PI* locus from other phylogenetically distant "spiny" solanums from the secondary (*S. dasyphyllum*) and tertiary (*S. elaeagnifolium*) gene pools confirmed that this locus is evolutionarily conserved in the subgenus *Leptostemonum*. Three candidate genes in the *PI* locus region were identified based on their putative biological function.

The study of the genetic control of the prickliness trait in eggplant using introgression breeding approach provides insights into the evolution of the trait and identifies potential candidate genes for future breeding programs.

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15 Poster presentation

TITLE: QUALITY TRAITS EVALUATION OF EXPERIMENTAL HYBRIDS BETWEEN SPANISH TRADITIONAL PEPPERS (*CAPSICUM ANNUUM* L.) AND A RESISTANCE VIRUS (L4 AND TSW) BREEDING LINE UNDER ORGANIC FARMING

Authors: Jiménez-Pérez, M.*[1], Adalid-Martínez, A.M.[1], Moreno-Peris, E.[1], Fita, A.[1], Rodríguez-Burruezo, A.[1]

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Abstract: Spain has a wide range of traditional pepper ecotypes (*Capsicum annuum* L.), highly valued by consumers due to their internal quality properties, but susceptible to many pathogens. Thus, three experimental hybrids (BGV5126x275, BGV10582x275 and BGV4348x275), derived from a breeding program with the aim of introducing *Tsw* and *L4* resistance genes (against TSWV and PMMoV, respectively) from the breeding line L275 into Spanish pepper landraces (BGV5126, BGV10582, BGV4348), were evaluated under organic conditions in Valencia, Spain. Total red and total yellow-orange carotenoids, ascorbic acid as well as fructose and glucose levels were analysed. In general, carotenoids were statistically lower in hybrids respect to their respective parent landraces, highlighting a reduction of 63.1% and 79.5% of yellow-orange carotenoids content in BGV4348x275 and BGV5126x275 compared with BGV4348 and BGV5126, respectively. This reduction seemed to be produced by the low carotenoid contents in L275. However, ascorbic acid showed a statistical increase of 29.4% and 36.3% in BGV5126x275 and BGV4348x275 compared to their corresponding landraces, respectively. Regarding soluble sugar levels, BGV5126x275 showed a significant increase of 29.9% and 24.4% in fructose and glucose contents respect to BGV5126, whereas the contrary result was observed in BGV10582x275, which showed a statistically reduction in its sugar profile respect to BGV10582. These results show the interesting ascorbic acid and/or sugar content of BGV5126x275 and BGV4348x275 under organic farming compared to their corresponding landraces,

also incorporating both resistance genes. However, further studies on the first backcross generation with the respective landraces would be necessary to assess whether the carotenoids content increases.

This work was supported by Agencia Estatal de Investigación (AEI) through the project PID2019-110221RR-C32 and the grant CIPROM/2021/020 funded by Generalitat Valenciana (GVA). Finally, M. Jiménez thanks to Ministry of Universities for her grant FPU20/03486.

16 Poster presentation

TITLE: STUDY OF SPANISH WAX PEPPERS (*CAPSIUM ANNUUM* L.) AND THEIR EXPERIMENTAL HYBRIDS UNDER ORGANIC CONDITIONS

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Abstract: In the Spanish market a great variability in pepper fruits (*Capsicum annuum* L.) can be found. In certain regions of Spain, some landraces that respond to the Hungarian wax pepper type are consumed. Thus, four landraces (PL1, PL2, PL3 and PL4) provided by cooperatives from Alicante and Barcelona were evaluated under organic farming with their experimental hybrids (H1-2, H2-3, H2-4). Flesh thickness (FT), total soluble sugars (TSS), ascorbic (AA) and dehydroascorbic acids (DHA) and kaempferol (KP) levels were evaluated by HPLC analysis. FT varied between 4 and 5.8 mm, highlighting the hybrid H2-4, with a thickness statistically higher than their parent lines PL2 and PL4, followed by PL3. TSS varied from 17.2 to 24.3 g·kg⁻¹ fresh weight (fw), corresponding the highest content to PL3, followed by H2-4, with a content statistically higher than PL2 and PL4. AA and DHA ranged between 156 - 922 mg·kg⁻¹ fw and 195 - 419 mg·kg⁻¹ fw, respectively. Though H2-4 showed a statistically lower AA levels in comparison to their parent lines, H1-2 showed the contrary results, showing a significant increment respect to PL1 and PL2. DHA was particularly higher in PL2, statistically similar to the content found in their hybrids H2-3 and H2-4, whereas PL3 and PL4 showed the lowest DHA levels. Finally, KP was comprised between 7.1 and 10.9 mg·kg⁻¹ dry weight (dw), highlighting PL1 and PL2. This study reveals the potential of these landraces to be used as parent lines of future hybrids and/or donors of a range of quality traits.

This study forms part of the AGROALNEXT programme, supported by MCIN with funding from NextGenerationEU (PRTR-C17.11) and by Generalitat Valenciana (GVA), subproject AGROALNEXT/2022/027. This work was also supported by the grant CIPROM/2021/020, funded by GVA and the grant FPU20/03486 of M. Jiménez (M.Universities).

17 Poster presentation

TITLE: MORPHOLOGICAL CHARACTERIZATION OF ANTHR-DERIVED PEPPER LINES (*CAPSICUM ANNUUM* L.)

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Abstract: Pepper (*Capsicum annuum* L.) is one of the most important and grown vegetables on Balkans. To develop new varieties rapidly or to improve specific characteristics the combination of conventional breeding methods with biotechnological tools are widely used. Anther culture is one the most popular of the in vitro techniques applied to induce haploid and double haploid plants in a short period. The current study aimed to evaluate the morphological characteristics of anther-derived pepper plants. During the experimental period, the embryogenic reaction in anther culture of 50 pepper genotypes was studied. The genotypes belong to three groups – local accessions, kapia-type, and BC progeny. The summarized results showed that the highest androgenic potential was observed in the BC progeny group, where on average 18,3 of the cultured anthers reacted with embryo induction. Also, the highest number of embryos (127,3) and plant-regenerants (7,7) were formed in this group. However, the highest conversion ratio from embryos to plant-regenerants was found in the kapia-type group, followed by local accessions and BC progeny groups. Variation in plant and fruit characters was observed between double haploid lines. Morphological evaluation and comparison to control variety Stryama showed that three lines 251, 255, and 257 are superior and recommended them to include in pepper breeding program.

Acknowledgements: The research leading to these results has received funding from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

18 Poster presentation

TITLE: PRELIMINARY FIELD EVALUATION OF BC₁F₂ AND BC₂ PEPPER LINES ORIGINATING FROM LOCAL CULTIVARS

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Abstract: Pepper is one of the most popular vegetables on the Balkan peninsula, particularly in Bulgaria. Due to its climate and soil conditions, the region is regarded as a secondary center of diversity, and as such, it possesses sweet pepper of various shape and size. Although popular, the local cultivars and forms are vulnerable to viral diseases, namely tobamoviruses. Breeding efforts in Maritsa VCRI during previous years were directed towards the development of lines resistant to *Pepper mild mottle virus* (PMMoV) in the background of traditional cultivars. The primary goal of the program was to introduce L^4 gene in three local elite pepper cultivars (Ivaylovska Kapia, Sivriya 600 and Stryama) belonging to Kapia, Sivriya and conical fruit type, respectively. F_1 generations were made by crossing the three cultivars with the L^4 donor line – Stryama x E49, Ivaylovska Kapia x Belcanto F_1 , Sivriya 600 x Belcanto F_1 . Next generations were obtained following a backcross scheme with the respective traditional cultivars and self-pollination. A total of 18 pepper lines at BC₁F₂ and BC₂ stages were assessed for presence of L^4 gene for resistance to PMMoV by biological and molecular methods as well as field evaluated for some agronomic traits. A pilot evaluation of these 18 lines for five quantitative fruit traits (weight, length, width, usable part and wall thickness) and two plant traits (number of fruits per plant and fruit yield in kg per plant) was performed in open field conditions. Results were compared to the standard of the respective recurrent genitor and differences were evaluated according to Duncan's Multiple Range Test. Generally, individuals from lines 333-1 and 333-6 in Ivaylovska Kapia background exceeded the standard. Most notable statistically significant differences ($p \leq 0.05$) were found in fruit weight and wall thickness. BC₂ individuals in Stryama background did not surpass the standard. Individuals from two lines (1001 and 1055) out of five in Sivriya 600 background

significantly exceeded the standard for fruit weight, usable part and wall thickness.

Acknowledgements: The research leading to these results has received funding from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

19 Poster presentation

TITLE: THE EFFECTS OF OPEN SUN DRYING AND OVEN DRYING ON DRIED FRUIT QUALITY CHARACTERISTICS IN PURE LINES DEVELOPED FOR DRIED PEPPER BREEDING

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Abstract: Türkiye is the fourth biggest pepper producer in the world with 7.3% share of production. Dried pepper is 8.17% of this production and fresh pepper is 91.83% . Although dried pepper has a unique and important place in traditional Turkish cuisine, there are no Turkish high quality commercial dried pepper F1 hybrids. A number of studies have been carried out to evaluate the collection of local dried pepper genotypes and pure lines were developed using Doubled Haploid: DH technique. The subject of the current study were 20 pure pepper lines selected from more than 200 pure lines. Evaluation trials were set up in 3 different locations, Adana, Antalya and Manisa. Conventional drying method (under 50% shade net in sunny weather) and drying method in tunnel type ovens were used to determine the drying performance of DH pepper lines whose pre-washing and filtration processes were completed. In the oven drying process, the samples were laid on the baking tray and kept at 65°C for 6 hours. In fruit samples tests, the values of parameters such as moisture, ash, oil, pH, processing efficiency, sensory evaluations (color, juiciness, crispness, flavor, general acceptability) and dry product shelf life were calculated separately for both sun drying and oven drying. On the 32nd, 48th and 64th days, weights, color parameters (L^* (brightness), a^* (redness), b^* (blueness)), shape and taste characteristics of the products were evaluated as packed in vacuum and normal bags. The most suitable pepper for stuffing and drying were Tokat, Dolma and genotype 28 and genotypes 6, 26A, Erkenci Urfa and Samandağ Type I were determined as suitable for slicing and drying.

20 Poster presentation

TITLE: EVALUATION OF MORPHOLOGICAL CHARACTERISTICS OF CAPIA PEPPER USING PHYSICAL MUTAGENESIS

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Abstract: Pepper is among the most used vegetables in the Eastern Europe region, including Albania. Our investigations aim to improve some physiological, morphological and yield traits by physical mutagenesis. In the current study, we were focused on the germination ability and chlorophyll mutations in M1 generation of Capia pepper variety (*Capsicum annuum*). Seeds were collected from central part of Albania. The seeds were treated with physical mutagens Cs-137 gamma radiation (661.7 keV) and were irradiated with 50 Gy, 100 Gy, 150 Gy, 200 Gy and 300 Gy in the Institute of Applied Nuclear Physics facility in Tirana. After irradiation, the seed material, including control, were planted in the greenhouse (Tirana region) and carefully observed during four weeks to select the appropriate doses based on radio sensitivity. At the end of 4th week only 10% of the plants treated with 200 Gy dose survived, while those treated with 300 Gy survived only to 2%. Three doses were selected to continue the field experiment – 50 Gy, 100 Gy and 150Gy. The following traits were assessed: germination ability, pigment change in leaves, chlorophyll content and morphological characteristics. Different types of mutations were also identified and classified using Gustafson classification. Many chlorophyll mutations were observed in plants irradiated with 100 Gy. Chlorophyll depigmentation was observed in many leaves, 20 – 30 days after the germination in experimental field. The levels of photosynthetic leaves pigments in plants treated with 50 Gy and 100 Gy increased at least 10%.

Acknowledgements: The research leading to these results has received funding from International Atomic Energy Agency Technical Cooperation project RER5024/ Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia.

21 Poster presentation

TITLE: DETERMINATION OF CAPSAICIN, TOCOPHEROLS AND PHENOLICS ACIDS IN DIFFERENT CHILLI CULTIVARS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Abstract: Chilli (*Capsicum spp.*) is consumed as a spice and condiment in Mauritius. Colour, shape, pungency, and biochemical compounds are important attributes that determine the fruit quality of a chilli cultivar. The aim of this study was to determine the content of different biochemical compounds for ten different chilli cultivars. The content of capsaicin, tocopherols (vitamin E) and selected phenolic acids for ten different chilli cultivars namely Indam 5, Sizzler, KSP 1471, KSP 1470, KSP 1350, KSP 1251, NS 203, AVPP 0514, APC 4 and KSP 1472 were determined by HPLC MS. Red dried fruits of the cultivars were utilized for analysis. Capsaicin and tocopherols were extracted using 96% ethanol while phenolic compounds with 50% ethanol. Significant difference was observed for the capsaicin content between the different cultivars which ranged from 2.13 to 7.76 mg/g. KSP 1471 had the highest capsaicin content (7.76 mg/g) and KSP 1350 the lowest (2.13 mg/g) per dry matter. Results showed that the content of gamma tocopherol varied from 0.037 to 0.061 mg/g with no significant difference. There was significant difference for alpha tocopherol between the cultivars which ranged from 0.20 to 0.35 mg/g per dry matter. Chlorogenic, p-hydroxybenzoic, synapic, ferulic, salicylic and cinnamic acids had significant difference between cultivars whereas caffeic, syringic and

p-coumaric acids were not significantly different. KSP 1471 had the highest chlorogenic acid (6.1 $\mu\text{g/g}$), p-hydroxybenzoic (20.5 $\mu\text{g/g}$), syringic (3.10 $\mu\text{g/g}$) content per dry matter. KSP 1470, Indam 5 and NS 203 had the highest ferulic acid (13.70 $\mu\text{g/g}$), salicylic acid (3.40 $\mu\text{g/g}$) and cinnamic acid (0.40 $\mu\text{g/g}$) content per dry matter respectively. The variability for the biochemicals observed in the germplasm can be exploited in a breeding programme aiming to improve the quality of chilli pepper fruits.



Session:

CLIMATE CHANGE
RESILIENCE – BREEDING
FOR BIOTIC AND ABIOTIC
STRESS RESISTANCE



Keynote talk 4

TITLE: DECIPHERING THE PEPPER-PATHOGEN-ENVIRONMENT INTERACTIONS TO REDUCE PESTICIDE USE AND COPE WITH CLIMATE CHANGE

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Abstract: Occurrence and severity of plant diseases are the result of tripartite interactions between the host plant, the pest or pathogen, and the environmental conditions. Management practices can affect the edges of this disease triangle for a wide variety of pests and pathogens causing significant yield losses in pepper crops (*Capsicum annuum* L.). Regarding the host plant, biparental progeny analyses and genome-wide association studies were conducted to identify genomic regions and candidate genes involved in plant immunity to a set of diseases as well as immunity durability traits. To uncover the factors of pathogen adaptation, the evolutionary potential of *Phytophthora capsici* and Potato Virus Y (PVY) is being characterized using pepper lines contrasted for alleles at resistance quantitative trait loci (QTLs). Mutational events responsible for evolution of pathogen virulence and fitness changes are being identified. Studying the influence of the host plant on the gene expression of *P. capsici* led to the identification of an RxLR effector that partially triggers quantitative resistance. With respect to the environment, the effects of temperature variation on immunity to *P. capsici* and PVY were assessed in a core collection of pepper, and QTLs affecting quantitative estimators of immunity robustness were identified. The genetic architectures of immunity, durability and robustness of immunity in various environments will be examined, to highlight key genomic regions for sustainable crop health, aimed at reducing pesticide use and coping with climate change.

Keynote talk 5

TITLE: BREEDING FOR BIOTIC STRESS RESISTANCE IN CHILLI (*CAPSICUM ANNUUM* L.)

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Abstract: Global area and production of hot pepper increased considerably over last 20 years. India dominates the global dry chilli market with an annual production of two million MT. Chilli is native to Central and South America and got introduced to the country by the Portuguese traders about 550 years ago. It got well adapted to the tropical and sub-tropical climatic conditions. Chilli is mainly grown for its fruits and due to its pungency (capsaicinoids), colour (carotenoids) and flavour it has become an indispensable commodity in Indian cuisine. Because of its long history of cultivation, out-crossing nature and popularity of the crop, large genetic diversity including several landraces has been evolved in India. Due to the raise in demand, continuous cultivation and climate change, the chilli crop is being affected by various emerging pests and diseases arising the need to breed for resistance to major biotic stresses. Major biotic stresses affecting chilli yield in India are viruses, fungal diseases and sucking pests. The existing genetic resources have been exploited in developing several improved varieties/ F_1 hybrids with resistance/tolerance to biotic and abiotic stresses and enhanced quality traits for commercial cultivation. Wild germplasm pool has been very limitedly exploited, which can be explored in breeding as they are potential sources of novel alleles against various stresses. Exploring accelerated breeding approaches like double haploids, speed breeding techniques and use of molecular markers will save time in chilli crop improvement program. With new generation sequencing technology, hot pepper genomic sources are readily available, enabling development of molecular markers tightly linked to useful traits to enable in marker assisted breeding. Achievements made in chilli improvement, illustrate further crop research and their opportunities in international markets. Application of genomic tools coupled with conventional breeding approaches and enhanced multidisciplinary approaches will enable accelerated breeding to develop improved lines to overcome major biotic stresses and suit varied climatic conditions.

Oral presentations

15 Oral presentation

TITLE: BEGOMOVIRUS RESISTANCE GENE *PEPY-2* ENCODES DFDGD-CLASS RNA-DEPENDENT RNA POLYMERASE IN *CAPSICUM*

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Abstract: The whitefly-transmitted begomovirus causes massive yield losses in pepper (*Capsicum* spp.) production around the world. Although introgression of the genetic resistance against begomovirus to commercial cultivars is strongly required, the recently discovered recessive resistance gene *pepy-1*, which encodes the messenger RNA surveillance factor Pelota, is the only begomovirus resistance gene identified in *Capsicum* so far (Koeda et al., 2021). In this study, we fine-mapped another begomovirus resistance gene from PG1-1 (*C. annuum*), which is resistant to bipartite begomoviruses pepper yellow leaf curl Indonesia virus (*PepYLCIV*) and pepper yellow leaf curl Aceh virus (*PepYLCIV*), to further speed up the marker-assisted breeding of begomovirus resistance in peppers. A single dominant locus, *Pepy-2*, conferring resistance against *PepYLCIV* in PG1-1 was identified on chromosome 7 by screening recombinants from the F₂ and F₃ segregating populations derived from a cross between PG1-1 and begomovirus susceptible SCM334. In the target region spanning 722 kb, a strong candidate gene, the RNA-dependent RNA polymerase 3a (*CaRDR3a*), was identified. The whole-genome and transcriptome sequences of PG1-1 and SCM334 revealed a single Guanine (G) deletion in *CaRDR3a* first exon, causing a frameshift resulting in loss-of-function in SCM334. In addition, multiple loss-of-function alleles of *CaRDR3a* were identified in the reference sequences of *C. annuum*, *C. chinense*, and *C. baccatum* in the public database. Furthermore, virus-induced gene silencing of *CaRDR3a* in PG1-1 resulted in the loss of resistance against *PepYLCIV*. PG1-1 and the DNA marker developed in this study will be useful to breeders using *Pepy-2* in their breeding programs.

16 Oral presentation

TITLE: FIELD EVALUATION OF *PEPY-1* CONFERRED BEGOMOVIRUS RESISTANCE IN PEPPER *CAPSICUM ANNUUM*)

Authors: Pohan, N. S. [1], Alfian, G. [2], Khalil, M. [2], Bahagia, P. [3], Hayati, R. [3], Haidar, Y. [3], Hadisah, N. [3], Onouchi, M. [1], Shirono, R. [4], Kohno, Y. [4], Hamada, A. [4], Maruishi, T. [4], Hachisu, S. [1], Homma, K. [1], Zakaria, S. [2,3], Kesumawati, E. [2,3], Koeda, S.* [1,4].

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Abstract: Pepper (*Capsicum annuum*) is an essential condiment and vegetable crop cultivated for innumerable purposes for food and non-food products. Among numerous limiting factors in pepper production, a whitefly-transmitted begomovirus has become a major constraint. The management of begomovirus mainly relies on suppressing the insect-vector population by implementing intensive chemical insecticide application, which is found to be partially effective. A recently identified recessive resistance gene *pepy-1* from BaPep-5 (*C. annuum*) showed a significant low disease progression and viral DNA accumulation during the infection of pepper yellow leaf curl Indonesia virus (PepYLCIV) and pepper yellow leaf curl Aceh virus (PepYLCAV) (Koeda et al., 2021). In this study, to evaluate the begomovirus resistance conferred by *pepy-1* in actual field conditions, multiple years of field study employing begomovirus resistance BaPep-5 were conducted in Indonesia, an area where begomovirus is affecting pepper productions. BaPep-5 showed a significantly lower disease incidence than other commonly six pepper cultivars throughout 135 days of field survey. Begomovirus resistance conferred by *pepy-1* was expressed in the mode of slowing down the disease onset and progression. As the result, BaPep-5 produced significantly higher fruit productivity in comparison to BaPep-4, a susceptible control. Moreover, the evaluation using F2 offspring showed that the

resistant genotype plants (*pepy-1/pepy-1*) had a significant reduction in disease symptoms and produced a significantly higher total fruit yield. Despite the most inevitable mixed infection of multiple begomoviruses in the field by the predominant bipartite PepYLCIV, PepYLCAV, tomato yellow leaf curl Kanchanaburi virus (TYLCKaV), and a monopartite ageratum yellow leaf curl virus (AYVV), the field study showed that *pepy-1* begomovirus resistance attenuates the symptoms progressions enabling *pepy-1*-harboring plants to produce higher fruit productivity, twice to that of susceptible plants. This present study highlights the importance of production scale-based evaluation for an identified begomovirus resistance source.

17 Oral presentation

TITLE: SELECTION FOR DROUGHT AND HEAT STRESSES TOLERANCE IN A F₃ POPULATION DERIVED FROM AN INTERSPECIFIC CROSS BETWEEN *S. MELONGENA* AND *S. INSANUM*

Authors: Cebeci, E. *[1], Boyaci, H.F.[2], Toppino, L.[3], Rotino, G.L.[3], Sahin, M.[4]

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Abstract: Climate change often results in extreme weather events as raises in temperatures, scarce and erratic precipitations, expansion of lands that suffer from floods or water deficit. These extreme weather events directly affect plant growth and crop yields. Eggplant, whose popularity raised in recent years, is widely produced and consumed in Asia, Middle East, and some Mediterranean countries which are among the regions most affected by heat and drought stresses. Heat and drought, individually or combined, are among the most strong and widespread abiotic stresses, which can raise agricultural losses, reduce the crop productivity and yield leading to lower income for farmers. Although cultivated eggplant is known to better tolerate the stresses with respect to other crops, its quality and yield are negatively affected by the environmental stresses. Among the eggplant crop wild relatives, *Solanum insanum* is considered highly tolerant to abiotic stresses. In this study, 100 progenies at F₃ level derived from an interspecific cross between the wild relative of *S. insanum* L. and the pure line BATEM-TDC47 I were used as plant materials. For each F₃ progeny, 12 plants were exposed to drought stress and 4 plants used as controls, thus 1600 plants were evaluated for their drought tolerance. Seedlings with 3-4 true leaves were subjected to drought stress in greenhouse under semi-controlled compartment conditions.

Control plants were watered by required irrigation at 100% ETP, while water deficit was applied as 75% level of required irrigation. The stress symptoms on plants were determined by 0-5 visual scale on the 25th day of the experiment. A total of 400 drought tolerant plants were selected and transferred to the greenhouse to further confirm their heat tolerance levels. After the evaluation, 24 F3-plants were found to be also heat tolerant, while 59 and 17 plants were classified as heat moderate tolerant and sensitive, respectively.

18 Oral presentation

TITLE: INSECT PROBLEMS IN A CHANGING WORLD: BREEDING FOR INSECT RESISTANT PLANTS UNDER SALINITY STRESS

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Abstract: Climate change has profound effects on crop production, for example through salt intrusion in deltas. In addition, pest pressure will change. Both salinity and insects can cause yield losses in plants. Importantly, abiotic and biotic stresses interact, and should not only be considered as independent problems. In this study, our objective was to identify pepper (*Capsicum* sp.) varieties that are salt-tolerant, and/or resistant to the silver leaf whitefly, *Bemisia tabaci*. In addition, we aimed to study the interaction of salinity stress and insect resistance. To this aim, we grew 26 pepper accessions in the greenhouse, applied a salt treatment for two weeks, and then evaluated the plants for resistance to *B. tabaci*. To study the effects of salinity stress on plants, plant weight and height were measured. Salt treatment had a clear effect on growth of plants, and resulted in biomass reduction for almost all pepper accessions. Three accessions show no or very little growth reduction when grown under salt, and could be further studied as potential salt-tolerant plants. Plant resistance to *B. tabaci* was investigated by measuring survival and reproduction. Significant differences were observed among accessions for adult survival and reproduction and five accessions with low survival of *B. tabaci* were selected as resistant. However, the salt treatment invariably resulted in increased survival and reproduction of whiteflies on all plants, meaning plants that were resistant to whiteflies became susceptible when grown under salinity stress. Currently, we are studying if crosstalk between phytohormones is involved in the reduction of plant defence under salinity stress. In conclusion, for both salt tolerance and whitefly resistance there is genetic variation available that may be used to breed new cultivars with improved performance. Studying the interaction between both stresses may result in tools to grow plants that are stable resistant in changing conditions.

19 Oral presentation

TITLE: EFFICIENCY OF VISUAL SELECTION TO RESISTANCE TO *VERTICILLIUM WILT* FOR BREEDING PROGRAMS IN EGGPLANT

Authors: Boyaci, H.F.*[1], Gumrukcu, E.[2], Cebeci, E.[2]

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Abstract: *Verticillium* is one of the most destructive fungal pathogens that restrict eggplant production throughout the world. In order to maintain eggplant cultivation, the plant breeders have made an effort to improve disease-resistant cultivars.

The selection of resistant plants in classical breeding programs is commonly based on visual observations in the field. Since the number of plants in the population can be practically reduced by visual selection, breeders prefer to use in their programs. Visual observation used in the field is based on scale values for the presence of disease symptoms. This method is also used for improvement of resistance to *Verticillium* wilt in eggplant, and a scale of 0-5 is generally preferred. However, there is no sufficient available data on the effectiveness of this method.

In this study, a comparison was made by investigating both the visual observations of the plants and the development of stem infection and degree of fungal colonization in eggplants classically inoculated with *Verticillium*. For this purpose, a total of 10 genotypes, including inbred lines and wild relatives, were tested with *Verticillium dahliae* Kleb. The resistant and susceptible genotypes were used as control in the study. The pathogen was cultured on Vd specific medium. Plants at 4-5 leaves stage were inoculated by using the root-dip method for inoculation. After the inoculation they were transferred in plastic pots filled with sterile peat-perlite mixtures (1:1 v/v) to prevent contamination with the other pathogens causing wilt and grown for two months in a glasshouse compartment in which temperature and humidity partially controlled. Visual numeric scales were used

to correlate appearance of the wilt symptoms of individual plants. Disease severity index was calculated based on visual scale value. At the same time, the stems of these plants were cut longitudinally and fungal colonization and necrosis formation were examined. The plants were evaluated by measuring the length of each necrotic lesion. Although the differences were found among the genotypes, and they showed a wide range of disease reactions from highly resistant to very susceptible, the significant correlation was observed between visual dieback assessment scores and the length of stem necrosis. According to the finding, it was concluded that the visual scale evaluation was highly effective method for eggplant breeding programs.

20 Oral presentation

TITLE: SCREENING OF A PEPPER COLLECTION FOR RESISTANCE TO POWDERY MILDEW CAUSED BY *LEVEILLULA TAURICA*, A WORLDWIDE THREAT

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Abstract: Cultivated peppers (*Capsicum annuum* L.) are under constant pressure from various pathogens. Among them, the ascomycete *Leveillula taurica*, the causal agent of powdery mildew, is responsible for severe infections worldwide, resulting in yield losses of up to 40%. The genetic leverage contributes to the current challenge aiming to reduce the use of phytosanitary products and their impact on the environment and human health. Both monogenic and polygenic resistances to *L. taurica* have been described in *C. annuum* and related species. However, either resistance remains ineffective under heavy infection, or the genetic determinants have not been sufficiently characterized to assist breeding. Therefore, we aim to establish a catalogue of resistance loci to *L. taurica*. For this purpose, core collections of *C. annuum* containing more than 600 accessions altogether were screened in different geographical regions with artificial inoculation to increase the disease pressure. The doubled-haploid line HV12 carrying resistance QTLs and a line carrying the PMR1 resistance gene were used as controls in experiments. PMR1-carrying plants showed significant symptoms, with the presence of sporulation, while HV12 plants displayed one of the highest levels of resistance. Our results suggest that the PMR1 gene used by breeding companies is not sufficient to control the disease. The data collected were used to perform a genome-wide association study (GWAS) to help identify novel genitors for powdery mildew resistance QTLs useful for pepper breeding programs.

21 Oral presentation

TITLE: ASSESSMENT RESULTS OF SALINITY STRESSED F2 POPULATION ORIGINATED FROM INTERSPECIFIC HYBRIDIZATION OF EGGPLANT WITH WILD RELATIVE *S. INCANUM* L.

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Abstract: Salinity, which is one of the major abiotic stresses, prevailing in mostly arid and semiarid areas that is nearly 20 % of the world's cultivated area. The existence of excessive amount salt around the plant root zone is detrimental for vegetative growth and economic yield. Today salinization is still severely expanding and posing a great threat to development of sustainable agriculture. Although eggplant (*Solanum melongena* L.) is considered moderate sensitive, soil salinity mitigates strictly the growth and yield of eggplant. Eggplant has significant crop wild relatives (CRWs) which are thought to be more tolerant to abiotic stresses and it is substantial to exploit their potential against salinity in hybrid breeding studies. Previously it is proven that *Solanum incanum* L. has tolerance to salinity stress. In this study, it was aimed to improve salinity tolerant pure eggplant lines. Therefore, acquired F2 population from interspecific hybridization between the pure line (BATEM-TDC47) with distinctive features from BATEM eggplant genepool and *S. incanum* L., were subjected to salinity stress at 150 mM NaCl level with its parents and F1 plants. On 12th day after salt treatment, plants were evaluated using 0-5 visual scale and 50 F2 individuals were determined as salt tolerant among stressed 256 plants. Additionally, some of their morphological and physiological features, such as plant height, stem diameter, number of leaves, anthocyanin presence, MDA (malondialdehyde) and proline levels, were studied and compared to their parents and F1 plants. While

plant height and stem diameter were decreased dramatically under salt stress, number of leaves per plant was not affected. According to analysis, average MDA and proline levels of F2 population were identified as $10.82 \mu\text{mol g}^{-1}\text{FW}$ (fresh weight) and $7.44 \mu\text{g g}^{-1}\text{FW}$. The 50 F2 plants that have an increased salinity tolerance were transferred to greenhouse conditions and self-pollinated to produce F3 generations.

22 Oral presentation

TITLE: EVALUATION OF THE EFFECTS OF GRAFTING AND VERMICOMPOST APPLICATIONS ON THE MORPHOPHYSIOLOGICAL PROPERTIES OF EGGPLANT UNDER DROUGHT STRESS WITH PRINCIPAL COMPONENT ANALYSIS

Authors: Kiran, S.[1], Boyacı H.F.*[2], Demir, Z.[1], Aydınşakir K.[3], Kuşvuran, Ş.,[4], Zengin, S.[5], Ellialtıoğlu, Ş.Ş.[6]

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Abstract: The scope of the study: it consists of the evaluation of the effects of grafting and vermicompost applications on the morphophysiological properties of eggplant under drought stress by principal component analysis (PCA). In the study carried out under greenhouse conditions, the grafting and non-grafting plants were grown in conditions containing different levels of vermicompost - V (0, 1, 2, 3%) under drought stress (control; 100%, mild stress-MS; 70% and severe stress-SS; 30% irrigation). In the study, seedlings of the eggplant variety Aydın Siyahı, grafted on Köksal and ungrafted, were used. In terms of traits studied (shoot fresh weight -SFW and root fresh weight-RFW, shoot dry weight -SDW and root dry weight- RDW, shoot length-SL and shoot diameter-SD, leaf area-LA, chlorophyll content (SPAD value), stomatal conductivity-Gs, relative water content-RWC), the first two of the components had 94.39% of the total variation in the grafted plants according to the bi-plot. PCA showed that 'V (3%) + MS' and 'V (2%) + MS' were in significant and positive correlations with SPAD, LA, RWC, SDW and RFW. 'V (3%) + MS' and 'V (2%) + MS' applications; SL correlated positively and weakly with gs, SFW, SD,

and RDW. However, the correlations between V applications and morphophysiological features in SS conditions was not significant. Looking at the relationships between the variables; in general, the correlations of all the examined features with each other was found to be significant and positive. Especially; the positive and strong correlations between SPAD value and LA and RWC, SDW and RFW, Gs and SL and SFW, and RDW and SD were remarkable. As a result, the use of grafted plants and V applications under MS condition in eggplant positively affected the morphophysiological properties of the plants and increased their tolerance to stress.

Poster presentations

22 Poster presentation

TITLE: SCREENING OF PEPPER (*CAPSICUM ANNUM* L.) GENOTYPES FOR RESPONSE TO PATHOGENS AND PESTS UNDER CONDITIONS OF CONVENTIONAL AND ORGANIC FIELD PRODUCTION

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Abstract: Monitoring of eight pepper genotypes was conducted at Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, during two successive years, under different diseases and pests' management systems. The surveys were carried out, using the standard field inspection methods to detect virus diseases (*Tobacco mosaic virus* - TMV, *Cucumber mosaic virus* - CMV and *Tomato spotted wilt virus* - TSWV, stem rot (*Phytoplasma solani*), verticillium wilt (*Verticillium dahliae* Kleb.), brown spots (*Alternaria solani*), the pests cotton bollworm (*Helicoverpa armigera* Hubn.) and aphids (*Homoptera:Aphididae*). During the survey, no plants with symptoms of verticillium wilting were reported in all studied genotypes. The mean score of virus attack was below 12%. The average rate of brown leaf spots ranged between 29.05% for line K992 to 36.26% for line K995. The established highest *Phytoplasma solani* attack averaged from 24.10% for Stryama to 32.15% for Kapia UV. Single plants with thrips damage were observed during seedling production and immediately after transplanting in the field. At maturity stage, an infestation of cotton bollworm (*Helicoverpa armigera* Hb) was observed, while aphids' population was low with no colonies established. The lowest infestation rates by *H. armigera* were reported in pepper genotypes K992 (0.92%) and K995 (0.49%) grown under organic production conditions.

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23 Poster presentation

TITLE: IDENTIFICATION OF QTLS IN EGGPLANT INTROGRESSION LINES UNDER IRRIGATED AND WATER-STRESSED CONDITIONS

Authors: Flores-Saavedra, M.*[1], Gramazio, P.[1], Vilanova, S.[1], Mircea, D.M.[1,2], Ruiz-González M.X.[1], Vicente, O.[1], Prohens, J.[1], Plazas, M.[1]

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Abstract: Climate change has led to a decrease in rainfall in many regions, resulting in irrigation water limitations and necessitating the development of water-stress tolerant genotypes. We evaluated a set of eggplant (*Solanum melongena*) lines with introgressions from the wild parent, *S. incanum*. Plants at the vegetative phenological stage were subjected to two levels of irrigation: 100% of field capacity for control conditions and 30% for water deficit conditions. After 14 days of treatment, growth parameters and biochemical markers were evaluated. Reduced irrigation resulted in reduced growth and increased proline and malondialdehyde in the plants. Most of the introgression lines showed lower growth levels than the cultivated parent in both irrigation conditions. However, two QTLs were found to promote growth under drought: *dwt8*, which produces higher stem biomass, and *dwr6%*, which promotes increased root development under drought. In addition, positive alleles at QTLs were identified for leaf water content (*wc12%*), water use efficiency (*wue1*) and chlorophyll content (*chl2* and *chl8%*), which are associated with higher productivity under water stress. These results suggest that introgression of relevant genomic regions from drought-tolerant wild genotypes could be a practical approach to improve drought tolerance in eggplant. However, fine-mapping of tolerance QTLs and minimising linkage drag will be critical to significantly improve drought tolerance in eggplant through introgression breeding.

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24 Poster presentation

TITLE: CURRENT STATUS OF PEPPER VIRUSES IN BULGARIA

Authors: Pasev, G.*[1], Radeva-Ivanova, V.[1], Nankar, A.[2], Kostova, D.[2]

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Abstract: Pepper (*Capsicum annuum* L.) is a traditional vegetable, part of the Bulgarian cuisine. It is consumed fresh and processed, because of its valuable taste, mineral and vitamin content. However, the crop is vulnerable to many dangerous viruses that have severe impact on the quality and quantity of the production. The study attempts to present the current status of the viruses on pepper in the country. For the period of 2019-2022 more than 200 pepper samples were collected from leaves and fruits. These samples originated from commercial fields and small private gardens from pepper growing regions in Thracian valley and central regions of northern Bulgaria. Using DAS/TAS-ELISA 6 viral pathogens were identified in single or mixed infection (*Broad bean wilt virus 1*, BBWV1; *Broad bean wilt virus 2*, BBWV2; *Beet western yellows virus*, BWYV; *Cucumber mosaic virus*, CMV; *Potato virus Y*, PVY and *Pepper mild mottle virus*, PMMoV). Analysis with group-specific antisera identified viruses belonging to genus *Tobamovirus* and *Orthospovirus*. Most commonly occurring virus was CMV presented in 61% of the cases, followed by BBWV2 32%, *Orthospovirus* 22% and PVY 20%. Identification of isolates by RT-PCR revealed CMV samples with subgroup I and II as well as isolates with satellite RNA. Similarly, isolates of BBWV2 were found to belong to the group II of the virus.

These results suggest a careful consideration of the status quo of the problem as well as future tendencies in pepper breeding.

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25 Poster presentation

TITLE: *IN VITRO* ANTHOR CULTURE FOR DEVELOPMENT OF DOUBLE HAPLOIDS FOR RESISTANCE TO CUCUMO AND TOBAMOVIRUSES

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

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Abstract: *Pepper mild mottle virus* (PMMoV), *Tobacco mosaic virus* (TMV) and *Cucumber mosaic virus* (CMV) are among the diseases that seriously reduced pepper production. Unfortunately, producing of resistance lines with good horticultural characteristics is a long process by classical breeding methods, especially in open-pollinated vegetable species, and this period can be up to 8-10 years. The doubled haploid technology by anther culture is an important plant breeding tool that allows rapid developed of 100% homozygous pure lines in 1 or 2 years. For that reason, the experiments were conducted to obtain pepper lines resistant to CMV, TMV and PMMoV using anther culture technique.

Six segregating populations derived from local accessions that showed resistance to CMV and TMV in preliminary experiments and three F₁ genotypes with L4 allele for resistance to PMMoV were used as initial material. Total 155 plants were obtained by anther cultivation in vitro. The ratio of haploid:diploid plants was 1:1,4. Resistance to TMV by Detached Leaf Test was established in 23 of the adapted diploid plants. Biological testing with CMV and creation of F₁ hybrids with susceptible genotypes for further development of mapping populations was also presented.

Possible inclusion of obtained double haploids as an initial material for hybrid breeding is discussed.

Acknowledgements: The research leading to these results has received funding from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

26 Poster presentation

TITLE: EVALUATION OF PEPPER CORE COLLECTION FOR RESISTANCE TO PEPPER MILD MOTTLE VIRUS

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Abstract: A pepper core collection of 412 accessions, developed in the G2P-SOL project, comprising of four species (*Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*) was assessed for resistance to Pepper mild mottle virus P_{1,2} (PMMoV, genus *Tobamovirus*). The screening was performed with 12 plants total for each accession. The experiment included two independent trials with two blocks per trial. Local and systemic symptoms were assessed visually for each accession. The number and size (mm²) of lesions of the resistant accessions were measured. According to the obtained results accessions were divided into five groups. The first group included 8 resistant accessions showing hypersensitivity as necrotic lesions only on the inoculated leaves. Seven of them were *C. chinense* and one was *C. annuum*. The second group comprised of three accessions also classified as resistant – two *C. chinense* and one *C. annuum*. They reacted with systemic necrosis followed by leaf abscission. The third group included accessions with systemic mosaic and slow development of necrosis, not related to hypersensitive response. In some plants the virus caused top necrosis and wilting followed by death. The fourth group consisted of heterogenic accessions in which some plants reacted with mosaic and systemic necrosis while other plants developed only mosaic. As expected, the largest group (78% among the four species) represented all susceptible accessions - all individuals developed severe systemic mosaic, in some cases accompanied with leaf deformation. Presented data shows the variability of the core collection's response to PMMoV and highlights possible sources of resistance.

Acknowledgements: This work was funded by the European Commission, Horizon 2020 G2P-SOL project (grant agreement no. 677379). The authors also acknowledge financial support received from Horizon 2020 Teaming Project PlantaSYST (Grant agreement no739582).

27 Poster presentation

TITLE: GROWTH VARIABILITY IN *SOLANUM MELONGENA* AND TWO WILD RELATED SPECIES UNDER SALT STRESS

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Abstract: Plant production under salt stress is one of the main targets in new breeding projects due to the increase in soil salinity worldwide. Actual breeding programs investigate the genetic resources available in wild species to develop new improved varieties. In this sense, growth parameters during plant development are crucial for final good production. *Solanum melongena* limits its growth and productivity in salt presence. However, related wild species have been studied by their tolerance to different abiotic stresses. Therefore, this work studies the performance of one cultivated eggplant genotype (*Solanum melongena*) and two wild-related genotypes (*Solanum dasyphyllum* and *Solanum macrocarpon*) under salt stress. Measurements of plant height, leaf number, root length, leaf surface, and leaf and root fresh weight were taken in four-leaf stage plants in control, 200 mM, and 400 mM NaCl conditions. Results showed that the three genotypes were affected by salt stress. The three genotypes reduced leaf and root biomass, root length, plant height, leaf number and leaf surface. Interestingly, *S. macrocarpon* did not reduce leaf surface compared to *S. melongena* and *S. dasyphyllum* at 200 mM. These findings support the hypothesis that cultivated eggplant is relatively tolerant to abiotic stresses, but still there are few differences in the response of some wild-related species that may be useful for breeding.

The aim of this work is to establish the basis for new breeding programs for future environmental and soil changes in eggplant through the

study of development parameters in young and adult genotypes of cultivated and wild eggplant species.

Acknowledgements: This work is supported by project AICO/2020/174, CIPROM/2021/020 funded by Conselleria d'Innovació, Universitats, Ciència i Societat Digital (Generalitat Valenciana, Spain), and contract FPU19/04080 funded by Ministerio de Educación y Formación Profesional (Gobierno de España).

28 Poster presentation

TITLE: UNRAVELING THE QUANTITATIVE PEPPER - *PHYTOPHTHORA CAPSICI* INTERPLAY: GLOBAL ASSESSMENT OF ISOLATE AGGRESSIVENESS

Authors: Merino Martin, G.*[1,2], Massire, A.[2], Bachellez, A. [2], Gabriels, S.[1], Vleeshouwers, V. [1], Bai, Y.[1], Lefebvre, V.[2]

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Abstract: Pepper is one of the most economically important crops worldwide, but its production is often challenged by plant pathogens such as *Phytophthora capsici*. This oomycete can cause devastating losses, and its high level of global genetic diversity poses a significant challenge for breeding programs aimed at developing resistant pepper varieties. It is acknowledged that resistance to *P. capsici* mostly follows a nested pattern. However, to better understand the interactions between pepper and *P. capsici*, we conducted a study to investigate how different *P. capsici* isolates from various regions of the world interact with different pepper accessions. We inoculated 11 *P. capsici* isolates from France, Mexico, Korea, China, the USA, and the Asian Pacific on 9 pepper accessions (*Capsicum annuum*) with varying degrees of resistance to the pathogen. We then assessed the symptoms of the disease. Our results revealed two key findings. Firstly, we observed a quantitative aspect to the plant-pathogen interaction, with some pepper accessions being more resistant to certain *P. capsici* isolate than others. Secondly, we observed a diverse range of symptom intensities, demonstrating that some *P. capsici* isolates have high aggressiveness and can infect a broad range of pepper accessions, while others have low aggressiveness and infect fewer accessions. These findings provide important insights on the *P. capsici* infectivity patterns and have significant implications for breeding programs aimed at developing resistant pepper varieties. By characterizing the isolates and accessions involved in these interactions, we can begin to better understand the complex dynamics of plant-pathogen interactions and develop more targeted breeding strategies to combat this devastating pathogen. In summary, our study highlights the challenges posed by *P. capsici* to pepper production and provides valuable insights into the complex interactions between this pathogen and its host.

29 Poster presentation.

TITLE: THE EFFECT OF SILICONE FOLIAR FERTILIZATION ON LIMITING THE GROWTH OF STEM NECROSIS IN PEPPER (*CAPSICUM ANNUUM L.*)

Authors: Karadzhova, N.*[1], Todorova, V. [1]

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Abstract: *Botrytis cinerea* and *Sclerotinia sclerotiorum* are important fungal pathogens of a wide range of hosts. Infection prevention and control can be extremely difficult. There are no effective fungicides to control this soil-borne pathogens, causing gray and white rot on pepper. The results of a study of the influence of liquid fertilizer "Optysil" (Intermag, Poland) are presented: containing silicon: (SiO_2 – 200 g/L) and iron (Fe 16.5 g/L) on the pepper resistance to attack by *Botrytis cinerea* and *Sclerotinia sclerotiorum*. The experiment was carried out in 2022, in field conditions at the Maritsa VCRI with the "Stryama" variety, planted according to a scheme (60/25 cm) on the area with mineral fertilization (N220 P160 K200). The fertilizer "Optysil" was applied by spraying as an aqueous solution in a concentration of 0.05 ml per L of water three times: one week after transplanting, twice during mass flowering with an interval of 14 days. Plants were artificially infected with pure cultures of the pathogens *Botrytis cinerea* and *Sclerotinia sclerotiorum* by the method of decapitation of the main stem, application of a 7-day-old pure culture of each isolate and wrapping the wounded area with aluminum foil. An increase in necrotic areas was observed in the variants treated with "Optysil" and the control variants without treatment. Infection of the young plants was carried out in the beginning of flowering phase. Temperature during the trial period: daytime 27-32°C; night – 13–17°C. The length of the necrotic lesions in the decapitated plants was measured up to the 10th day after transplanting. A tendency to enhance the immune response of "Optysil" - treated pepper plants to infection with the studied fungal pathogens was found. The effect of "Optysil" application against the growth of necrosis from gray rot is 43%, against white rot - 41%. The results show that the silicon acts as an immunostimulant, blocking the rapid growth of stem necrosis caused by infection with *Botrytis cinerea* and *Sclerotinia sclerotiorum*.

Acknowledgements: The authors also acknowledge financial support received from EU Horizon 2020 Teaming Project PlantaSYST (Grant agreement no739582).

30 Poster presentation.

TITLE: HETEROGENITY OF TOMATO SPOTTED WILT VIRUS (TSWV) RESISTANCE BREAKING (RB) AND WILD TYPE (WT) STRAINS COLLECTED IN KECSKEMÉT FROM PEPPER CULTIVARS HOMOZYGOUS OR HETEROZYGOUS FOR *TSW* GENE

Authors: Almási, A. [1], Timár, Z.*[2], Palotás, G.[2], Salánki, K.[1]

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Abstract: Tomato spotted wilt virus (TSWV) causes severe epidemics and economic losses in pepper production worldwide. The most effective way to control the virus is resistance breeding. However, still there is only one dominant resistance gene in pepper cultivars that confer resistance against TSWV: the *Tsw* gene. The breeding resistant pepper cultivars harboring the *Tsw* gene led to the rapid emergence of resistant breaking (RB) isolates. The NSs protein encoded by the S RNA of the TSWV genome was identified as the viral effector of hypersensitive response in pepper plants, and point mutations in its amino acid sequence are responsible for the resistance breaking phenotype. Therefore, it is important to study the composition of TSWV isolates of RB and wild type (WT) strains and the interaction with different resistant pepper cultivars.

During 2020-2022 15 samples were collected from different susceptible and resistant pepper cultivars homozygous or heterozygous for *Tsw* gene in the pepper producing region in Kecskemét, Hungary. Biological assays were carried out by mechanical inoculation on resistant and susceptible pepper cultivars. Total RNAs from the infected plants were extracted, the NSs coding region was amplified by RT/PCR and the nucleotide sequences were determined of the collected isolates. Based on the nucleotide and deduced amino acid sequences of the NSs region, maximum likelihood phylogenetic trees were composed and phylogenetic analysis was performed.

There were differences in the symptom development among the distinct virus-host combinations and more than half of the TSWV isolates showed RB phenotype. In the amino acid sequences there were various point mutations detected among the isolates. According to the phylogenetic analysis, most of the collected isolates located on clade-I, but some of them grouped to the clade-II with the formerly identified HUP1-2012-RB and HUP4-2012-WT Hungarian strains (Almási et al. 2015).

Almási, A., Csilléry, G., Csömör, Z., Nemes, K., Palkovics, L., Salánki, K., & Tóbiás, I. (2015). Phylogenetic analysis of Tomato spotted wilt virus (TSWV) NSs protein demonstrates the isolated emergence of resistance-breaking strains in pepper. *Virus Genes*, 50, 71-78. DOI 10.1007/s11262-014-1131-3"

31 Poster presentation.

TITLE: CHILI PEPPER BREEDING WITH *GDS* GENE BASED ON TISSUE RETENTION

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Abstract: It is now a basic requirement for pepper varieties for open field cultivation to include resistance to *Xanthomonas euvesicatoria* bacterium. Bacteria-resistant chili pepper varieties in cultivation without exception contain *Bs-2* gene based on tissue destruction (hypersensitive reaction, HR). In case of a strong bacterial infection, bacterial spots appear on the leaves of certain resistant varieties. The *gds* resistance gene based on tissue retention (non-HR), described by Gábor Csilléry and János Szarka in 1995, is contained in only one cherry-type pepper variety. Based on processors' requirement, breeding of a chili pepper variety was carried out that contains the *gds* gene. Basic crossings were started in 2016 in a heated greenhouse using chili-type lines with good nutritional characteristics and *gds*-resistant lines. For selecting the *gds* gene, starting from F₂ generation, the plants at approx. 8-leaves-stage were inoculated with a suspension of *X. euvesicatoria* at a concentration of 10⁸ cells/mL. On the 7th day-after-inoculation (DAI), resistant lines were selected based on the symptoms. The selections for the desired continuous growth type, hanging fruit position and high dye content were carried out in a heated greenhouse up to the F₆ generation. In the F₇-F₉ generation, further phenotypic and nutritional characteristics selections were carried out in open field. End of 2020, the selected line was submitted for state registration with variety name "Borbási". Based on novelty research, we were the first in the world to announce a chili pepper variety with *gds*-resistance. In 2021, "Borbási" was tested in direct sowing cultivation under large-scale conditions on sandy soil in Szentkirály, Hungary. Its yield was 32.5 t/ha, average fruit weight 25.6 g, dry matter content 16%. The dye content of the post-ripened fruits was 183-210 ASTA. Based on our assays, its yield was similar to that of the reference variety in Hungary "Meteorit", but dye content is significantly higher.

32 Poster presentation.

TITLE: COMPARATIVE EVALUATION OF RESISTANCE TO TOMATO SPOTTED WILT VIRUS IN PEPPER WITH CAPS MOLECULAR MARKER AND SIMPLE PROBE (FRET TECHNOLOGY) IN REAL TIME PCR

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Abstract: Tomato spotted wilt virus is a dangerous virus that negatively affects agricultural production. One of its important hosts is pepper therefore the use of genetic resistance to prevent virus-induced losses is important for the environment and human health. Resistance for TSWV in pepper is controlled by the single dominant gene *Tsw*. In the study, 96 pepper genotypes with different fruit (capia, bell, jalapeno, charliston) types were tested using classical PCR and real-time PCR methods based on fluorescently labeled sequence-specific simple probe (FRET Technology). Firstly, cleaved amplified polymorphic sequence (CAPS) marker SCAC568 molecular marker, Taq1 and Xba1 restriction enzymes were used. In this method, homozygous resistant, heterozygous resistant and susceptible genotypes were successfully determined. Secondly, in order to determine the resistance status of the same genotypes, resistance was tested for the *Tsw* gene in a real time PCR device with a simple probe-based molecular kit. Similar results were obtained in both methods. It can be used as a useful method for the determination of commercial varieties and breeding lines, especially in the capia pepper population.

33 Poster presentation

TITLE: THE BIOSTIMULANT SUPER FIFTY PRIME® ENHANCES THE YIELD IN EGGPLANT AND PEPPER

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Abstract: With the global warming and consecutive climate change, agricultural species worldwide experience worsening of the growth conditions and increased abiotic stress. To combat this, a whole new branch of biologically sound substances, called biostimulants, emerged. SuperFifty Prime® is a commercially available biostimulant that contains highly concentrated *Ascophyllum nodosum* (brown seaweed) extract. Previously, it has been demonstrated that it successfully reduces abiotic stress and increases plant performance in some crops like tomato, rice and wheat. However, its effect on other economically important species, such as eggplant and pepper, is still unknown. To address this question, we have investigated the influence of Super Fifty Prime® on eggplant (*S. melongena* var. Black Pearl F1) and pepper (*C. annuum* var. Amareta F1) plants grown in open field conditions. During the flowering stage, they were sprayed two times with an optimal concentration of the biostimulant. To monitor the progression of fruit development in treated and untreated plants, fruit diameter was measured at the cell division, cell expansion, and maturation stages. Our results, gathered from two independent experiments, with identical conditions and treatments, but conducted on separate geographic locations, demonstrate that Super Fifty Prime® improves the overall plant growth, increases the fruit number at every studied developmental stage, and greatly enhances the fruit size and mass in both analyzed crops. We have been also able to determine the time period, during which this biostimulant retained its functional properties.

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Session:

INTEGRATED OMICS - NEW TOOLS AND APPLICATIONS FOR CROP IMPROVEMENT



Keynote talk 6

TITLE: COMPARATIVE GENETIC ARCHITECTURE OF FRUIT TRAITS IN THE THREE MAJOR SOLANACEAE FRUIT CROPS

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Abstract: Pepper, eggplant and tomato are among the most important horticultural crops worldwide. Their fruits are essential ingredients in many culinary cultures and constitute a good source of antioxidant compounds and vitamins. All three species belong to the Solanaceae family, and their diploid genomes exhibit very similar gene numbers and extensive synteny. Despite these similarities, the three species exhibit a large diversity of morphological and compositional traits, as well as different (climacteric vs non-climacteric) modes of ripening.

In the G2P-SOL project, we constructed core collections of the three species, representative of their worldwide genetic diversity. These collections were genotyped/resequenced and subjected to phenotypic characterization in multiple locations, as well as to metabolic profiling of fruits. GWA analyses identified hundreds of QTLs controlling fruit morphology and composition in each species, many of which are new and exhibit high LOD scores.

The genetic architecture of these traits in the three genomes will be discussed, in order to uncover common and species-specific genetic determinants controlling fruit characteristics in these species.



Keynote talk 7

TITLE: EXPLORING IMPAIRED SUSCEPTIBILITY (S) GENES IN PLANT RESISTANCE BREEDING: 10 YEARS FROM A CONCEPT TO RESISTANT CULTIVARS

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Abstract: Plants have evolved complex defence mechanisms to avoid invasion of potential pathogens. Despite this, adapted pathogens deploy effector proteins to manipulate host susceptibility (S) genes, rendering plant defences ineffective. The identification and mutation of plant S genes exploited by pathogens are important for the generation of crops with durable and broad-spectrum resistance. Application of mutant S genes in the breeding of resistant crops is limited because of potential pleiotropy. New genome editing techniques open up new possibilities for the modification of S genes. In this talk, I will give an overview on progresses in resistance breeding by applying the S gene concept. Using examples, I will illustrate how the S gene concept in combination with breeding techniques was accepted as a novel breeding strategy to produce new cultivars with improved resistance to different pathogens.

Oral presentations

23 Oral presentation

TITLE: EXPRESSION OF ANTHRACNOSE RESISTANCE GENES IDENTIFIED IN *CAPSICUM BACCATUM* 'PBC80'-DERIVED RECOMBINANT INBRED LINES

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Abstract: Chili anthracnose caused by *Colletotrichum* spp. has long been a threat to chili production worldwide, especially in the tropics. *Capsicum baccatum* 'PBC80' is an excellent source of resistance to the most aggressive species in Asia, *Colletotrichum scovillei*. Recently a QTL for ripe fruit resistance from the 'PBC80'-derived recombinant inbred lines (RILs) was located on chromosome 4. The physical size of the QTL was 123 Mbp housing over 80 defence-related genes. To identify the genes most related to anthracnose resistance, fine mapping of the QTL region was achieved by adding 80 additional markers to the region. Single-marker analysis identified two markers most linked to the anthracnose resistance gene, which were physically 1.12 Mbp apart. Nine putative genes were selected from the new QTL for their expression 0 to 12 h post-fruit inoculation in two different RIL genotypes, RR and SS. The RR exhibited resistance, while the SS showed susceptibility in both fruit stages. Five genes were up-regulated only in the RR genotype, and one up-regulated in both chili genotypes; however, the expression was relatively and constantly low in the SS. Most up-regulated genes reached the highest peak (2.3-4.5 folds) at 6 h, except for one having the highest peak at 12 h (6.4 folds).

24 Oral presentation

TITLE: A WORLDWIDE EGGPLANT CORE COLLECTION TO STUDY AGRONOMICALLY RELEVANT TRAITS AND FRUIT QUALITY-RELATED METABOLITES

Authors: Gaccione, L.*[1], Toppino, L.[2], Sulli, M.[3], Tumino, G.[4], Alonso, D.[5], Aprea, G.[3], Tassone, M.R.[2], Boyaci, H.F.[6], Lin, Y.P.[7], Lanteri, S.[1], Prohens, J.[5], Portis, E.[1], Rotino, G.L.[2], Giuliano, G.[3], Barchi, L.[1]

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Abstract: Within the *Solanaceae* family, eggplant (*Solanum melongena* L.) represents the second most important berry-producing crop after tomato, with an estimated global production of 59 Mt (FAOSTAT 2023). Compared to tomato and pepper, eggplant stands out for its high content in phenolics, mostly anthocyanins in the purple-coloured peel, and chlorogenic acid in the flesh, and it is a good source of minerals such as K, Mg and Cu.

In the frame of the EU G2P-SOL project (<http://www.g2p-sol.eu>) a core collection including 321 *S. melongena* accessions representative of the genetic diversity of over 3,600 accessions maintained in genebanks, as well as members of wild species belonging to its primary to tertiary genepools, was established. The core collection was phenotyped for 46 agronomic traits at Montanaso Lombardo (Italy), Valencia (Spain) and Antalya (Turkey). Peel and flesh tissues were separately sampled from berries at commercially ripe stage in Italy and Spain; their analysis through LC-PDA-HRMS allowed the identification and relative quantification of 80 and 82 semi-polar metabolites, respectively.

The core collection was also resequenced at 20X, resulting in >51M SNPs and >1.9M structural variations (SVs). Sequencing data were also used to build a *k-mers* presence/absence table.

After quality filtering, a genome-wide association study (GWAS) for both agronomic and metabolic traits was performed on 309 eggplant accessions. Using >1.2M SNPs, >71k SVs and >4M *k-mers*, it was possible to identify hundreds of quantitative trait loci (QTLs) for each trait category, the majority of which were concordant in SNP-, SV-, and *k-mer*-based GWAS. Furthermore, selective sweeps (SSs) putatively involved in selection signatures for key agronomic and metabolic traits were also detected. Candidate genes associated with QTLs and SSs are under investigation.

Acknowledgment: This work was supported by the European Commission, Horizon 2020 G2P-SOL project (grant agreement no. 677379) and by the “Promoting a Plant Genetic Resource Community for Europe (PRO-GRACE)” project (grant agreement no. 101094738).

25 Oral presentation

TITLE: EFFECTS OF NITROGEN FORM SUPPLY ON THE RESPONSE TO *FUSARIUM OXYSPORUM* F. SP. *MELONGENAE* IN A PARTIALLY RESISTANT EGGPLANT LINE

Authors: Tassone, M.R.*[1], Sirangelo, T.M.[1], Toppino, L.[1], Mercati, F.[2], Puccio, G.[2], Mauceri, A.[3], Sunseri, F.[3], Abenavoli, M.R.[3], Rotino, G.L.[1]

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Abstract: *Fusarium oxysporum* f. sp. *melongenae* (Fom) is a soil-borne fungus that invades eggplant (*S. melongena*) roots resulting in vascular wilting and significant yield losses in both field and greenhouse conditions. It has long been shown that nitrogen (N), in its different forms, might influence plant physiological processes that affect plant-microbe interactions and disease development. In this work, the effects of different N-forms (NO_3^- , NH_4^+) fertilization compared to no supply (N0) on the efficiency of response to Fom in the partially resistant eggplant line 'AM199' were elucidated. Disease symptoms evaluation after inoculation revealed a reduced disease incidence in plants supplied with NO_3^- compared to N0 in opposite behaviour with respect to seedlings supplied with NH_4^+ , showing more evident symptoms mainly 15 days after inoculation. Root samples were harvested at different timepoints (0 - T0, 4 hours - T4h, and 15 days - T15d), after artificial Fom or mock (water as control) inoculation in seedlings supplied with different N-forms and RNA-Seq libraries were constructed. The filtered reads were mapped to the latest V4.1 version of the '67/3' genome and differentially expressed genes (DEGs) analysis was conducted through appropriate pairwise comparisons, considering different supplies and timepoints after Fom inoculation vs mock-infected controls. WGCNA (Weighted-Gene Correlation Network Analysis) and GO Enrichment analyses allowed to extract hub genes

and key pathways associated to the treatments applied. Interestingly, preliminary results showed that NO_3^- treatment boost a better plant response (tolerance) to *Fusarium* compared to NH_4^+ . Indeed, a limited decrease of R genes expression level, comparing T4h to T15d after Fom inoculation was observed, while an opposite trend was found in NH_4^+ -supplied plants. In agreement, the overexpression of many Disease resistance proteins RPP13 and LRR receptor-like serine/threonine-protein kinases, as well as of CYP71D55: Premnspiropodiene oxygenase, a strong antifungal phytoalexin, improved the eggplant tolerance only under NO_3^- treatment.

26 Oral presentation

TITLE: GENOMIC AND TRANSCRIPTOMIC DISSECTION OF RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *MELONGENAE* IN THE '305E40' EGGPLANT LINE

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Abstract: Cultivated eggplant (*S. melongena* L.) is severely affected by *F. oxysporum* f. sp. *melongenae* (Fom), causing heavy yield losses both in field and greenhouse. Eco-friendlier options to chemical treatments include development of resistant lines. In this work, '305E40', an eggplant doubled haploid line from somatic hybridization with *S. aethiopicum* carrying an introgression fragment hosting full resistance to Fom, was investigated through a multi-omics strategy. A mapping approach based on a segregating Recombinant Inbred Lines (RILs) population from the cross '305E40'x'67/3' allowed identification of a major QTL associated with the Fom resistance trait on CH02. Through Oxford Nanopore Technology (ONT) de novo sequencing, assembly and annotation, a new high-quality genome of '305E40' was developed and employed as reference for deep characterization of the introgressed region aimed at identifying the candidate gene(s) responsible of the resistance. Genome comparison between '305E40' and '67/3' revealed the extension of the introgressed fragment in which unique *S. aethiopicum* specific DNA sequences are present compared to '67/3'. A targeted BSA-Seq was employed, by aligning two high-quality reads bulks of RILs, clustered according to their resistance score, to the reference '305E40', revealing differentially enriched

regions between resistant vs. susceptible RIL bulks. By combining different strategies as evaluation of differential reads coverage and annotation sources, promising candidate genes were identified. To further provide evidence of their involvement in Fom resistance, a RNASeq DEGs analysis was performed after root Fom inoculation of two lines with diverging behaviour, '305E40' and 'Tal1/1' (an eggplant line fully sensitive to Fom, employed as recurrent parent to obtain '305E40'). Relevant DEGs lying on CH02 with a very low or absent gene expression in 'Tal1/1' were identified, highly consistent with BSA-seq prediction, allowing to select a set of best candidates deserving functional analyses for the identification of the key resistance gene(s) in eggplant.

27 Oral presentation

TITLE: CRISPR-CAS9 - MEDIATED EDITING OF *AUCSIA2* GENE INDUCES PARTHENO-CARPIC FRUIT DEVELOPMENT IN EGGPLANT

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Abstract: Parthenocarpy is a desirable trait in vegetable crops because it results in seedless fruits with added value for direct consumption and industrial processing. In the case of eggplant (*Solanum melongena* L.), harvesting of commercially ripe fruit must take place long before physiological maturity, which coincides with the presence of mature seeds that reduce the value of the berries or even make them unmarketable, as consumers dislike their occurrence. Seeds development decreases the quality of the eggplant flesh for any use, as it induces its progressive softening and browning as they mature; for this reason, obtaining parthenocarpic lines of this crop would both increase the value of the fruits and increase the time window for their harvesting. Silencing of a pair of paralogous *AUCSIA* genes in tomato by RNA interference resulted in parthenocarpy (Molesini et al, 2009) linked to increased auxin content in the flower buds, making these genes prime targets for genome editing in *Solanaceae*. Here, we report on the study of the induction of parthenocarpic fruit development in eggplant by means of CRISPR/Cas9 editing of the two *AUCSIA* genes. Homozygous ge*AUCSIA2* T1 plants with different frameshift mutations yielded parthenocarpic fruits. Expression analyses in these lines confirmed presence of the edited transcript and its nonsense mediated decay, with no off-target effect on the *AUCSIA1* gene. Analysis of the auxin dosage in fruits in pre-anthesis flower buds of these plants is ongoing. Moreover, recently obtained ge*AUCSIA1* T1 plants with analogous mutations are currently being grown to verify the presence of the parthenocarpic trait and will be subjected to the same analyses. These lines will be used to generate a ge*AUCSIA1/2* double mutant.

28 Oral presentation

TITLE: APPLICATION OF OBJECT DETECTION AND INSTANCE SEGMENTATION BASED ON DEEP LEARNING MODELS TO EGGPLANT BREEDING

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Abstract: Although it is recognized that there are large differences among cultivars in the early growth of eggplant, the genetic diversity of these differences is not always clear. Time series monitoring is necessary to accurately quantify the early growth of each individual, but it is not easy to record the phenotypes in a large number of genetic resources. In recent years, rapid improvements in deep learning models have led to various attempts to obtain useful trait data about plants from images. In this study, I investigated methods to obtain useful trait data on early growth of eggplant from fixed-point time series images. Tablet terminals installed in the upper part of the greenhouse were used to take images of the growth of the eggplant mini-core collection for about one month after planting. A portion of the captured images was used to train YOLO's object detection and instance segmentation models. Plant individuals in each image were detected with relatively high accuracy. It is expected that the size of each individual can be approximated by the area of the plant body in the image, but further study is needed for practical use. Currently, multi-object tracking of individual plants in time-series images is underway with the aim of obtaining phenotypes related to early growth that will be useful in breeding.

Poster presentations

34 Poster presentation

TITLE: EVALUATION OF DIFFERENT CULTURE MEDIA FOR REGENERATION OF PEPPER THROUGH DIRECT ORGANOGENESIS AND PLOIDY ANALYSIS

Authors: Martínez-López, M.*[1], García-Pérez, A.[2], Gimeno-Páez, E.[1], Prohens, J.[1], Vilanova, S.[1], García-Forteza, E.[2]

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Abstract: The regeneration of pepper (*Capsicum* spp.) through *in vitro* culture is challenging due to their recalcitrance. In this study, the effect of various combinations of auxins, cytokinins, and micronutrients on direct organogenesis in cotyledon and hypocotyl explants of *C. annuum*, *C. baccatum*, and *C. chinense* was investigated. The study found that there was variation in regeneration response among different species and explant types. Specifically, for *C. annuum*, the average number of shoots per cotyledon and hypocotyl explant were 1.44 and 0.28, respectively, while for *C. baccatum*, these numbers were 4.17 and 3.20. In contrast, for *C. chinense*, the average numbers of shoots per cotyledon and hypocotyl explant were very low at 0.08 and 0.00, respectively. Among the media tested, the one containing 5 mg/L BAP, 0.5 mg/L IAA, and 0.47 mg/L CuSO₄, followed by a subculture in the same medium with 10 mg/L AgNO₃, resulted in the best overall regeneration rates. This medium was most effective for *C. baccatum* using cotyledon explants, producing 8.87 shoots per explant. The explants grown in this medium were greener, with hypocotyl explants being greener than those of cotyledon. Our findings indicate that there is a significant variation in regeneration rates among different *Capsicum* species and suggest that a synergistic effect of copper and

silver enhances regeneration. The regenerated shoots were transferred to different media for elongation, rooting, and acclimatization. Cytometry results revealed a higher number of polyploid cells in hypocotyl tissue compared to leaves and cotyledons. Additionally, a tetraploid plant was obtained from hypocotyl tissue, suggesting that *in vitro* regeneration can produce polyploid pepper plants without antimetabolic agents. This study improves *Capsicum* regeneration protocols by identifying specific combinations of medium, explant, and genotype, allowing for efficient genetic transformation and gene editing technologies for other crops.

Acknowledgements: FPU21/02288 (to MM-L; Spanish Ministerio de Universidades)

35 Poster presentation

TITLE: WHOLE-GENOME RESEQUENCING OF THE EIGHT PEPPERS (*CAPSICUM ANNUUM* L.) ACCESSIONS FOUNDING A NOVEL MAGIC POPULATION

Authors: Martina, M. [1], Gianoglio, N. [2], Barchi, L. [1], Milanese, C.[2], Acquadro, A.[1], Portis, E.*[1]

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Abstract: The need for increasing worldwide agricultural production necessitates, among others, the development of novel breeding strategies to exploit the diversity within plant genetic resources. Multi-parent Advanced Generation Inter-Cross (MAGIC) populations, which are constituted of recombinant inbred lines (RILs) that possess a genetic mosaic of multiple founder parents, have emerged as a promising approach. MAGIC populations exhibit distinctive advantages over traditional bi-parental and germplasm populations due to their ability to combine substantial levels of genetic recombination, absence of genetic structure, and exceptional genetic and phenotypic diversity. In this study, we selected eight accessions based on their phenotypic and genetic diversity and designated them as founder parents for a pepper MAGIC population. The morpho-agronomic characterization of parents was performed using 23 key agronomic descriptors. Furthermore, their genetic diversity was evaluated by means of a whole-genome resequencing of these accessions at an average depth of 25x. By using the high-quality genome “Ca_59” (Liao et al. 2022, Nature Communications 13:3479) as reference, around 15 million high-reliable polymorphisms were identified, including ~14.4M (94.9%) being single nucleotide polymorphisms (SNPs) and ~0.7M (5.1%) being InDels. Overall, the comprehensive morpho-agronomic and genetic characterization of these accessions will be of great significance for the genetic analysis of the MAGIC population. Furthermore, the results provide valuable knowledge and tools for the pepper community for genetic and genomic studies and for breeding purposes.

36 Poster presentation

TITLE: IMPACT OF COVERAGE DEPTH ON SNP DISCOVERY IN EGGPLANT: RECOMMENDATIONS FOR A SKIM WHOLE GENOME SEQUENCING PIPELINE

Authors: Baraja-Fonseca, V. [1], Arrones, A. [1], Vilanova, S. [1], Plazas, M. [1], Prohens, J. [1], Bombarely, A. [2], Gramazio, P.*[1]

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Abstract: Sequencing technology is a powerful tool that enables the development of genetic resources such as the identification of massive SNP cohorts. Reduced representation sequencing and whole genome resequencing are the most commonly used methods for SNP identification and high-throughput genotyping. However, skim whole genome resequencing is a new promising approach that can achieve an unbiased representation of the genome using very low sequencing coverage. Five sequencing coverages (1X-5X), obtained in silico from the whole genome resequencing at 5X, were evaluated in a *S. melongena* genotype. After mapping against the eggplant reference genome 67/3 and performing the SNP calling, a range of minimum coverage filters (from 1 to 10X) was applied to survey the size of each SNP set. The number of variations identified decreased as the minimum filtering coverage was increased, unlike the confidence in their status as true positives. Nevertheless, low sequencing coverage can increase the risk of false positives and artefacts, affecting data reliability. To overcome this issue, the SNP sets were compared with a gold standard set, built from the resequencing of the same genotype at 20X. The gold standard was filtered at maximum mapping coverage of 40X to avoid overrepresented regions, minimum coverage after the SNP calling at 10X to confidently call heterozygous variants, the minimum frequency of the alternative allele at 0.3, and removal variants in chromosome 0. It was observed that a sequencing coverage

of 1X is sufficient to achieve a high level of genotype concordance (69% using 1X minimum filtering coverage) with the gold standard. This study provides valuable recommendations for the design of high throughput genotyping pipelines, enabling fingerprinting a larger number of accessions at reduced costs and thus fostering genetics and breeding studies. However, the best combination of sequencing coverage and variant filtering should be evaluated on a case-by-case basis, considering available resources and study objectives.

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37 Poster presentation

TITLE: OPTIMIZATION OF A CRISPR/CAS AGROBACTERIUM-MEDIATED PROTOCOL IN EGGPLANT

Authors: Martínez-López, M.[1], Arrones, A.[1], Vilanova, S [1], Plazas, M [1], Prohens, J.[1], Gramazio, P.*[1]

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Abstract: Recently, several intra and interspecific experimental populations have been developed in eggplant (*Solanum melongena*) to associate morphoagronomic traits with genomic regions, which usually contain several genes candidates to control the trait. Advanced genetic engineering techniques like CRISPR/Cas-mediated gene editing are required for the functional evaluation of these genes. The CRISPR/Cas technique has emerged as the preferred approach due to its easy implementation and the generation of heritable edits. However, its use is largely limited by transformation protocols mediated by *Agrobacterium tumefaciens*, especially in recalcitrant crops such as eggplant. This study aims to optimize the CRISPR/Cas gene editing process in eggplant by evaluating the effect of multiple factors and compounds on regeneration efficiency. These factors include genotypes, candidate genes, gRNAs, Cas proteins, *Agrobacterium* strains, bacterial growth, incubation times, types and concentrations of antibiotics, hormones and antioxidants, and manipulation techniques. All the factors assessed were based on the differences found among several organogenesis and *Agrobacterium* transformation protocols in eggplant to investigate their impact on the whole process plus other factors that resulted to be beneficial in other recalcitrant species. Preliminary results confirm the high recalcitrance of the crop and indicate that no single factor is determinant in the success of this process, although the combination of several can significantly increase the positivity rate. The highest rate of transformed explant regeneration has been achieved in cotyledons by utilizing 2 mg/L zeatin-riboside, 75 mg/L kanamycin, and 200 mg/L carbenicillin. This study provides a step forward to dissect the factors that impact most

the *Agrobacterium*-mediated transformation processes in eggplant making a relevant contribution in overcoming the high recalcitrance of this economically important crop.

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Session:

BREEDING FOR
ORNAMENTAL/URBAN
GARDENS AND ORGANIC
SYSTEMS



Oral presentations

29 Oral presentation

TITLE: THE USE OF *TTI* AND *PCX* GENES DURING THE BREEDING OF HANGING ORNAMENTAL “AMPOLNA” TYPE PEPPER VARIETIES

Authors: Csilléry, G.*[1], Ruskó, J.[2], Pápai, B.[3], Kovács, Zs.[3], Fári, M.[4], Szamosi, Cs.[5]

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Abstract: Cultivated varieties of pepper and the majority of wild *Capsicum* species have erect stems. However, the stem of several *Solanaceae* species is prostrate. A well-known example is tomato, where this trait is used in the development of modern greenhouse tomato cultivation technology. On the other hand, pepper cannot even tolerate laying down the plants at 45 degrees without fruit quantity and quality reduction. Based on our research so far, we consider the *tti* and *Pcx* pepper mutants may solve this problem.

Without any support system, the tortuous (*tti*) mutants and the negative geotropism type *Pcx* plants are developing hanging stems like ornamental plants (e.g., Pelargonium).

The recessive *tti* - tortuous internode and the semi-dominant *Pcx* - Procumbent plant mutant genes were introduced not only into the ivory conical “Cecei” type, but also into sweet and hot snack programs. In addition, the dual purpose “eatable ornamental pepper” option also did not escape our attention. Due to the ornamental plant aspects,

we also included the following mutants in our crossing programs: lutescens leaves (lux), mosaic leaves (mol), filiform leaves (fil), ilex leaves (ixl), heart leaves (Htl), items with purple leaves (Lil), purple flowers (Lif), purple fruits (Leb), and yellow spotted flowers (Ysf). The plants were grown in hanging 5 l pots filled with peat moss using drip irrigation.

The internode of the tti mutant is longer than usual. The stem structure is slightly twisting and tortuous. Stem covers the sides of the pots, hanging, but the tip of the shoot is erect.

The growing habit of the Pcx mutants are more generative. In contrast to the tti mutant, the shoot tip of Pcx is drooping, stem covering well the sides of the pots, making it an ideal hanging "ampolna" plant.

Poster presentations

38 Poster presentation

TITLE: INSECTICIDAL ACTIVITY OF SOME BOTANICAL PRODUCTS AGAINST GREEN PEACH APHID (*MYZUS PERSICAE* SULZER) AND THRIPS (*THRIPS TABACI* LINDEMAN, *FRANKLINIELLA OCCIDENTALIS* PERG.) IN GREENHOUSE PEPPER PRODUCTION

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Abstract: Global trends for ecological agriculture require looking for an alternative to conventional production. In recent years, crop protection has relied to a great extent on synthetic chemical pesticides, but their availability is now declining as a result of new legislation requirements and the development of resistance in pest populations. Synthetic chemical insecticides pose risks to the environment and to consumers of produce. Biopesticides are products for control of pests based on microorganisms or natural products. Botanical insecticides, which are of plant origin belong to biopesticides. They have a proven potential to control pests and are used all over the world. Studies were carried out in the greenhouses of the Maritsa Vegetable Crops Research Institute-Plovdiv to determine the biological activity of some botanical products, as well as a combination of them, to control the green peach aphid (*Myzus persicae* Sulzer) and thrips (*Thrips tabaci* Lindeman, *Frankliniella occidentalis* Perg.). The bioproducts Krisant EC (a. i. pyrethrins) and Neem Azal T/S (a. i. azadirachtin) were tested. The products Krisant EC 75 ml/da and the combination Krisant EC 75 ml/da+Neem Azal T/S 0.3% show very good effectiveness over 80% against sucking pests aphids and thrips.

Acknowledgements: The research leading to these results has received funding from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

39 Poster presentation

TITLE: A NEW MINIATURE DWARF EGGPLANT AS A POTENTIAL ORNAMENTAL AND MODEL PLANT

Authors: Plazas, M.*[1], Martínez-López, M.[1], García-Fortea, E.[1], Gramazio, P.[1], Prohens, J.[1], Vilanova, S.[1]

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Abstract: Eggplant (*Solanum melongena*) is a globally important crop appreciated for its nutritional and culinary properties. Despite its primary agricultural use, eggplant is also an aesthetically pleasing ornamental plant with considerable decorative value for gardens and landscaping. Its diverse fruit shape and color variations, including dark purple, white, green and others, make it an attractive choice for ornamental horticulture. Traditionally, eggplant has been grown as a vegetable crop in open fields; however, some materials may have interest as ornamentals, including indoors cultivation. Controlled environmental conditions enable eggplant to bloom profusely and to achieve a lush foliage, making it an excellent option for interior and exterior decoration. In the context of eggplant breeding, pre-breeding programs have played a pivotal role in the development of novel genotypes with enhanced adaptive characteristics. These programs have made use of wild crop relatives of eggplant, which harbor desirable traits such as disease resistance, drought tolerance, adaptation to varying environments, and novel phenotypes. Notably, as a result of our eggplant pre-breeding efforts we have developed a small-fruited line derived from a backcross between cultivated eggplant and the wild species *S. anguivi*. This new line exhibits unique morphological and agronomic features that render it a promising candidate for breeding programs. Specifically, it is characterized by a small plant size, semi-determinate and rapid growth, abundant purple flowers, and numerous small white fruits. Its unique characteristics make it a promising model plant for studies on eggplant genetics and physiology, as well as a potential ornamental crop.

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