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XXI EUCARPIA MEETING OF THE TOMATO WORKING GROUP

10–13
June 2025
Grand Hotel Plovdiv
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BOOK OF ABSTRACTS

XXI EUCARPIA MEETING
OF THE TOMATO WORKING GROUP

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Meeting venue:

Grand Hotel Plovdiv,
2, "Zlatyu Boyadzhiev" Str.
4003 Plovdiv
BULGARIA

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Session:

GENETIC RESOURCES

KEYNOTE TALK 1

TITLE: TRANSFIGURE AND CONQUER: A BRIEF HISTORY OF TOMATO FRUIT SHAPE

Authors: Giuliano G.*[1], Ferrante P.[1], Aprea G.[1], Fain V.[1], Blanca J.[2], Alonso D.[2], Cañizares J.[2], Prohens J.[2], Pons Puig C.[2], Díez M.J.[2], Barchi L .[3], Schafleitner R.[4], Stein N.[5], Lefebvre V.[6], Zamir D.[7], Boyacı H.F.[8], Tringovska I.[9], Bovy A.[10]

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Abstract: It is commonly believed that tomato fruit shape diversified progressively, from small, round tomatoes in the center of domestication in South America, to larger, more complex shapes generated

by subsequent selection and breeding after their transfer in Europe. Contrary to this belief, most of the modern morphological diversity is already present in the South American center of domestication and was presumably generated in pre-Columbian times. Genetic analysis of 15,000 worldwide accessions identified 8 main groups, both in the center of domestication and post-transfer to Europe, with distinct genetic composition and fruit morphological characteristics. The findings were confirmed by analysis of historical genebank pictures, paintings, seed company catalogs and botanical texts spanning from the 15th to the 20th centuries. Genome-wide genetic diversity revealed the genetic bottlenecks and admixture events that occurred during the transitions between these groups. Deep sequencing and association analyses revealed both known and novel loci controlling fruit shape, and the relative role of each of them in the transitions.

Acknowledgments: We acknowledge funding from the EU (projects G2P-SOL, Grant agreement 677379 and PRO-GRACE, Grant agreement 101094738)

ORAL PRESENTATIONS

ORAL PRESENTATION 1

TITLE: TOMATO STOMATAL DEVELOPMENT: DIVERSE MECHANISMS OF ADAPTIVE FLEXIBILITY REVEALED BY MULTI-SPECIES ANALYSIS

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Abstract: Stomata are pores in the plant epidermis that control gas exchange between the plant and atmosphere. In Arabidopsis, stomatal development requires the bHLH transcription factor SPEECHLESS (AtSPCH) and perception of signals from adjacent cells, from other parts of the plant and from the environment. SPCH is thought to be a critical target for environmental inputs into development. Despite the power of Arabidopsis as a model for stomatal development, we found crop plants like tomatoes often lean on different cellular and genetic strategies to achieve optimal stomatal distributions. By making genetically encoded reporters of the stomatal lineage, and long-term confocal microscopy we tracked the developing epidermis of M82 (WT) and mutant tomato seedlings. We found that, like in Arabidopsis, tomato undergoes a series of asymmetric and symmetric cell divisions to produce stomata. However, we found one type of asymmetric division (ACD) was missing in the tomato epidermis, and other ACDs could be used to generate non-stomatal cells. These data suggest differences in ACD strategies that control stomatal production between plant species. Since SPCH serves as the major integrator of environmental information in stomatal development, we targeted the tomato

SPCH promoter for CRISPR-based mutagenesis. By screening lines in response to light, temperature, and drought we found putative SPCH cis-regulatory elements that indicate complexity in the regulation of developmental flexibility. Taken together these results further our understanding of the species-specific cellular and genetic pathways plants use to adapt to their environment.

ORAL PRESENTATION 2

TITLE: HARNESSTOMDB V1.0: A COMPREHENSIVE MULTI-OMICS RESOURCE FOR TOMATO GENETIC DIVERSITY

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Abstract: Over the past decades, significant advancements have been made in characterizing a series of tomato diversity panels at the genetic, phenotypic, and metabolic levels. However, this valuable information remains scattered across databases and labs, often lacking integration both among these levels and with essential (meta) data such as plant passport and experimental/environmental conditions. These gaps hinder the effective use of germplasm data in breeding efforts aimed at sustainable agriculture and food quality. To address these challenges, we developed HarnesstomDB, a comprehensive public platform based in Europe (accessible at https://gateway.harnesstom.eu/) that integrates tomato genetic resources with pre-breeding bioinformatics tools. The platform uses a BrAPI-compliant, JSON-based REST API, enabling integration with other databases and facilitating data exchange. HarnesstomDB serves as a centralized hub for storing, integrating, and exploring experimental metadata, accession details, phenotypic and metabolic data, images, genetic information, genotyping,

QTLs, and GWAS variants. Currently, HarnesstomDB consolidates data and metadata from major European projects like TRADITOM, TomGem, and G2P-SOL, along with data from selected INRA GWAS experiments. The database offers information of Multicrop Passport Descriptors for 17,000 accessions (including traditional, wild, and breeding lines), 170 pedigrees, 10,472 accession images, and 93,020 phenotypic observations across 79 variables. Additionally, it provides metadata for 872 metabolites, and genotypic data from five platforms (e.g., TRADITOM GBS, SPET panel, SolCap arrays across three genome versions) covering 127,809 markers, 3 million genotyping points, 21 QTLs, and 773 GWAS variants. HarnesstomDB also includes tools for exploring germplasm attributes, phenotypes, and genetic data, such as multi-filters, interactive plots, genome browsers, and the HarnessAPI. These features enable users to compare accessions, analyze genomic features, and investigate environmental and phenotypic variables in a user-friendly manner. By combining comprehensive data, advanced tools, and open access, HarnesstomDB has become the largest germplasm tomato database in the world, fostering knowledge sharing to address global challenges in agriculture and nutrition.

ORAL PRESENTATION 3

TITLE: IDENTIFICATION OF NOVEL SOURCES OF *TOMATO BROWN RUGOSE FRUIT VIRUS* RESISTANCE IN WILD TOMATO SPECIES

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Abstract: *Tomato brown rugose fruit virus* (ToBRFV) is a highly contagious pathogen that poses a significant threat to tomato production worldwide, including Canada. Given the economic importance of tomatoes and the potential devastation caused by ToBRFV, breeding for resistance to this virus has become a critical focus in agricultural research. Wild tomato species have been used as a source of natural resistance for many pathogens and bred into commercial tomato varieties. Since the breakdown of Tm-2 and Tm-22 resistance by ToBRFV in 2014, promising sources of resistance to ToBRFV have been identified in the wild tomato species *S. habrochaites*, *S. peruvianum*, *S. chilense*, *S. ochrantum*, and *S. pimpinellifolium*. The main objectives of this study were 1) to screen and identify new sources of wild tomato accessions resistant to ToBRFV and 2) to confirm previously reported ToBRFV resistance in wild tomato accessions. A total of twenty-four *S. pimpinellifolium* and seven *S. habrochaites* accessions were screened using a ToBRFV strain sourced from a greenhouse in Leamington, Ontario. No symptoms were visible on resistant plants for 21 days post infection. Additionally, no virus could be detected on leaf tissue using Agdia strips and RT-qPCR. Ten of the 31 accessions had an intermediate to high level of resistance, in which six were identified in *S. pimpinellifolium* and four from *S. habrochaites*. Two of the resistant *S. pimpinellifolium* lines did not possess the alleles associated with bipartite resist-

ance between the known quantitative trait loci on chromosome 11 and Tm-1 on chromosome 2. This suggested that the two sources of ToBRFV resistance identified in this study genetically map to different regions than the bipartite resistance. These new sources of resistance are currently being combined with the bipartite resistance to develop durable resistance for ToBRFV in tomato varieties adapted to Canadian environments.

ORAL PRESENTATION 4

TITLE: TOMATO GENETIC RESOURCES STUDIES AT THE AEGEAN AGRICULTURAL RESEARCH INSTITUTE

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Abstract: Tomato is native to the western coast of South America, where its wild relatives still occur naturally. Although introduced to Türkiye in the early 1900s, tomato cultivation has since spread all over Türkiye. Over time, many local varieties and different populations, such as Ayaş tomato, Çanakkale tomato, Yamanlar tomato have emerged in many parts of our country. However, our tomato genetic resources are under threat of extinction for many reasons such as the widespread use of modern tomato varieties and cross-pollination, as in many other crops. Vegetable genetic resources research studies have been carried out in AARI since 1963 to protect against this threat. Currently, over 1,000 tomato accessions collected from all over Türkiye are preserved in National Gene Bank. In this respect, collection, regeneration, characterization, evaluation and conservation of tomato genetic resources of our country are carried out in line with practices applied to other vegetable crops. These studies make significant contributions to the sustainable use of tomato genetic resources and their transfer to next generations. In addition, these tomato genetic resources are used in tomato breeding studies at AARI. As a result of these tomato breeding studies, F_1 tomato varieties Menemen, Ege Pembesi 50, 1915 and MASS 1001 were developed. In addition, ongoing breeding studies for the "Ayaş Tomato", a local variety that stands out in terms of taste and flavor, are underway.

POSTER PRESENTATIONS

POSTER PRESENTATION 1

TITLE: DEVELOPMENT OF NEW INTROGRESSION MATERIALS OF INTEREST FOR TOMATO BREEDING

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Abstract: Crop wild relatives (CWRs) are valuable genetic resources for broadening the genetic base of tomato (*Solanum lycopersicum*) to address future breeding challenges. This study aims at developing two distinct types of introgressed breeding populations in tomato: (1) introgression line (IL) sets with *S. galapagense* and *S. cheesmaniae*, and (2) neodomestication of *S. pimpinellifolium* by introgressing domestication-related traits into its wild genetic background. The introgression lines programs exploit the genetic diversity of both Galapagos Island species. *Solanum galapagense* is of particular interest for breeding, as it has type IV trichomes rich in acyl sugars, conferring whitefly resistance, and alleles for purple pigmentation, which enhance fruit quality and antioxidant properties. *Solanum cheesmaniae* exhibits tolerance to salinity, high micronutrient content, and moderate pest resistance. Using a refined speed breeding protocol recently developed by our team, which combines 6 L pots, an eight-day cold (14 °C) pre-treatment at the cotyledonary stage and extra K fertilization, combined with embryo rescue, we developed 15 BC2 families from *S. galapagense* and 25 BC2 families from *S. cheesmaniae* in a year and a half. Marker-assisted selection will begin at the BC2 stage to identify and

advance the generations to obtain a set of introgression lines each of which contains a single introgression from the respective wild donor into the cultivated tomato genetic background. The neodomestication approach has involved crossing *S. pimpinellifolium* with cultivated tomato to introduce domestication-related genes (fw2.2, lc, fas, ovate, and fw3.2) into the *S. pimpinellifolium* genetic background. Currently, 15 BC1 generations have been generated by crossing *S. pimpinellifolium* with parents carrying different allelic combinations for the target genes. Genotyping will be conducted to select lines with all possible combinations of these genes, creating a foundation for pyramiding traits and evaluating epistatic interactions. The goal is to produce lines that retain the adaptive resilience of *S. pimpinellifolium* while incorporating larger fruit size and different shapes. These two complementary approaches provide valuable new genetic resources that may be of interest for breeding and expanding the genetic base of tomato.

POSTER PRESENTATION 2

TITLE: FUNCTIONAL GENOMIC ANALYSIS OF TRICHOME-SPECIFIC GENE EXPRESSION IN *SOLANUM PENNELLII*

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Abstract: Trichomes are specialized epidermal structures present in the aerial organs of most plant species, playing crucial roles in responses to abiotic stresses like radiation and desiccation, and especially in biotic stress defense. Among *Solanum* species, seven trichome types are categorized into glandular (types I, IV, VI, and VII) and non-glandular (types II, III, and V) groups. Non-glandular trichomes create physical barriers against pests, while glandular trichomes synthesize specialized metabolites with toxic effects. Type IV glandular trichomes produce acyl sugars—non-volatile sugar esters that are both toxic and capable of immobilizing pests. These structures are abundant in wild species like *S. pennellii* L. but scarce in the cultivated tomato *S. lycopersicum* L. However, the mechanisms underlying trichome formation in *Solanum* species remain poorly understood, as well as the reason for the presence or absence of certain trichome types in different species. This study presents the phenotypic and molecular characterization of *S. pennellii* mutant lines generated via an enhancer trapping T-DNA insertional mutagenesis program. These mutants exhibit reporter *uidA* gene expression in type IV glandular trichomes, enabling iden-

tification of T-DNA-tagged genes through an anchor-PCR approach. Functional analysis of these genes is ongoing, using CRISPR-Cas9 to create knockout alleles in both wild and cultivated species. The findings will provide insights into the regulatory mechanisms of tomato glandular trichome development and offer potential strategies to enhance pest resistance in cultivated tomato.

Acknowledgements & Funding: This work was supported by research grants from the Spanish Ministry of Economy and Competitiveness and the UE-European Regional Development Fund (AGL2017-88702-C2-1-R) and by the PPIT-UAL, Junta de Andalucía-ERDF 2021-2027. Objective RSO1.1. Programme: 54.A. Juan Diego López Fábregas was funded by a PhD fellowship in the FPI program of the Spanish Ministry of Science and Innovation in Spain (PRE2018-083639).

POSTER PRESENTATION 3

TITLE: INTEGRATED APPROACHES TO CHARACTERIZE POTATO-LIKE LEAF ITALIAN LANDRACES FOR AGRIFOOD TRACEABILITY

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Abstract: Tomato in Italy is known for the wealth of its genetic diversity, justified by the high number of landraces distributed in many regions, involving different environments and traditional product uses. The setup of molecular strategies for varietal distinctiveness is crucial for the agri-food traceability. In this study, allelism test and genotyping was performed to assess the key-role of the potato leaf (C) gene in the potato-like leaf phenotype (PL) in tomato landraces. Four Italian PL landraces: 'Spagnoletta di Formia e Gaeta' (SPA) from southern Lazio, 'Giagiù' (GIA) and 'Patanara' (PTN) from Campania, and 'Pomodoro di Mola' (MOL) from Apulia were analysed, along with two American PL (c mutant gene) accessions, LA2374 (LA) and PC711571 (PC) and a Red Setter PL mutant line (entire mutant gene). Nagcarlang, was used as a wild-type leaf control. The allelism test confirmed the putative role in the C gene for PL phenotype determination in Italian landraces. Genotyping confirmed the richness of genetic diversity in Italian landraces. A SCAR marker targeting a retrotransposon Rider insertion in the C gene, previously reported in tomato heirlooms of North American and Centre European and known as the most representative mutation conferring the PL phenotype, was designed. The marker analysis distinguished the Italian landraces from LA and PC, carrying the Rider insertion. Whole-genome sequencing of SPA identified a private novel

nonsense SNP variant allele as also confirmed by dCAPS marker analysis. In addition, two novel C alleles responsible for missense variations in GIA/PTN and MOL were identified via Sanger Sequencing. In silico protein analysis suggested that the mutations may functionally affect the protein activity. These newly identified PL mutations will serve as suitable molecular tools for traceability in the agri-food chain for early differentiation, genetic distinctiveness of similar varieties, to support the growing interest in high-quality local food products.

POSTER PRESENTATION 4

TITLE: ENRICHMENT OF BULGARIAN TOMATO GENEBANK COLLECTION AND INFORMATION ACCESS TO CONSERVED BIODIVERSITY

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Abstract: The Global Plan of Action for Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture is implemented in Bulgaria by IPGR-Sadovo. Ex situ conservation is an essential approach for preservation of agrobiodiversity. Tomato is the most important vegetable crop in the Bulgarian market, andthe success of its breeding programmes largely depends on diverse initial material. The decreasing number of landraces in the last forty years indicates the need for monitoring the genetic erosion of traditional varieties. During the period 1982-2024 the fund of tomato in the National Genebank at the Institute in Sadovo was enriched with 1,518 *Solanum lycopersicum* accessions, of which 905 introduced from foreign genebanks by international exchange. 613 accessions are of Bulgarian origin, local forms acquired through collecting missions, advanced cultivars or breeding lines. A great diversity of traditional varieties from home gardens are collected. An important and necessary part of genebank activities is the maintenance and development of databases, consisting information, synchronized at the European level. The electronic register of IPGR-Sadovo includes passport data, according to FAO/Bioversity descriptors. This standard allows the Bulgarian ex situ collection to be published open access on the European Search Catalogue for Plant Genetic Resources – EURISCO. The study aims to present the status of the tomato collection in the Bulgarian genebank,

to facilitate their utilization. Since 2024, an intelligent system for managing the Bulgarian plant gene pool, conserved in the genebank of IPGR-Sadovo, is being established. The results are expected to lead to significant improvements in the storage and digitalization of plant genetic resources, thereby supporting the long-term conservation of plant gene pools as a public resource used by the scientific community, breeding companies and other users.

POSTER PRESENTATION 5

TITLE: SPET GENOTYPING OF OVER 15,000 TOMATO ACCESSIONS REVEALS THE GENETIC STRUCTURE AND HISTORY OF TOMATO AT WORLD LEVEL

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Abstract: The large diversity in tomato fruit shape and size is a paradigm of the morphological diversification introduced by human

domestication. It is generally believed that this morphological diversity evolved gradually through centuries of selective cultivation by growers. To verify this hypothesis, we used an interdisciplinary approach, by genotyping 15,490 tomato accessions from 124 countries, and analyzing 2,776 genebank images, 260 historical sources and 71 paintings describing tomato fruit morphology. We find that most of the modern morphological diversity is already present in the South American center of domestication and that, contrary to the general belief, until the late 19th century the morphological diversity outside the center of domestication was surprisingly limited, encompassing only a few cultivated types. The diversity observed today was predominantly re-created in the latter half of the 19th century by professional Italian and US breeders, to serve the demands of rapidly growing markets. Whole genome resequencing of genotypes representative of the various groups narrowed down many of the different genomic regions selected by these breeders. This interdisciplinary approach could be used in other culturally and economically relevant crops.

POSTER PRESENTATION 6

TITLE: GENETIC DIVERSITY IN BULGARIAN TOMATO GENOTYPES: INSIGHTS FROM MOLECULAR AND BIOCHEMICAL ANALYSES

Authors: Todorovska E.*[1], Ganeva D.[2], Grozeva S.[2], Tsonev S.[1], Christov N.[1], Radkova M.[1], Tomlekova N.[2], Aziz S.[2]

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Abstract: Genetic diversity in commercial vegetable crops, including tomato, is limited due to domestication and selection processes. Genetic variation in eight Bulgarian tomato varieties and breeding lines, differing in their morphological and biochemical composition, was assessed using 182 simple sequence repeat (SSR) and 17 single nucleotide polymorphism (SNP) markers in 19 genes involved in diverse metabolic pathways associated with quality and other trait. The analysed plant material included tomato varieties Plovdivska karotina and IZK Alya, as well as breeding lines L1116, L975, L984, L1140, L21β and L53β. The total number of the amplified SSR alleles was higher than the total number of SNP alleles identified in this study. The genetic diversity within the collection based on SSRs was relatively low (0.2139) but similar to that observed with SNP markers (0.2337). Nei's genetic distance based on both marker types varied from 0.1181 between Plovdivska karotina and L21β to 0.5836 between IZK Alya and L984. Cluster analysis using the un-weighted pair group method with arithmetic mean (UPGMA) method indicated that the studied tomato genotypes are grouped in several clusters, which is to some extent consistent with the morpho- and hemo-types of

the studied tomatoes. The observed grouping of Plovdivska karotina and L21β in a common sub-cluster reflects their similar morphological and biochemical composition. The genetic distance information from this study could be useful for further implementation in tomato breeding strategies in Bulgaria.

Acknowledgments: DG, SG, NT, SA acknowledge funding from the Bulgarian Agricultural Academy project ZEMDKT 17.

POSTER PRESENTATION 7

TITLE: SENSORY EVALUATION OF TOMATO VARIETIES: A CONSUMER-CENTERED APPROACH TO BREEDING

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Abstract: Consumer preferences play a crucial role in the development of new tomato varieties, guiding breeders toward cultivars that combine both agronomic performance and desirable sensory attributes. To assess consumer perceptions of tomato quality, a tasting event, "Your Opinion Matters: Tomato Tasting," was organized on August 9, 2024, at the Vegetable Research and Development Station Buzău. The event aimed to evaluate taste, texture, and overall appeal of 40 tomato varieties, including 14 certified cultivars, 16 breeding lines, and 10 F1 hybrid combinations. Participants assessed each variety based on key sensory attributes such as size, color, firmness, juiciness, taste, and aroma, using a standardized scoring system ranging from 1 to 5. The results provided valuable insights into consumer preferences, with the Andrada variety—a "Ox heart" type—emerging as the most appreciated due to its large size, fleshy texture, and balanced sweetness. Other highly rated varieties included the pink tomato Florelia and the orange-fruited Nectaria, highlighting a preference for distinct fruit characteristics. Additionally, six of the newly developed hybrid combinations ranked among the top 15 selections, demonstrating their commercial potential. Statistical analyses of the collected data confirmed clear consumer trends, reinforcing the importance of sensory testing in the breeding process. The findings will help refine selection strategies for future tomato improvement, ensuring that newly developed varieties align with market demands.

Beyond research implications, the event also contributed to consumer education, raising awareness of tomato diversity and promoting interest in high-quality, locally grown produce. Moving forward, integrating consumer-driven sensory evaluations with genetic selection will be essential for developing superior tomato varieties tailored to both growers and consumers.

Acknowledgements: The study was financially supported by Ministry of Agriculture and Rural Development ADER 6.2.2.

POSTER PRESENTATION 8

TITLE: NATURAL RESOURCES, CULTURAL PHENOMENON, AND SCIENTIFIC ACHIEVEMENTS IN TOMATO CULTIVATION IN BULGARIA

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Abstract: The cultivation of tomatoes as a cultural crop in Bulgaria has a centuries-old history. The Central Agricultural Library of the Agricultural Academy preserves numerous valuable specimens that serve as carriers of rich cultural and historical information. This archival collection enables a historical analysis of the development of tomato production, illustrating the interplay between scientific advancements, agricultural practices, traditions, and cultural influences shaped by the country's favorable natural and climatic conditions. A testament to the integration of science, tradition, and nature is the fact that more than half of the cultivated area and tomato production in Bulgaria is concentrated in the Southwestern Central Region. The selection of the Maritsa Vegetable Crops Research Institute as a key site for international collaboration is a well-deserved recognition of Bulgarian scientific achievements in advancing global tomato breeding as an economically significant vegetable crop.

Session:

BREEDING FOR AGRONOMIC AND FRUIT QUALITY TRAITS

KEYNOTE TALK 2

TITLE: GENETIC CONTROL OF TOMATO FRUIT QUALITY IN CHANGING ENVIRONMENTS: FROM GWAS TO BREEDING

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

ORAL PRESENTATION 5

TITLE: METABOLOMIC CHARACTERIZATION AND FINE MAPPING OF QTLS ASSOCIATED WITH VOLATILE COMPOUNDS AND SUGARS FOR TOMATO FLAVOR IMPROVEMENT

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Abstract: Tomato (*Solanum lycopersicum*) is a globally consumed crop, and improving its organoleptic quality is a key objective in breeding programs. Flavor, a critical component of fruit quality, is influenced by volatile organic compounds (VOCs), sugars, and acids. In this study, we conducted fine mapping of quantitative trait loci (QTLs) associated with soluble solids (°Brix), benzyl alcohol, and 3-methylbutanoic acid. Introgression lines (ILs) derived from interspecific crosses between *S. lycopersicum* cv. Moneymaker and *S. pimpinellifolium* accession TO-937 were characterized using high-resolution genotyping techniques and metabolomic profiles assessed via gas chromatography/mass spectrometry (GC/MS). Fine mapping allowed the identification of genomic regions associated with QTLs for benzyl alcohol and °Brix on chromosome 9, revealing potential colocalization. Similarly, the QTL region associated with 3-methylbutanoic acid was mapped on chromosome 11. Metabolomic analyses highlighted clear differences between ILs carrying alleles from *S. pimpinellifolium* and *S. lycopersi-*

cum. Principal component analysis (PCA) of metabolic traits confirmed significant discrimination between samples. Heatmaps illustrating allele effects demonstrated positive contributions of *S. pimpinellifolium* alleles to VOCs and sugar content. In addition, interactions between QTLs, which play a key role in the expression of quantitative traits in tomatoes, such as VOC and sugar content, were studied. QTLs exhibited additive, dominant, or overdominant modes of action, depending on the contribution of each allele and the influence of heterozygotes. Furthermore, epistatic interactions between QTLs revealed how these loci combine to enhance, reduce, or have no significant effect on the levels of these compounds.These findings provide valuable insights into the genetic architecture of flavor-related traits in tomato and identify candidate genomic regions for breeding programs focused on improving organoleptic quality.

Acknowledgments: This work was funded by the Ministry of Higher Education, Science, and Technology (MESCYT) and the European HARNESSTOM project (Grant Agreement No. 101000716).

ORAL PRESENTATION 6

TITLE: SIKNOXII AND SIBIH1-LIKE GENES CONTROL THE COORDINATION BETWEEN FRUIT PIGMENTATION AND SOFTENING DURING RIPENING

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Abstract: Ripening, defined as a fruit's ability to change pigmentation and soften, is a distinct characteristic of fleshy fruits, which are an essential component of the human diet. This process determines economically significant fruit quality and preservability characteristics and has been extensively studied. These studies have indicated that the progression of the ripening process is tightly coordinated via hormonal and complex transcriptional networks. In our previous research, we found that the four tomato CLASS-II KNOX (TKN-II) genes are jointly involved in coordinating the ripening progression from internal locular tissue to the external pericarp domain. Recent studies in the tomato cultivar Micro-Tom demonstrated that TKN-II

proteins form heterodimers with three fruit-expressed tomato BLH1-like proteins (SLBLHL1). It was also shown that the triple blhl1 mutant shows fruit coloration phenotypes comparable to those observed in the tknII3 mutant fruits. Our experiments in a different cultivar, M82, support these observations while also demonstrating that in the high-order tknII mutant fruits, the delay of chlorophyll degradation is significantly stronger than in the triple blhl1 mutants. Moreover, we found that while the delay in pigmentation change of the high-order tknII mutants is strongly associated with the inhibition of pericarp softening, the pericarps of the triple blhl1 mutants become significantly softer than the control with the onset of ripening. These results suggest that TKN-II and fruit-expressed BLHL1 genes are likely involved in controlling the coordination between the two key processes defining fruit ripening: change in fruit pigmentation and progression of pericarp softening.

POSTER PRESENTATIONS

POSTER PRESENTATION 9

TITLE: REVISITING THE CRUCIAL ROLE OF *TOMATO ARGONAUTA7* (*SLAGO7*) IN PLANT DEVELOPMENT AND GROWTH

Authors: Barragán-Lozano T.[1], Lebrón R.[1], Jáquez-Gutiérrez M.[2], Fonseca R.[1], Ibañez T.[1], Atarés A.[2], Moreno V.[2], Yuste-Lisbona F.J.*[1], Lozano R.[1]

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Abstract: Gene regulation of post-transcriptional RNA silencing mediated by ARGONAUTE (AGO) proteins is a key process for plant growth and development. Phylogenetic analysis and expression profiles of the tomato (*Solanum lycopersicum* L.) AGO genes (SlAGO), grouped them into three major clades (i.e., SlAGO1/5/10, SlAGO2/3/7 and SlAGO4/6/15), whose members show tissue- and stage-specific profiles suggesting that SlAGO have different putative roles in tomato development. Among members of SlAGO gene family, SlAGO7 plays a crucial role in ensuring a proper development in tomato, either at vegetative and reproductive stages. Indeed, both Slago7 and CRISPR/Cas9 loss-of-function alleles result in a wiry phenotype characterized by severe stunted plant growth, needle-like leaves, poorly developed floral organs, and small, seedless fruits. Grafting experiments revealed that the roots of plants lacking SlAGO7 were underdeveloped compared to wild-type plants. However, this limited root development was

not responsible for the abnormal growth observed in mutant plants, indicating that SlAGO7 function is required throughout the entire development of the plant. This observation aligns with the preferential expression of SlAGO7 in meristematic tissues. Furthermore, it is noteworthy that although SlAGO7 is the member of the gene family with the lowest expression level, its role is essential for proper plant growth. Overall, our findings not only highlight the critical function of SlAGO7 but also underscore the challenge of modulating its expression to enhance growth and productivity in tomato crops.

Acknowledgments: This research was funded by grants PID2023-151867OB-C31 and PID2023-151867OB-C32 of the Spanish Ministry of Science and Innovation (MICI/AEI/FEDER, UE). TB-L is recipient of a PhD contract from the FPI Program of the Ministry of Science and Innovation (ref. PRE2020-096013).

POSTER PRESENTATION 10

TITLE: FLORAL ORGAN GROWTH REQUIRES SPECIFIC RIBOSOME BIOGENESIS REGULATED BY TOMATO *ЯUFD* LOCUS

Authors: Ortiz-Atienza A.[1], Lebrón R.[1], Capel J.[1], Moreno-García I.[2], Pineda B.[2], Moreno V.[2], Angosto T.*[1], Yuste-Lisbona F.J.[1], Lozano R.[1]

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Abstract: During floral development, specialized structures allowing reproduction success are generated. While the genetic and molecular mechanisms involving MADS-box and other transcriptional regulators have been well characterized, the contribution of ribosome biogenesis and rRNA modifications remains underexplored. Eukaryotic ribosomes, composed of two subunits forming the 80S ribosome, are assembled through a complex process that requires ribosomal proteins and biogenesis factors. rRNA undergoes extensive modifications, such as 2′-O-methylation and pseudouridylation, to enhance folding, assembly, and translation efficiency, though the functional roles of many ribosome modifications remain unclear. Ribosomes are essential players for plant development, as defects in their biogenesis lead to abnormalities in embryogenesis, germination, vegetative growth and flower formation. This study identified the unfinished flower development (*ufd*) mutation in tomato, which disrupts pre-rRNA processing and halts flower development while leaving vegetative growth unaffected. Northern blot hybridization analysis with specific RNA probes revealed that intermediate ribosomal pre-RNA levels were higher in reproductive tissues compared to vegetative tissues in both wild-type

and *ufd* mutant plants. Notably, the ITS1 probe detected a decrease in intermediate rRNA levels in the reproductive tissues of *ufd* floral buds. Ribosome profiling revealed identical sedimentation profiles of wild-type and *ufd* mutant leaves, but significant differences in floral buds, including increased levels of mature ribosomal subunits were detected. Further phenotype and molecular analysis of *ufd* and CRISPR/Cas9 loss-of-function alleles corroborates that UFD function is absolutely necessary for pre-rRNA processing. Taken together, our research underscores the key role of UFD in promoting flower-specific ribosome biogenesis, a process crucial for the development of reproductive organs in higher plants.

Acknowledgments: This research was supported by grants PID2023-151867OB-C31 and PID2023-151867OB-C32 funded by the Spanish Ministry of Science and Innovation (MICI/AEI/FEDER, UE).

POSTER PRESENTATION 11

TITLE: PIVOTAL ROLE OF TOMATO GASSHO RECEPTOR KINASE IN EMBRYO DEVELOPMENT AND SHOOT APICAL MERISTEM ACTIVITY

Authors: Fonseca R.[1], Quispe J.L.[1], Barragán-Lozano T.[1], Moreno-García I.[2], Pineda B.[2], Moreno V.[2], Yuste-Lisbona F.J.[1], Lozano R.*[1]

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Abstract: The epidermis is an essential barrier that provides protection against environmental challenges and maintains structural integrity during plant development. Despite the advances achieved in the characterization of Arabidopsis thaliana L. regulatory mechanisms governing epidermis differentiation, these remain poorly understood in crop species like tomato (*Solanum lycopersicum* L). Here, we describe the functional role of TOMATO GASSHO (TGSO) receptor kinase, a leucine-rich repeat receptor-like kinase (LRR-RLK), in embryonic epidermis and shoot apical meristem (SAM) differentiation by means of a reverse genetics strategy that included the characterization of a tgso mutant showing altered development of both organs. Both *tgso* and CRISPR/Cas9 knock out mutants exhibited SAM disorganization, cotyledon adhesion, and abnormal embryonic epidermis permeability associated with defects in cuticle formation. Mapping-by-sequencing identified a single nucleotide mutation in the GSO1 homologous gene of Arabidopsis as the causal factor of the mutant phenotype. Transcriptomic analyses revealed several differentially expressed genes (DEGs), including those related to cell wall biogenesis and cuticle biosynthesis. However, key protodermal regulators

were not differentially expressed, suggesting that TGSO functions in a distinct regulatory pathway. Comparative phylogenetic and structural analyses indicated that TGSO has diverged functionally from its paralogue TGSO-Like (TGSOL), which lacks a kinase domain. This work highlights TGSO as a pivotal regulator of epidermal differentiation and SAM organization in tomato, bridging embryonic and post-embryonic development. These findings broaden our knowledge of the role of LRR-RLK-mediated signaling in crop species development.

Acknowledgments: This research was funded by grants PID2023-151867OB-C31 and PID2023-151867OB-C32 of the Spanish Ministry of Science and Innovation (MICI/AEI/FEDER, UE). TB-L is recipient of a PhD contract from the FPI Program of the Ministry of Science and Innovation (ref. PRE2020-096013).

POSTER PRESENTATION 12

TITLE: ENHANCING NUTRITIONAL QUALITY OF TOMATOES UNDER DROUGHT STRESS THROUGH TRADITIONAL LANDRACES

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Abstract: Drought stress reduces tomato (*Solanum lycopersicum*) productivity; however, moderate stress can improve fruit quality, addressing one of modern agriculture's key challenges. Genetic erosion in tomatoes underscores the importance of locally adapted landraces in improving fruit quality under adverse conditions. This study evaluated two southeastern Spain landraces, Negro Yeste (NY), known for its dark-red fruits, and Verdal (V), which produces non-red ripe fruits, comparing their agronomic, physiological, and metabolic responses to the commercial variety Moneymaker (MM) under control and drought stress (50% reduced irrigation) over 86 days. While drought stress reduced fruit yield by ~40% in all varieties, its impact on nutritional quality was markedly superior in the landraces compared to MM. Stress accelerated fruit ripening in the landraces, which exhibited higher maturity indices than MM, marked by reduced titratable acidity while maintaining similar soluble solids content. Interestingly, ripe fruits of NY and V retained high chlorophyll levels, a feature absent in ripe MM fruits, and drought further increased chlorophyll content in both landraces. Metabolomic analysis revealed distinct carotenoid profiles: NY accumulated significantly higher carotenoid levels than MM under

drought, whereas V showed an absence of lycopene—the main carotenoid in ripe commercial tomatoes—highlighting its unique metabolic profile. Drought also enhanced ascorbic acid, phenolic compounds, and flavonoids in all varieties, with V showing the highest levels, particularly in flavonoids. Additionally, V accumulated macronutrients like Ca^{2} , Mg^{2} , and K , with concentrations significantly higher than in MM and NY under drought conditions. These findings underscore the potential of traditional tomato landraces to produce fruits with superior nutritional quality under drought stress, offering sustainable solutions for agriculture in Mediterranean climates.

Acknowledgments: This work was supported by TED2021-131400B-C31 and TED2021-131400B-C32 grants (MCIN/AEI/10.13039/501100011033 and European Union NextGenerationEU/PRTR) AGROSIMBIOSIS project (Biodiversity Foundation, Ministry for Ecological Transition and Demographic Challenge).

POSTER PRESENTATION 13

TITLE: CREATION OF BREEDING MATERIAL FOR CHERRY TOMATOES WITH DIFFERENT FRUIT COLORS AND HIGH CONTENT OF DRY SOLUBLE SUBSTANCES

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Abstract: This study explores the use of parental lines with diverse fruit colors to enhance organoleptic properties in cherry tomato F_1 hybrids, focusing on inheritance patterns of fruit color (FC) and total soluble solids (TSS). A diallel crossing scheme (9×9) was employed, utilizing parental lines with red (11.2–12.1°Bx), yellow (10.1°Bx), pink (11.1°Bx), light-yellow (9.4°Bx), orange (10.2°Bx), crimson (7.2°Bx), brown (11.5°Bx), and purple (9.1°Bx) fruit. Sixty-one F_1 hybrids were generated, including 36 red, 13 orange, and others with mixed colors. FC analysis revealed dominance of pink fruit in crimson × pink crosses and brown fruit in crimson × brown hybrids. Purple coloration required both parental lines carrying the trait, as single parent crosses failed to express it. TSS content in hybrids averaged 9.7°Bx (range: 8.3–13.4°Bx). Highest TSS levels were observed in pink × red, pink × brown and red × pink hybrids. Parent-specific TSS contributions varied: red (8.8–13.4°Bx), yellow (9.0–11.2°Bx), crimson (8.1–10.0°Bx), and brown (8.6–12.4°Bx). The analysis of the inheritance pattern of the soluble solids content in fruits showed that in 26.2% of the combinations, the trait corresponded to dominance and only in 9.8% to overdominance. However, when using paternal lines with higher soluble solids content, the frequency of combinations demonstrating dominance and overdominance effects increased to 30.7% and 23.1%, respectively. The results obtained demonstrate the possibility of improving the taste characteristics of the fruit by selecting the original parent forms of cherry tomatoes with different colors.

POSTER PRESENTATION 14

TITLE: GENETIC STRUCTURE OF YELLOW SHOULDER DISORDER TRAIT IN TOMATO (*SOLANUM LYCOPERSICUM*)

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Abstract: Yellow shoulder disorder (YSD) is a severe physiological disorder affecting tomato fruit development and ripening. Externally, it is manifested as a yellowish-decolorated area surrounding the peduncle at ripe stage, which is usually accompanied by irregular ripening areas inside the fruit that reduce drastically quality and marketability. YSD appearance and severity is multifactorial involving environmental (high temperature and sun radiation), nutritional (K deficit) and genetic (Uniform locus) triggering factors. A genetic study on YSD susceptibility in a RIL population between *S. lycopersicum* var. 'Moneymaker' (*lyc*) x *S. pimpinellifolium* acc. TO-937 (*pim*) identified 5 QTL with additive effects of different sign: <i>*ysd4.1*, *ysd8.1* and *ysd10.1* with *pim* effect and *ysd3*.1, and *ysd11.1* with *lyc* effect. Interestingly, neither the *lyc* nor the *pim* parental of the population presents susceptibility to YSD, however, the phenotype was present, with varying severity degree, in 40% of the RIL. Of the identified QTL, *ysd10.1* collocates with *Uniform*locus, where dominant *U pim* allele confers a green shoulder phenotype increasing YSD susceptibility and *ysd3.1* collocates with *fw3.2* and *cm3.2*, major QTL for fruit weight and cuticle mass respectively. The other three ones do not colocalize with previously reported tomato QTL. The isolated and combined effect of the identified QTL was estimated on

isogenic lines (IL) obtained from the same *lyc* x *pim* cross (IL-QTL) and their derived double-introgression lines obtained by directed crosses and marker assisted selection. The IL-QTL (*pim* introgressions in *lyc*-genetic background) revealed that in the genetic context of *lyc*, only lines carrying *ysd10.1*, which also developed green-shoulder phenotype, where susceptible to YSD. However, double introgression lines carrying the combination *ysd4.1+ysd10.1* or *ysd8.1+ysd10.1* presented an exacerbated YSD phenotype, confirming the additive effects.

Acknowledgments: Financed by AEI/PID2021-126604OB-C21, Science and Innovation Ministry (Spain) and Emergia Program DGP_EMEC_2023_00020 Junta de Andalucía (Spain).

POSTER PRESENTATION 15

TITLE: PHENOLIC CONTENT AND COLOR OF FRUIT CUTICLES OF *SOLANUM GALAPAGENSE* AND *S. CHEESMANIAE* WILD TOMATOES

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Abstract: Cuticle phenolics have been known to confer several desirable traits to the cuticle that have a positive impact on fruit quality; from mechanical resistance to UV photoprotection together with participating in the final red color of tomato. *Solanum cheesmaniae* and *Solanum galapagense* are two wild species that can be easily crossed with the domesticated tomato and, therefore, can be great sources of interesting traits. Analysis of mature fruit cuticle phenolics within these two species displayed notable differences. Whereas fruits from all the 26 accessions of *S. galapagense* studied had cuticles with a very high fraction of phenolics (mainly naringenin chalcone), notable higher than the domesticated tomato, within the 36 accessions of *S. cheesmaniae* analyzed, a remarkable variability for this trait was observed. Thus, regarding objective color measurement in the CIE L*C*h space color by using a colorimeter, isolated cuticles of fully-ripen fruits from all *S. galapagense* accessions had a lower color hue (h) value compared to those from the domesticated tomato. This corresponded to cuticles more orange due to the naringenin chalcone contribution to color. However, in *S. cheesmaniae* accessions cuticles varied from very low fraction of phenolics, and hence a high h, to phenolic contents higher

than the domesticated tomato, so displaying a much lower h. An interspecific *S. galapagense* x *S. cheesmaniae* F₂ population derived from the cross of two accessions deeply contrasting their phenolic contents and color hues was developed. Phenotyping in the F₂ population of phenolic content and objective color parameters of isolated fruit cuticles along with genotyping with SPET markers are currently ongoing to study QTLs related to these traits.

Acknowledgments: Financed by AEI/PID2021-126604OB-C21, Science and Innovation Ministry (Spain) and Emergia Program DGP_EMEC_2023_00020 Junta de Andalucía (Spain).

POSTER PRESENTATION 16

TITLE: MULTI-TRAIT ANALYSIS OF TOMATO HYBRIDS REVEALS PROMISING GENOTYPES FOR FRUIT QUALITY IMPROVEMENT

Authors: Marovska G.*, Asenova V., Stoeva V., Grozeva S., Ganeva D., Tringovska I.

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Abstract: The assessment of fruit quality traits in breeding programs is critical for the selection of superior cultivars. This study evaluated a set of tomato hybrids and their parental lines for a range of physico-chemical and biochemical fruit quality traits. A total of 17 hybrids, suitable for fresh market, derived from controlled crosses among 13 parental lines, were grown in two consecutive years (2023 and 2024). Key fruit quality parameters, including dry matter content (DM), soluble solids content (TSS), titratable acidity (TA), firmness, and fruit color, vitamin C content, antioxidant capacity (FRAP), and total polyphenols were measured. Significant variation was observed among hybrids for all traits, indicating the presence of substantial genetic diversity. Hybrid 171×174 consistently exhibited superior performance across multiple traits, including TSS (5.7 °Brix), dry matter (7.0%), vitamin C (43.0 mg/100g), total polyphenols (749.5 mg GAE/100 g FW), firmness (24.8 Shore degrees) and fruit color (redness, a* = 27.7) suggesting its potential as a high-quality hybrid in terms of both nutritional value and taste. Other promising hybrids such as 343×352, 347×352 and 326×185 also showed favourable profiles for antioxidant content, firmness, and acidity. Selection of the best-performing hybrids was primarily based on trait values rather than heterosis alone. However, mid-parent heterosis estimates supported hybrid vigour in several

crosses, particularly in traits such as antioxidant activity, vitamin C, TA, DM and firmness. The study underscores the importance of multi-trait evaluation in identifying hybrids that meet both nutritional and sensory quality standards. These findings provide valuable insights for tomato breeding efforts in Bulgaria and offer a solid foundation for selection of elite genotypes well-suited to local growing conditions, market demands, and future genetic improvement programs.

Acknowledgments: The Research leading to this results has received funding from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

POSTER PRESENTATION 17

TITLE: TOMATO BREEDING IN BULGARIA AT MARITSA VEGETABLE CROPS RESEARCH INSTITUTE: TRADITION, INNOVATION, AND SUSTAINABILITY

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Abstract: The tomato breeding program at the Maritsa Vegetable Crops Research Institute (MVCRI) has a long and rich history, continuously evolving to meet the challenges and opportunities of modern agriculture. Bulgaria possesses a valuable tomato genetic resources that includes local landraces, traditional heirloom varieties, and modern breeding lines characterized by genetic resistance or tolerance to a broad spectrum of biotic and abiotic stress factors, as well as high adaptability to the country's diverse soil and climatic conditions. The Institute maintains an extensive collection of over 2,000 accessions, most of them thoroughly characterized both phenotypically and genotypically. This rich genetic pool is actively utilized in contemporary breeding programs focused on developing new varieties and hybrids. The efforts of breeders are directed toward sustainability, high productivity, environmental responsibility, and alignment with both local and global agricultural trends. Modern tomato breeding in MVCRI also aims to improve local and heirloom varieties in terms of yield and resistance to various stresses, preserving their excellent fruit quality and thus ensuring their continued relevance and competitiveness. Breeding at the Institute combines classical methods such as hybridization and individual selection with advanced biotechnological tools, including molecular marker-assisted selection, significantly enhancing overall efficiency. One of the big achievements of Bulgarian breeding

is the exceptional quality of tomato fruits, which combine high nutritional value with outstanding taste and aroma. These ongoing efforts ensure that Bulgarian tomato varieties such as *Kopnezh* F$_1$, *Rozovo sartse*, *Aleno sartse*, *Dara*, *Rozovo Siyanie*, IZK *Alya*, *Mini miss*, *Salzitsa*, *Slantse*, *Plovdivska karotina*, and many others continue to be highly valued on both local and international markets. Their popularity stems not only from their agronomic qualities but also from their ability to meet the growing consumer demand for healthy and flavourful food.

Acknowledgments: The Research leading to this results has received funding from the Bulgarian Agricultural Academy project ZEMDKT 17. The authors acknowledge the support from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

POSTER PRESENTATION 18

TITLE: GEOSEMSELECT: PIONEERING PRIVATE TOMATO BREEDING IN BULGARIA

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Abstract: Geosemselect is the first private company in Bulgaria devoted exclusively to tomato breeding and seed production. Established in 1990 by Prof. Hristo Georgiev – an internationally recognized tomato breeder – the company has built on a strong foundation of genetic expertise and dedication to fruit quality. Today, as a family-owned enterprise, Geosemselect continues to advance Prof. Georgiev's legacy by combining classical breeding techniques with modern molecular tools, with a clear focus on flavor, earliness, and adaptation to diverse growing conditions. The breeding program serves both hobby growers and market-oriented producers, offering varieties tailored to the needs of small-scale and semi-professional cultivation. Long-standing hybrids such as Rila F_1 and Kalina F_1 are staples among Bulgarian growers, while Prekos F_1 and Parris F_1 – extremely early semi-determinate varieties–have become reference standards for quality in Romania. Rugby F_1, known for its distinctive shape, color, taste, and broad adaptability, has achieved commercial success in both domestic and international markets. Geosemselect's strategic direction includes maintaining the identity and excellence of Bulgarian tomato breeding while expanding its presence in Central Europe, the Balkans, and the United States. Current efforts focus on integrating marker-assisted selection and resistance breeding without compromising fruit flavor–a hallmark of the company's varieties. By aligning traditional strengths with cutting-edge technologies, Geosemselect contributes to the development of competitive, high-quality tomato germplasm for diverse agroecological and market needs.

Session:

BREEDING FOR BIOTIC STRESS RESISTANCE

KEYNOTE TALK 3

TITLE: SEQUENTIAL BREAKDOWN OF LEAF MOULD RESISTANCE IN TOMATO BY *FULVIA FULVA* AND APPROACHES FOR DURABILITY

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Abstract: Leaf mould, caused by *Fulvia fulva*, is a devastating disease of tomato plants. In many commercial tomato cultivars, resistance to this disease is governed by dominant *Cf* genes that encode receptor-like proteins (RLPs). The most widely deployed resistance locus, *Cf-9*, encodes five RLPs, of which two confer resistance: Cf-9C recognises the *F. fulva* effector Avr9 and provides resistance during all plant growth stages, while Cf-9B recognises the effector Avr9B and provides mature plant resistance only. In recent years, *F. fulva* strains have emerged that have sequentially broken down resistance conferred by the *Cf-9* locus,

which has prompted breeders to deploy the novel resistance locus, *Cf-6*. To date, the identity of *Cf-6*, as well as the gene encoding the matching Avr6 effector from *F. fulva* have remained unknown. Strikingly, however, *Cf-6*-mediated resistance already appears to have been overcome in several locations worldwide. Using whole genome sequencing of *Cf-6*-breaking *F. fulva* strains, we revealed that the known secreted effector Ecp5 is Avr6. Using Avr6-triggered hypersensitive responsiveness as a marker for disease resistance, we mapped the *Cf-6* locus to the short arm of chromosome 12 in tomato. In this talk, I will present the sequential breakdown of resistance conferred by *Cf-9* and *Cf*-6, and discuss new resistance sources for durable leaf mould resistance in tomato.

ORAL PRESENTATIONS

ORAL PRESENTATION 7

TITLE: ANALYZING THE IMPACT OF TOMATO LINES CONTAINING VARIOUS TY GENE COMBINATIONS ON ATTAINING RESISTANCE AGAINST DIVERSE TOMATO YELLOW LEAF CURL VIRUS STRAINS

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Abstract: Tomato yellow leaf curl disease (TYLCD), caused by begomoviruses, poses a major challenge to tomato production in tropical and subtropical regions. Symptoms include leaf curling, yellowing, mottling, and reduced plant size, with severe cases leading to stunted growth and potential yield loss, especially when infections occur early. Without resistant varieties, farmers often resort to frequent insecticide applications to manage whitefly vectors in open-field production. Begomoviruses' rapid spread and evolution complicate control efforts, with over 70 species infecting tomatoes globally. Many begomoviruses spread beyond their native regions, displacing or recombining with local strains and thereby increasing TYLCD incidence and severity.

Six primary resistance genes (Ty genes) are available for TYLCD, allowing for the pyramiding of multiple Ty genes in a single variety, which can provide stronger and more durable resistance. The efficacy of individual and combined Ty genes was evaluated through multilocation trials in disease hotspots. In collaboration with research partners, 11 tomato lines with homozygous Ty gene combinations were tested under various conditions. Lines containing one or two Ty genes exhibited reduced TYLCD symptoms compared to those without Ty genes, with significant field resistance observed in lines with TY1, TY3, and TY3a combinations. Collaboration with the University of Valencia (Spain) confirmed that the TY2 gene provides resistance to the TYLCV-IS76 strain. Additionally, lines containing only ty5 and TY6 genes demonstrated tolerance similar to that of lines with TY1/3. Another trial in Varanasi, India—a hotspot for Tomato leaf curl New Delhi virus (ToLCNDV) infections—found that lines with Ty-1 or ty5+Ty6 exhibited moderate resistance, while lines with Ty3a+Ty-2 or TY3+TY2, with or without ty5, showed strong resistance and slower symptom progression. This finding emphasizes the importance of combining resistance markers, as additional components like ty5 can significantly enhance resistance.

ORAL PRESENTATION 8

TITLE: TRADE-OFF BETWEEN TOMATO FERTILITY AND VIRUS RESISTANCE

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Abstract: The tomato industry faces a significant challenge in adapting to changing climatic conditions. Rising temperatures exacerbate the incidence of plant diseases caused by viruses such as *Tomato yellow leaf curl virus* (TYLCV), a single-stranded DNA begomovirus transmitted by whiteflies, which can result in crop losses of up to 100%. Efforts to protect cultivated tomatoes against TYLCV have largely focused on the dominant resistance genes, Ty-1/Ty-3. However, while Ty-1/Ty-3-based resistance can prevent disease symptoms, it still permits viral accumulation, leading to strong selective pressure and enabling TYLCV to overcome this resistance in the field. Consequently, anti-TYLCV strategies should incorporate other resistance genes, such as ty-5, a recessive resistance allele mapped to the PELOTA (PELO) gene, which is among the strongest TYLCV resistance alleles. Our research demonstrates that while pelo loss-of-function mutants exhibit significant resistance to TYLCV, they suffer from severe yield loss due to compromised fertility. Therefore, deepening our understanding of PELO's role in balancing plant development and stress response is crucial for developing more resilient tomato cultivars. Utilizing CRISPR-Cas9 technology, we have created tomato lines with varying levels of PELO expression to fine-tune its expression and strike a balance between enhancing viral resistance and maintaining plant fertility. Additionally, we are uncovering new insights into the molecular mechanisms through which PELO regulates plant fertility and virus accumulation.

ORAL PRESENTATION 9

TITLE: NEW SOURCES FOR RESISTANCE TO IMPORTANT TOMATO DISEASES AND NEMATODE

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Abstract: The last two decades after screening many cultivated tomato lines having in their pedigree wild species (*Solanum peruvianum*, *S. chilense*, *S. habrochaites*, *S. pennellii* and *S. pimpinellifolium*) new resistances to the following pathogens have been found: *Verticilium dahliae* race 2 - Lines 505 and 501 showed the same level of resistance as our previously reported Veda line - the only source for resistance to race 2 of the pathogen. *Pyrenochaeta lycopersici* - We selected two lines - Violet 270 and Violet 280, without root symptoms like our line Pirelly with dominant resistance to Corky root. *Fusarium oxysporum* f. sp. *lycopersici* race 3 - Several lines were found resistant to the California isolate of the fungus. *Fusarum oxysporum* f. sp. *radicis – lycopersici* - New sources of resistance, different from the Japanese IRB - 301 were selected. *Fusarium falciforme* - Resistance to this new *Fusarium* in California was found in lines having two wild species in their pedigree. *Phytophthora capsici* - Lines Viola 1170 and Gilroy 30 showed high level of resistance to *Ph. root rot. Botrytis cinerea* - Lines Ninel and Boryana were selected for their dominant resistance to stem infection and tolerance to leaf and fruit infection. After screening lines with bacterial pathogens - *Clavibacter michiganensis* sp. *michiganensis*, *Pseudomonas syringae* pv. *tomato* and *Xanthomonas vesicatoria* we found resistance or tolerance to all three pathogens. Special attention was paid to searching resistance to *Sw*-5 breaking strain of *Tomato Spotted Wilt Virus*. Lines with one, two and three wild species in their pedigree were screened and high level of resistance were found in lines Feres, Bogi and Komo. *Meloidogyne incognita* - Lines Namaste and Fortara were selected for their resistance to the *Mi*-1 breaking strain of the nematode.

ORAL PRESENTATION 10

TITLE: THE *TOBAMOVIRUS* RESISTANCE GENE *TM-1* CONTRIBUTION IN CONTROLLING TOBRFV RESISTANCE IN TOMATO

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Abstract: *Tomato brown rugose fruit virus* (ToBRFV), a newly identified *Tobamovirus*, is a rapidly spreading pathogen posing a significant threat to tomato production worldwide. ToBRFV-infected tomato plants show a mosaic pattern on leaves accompanied by narrowing of leaves and yellow-spotted fruit. ToBRFV induces severe losses due to reduction in plant vigor and fruit quality and is able to overcome the tobamovirus resistance gene *Tm-22*. Thus, the virus has become a major limiting factor for the production of fresh-market tomatoes. We have previously identified a locus on tomato chromosome 11 controlling tolerance to the virus. We further established that combining this locus with one that maps to the Tm-1 region on chromosome 2 confers resistance to the virus. Here we sought to determine whether, and how, the *Tm-1* gene itself is involved in ToBRFV resistance. Overexpression of *Tm-1* in a tolerant genotype significantly reduced viral accumulation, conferring resistance to ToBRFV. On the other hand, overexpression of *Tm-1* in a susceptible genotype only delayed symptom appearance. Moreover, effective RNAi-silencing of *Tm-1* in the resistant genotype yielded susceptible plants. These findings show that the *Tm-1* gene interacts genetically with the locus controlling tolerance on chromosome 11 and that this interaction is critical for achieving effective resistance to ToBRFV.

ORAL PRESENTATION 11

TITLE: DETERMINATION OF *TORRADO TOMATO VIRUS* AND *TOMATO SPOTTED WILT* VIRUS RESISTANCE IN TOMATO LINES AT DIFFERENT BREEDING STAGES

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Abstract: Tomato (*Solanum lycopersicum* L.) originated in South America. Tomato plants show wide climatic tolerance and are grown in both tropical and temperate regions around the world, in open-field or greenhouses. Tomato production is highly affected by viral diseases. *Tomato spotted wilt virus* (TSWV) causes major economic losses globally due to an extremely wide host range involving more than 900 species of plants in 90 families that include important ornamental, fruit, and vegetable crops such as tomato. The most commonly used resistance gene in tomato is the Sw-5 located on chromosome 9. The gene originates from wild relatives of tomato *Solanum peruvianum*. Many commercial tomato varieties have been released containing this resistance gene. Several viral species belonging to different families emerge as new viruses of tomatoes. These are *Tomato torrado virus* (ToTV) (Torradovirus, Secoviridae) spread in Spain, Canary Islands, Panama, Poland, Hungary, and Australia. The presence of the virus in Türkiye has not yet been reported. However, ToTV is an emerging disease of tomato crops, the EPPO Panel on Phytosanitary Measures recommended its addition to the Alert List. The symptoms of ToTV resemble those caused by *Tomato spotted wilt virus* (TSWV) and *Tobacco streak virus* (TSV), but samples from symptomatic plants tested negative for these and other known tomato viruses. Initial disease symptoms consist of necrotic spots at the base of the leaflets. At a later stage, leaves, stems, and fruits can display severe necrosis in tomato plants. Tightly

linked molecular markers associated with resistance genes improve selection efficiency as an independent of environmental conditions. In the study, tested 96 genotypes (60 genotypes in F4 determinate, suitable for fresh consume tomato lines, 30 F3 determinate, with pink skin, beef type, suitable for fresh consume tomato lines and 4 commercial registered varieties (2 open pollinated variety, 2 hybrid variety) and controls tested with a simple probe-based molecular markers. in a real time PCR.

POSTER PRESENTATIONS

POSTER PRESENTATION 19

TITLE: PRESERVING TRADITION AND RESILIENCE: ACCELERATED BREEDING FOR MULTI-RESISTANT *TOMATA VALENCIANA* VARIETIES

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Abstract: The *'Tomata Valenciana'* (*Solanum lycopersicum*) is a traditional tomato variety highly valued in the Mediterranean region of Valencia, Spain, for its exceptional flavor, texture, and quality. However, its cultivation has been increasingly challenged by biological threats, including diseases such as verticillium wilt, downy mildew, and tomato mosaic virus (ToMV), alongside pests like root-knot nematodes (*Meloidogyne* spp.) and whiteflies. These challenges underscore the urgent need for integrated pest management strategies and the development of resistant varieties. This project focuses on a speed breeding program to develop multi-resistant varieties of *'Tomata Valenciana'* by incorporating resistance genes for ToMV, *Fusarium oxysporum* f. sp. *lycopersici*, and *Meloidogyne* spp. The breeding strategy is based on classical backcrossing combined with molecular marker-assisted selection to accelerate the introgression of resistance alleles. Additionally, genotyping will ensure the preservation of the *'Tomata Valenciana'* genetic background by selecting plants with the highest genomic similarity to the original variety. After obtaining the F_1 and making the first backcross with the *'Tomata Valenciana'* recurrent parent, a total of 300 BC_1

seeds were sown, and 250 of those seeds were taken to the field. The 250 BC_1 plants were genotyped using molecular markers to identify individuals carrying resistance alleles against the three pathogens. Finally, 19 individuals were selected, along with three samples from each progenitor and three F_1 plants, and subjected to bulk genotyping to select according to the genetic background recovered from the donor parent. This approach aims to streamline breeding cycles, maintain the genetic identity of this traditional variety, and safeguard its cultural and agricultural significance in the face of modern challenges.

POSTER PRESENTATION 20

TITLE: EVALUATION OF BULGARIAN TOMATO VARIETIES, LINES AND HYBRIDS FOR RESISTANCE TO FUNGAL PATHOGENS

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Abstract: The analysis of breeding genetic materials for disease resistance is a complex process that includes annual monitoring of field (horizontal) resistance (phenotypic selection) and selection of promising lines on an artificially created infectious background (genotypic selection). The aim of this work is to select donors based on resistance to fungal pathogens common in Bulgaria and to create varieties with complex resistance. During the period 2019-2021, as a result of phytopathological observations of tomato collection lines, varieties and hybrids with different production directions, samples with horizontal complex resistance to tomato late blight (*Phytophthora infestans*) and tomato early blight (*Alternaria solani*) were selected. In field conditions, the ratio of resistant to susceptible F1 hybrids is 2.5:1, and for lines 2:1. The resistance of promising lines, varieties and hybrids of tomatoes to *Phytophthora infestans*, *Alternaria solani*, *Fusarium oxysporum* and *Cladosporium fulvum* was additionally assessed on an artificial infectious background using classical express methods - "detached leaf assay" and "in infected soil". It has been established that the tomato varieties "Ideal", "Milyana", "Prometey", "IZK Alya" and "Plovdivska karotina" are weakly susceptible to the F0 and F1 races of *Fusarium oxysporum* f. sp *lycopersici* and the aerogenic pathogens *Cladosporium fulvum* and *Alternaria solani*. The variety "Prometey" has the highest resistance to the F0 and F1 races of *Fusarium oxysporum*, with a disease incubation period of 25 days af-

ter infection with the F0 race and 12 days after infection with the F1 race of the pathogen. The percentage of diseased plants is 2.67% and 7.40%, respectively. The resistance of the varieties to *Phytophthora infestans*, *Cladosporium fulvum* and *Alternaria solani* is expressed in a hypersensitivity reaction and lack of sporulation of the pathogen. Based on the conducted studies, the resistance of the varieties to the main fungal diseases of tomatoes – tomato late blight, tomato early blight, tomato leaf mold and fusarium wilt has been established.

Acknowledgments: The Research leading to this results has received funding from the Bulgarian Agricultural Academy project ZEMDKT 17. NK acknowledge the support by the Bulgarian Ministry of Education and Science under the National Program "Young Scientist and Postdoctoral Students – 2".

POSTER PRESENTATION 21

TITLE: SCREENING A COLLECTION OF TOMATO HYBRIDS FOR INFESTATION BY PESTS UNDER FIELD CONDITIONS

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Abstract: Tomato is a crop that is infested by a large number of pests throughout the growing season from seedling production to harvest. An interesting alternative to minimize the problems arising from the application of chemical insecticides and maintain pests populations below the thresholds of economic harm is the cultivation of tomato plants that show resistance to pests and mites. An assessment for pest infestation of a collection containing 152 hybrids of tomato grown in the field was made in a natural background. Differences in the response of the studied hybrids to the pests were observed. Test pests included in the study: tomato leaf miner (*Tuta absoluta* Meyrick), thrips (*Frankliniella occidentalis* Perg. and *Thrips tabaci* Lindeman), twospotted spider mite (*Tetranychus urticae* Koch.), greenhouse whitefly (*Trialeurodes vaporariorum* Westw.), cotton bollworm (*Helicoverpa armigera* Hubn.). Comparatively low infestation by the five pests were established in the following twelve hybrids: LYC-16 x LYC-3; LYC-22 x LYC-1; LYC-22 x LYC-23; LYC-22 x LYC-171; LYC-23 x Prometey; LYC-24 x LYC-3; LYC-49 x LYC-150; LYC-50 x LYC-152; LYC-51 x Prometey; LYC-117 x 178 GPT; LYC-121 x 178 GPT; LYC-152 x Prometey. The hybrids LYC-51 x Prometey and LYC-117 x 178 GPT are free of pests infestation. The highest percentage in these hybrids was recorded in the group of Indeterminate, high-rounded, for processing (50%), followed by the group Cherry type (25%), Indeterminate, round, fresh consumption

(17%) and Determinate, for processing, high- rounded (8%). These tomato hybrids are of interest for future breeding programs as an eventual source of complex resistance to pests.

Acknowledgment: The authors acknowledge the support from EU Horizon 2020 Teaming Project PlantaSYST (Grant 739582).

POSTER PRESENTATION 22

TITLE: RESPONSE OF YOUNG TOMATO PLANTS TO FERTILIZATION UNDER *TOMATO BROWN RUGOSE FRUIT VIRUS* (*TOBRFV)* INFECTION: GROWTH, NUTRIENT UPTAKE, AND PHENOLIC CONTENT

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Abstract: *Tomato brown rugose fruit virus* (*ToBRFV*), a *Tobamovirus* member, cause a significant threat to tomato crops worldwide. While fertilizers do not affect viral spread, proper use can improve crop quality, resilience and symptom tolerance. The present study aimed to assess the impact of mineral nutrition on three different tomato varieties infected with *ToBRFV*. The varieties tested differ in the absence or presence of ToMV resistance genes (Tm1 and Tm2^2). Plants were mechanically inoculated at the first true leaf stage and grown until flowering under four different fertilization treatments (0, 0.5, 1, and 2 times the optimal dose), alongside a control group of non-inoculated plants. Plant growth parameters, macronutrient (N, Na, Mg, Ca, K) accumulation, chlorophyll content, and total phenolic content were evaluated. *ToBRFV* infection was confirmed in the three tested varieties 30 days post-inoculation with distinct symptoms and changes in morphology, chlorophyll levels and phenolic content. The findings suggest that fertilization improved growth performance in *ToBRFV*-infected tomato plants as shown by increased biomass, height, and leaf number. While macronutrient accumulation remained largely unaffected by infection status, the chlorophyll content and total phenolic content

were significantly influenced by both fertilization and viral infection. Chlorophyll content remained lower in *ToBRFV* infected plants, while total phenolic content was increased in inoculated plants under 0.5× and 1× fertilization treatments, with the highest levels at 0× and the lowest at 2× fertilization. These findings highlighted the complex interaction between viral stress and mineral nutrition, suggesting that low fertilization may enhance defense-related phenolic responses, while higher fertilization supports vegetative growth.

Acknowledgment: The authors acknowledge the support from the Bulgarian Agricultural Academy, project ZEMDKT 17.

POSTER PRESENTATION 23

TITLE: SCREENING OF $F_1 \times F_1$ TOMATO CROSSES FOR *TOMATO MOSAIC VIRUS* AND *TOMATO SPOTTED WILT VIRUS* RESISTANCE

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Abstract: *Tomato mosaic virus* (ToMV) and *Tomato spotted wilt virus* (TSWV) are among the most common threats to local tomato landraces. The aim of this study was to screen tomato hybrid populations with local germplasm to identify resistance to these pathogens. The experiment was carried out using 42 $F_1 \times F_1$ red and 16 $F_1 \times F_1$ pink tomato crosses. Line 1627, carrying the *Sw-5* gene was used as a source of TSWV resistance in red tomato crosses, while line 19, carrying $Tm2^2$ was used as a donor of ToMV resistance in pink tomato crosses. The screening was conducted by codominant markers Sw-5–2 and NCTm-019 for *Sw-5* and $Tm2^2$, respectively. Stable TSWV resistance (*Sw-5*/*Sw-5*) was found in 38 individuals belonging to 15 red tomato crosses. Homozygous ToMV resistance ($Tm2^2$) was identified in six individuals from six pink tomato crosses. Individual resistant plants with the highest productivity and average fruit weight (g) were selected and will be used for subsequent selection and crosses, focusing on agronomic, morphological and biochemical traits.

Acknowledgment: The authors acknowledge the support from the Bulgarian Agricultural Academy, project ZEMDKT 17.

POSTER PRESENTATION 24

TITLE: DEVELOPMENT OF A MAPPING POPULATION DERIVED FROM *SOLANUM PENNELLII* (LA0716) FOR THE IDENTIFICATION OF QTLS CONFERRING TOLERANCE TO TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* (ACARI: *TETRANYCHIDAE*) IN THE TOMATO GENOME

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Abstract: Tomato is one of the most cultivated vegetables in the world. Although tomato is cultivated in large areas with diverse climate and soil types due to high adaptation capacity, biotic and abiotic stresses effect tomato production negatively. Two spotted spider mite *Tetranychus urticae* (Acari: *Tetranychidae*) is among the most important pests. The pest is widespread in tomato cultivation areas, kills the effected plants and reduce tomato production. Breeding for *T. urticae* tolerance breeding is difficult due to quantitative nature of the trait. Initial studies performed by project team revealed that *Solanum pennellii* (LA0716) has great potential for *T. urticae* tolerance. Mapping quantitative trait loci (QTLs) originating from *Solanum pennellii* (LA0716) in the tomato genome is essential for development of mo-

lecular breeding tools for two spotted spider mite tolerance. The initial phase of such mapping studies involves the development of mapping populations. In this context, the present study describes the creation of an F_2 mapping population resulting from a cross between the tolerant *S. pennellii* (LA0716) and the susceptible AK2082 (*S. lycopersicum)*. The population will be genotyped using Genotyping-by-Sequencing (GBS). The assessment of *Tetranychus urticae* adult preferences in the F_2 population will be conducted through the simultaneous implementation of two different experimental methods. A free-choice' assay will be employed to evaluate plant resistance based on the mites' preference or avoidance. In this method, 200 female adult mites (1–2 days old) will be released in the center of tray with a soft brush, allowing them to interact freely with all genotypes. Following data collection, a QTL mapping approach will be used to identify loci associated with *T. urticae* tolerance. The QTL map constructed in this project will provide valuable molecular resource for Marker-Assisted Selection (MAS), facilitating the development of tomato cultivars with enhanced resistance to *T. urticae*.

Acknowledgements: This project is supported by The Scientific and Technological Research Council of Turkey (TUBITAK) under project number 124O825.

Session:

BREEDING FOR ABIOTIC STRESS TOLERANCE

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KEYNOTE TALK 4

TITLE: DISSECTING THE CORE FACTORS INVOLVED IN THE ACCLIMATION CAPACITY OF TOMATO TO HEAT

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Abstract: Tomato is highly sensitive to elevated temperatures, particularly during reproductive development. As climate change increases the frequency and severity of heat stress episodes, improving thermotolerance has become a key breeding objective. Our group explores the core molecular, cellular, and physiological mechanisms underlying the acclimation capacity of tomato to heat stress. We identify key regulatory nodes such as Heat Shock Factors (HSFs) and chaperone networks that orchestrate protective responses during both acute and sustained heat exposure. In this talk, I will demonstrate how specific HSFs contribute to thermotolerance through dynamic interactions with co-regulators, enabling rapid and sustained activation of heat-responsive genes, as well as how they contribute to the timely attenuation of the response during recovery from the stress. I will also highlight how these regulatory circuits differ across tissues and developmental stages, providing insights into core mechanisms for thermotolerance in reproductive tissues. Understanding these core mechanisms provides a framework for targeted strategies to enhance heat resilience in tomato through breeding or biotechnological approaches.

INVITED TALKS

INVITED TALK 1

TITLE: FLOWER BUD COOLING PROTECTS PLANT REPRODUCTION AGAINST HEATWAVES

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Abstract: Pollen development is a critical bottleneck for plant fertility under heat stress. We have identified natural variation in pollen heat tolerance across several species and mapped loci associated with this trait. One such locus, *qPV11* in tomato, enhances pollen viability during heatwaves by promoting stomatal opening on sepals, which facilitates transpirational cooling of developing flower buds. This flower bud cooling mechanism appears to be conserved in other plant species as well. Our findings provide valuable genetic targets for breeding heat-resilient crops, contributing to improved agricultural productivity under climate change.

INVITED TALK 2

TITLE: DISSECTING GENES CONTROLLING STIGMA POSITION IN CULTIVATED TOMATO BY OMIC ANALYSES OF CONTRASTING LANDRACES

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Abstract: Domesticated tomato (*Solanum lycopersicum* L.) is characterized by a pistil that is usually shorter than the staminal cone, resulting in an inserted stigma position (SP). Inserted SP ensures fertility and autogamy and is a fixed trait in modern breeding material. However, several traditional and vintage genotypes show an SP at the level of the staminal cone or even slightly exerted. Moreover, also flowers with inserted SP can undergo stigma exertion as a response to high temperatures. Shift in stigma position thus represents an important breeding issue because the SP exsertion increases cross-pollination, generates fertility problems, and decreases crop yield. Identification of genetic and physiological mechanisms controlling SP is desirable to aid breeding of more productive and resilient varieties. Here, we perform differential analyses on two landraces phenotypically similar, but contrasting for having stably inserted (Scatolone di Bolsena, SCA) or exserted (Marmande ancienne, MAR) SP. F_2 plants segregating from the SCA x MAR cross were pooled to obtain an "inserted" and "exserted" DNA bulk. Resequencing (20X) of the bulk samples and of the parental lines yielded a SNP dataset of ≈400 k. Data analysis by the QTLseqR package showed significant polymorphic regions between bulks on Chr03, 05, and 11. Several of the SNP identified co-localized in QTL regions obtained from previous GWAS analyses. In addition, SCA and MAR stigma/style samples dissected from developing pistils were

analysed by RNAseq. Overall, 1840 differentially expressed genes were found, of which 842 up- and 998 down-regulated in the exerted genotype. Enriched biological process categories were found for genes involved in “response to hormones” (“response to auxin” overall), “anion transport” and “cell wall”, among others. Intersect analysis of the results of the different approaches, as well as quantitative validation of candidate genes will be presented to gain novel insights on SP control in cultivated tomato.

INVITED TALK 3

TITLE: EXPLORING THE IMPACT OF LIGHT QUALITY ON ONE *S. PENNELLII* INTROGRESSION LINE

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Abstract: Climate change represents a significant challenge for Mediterranean agriculture, necessitating the implementation of effective solutions and the diversification of crop varieties. A promising approach involves the identification of tomato cultivars capable of thriving within the context of intercropping systems (IC), a practice involving the cultivation of two crops in close proximity. Since leaves and other photosynthetic tissues absorb red light (R) but not far-red light (FR), under IC the light reflected from the leaves of neighbouring plants has a much lower R/FR ratio than direct sunlight. This low R/FR signal in shade-avoider plants results in elongated growth, increased apical dominance, reduced leaf number, reduced leaf size, premature flowering and degradation of photosynthetic pigments, including carotenoids. Here the response to white (W) + FR of different tomato genotypes at early stages of development was compared allowing the identification of one *S. pennellii* introgression line with a shade-tolerant phenotype. Another experiment conducted during long-term exposure to W + FR up to the fruit-bearing stage, confirmed the shade-tolerant phenotype of the introgression line. It was also demonstrated

that the W + FR treatment improved tomato fruit quality by increasing °Brix and accumulation of ascorbic acid and carotenoids in both IL and the parental line M82. Field trials under high density conditions further confirmed the shade-hyposensitive phenotype of the introgression line that also showed improved fruit yield. Transcriptomic investigations are under way that will allow for a more comprehensive characterisation of the response exhibited by the identified introgression line.

INVITED TALK 4

TITLE: PERSPECTIVES ON HARNESSING EPIGENETIC VARIATION FOR HEAT-STRESS RESILIENCE IN TOMATO

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Abstract: Heat stress is a major environmental factor limiting crop productivity, posing a significant threat to global food security. Various approaches are taken in order to develop crops with enhanced tolerance to heat stress conditions, for this aim, understanding the molecular mechanisms underlying heat stress tolerance is essential. Since epigenetic mechanisms, including DNA methylation, were shown to play a regulatory role in modulating plant responses to environmental stresses, we set to investigate the involvement of DNA methylation in the response to heat stress in tomato (*Solanum lycopersicum*), a major vegetable crop. We utilized the *ddm1a* and *ddm1b* DNA methylation-deficient mutants. Despite minimal phenotypic effects under normal conditions, both *ddm1* mutants exhibited enhanced seedling survival under a short-term heat stress. However, under Moderate Chronic Heat Stress conditions (MCHS), detailed phenotypic and transcriptomic characterization revealed notable differences between the *ddm1a* and *ddm1b* lines. In addition, the *ddm1b* line presented higher fruit set and seed set rates. In search for genes underling the heat stress tolerance of this line, we carried out a transcriptomic analysis, which highlighted a unique transcriptional response. Coupled with DNA methylation analyses, we identified several Differentially Methylated Regions

(DMRs), including in the promoter of a specific heat stress transcription factor that was transcriptionally upregulated in the mutant under normal conditions, suggesting a priming-like mechanism for heat stress tolerance. Our findings highlight the potential of targeting epigenetic variation for improving crop stress resilience. We continue to explore the utility of these methylation changes as a basis for epibreeding strategies aimed at developing heat-tolerant tomato cultivars.

Acknowledgements: We would like to thank Dr. Tzahi Arazi (Volcani Institute) and Dr. Nicolas Bouché (Université Paris-Saclay) for providing the *ddm1* lines and methylome data used to initiate this study. We also acknowledge the Crown Genomics institute of the Nancy and Stephen Grand Israel National Center for Personalized Medicine at the Weizmann Institute of Science for genomic analyses.

INVITED TALK 5

TITLE: IMPROVEMENT OF LOCAL TOMATO VARIETIES BY USING OMICS TECHNOLOGIES

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Abstract: Tomato production in Greece has been dominated by hybrid seeds without taking into consideration the extensive variation in desirable traits of local varieties and landraces which have been acclimated to local conditions. n-Tomatomics is a multidisciplinary project aimed at assessing, characterizing, and improving with useful traits the main collection of Greek local tomato varieties that exist in Greek Seed Banks and plant breeding laboratories. Local varieties like Santorini, Kardia Bodiou, Pantarosa, Aspros Lotos, and others, are grown in commercial greenhouse and field conditions to improve yield potential, disease resistance, fruit quality traits, appearance, and postharvest behavior. Initially these local varieties were characterized by using phenomic, transcriptomic, and metabolomic approaches. Several traits have been selected to comprise the core of genes to be introgressed for yield and quality: the *rin* mutant initially for increased

shelf life, and the sp, sft mutant combination for higher yield and uniform ripening. For disease resistance, five dominant genes have been selected: *I, Sw-5b, Tm-2a, Ty-1* and *Ve* to cover a wide range of pathogens. Considering the nature, location, and number of loci, a breeding strategy was conceived to reduce the number of backcrosses and maximize the benefit from these traits. Thus, each traditional variety was split into two parents that each will carry half of the genes and at the end will be crossed together to create the final hybrid lines. In addition, the physiological significance of cell glycoproteins in fruit ripening was investigated as well as the 3D-structure of tomato fruits of various shapes.

ORAL PRESENTATION 12

TITLE: UNDERSTANDING DIFFERENTIAL RESPONSES TO HEAT STRESS IN TOMATO GENOTYPES THROUGH INTEGRATED PHYSIOLOGICAL AND GENETIC INSIGHTS

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Abstract: Since tomato is a significant crop in the Mediterranean area, understanding its performance under high temperature conditions has become crucial to determine the elements affecting yield under abiotic stress. In this research, we cultivated two heat-tolerant genotypes (LA3120 and E42) in controlled settings at two different temperature regimes, to assess their physiological response at elevated temperatures. The findings indicated that heat stress impacted gas exchange and fluorescence parameters differently, along with the accumulation of secondary metabolites in both genotypes. Notably, the E42 genotype demonstrated an efficient regulation of the photosynthetic apparatus under heat stress by adjusting the electron transport chain, whereas the LA3120 genotype focused on modifying the secondary metabolism under similar conditions, enhancing flavonoid content. Genotyping information acquired from a GBS (genotyping by sequencing) analysis enabled exploration of the genetic diversity between the two genotypes. Candidate genes that may control the stress response were identified, focusing on those with HIGH and MODERATE polymorphisms known to be involved in

the photosynthetic process. This strategy allowed us to identify 22 candidate genes among which 13 presented variants exclusively in E42 and seven in LA3120. These findings further enrich the understanding of the physiological mechanisms involved in the heat stress response in tomatoes. They also illustrate how these tomato genotypes can serve as a valuable resource for traits related to adaptation to abiotic stress.

ORAL PRESENTATION 13

TITLE: GENETIC DISSECTION OF DROUGHT TOLERANCE IN THE TOMAGIC POPULATION

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Abstract: The ToMAGIC population is a Multi-parent Advanced Generation Inter-cross (MAGIC) population consisting of 375 inbred lines developed from a cross between four accessions of *Solanum pimpinellifolium* (SP) and four of *S. lycopersicum* var. *cerasiforme* (SLC). The founders of this population exhibit significant diversity in their adaptation to varied environments, ranging from deserts to tropical forests and altitudes from sea level to over 1500 meters. Developed using a funnel scheme including three cycles of intercrossing, ToMAGIC has undergone extensive recombination of the genomes of the eight founders. A subset of 138 lines, capturing the genetic variance of the population, was screened over two years under drought stress (irrigation halted at two stages) and control conditions in a greenhouse. Ninety-five traits have been phenotyped, including inflorescence traits, leaf characteristics, biometric parameters, yield, fruit quality, and leaf senescence, were collected. Repeated measures statistical analysis revealed that reduced irrigation began to impact inflorescence traits

such as fruit number, flower number per inflorescence, and fruit setting starting from the second inflorescence. In contrast, earliness was affected starting from the fifth inflorescence. A genome-wide association study (GWAS) identified eight genomic regions across seven chromosomes (1, 2, 3, 7, 8, 9, and 12) associated with traits linked to drought tolerance. Clusters of genes associated with abscisic acid (ABA) signalling, reactive oxygen species (ROS) regulation, and osmolyte biosynthesis pathways have been identified within each of these genomic regions. Notably, our results indicate that drought tolerance in this population is a combined effect of genomic regions contributed by multiple donors, rather than a single source. This study underscores the importance of integrating diverse genetic contributions to enhance drought tolerance. Further, the best drought predictors have been identified, and a subset of traits including fruit weight, yield, plant height, wilting, total biomass production, fruit setting, and the number of flowers per inflorescence was selected based on their relevance. These traits have been used to perform genomic selection to select lines exhibiting superior drought tolerance and possessing the key genomic regions identified. Two lines exhibited a transgressive phenotype, outperforming all parental lines in both conditions. These lines hold potential for targeted crosses to integrate desirable traits into commercial cultivars.

ORAL PRESENTATION 14

TITLE: DECIPHERING WATER STRESS RESPONSES IN *SOLANUM LYCOPERSICUM* THROUGH GENETIC AND METABOLIC VARIATION FOR IMPROVED FRUIT YIELD AND QUALITY

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Abstract: Tomato fruit quality, a critical breeding trait, is influenced by complex polygenic factors and strongly impacted by water stress (WS). The pronounced genotype × environment interactions necessitate precise breeding strategies to enhance quality traits under WS. To explore the effects of WS on the natural variation in tomato fruit yield, quality, and metabolism, we conducted an augmented block design greenhouse study with a novel tomato association panel comprising 152 varieties, comparing control (well-watered, WW) and WS conditions. Our findings revealed substantial genetic variation (33–77% of phenotypic variance) and high heritability in fruit quality and yield traits, underscoring their potential for breeding. Under WS, blossom-end rot (BER) incidence increased, while total soluble solids (TSS) were enhanced. Conversely, fruit weight (FW) and total yield (TY) were significantly reduced. WS also triggered a notable accumulation of sugars, organic acids, amino acids, hydroxycinnamic acids, and flavonoid O-glycosides, particularly at various stages of fruit development. Key

adaptive metabolites included tricarboxylic acid (TCA) cycle intermediates, sucrose, melezitose, myo-inositol, chlorogenic acid derivatives, di-caffeoyl-quinic acid, kaempferol-3-glucoside-7-rhamnoside, isorhamnetin-3-O-rutinoside, naringenin chalcone, and kaempferol-3-O-rutinoside. To evaluate WS tolerance, a stress susceptibility index (SSI) was calculated for each accession, categorizing them into performance groups. Correlation analysis highlighted the interplay between metabolic pathways at the red ripe stage. High-performing accessions exhibited enhanced flux through sugar interconversions, glycolysis, and the flavonoid biosynthesis pathway, whereas lower-performing accessions showed disruptions in the TCA cycle and γ-aminobutyric acid (GABA) metabolism. GWAS analysis identified candidate genes linked to sugar metabolism, the phenylpropanoid pathway, and epigenetic regulation of metabolic networks during fruit development. This study provides novel insights into the genetic and metabolic mechanisms underlying WS tolerance in tomatoes, offering a foundation for breeding WS-tolerant varieties with enhanced fruit quality.

ORAL PRESENTATION 15

TITLE: UNRAVELING THE GENETIC ARCHITECTURE OF MULTI-STRESS TOLERANCE IN *SOLANUM PIMPINELLIFOLIUM*

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Abstract: Global climate change is driving an escalation of heat waves and pathogen pressures, posing critical challenges for crop resilience. In this context, Cucumber mosaic virus (CMV) and heat stress (HS) represent important threats to tomato, a crop highly sensitive to both biotic and abiotic stresses.

To dissect the genetic mechanisms underlying trade-offs between growth and stress tolerance, we studied a genetically diverse population of *Solanum pimpinellifolium*, a wild tomato species. In controlled greenhouse conditions, plants were phenotyped under four treatments: greenhouses classical parameters growth, HS, CMV inoculation, and combined HS+CMV stress. Multiple traits such as biomass, gas exchange, symptom severity, viral load, and untargeted metabolite profiles were measured to capture multifaceted responses. Preliminary findings reveal striking genotype-specific responses, highlighting the interplay between physiological adjustments and defense activation. Metabolite profiling identified possible biomarkers of tolerance, suggesting potential links between stress-induced metabolic shifts and

growth dynamics. These metabolic signatures provide a window into the biochemical pathways driving resilience and could serve as predictive tools for breeding programs. To further elucidate the genetic basis of these responses, we performed genome-wide association studies (GWAS), integrating ecophysiological and metabolomic data. This approach allowed to map key loci associated with stress tolerance, uncovering trade-offs between growth and defense mechanisms under combined stress. This study offers insights into the complex genetic and biochemical networks underlying multistress adaptation in *Solanum pimpinellifolium*. By integrating metabolomics, ecophysiology, and genetics, this work lays the foundation for innovative strategies to enhance tomato crop resilience in the face of global climate challenges. Supported by IdEx Bordeaux University, “Investments for the Future” program / GPR Bordeaux Plant Sciences.

ORAL PRESENTATION 16

TITLE: COMPREHENSIVE MORPHOLOGICAL AND IONOMIC CHARACTERIZATION OF A TOMATO ASSOCIATION PANEL REVEALS MECHANISMS OF SALINITY STRESS TOLERANCE

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Abstract: Soil salinity is a critical environmental stressor that significantly impairs agricultural productivity globally. Gaining a deeper understanding of plant responses to salinity stress is essential for developing resilient crop varieties. To investigate the impact of salinity stress on natural variability in plant morphology, particularly in leaves and roots, we conducted a study using a novel tomato association panel comprising 152 diverse varieties. The experiment was designed using an augmented block design and evaluated plant performance under both control and salinity stress conditions. Our findings revealed substantial phenotypic variation among the tomato varieties in response to salinity stress. Key traits such as plant height, shoot diameter, leaf size, root length, root mass, and ion accumulation exhibited significant correlations with overall plant performance. Notably, differential

ion accumulation in the root emerged as a critical determinant of salinity tolerance, underscoring the importance of ion homeostasis in stress adaptation. Using a genome-wide association study (GWAS), we identified several candidate genes linked to salinity tolerance. Many of these genes had not been previously associated with ion homeostasis under salinity stress, demonstrating the effectiveness of integrating phenotypic analysis with genetic approaches to uncover novel aspects of salinity stress responses. These insights provide valuable contributions to our understanding of the genetic and physiological mechanisms underlying salinity tolerance in *Solanum lycopersicum* L. This study establishes a foundation for future research into the genetic basis of salinity tolerance traits and offers a resource for breeding programs aimed at improving salinity resilience in tomatoes and other crops. By identifying key traits and candidate genes, our findings pave the way for targeted breeding strategies to enhance the agricultural productivity of tomatoes in saline environments.

Acknowledgments: The NatGenCrop team thanks the Horizon Europe program, called HORIZON-WIDERA-2022-TALENTS-01, for the provided funding (Grant Agreement number 101087091). Special thanks to Sintia Aneva and Valeria Tonova for their engagement in this project.

ORAL PRESENTATION 17

TITLE: IDENTIFICATION OF HUB GENES ASSOCIATED WITH DROUGHT STRESS AND PLANT STRESS MEMORY IN *SOLANUM LYCOPERSICUM* THROUGH TIME-SERIES AND WEIGHTED GENE CO-EXPRESSION NETWORK ANALYSES

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Abstract: To address recurrent environmental constraints, plants have evolved sophisticated mechanisms, including stress memory, where past stress experiences inform their responses to future stresses. Harnessing these mechanisms can unlock strategies for sustainable agriculture and environmental resilience. Focusing on cultivated tomato (*Solanum lycopersicum* L.), we compared the landrace Lucariello and the commercial variety Red Setter under two cycles of drought stress (water withholding) each followed by one week of recovery. The phenotypic evaluation revealed that Lucariello exhibited higher stress tolerance, with less penalty on yield. To explore their molecular responses, we generated ~1.05 Tb of RNA-seq data, which were used to identify genotype- and timepoint-specific transcriptional changes through gene-weighted co-expression network (WGCNA). A total of

nine modules (MEs) were identified, of which six were highly correlated with drought stress, recovery treatments, and phenotypic traits. MEblue contained up-regulated genes across the time points, and it was negatively correlated with transpiration and photosynthetic rates, stomatal conductance, and RWC. By contrast, an opposite trend was observed for the MEbrown, which was negatively correlated with stress conditions, and positively correlated with phenotypic traits. Hub genes belonging to specific modules were further identified, allowing us to identify different regulators associated with stress memory acquisition during rewatering and subsequent stress cycles that might contribute to protect plants from physiological damage under drought stress. Among them, a nuclear transcription factor Y subunit (NF-YA), an amino acid transporter, and a protein phosphatase 2C were particularly intriguing since they were commonly induced in both genotypes across different time points. Our results provide new insights into the plant stress memory in tomato, allowing the identification of target for their validation through genome editing approaches.

This work was carried out within the Agritech National Research Center, funded by MIUR- European Union PNRR – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 -SPOKE 1 INVESTIMENTO 1.4.

ORAL PRESENTATION 18

TITLE: EXPLORING ABA-MEDIATED FLOOD TOLERANCE MECHANISMS IN TOMATO: INSIGHTS FROM INTROGRESSION LINES

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Abstract: Global warming intensifies flooding, causing US$7.8 billion in annual agricultural damages, making it the second most destructive disaster after drought. Plant flood tolerance depends on structural and physiological traits like adventitious roots and hypocotyl hypertrophy, but tomato plants are particularly sensitive to flooding. Current tomato varieties lack sufficient flooding tolerance, leading to significant crop losses. Introgression lines (ILs) provide genetic diversity, precise trait mapping, and improved flood resilience, enabling stable production under changing conditions. We hypothesized that tomato ILs IL8-1 and IL11-4, with distinct drought responses, exhibit significantly different physiological and morphological reactions to flooding compared to the mother plant M82, likely due to differential abscisic acid-mediated stress regulation. Under oxygen deficiency (via nitrogen gas) and flooding for seven and fourteen days, parameters such as oxygen levels, redox potential, nutrient concentration, flood sensitivity index (FSI), transpiration rate, and adventitious root formation were measured. Flooding reduced midday

transpiration rates by 9.72% to 67.37%, with M82 showing the least reduction. M82's shoot dry weight was unaffected, while ILs decreased by 50%. M82 developed 10% stem hypertrophy and had the lowest FSI (27.35%). Despite forming more adventitious roots, ILs were more sensitive to flooding. This research highlights IL potential for breeding flood-resilient tomatoes.

ORAL PRESENTATION 19

TITLE: HIGHER STOMATAL DENSITY AND RATIO ARE ASSOCIATED WITH BETTER YIELDS IN TOMATO FRUIT FORMATION STAGE

Authors: Tulva I.[1], Koll B.*[2], Bender I.[2], Vaht A.[1], Mikhailava N.[1], Bērziņa N. M.[1], Dieviņš K.[1], Jakobson L.[2], Merilo E.[1], Hõrak H.[1]

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Abstract: Stomata are microscopic pores on the surface of plant leaves. When the air gaps are open, they absorb carbon dioxide from the air, and when closed, they limit excess water vapour through transpiration. The number of stomata and how they are spread out between the leaf surfaces (stomatal ratio) determines the leaves' maximum gas exchange potential. We aimed to investigate whether stomatal parameters affect tomato yield in greenhouse conditions. We analysed leaf stomatal conductance, stomatal anatomical traits, and yields from 16 tomato varieties grown under greenhouse conditions for two years (2021 and 2022) in Estonia. Results showed a statistically significant positive linear relationship between stomatal density and tomato yield in the fruit formation stage. There was no statistically significant difference between stomatal conductance and tomato yield. Higher stomatal ratios were also associated with higher yields in both study years. However, in vegetative growth stage no significant correlation was found between stomatal parameters and yield. Our results show that increasing stomatal density and ratio in dicot crops may provide a strategy towards yield improvement, at least under greenhouse conditions, where water is not limiting.

POSTER PRESENTATIONS

POSTER PRESENTATION 25

TITLE: STUDY OF REPRODUCTIVE BARRIERS BETWEEN CULTIVATED TOMATO *SOLANUM LYCOPERSICUM*, STRESS-RESISTANT TOMATO *SOLANUM CHILENSE* AND THEIR HYBRIDS

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Abstract: The domestication of tomato (*Solanum lycopersicum*) and its introduction to Europe have decreased its genetic variability, resulting in a genetic bottleneck. Wild tomato species from the *Lycopersicon* clade are a great source of resistance genes to increase genetic diversity and improve important agronomic traits such as stress tolerance. A promising species for this purpose is *Solanum chilense*, a wild tomato found primarily in the Atacama salt desert. However, the use of this species is hindered by reproductive barriers, both inter- and intraspecific. Intraspecific barriers manifest as gametophytic self-incompatibility, whereas *Solanum chilense* is a self-incompatible species while *Solanum lycopersicum* is self-compatible. This mechanism is getting increasingly understood in other *Solanaceous* species but is relatively unexplored in wild tomatoes specifically. Interspecific barriers manifest as unilateral incompatibility, where self-incompatible species cannot breed with self-compatible species. This barrier remains largely misunderstood. In this study, we investigated the reproductive barriers between *Solanum chilense*, *Solanum lycopersicum*, and their

hybrids. We characterized these barriers by analyzing pollen tube growth, fruit set, and seed set for crosses between *Solanum chilense*, *Solanum lycopersicum*, and their hybrids. We also examined expression of genes potentially involved in reproductive barriers in the tomato clade. We found several genes potentially involved in reproductive barriers in *Solanum chilense*. These findings provide insights into the genetic and molecular basis of reproductive barriers in tomatoes, paving the way for future use of wild tomato species like *Solanum chilense* for crop improvement.

POSTER PRESENTATION 26

TITLE: PROTEOMIC, METABOLOMIC, AND PHOTOSYNTHETIC DETERMINANTS OF TOMATO PLANT ADAPTATION TO PHOSPHATE DEFICIENCY

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Abstract: Phosphorus (P) is an essential macronutrient that ensures plant metabolism and determines to a large extent crop yield. Roots absorb phosphorus as inorganic phosphate (Pi) which, depending on the soil pH, tends to form insoluble complexes with extremely poor bioavailability. Thus, crops often grow in conditions of Pi deficiency in spite of the extensive use of Pi fertilizers. In the course of their evolution, plants have developed diverse adaptive mechanisms to improve Pi acquisition and fine-tune their growth in response to Pi starvation. In an attempt to uncover novel aspects of these intricate mechanisms, we screened Bulgarian tomato germplasm and identified two varieties with differential responses to Pi starvation. The comparative analysis of plants grown in low P medium showed that the cultivar with higher retention of Pi in the shoot was characterized

by more pronounced starch accumulation, dissipation of excess light energy in the leaves, and strigolactone levels in the roots. On the other hand, the cultivar with higher sensitivity to low P stress displayed a more prominent increase in the root:shoot ratio, pointing to morphological rather than physiological adaptations for better Pi acquisition. We also found specific differences between the two genotypes concerning the low P-induced changes in the root proteome and metabolome. Those differences revealed the essential role of particular Pi transporters, purple acid phosphatases, glutathione transferases, as well as organic acids in shaping the plasticity of tomato plants in conditions of Pi starvation. Altogether, the different magnitude of changes in particular modules from the core response to low P stress determines the unequal sensitivity of the studied genotypes to limited Pi availability, thus making these modules promising targets in future strategies for crop improvement.

POSTER PRESENTATION 27

TITLE: EXPLORING GENETIC DIVERSITY IN SOUTHEASTERN SPANISH TOMATOES FOR DROUGHT TOLERANCE

Authors: Egea-Sánchez J.M.[1], Quevedo-Colmena A.S.[2], Moya C.[1], Lebrón R.[2], Justo M.[1], Flores B.[1], Lozano R.[2], Egea I.[1], Yuste-Lisbona F.J.*[2]

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Abstract: Due to the effects of climate change, drought is projected to become the most restrictive abiotic stress on agricultural productivity and nutritional quality, particularly in arid and semi-arid regions such as the Mediterranean basin. Local varieties from these regions, cultivated and refined by traditional farmers over centuries, represent an invaluable resource for breeding programs, thanks to their distinctive genetic adaptations to water-deficit conditions. This study evaluated the physiological, transcriptomic, metabolic, and cellular responses of four traditional tomato varieties from southeastern Spain to drought stress under controlled conditions, compared to a commercial variety. The Murciano Rojo (MR) variety demonstrated the highest drought tolerance, characterized by efficient osmotic adjustment, reduced water loss through transpiration, and elevated soluble sugars and proline levels. Enhanced mesophyll cell size and structural integrity allowed superior water retention without altering stomatal index. Consequently, MR exhibited greater photosynthetic efficiency under drought, as demonstrated by higher chlorophyll content, reduced oxidative damage, and elevated antioxidant levels, including

carotenoids and proline, which offered robust protection against oxidative stress. Additionally, comparative transcriptomic analyses enabled the identification of two categories of drought-responsive genes: (i) basal drought-response genes, defined as those with a consistent transcriptional response across all genotypes, and (ii) genotype-specific drought-tolerant genes, characterized by distinct transcriptional behavior under water-deficit in each genotype. Notably, 544 genes were found to be differentially expressed exclusively in the leaves of MR under stress, with 376 up-regulated and 168 down-regulated, highlighting its unique transcriptional adaptation to water-deficit conditions. The study highlights the potential of traditional tomato varieties as a genetic resource for breeding programs and emphasizes their role as sustainable alternatives for fostering a resilient agricultural system.

Acknowledgments: This work was supported by TED2021-131400B-C31 and TED2021-131400B-C32 grants (MCIN/AEI/10.13039/501100011033 and European Union NextGenerationEU/PRTR) and AGROSIMBIOSIS project (Biodiversity Foundation, Ministry for Ecological Transition and Demographic Challenge).

POSTER PRESENTATION 28

TITLE: TMAO BIOSYNTHESIS AND ITS CONTRIBUTION TO DROUGHT STRESS RESPONSE IN TOMATO

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Abstract: Trimethylamine N-oxide (TMAO) is widely recognized as a quintessential protein-stabilizing osmolyte. It accumulates at high concentrations in tissues to counteract the denaturing effects of urea and salt, as well as to protect proteins and cellular structures from freezing temperatures. In plants, TMAO is widely distributed, including in crops such as wheat, barley, and corn, where its levels significantly increase in response to abiotic stresses such as water deficiency, low temperatures, and high salinity. This accumulation promotes plant tolerance to adverse environmental conditions by maintaining proper protein folding and acting as an activator of abiotic stress-induced gene expression. TMAO is synthesized through the N-oxygenation of trimethylamine, a reaction mediated by flavin-containing monooxygenases (FMOs). In this study, TMAO levels were measured in tomato (*Solanum lyscopersicum*) and their wild-relative species grown under drought conditions, including *S. pimpinellifolium*, *S. habrochaites*, *S. pennellii*, and *S. chilense*. Among these, *S. habrochaites* stood out due to its notably high TMAO content from the earliest days of water deficit. Furthermore, phylogenetic analysis identified six FMO genes (SlFMO) in tomato that encode proteins with high sequence similarity to the FMOs in Arabidopsis involved in TMAO biosynthesis. Expression analysis of these SlFMO genes under drought conditions revealed

that SlFMO3 is significantly upregulated in response to water stress. Enzyme activity assays demonstrated that among the proteins encoded by the SlFMO genes, SlFMO1 is the most efficient enzyme for TMAO production, highlighting its pivotal role in drought stress response. Further studies exploring the regulatory mechanisms of SlFMO3 expression and its interaction with other stress response pathways could provide valuable insights for developing stress-resilient tomato crops through metabolic engineering.

Acknowledgments: This work was supported by TED2021-132141B-C21 and TED2021-132141B-C22 grants, funded by MCIN/AEI/10.13039/501100011033 and European Union NextGenerationEU/PRTR."

POSTER PRESENTATION 29

TITLE: NON-DESTRUCTIVE EVALUATION OF PHYSIOLOGICAL STATUS OF DROUGHT-STRESSED TOMATO PLANTS

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Abstract: The objective of this research is to investigate the application of non-destructive analytical techniques for evaluating the impact of drought stress on the physiological status of tomato plants. The study employs advanced remote sensing methodologies, including multispectral imaging, chlorophyll fluorescence analysis, chlorophyll content assessment, and the CIE-LAB colorimetric test. Specifically, multispectral aerial imagery was acquired via the Pleiades-1A satellite at 0.5-meter resolution and utilized to compute the Normalized Difference Vegetation Index (NDVI), a key indicator of vegetation health. Additionally, chlorophyll fluorescence parameters (Fv/Fm), the Chlorophyll Content Index (CCI), and CIELAB colorimetric values were measured in tomato plants subjected to drought stress during the mass flowering phase. The findings demonstrate a pronounced impact of water deficiency on physiological traits. NDVI values exhibited a 21% reduction, indicating significant stress-induced alterations in plant vitality. Furthermore, chlorophyll fluorescence ratios Fv/Fm (maximum quantum yield of PSII) declined by 11%, corroborating a disruption in photochemical efficiency. The CCI decreased by 23%, signifying a reduction in chlorophyll concentration and, consequently, impaired photosynthetic capability. Moreover, shifts in CIELAB values and a decrease in Chroma were indicative of early chlorophyll degradation and declining leaf quality. These results underscore the efficacy of

non-destructive analytical techniques in providing rapid and reliable assessments of physiological status and photosynthetic performance in drought-stressed tomato plants. A comprehensive understanding of the interrelationships between these parameters can facilitate the development of adaptive agricultural strategies, ultimately enhancing crop resilience and sustainability amidst escalating environmental challenges.

Acknowledgements: SG, ET and IT have benefited by networking opportunities provided by COST Action RECROP (CA22157), co-financed by the Bulgarian National Science Fund under grant No KP-06-COST/10/22.05.2024.

Session:

EMERGING TECHNOLOGIES FOR SUSTAINABLE TOMATO CROP PRODUCTION

KEYNOTE TALK 5

TITLE: INTEGRATING MULTI-OMICS DATA AND AI MODELS TO ACCELERATE TOMATO CROP IMPROVEMENT

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Abstract: Tomato breeding has achieved major technical innovations based on artificial selection, hybrid breeding and molecular selection to date, where optimal and precise selection is currently being developed. Today, our most important goal is to obtain high-performance tomato varieties that are resistant to environmental conditions in a short time. In recent years, advances in omics techniques, including genomics, transcriptomics and metabolomics, have accelerated the process of tomato plant breeding. Genomic selection (GS) and genome editing approaches have emerged as innovative tools to assemble desired alleles by reducing the time and laborious selection processes. GS models for morphological and agronomic traits were developed using different tomato populations. The prediction accuracy tested by modifying the crucial parameters was highly dependent on the genetic structure of the population and the characteristics of the target trait. The establishment of effective GS protocols will promote the use of genomics-guided selection. In addition, system biology and data mining can synergistically help to discover key genes involved in environmental stress response. Omics datasets have been leveraged by AI algorithms to help improve disease resistance traits. The workflow combines supervised and unsupervised machine learning approaches to support gene prioritization and discover meaningful patterns within complex biological datasets. In addition, natural

language processing (NLP)-inspired strategies are applied to support domain prediction and protein classification. These methods provide new opportunities to detect resistance-related traits and strengthen the integration between omics data and AI models to annotate resistance genes in *Solanum lycopersicum* genome. Overall, we explored the developing approaches and challenges for omics big data integration. Integrating AI with “omics” tools can accelerate crop breeding programs by making “omics” analyses more scalable, interpretable, and applicable to crop breeding.

ORAL PRESENTATIONS

ORAL PRESENTATION 20

TITLE: PHYSIOLOGICAL, CHEMICAL AND METABOLITE PROFILING OF *PECTOBACTERIUM CAROTOVORUM* INOCULATED TOMATO PLANTS AND GROWN IN NUTRIENT AMENDED SOILS

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Abstract: This study evaluated the effects of a plant pathogenic bacterium *Pectobacterium carotovorum* inoculation and nutrient solution ($CaCO_3$ (2 mM), NaCl (1 mM) and $K_2Cr_20_7$ (0.001 mM)) on the growth, photosynthesis, nutrient uptake and metabolomics of tomato seedlings. The experiment had four experimental treatments (1. Nutrient solution + *P. carotovorum* inoculation; 2. Nutrient solution alone; 3. *P. carotovorum* inoculation 4. Control). Plant growth and photosynthesis were not affected, and differences in nutrient assimilation and metabolite profiles were clear-cut. Of the photosynthesis parameters, only water use efficiency was impacted, higher in the bacterium-only treatment, and unchanged in the rest of the other treatments. The boron, bismuth and nickel assimilation was affected in the different experiments, accumulating mostly in the "Nutrient solution + *P. carotovorum* inoculation" experimental set. Principal

component analysis of metabolomics data separated the treatments in three groups, the first one includes the double treatment, group two the nutrient solution treatment and finally group three *P. carotovorum* and control treatments. Correlation analysis of the data showed an assumed interdependence of plant factors. In conclusion the interaction between the bacterium, the plant and the nutrient solution is complex and more pronounced at the chemical and metabolite level than on growth and photosynthesis.

ORAL PRESENTATION 21

TITLE: MOLECULAR APPROACHES FOR THE BENEFIT OF CONSUMERS AND RESEARCHERS

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Abstract: One of the main tasks of modern society is the purity and quality of food, which in turn is associated with the development and implementation of precise methods for control. The aim of the study is to assess the potential of a molecular method, applying fragment analysis of PCR products from the region of the chloroplast genes trnH and psbA, for qualitative and quantitative identification of the composition of vegetable mixtures available on the Bulgarian market. For this purpose, primers from the spacer region between the trnH and psbA genes were selected with the ability to amplify DNA from four popular vegetables - tomato, potato, carrot and pumpkin after processing. Pure vegetable mixtures as well as those purchased from the market were subjected to fragment analysis by capillary gel electrophoresis. The proposed method could distinguish the presence of tomato, potato, carrot and pumpkin qualitatively, but not quantitatively. Additional efforts are needed to validate the procedure. The discriminative power of SSR primers for distinguishing tomato varieties with different fruit colors was also studied. The SSR 70 marker demonstrates the capacity to distinguish orange colored tomato from red colored.

POSTER PRESENTATIONS

POSTER PRESENTATION 30

TITLE: MACRO AND MICRONUTRIENT CONTENT IN TOMATO PLANTS INFECTED BY BROOMRAPE AND MYCORRHIZAL COLONIZATION WITH THE FUNGUS *RHIZOPHAGUS IRREGULARIS*

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Abstract: Tomato crop is one of the main hosts of the holoparasitic species of "broomrapes" *Phelipanche ramosa* (L.) Pomel and and *P. mutelii* (Schultz) Pomel. They cause significant yield reduction and imbalance in the nutritional status. On the other hand, symbiosis with arbuscular mycorrhizal fungi (AMF) improves the absorption of nutrients and positively affects growth and productivity under different environmental conditions, including stress conditions. The aim of this study was to determine the changes in the content of macro- and microelements in tomato plants infected by broomrape and the effect of mycorrhizal colonization with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (formerly *Glomus intraradices*). The study was conducted in a pots experiment. Tomato variety "Kalina" was grown in alluvial-meadow soil artificially infested with seeds of broomrapes and inoculated with spores of the AM fungus R. irregularis. Four treatments in three replications have been set. The concentrations of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu in the organs of tomato plants (leaves, stems and roots) were determined at 80 day after transplanting. It was found that the broomrape infection reduced the content of P in stems and

roots, K in leaves, Fe in stems, and Cu in leaves and stems of infected tomato plants. Higher levels of Ca, Mg and Fe were recorded in their leaves compared to uninfected plants. The parasite had low effect on the concentrations of N and Zn. Mycorrhizal colonization with *R. irregularis* had a beneficial effect on the mineral composition of both uninfected and broomrape infected plants. The concentrations of P, Ca, Fe and Mn in the leaves and stems were increased. The results obtained in the present study contribute to clarifying the role of AMF in the nutrition of tomato plants and the possibilities for their use to overcome the negative effects caused by the broomrape parasite.

POSTER PRESENTATION 31

TITLE: EFFECTIVENESS OF ARBUSCULAR MYCORRHIZAL FUNGI ON THE PROCESSES OF INFECTION OF TOMATO (*SOLANUM LYCOPERSICUM* L.) BY BROOMRAPE (*PHELIPANCHE RAMOSA* (L.) POMEL)

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Abstract: Tomato (*Solanum lycopersicum* L.) is a crop that forms an active symbiotic relationship with arbuscular mycorrhizal fungi (AMF) and is one of the main hosts of the root parasite “broomrape” which is a serious plant protection problem for tomatoes. AMF are key players in the productivity of agro-ecosystems and increase plant tolerance to pathogens and stress factors. The aim of the study was to determine the response of tomato plants to different types of AMF, individually and in combination of several species, as well as to evaluate their effectiveness on the processes of infection by broomrape (*Phelipanche ramosa* (L.). Tomato variety “Kalina” was grown under controlled conditions (temperature 22 °C, 16h/8h photoperiod) in pot experiments with substrate - sterile perlite. Pre-conditioned broomrape seeds and microbial inoculants were added. Commercial products containing different species of AMF were used as sources of AMF spores. Mono-mycorrhizals with the species Rhizophagus irregularis and Glomus etunicatum. Poly-mycorrhizal with the species *R. irregularis*, *G. etunicatum*, *G. clarum*, *G. mosseae* and *Acaulospora colombiana*. There were 12 treatments in three replications. The nutritional regime of the plants was provided by adding ½ concentrated Hogland solution. The colonization of roots with AMF (%), the degree of infection by broom-

rape (number of tubercles/root), and the % of germinated broomrape seeds were determined. It was found that the tomato variety used as a model plant in the present study responds specifically to the different AMF species and shows a stronger selectivity towards establishing a symbiotic relationship with the species *R. irregularis*. Mycorrhization with AMF had a negative impact of *P. ramosa* seeds germination, depressed parasite development and reduced the rate of infection, but did not prevent or eliminate the broomrape. Broomrapes infection significantly suppressed mycorrhiza formation and reduced root colonization by over 50%, regardless of the type of AMF.

POSTER PRESENTATION 32

TITLE: BIOLOGICAL ACTIVITY OF EXTRACTION PRODUCTS OBTAINED FROM SPECIES OF THE GENUS *NICOTIANA* AGAINST THE CAUSATIVE AGENTS OF SOME FUNGAL DISEASES OF TOMATO (*SOLANUM LYCOPERSICUM L.*)

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Abstract: Tomato is a crop that is attacked by a number of fungal pathogens that cause significant economic damage. Climate change favors their mass spread and increases the risk in their production. In modern practices for sustainable and precision agriculture, the role of biological products with high biological activity against plant pathogens is increasing. Interest in products derived from secondary plant metabolites is constantly growing. In this aspect, species of the genus *Nicotiana* have high potential. The aim of this work was an *in vitro* testing of the antifungal biological activity of two natural aromatic products, concrete and resinoid, derived from three species of *Nicotiana*: *N. tabacum* L. – oriental type, *N. alata* Link & Otto and *N. rustica* L. Test-pathogens were isolated from tomato plant tissues with typical symptoms of fungal diseases, by classical microbiological methods. Morphological characteristics of the colonies and microscopic diagnosis were made on each fungal isolate. The *in vitro* antifungal tests were performed by the diffusion method on 72-96 h cultures of the pathogen, according to the size of the inhibited zone (mm). Isolated

fungal pathogens were related to the phylum Ascomycota, genera Stemphylium (*S. lycopersici*), Alternaria (*A. solani*) and Fusarium (*F. solani*). Pathogens cause the widespread fungal diseases - Leaf blight, Early blight and Fusarium wilt on tomato plants. Different antifungal effects of tobacco aromatic products were found. Nicotiana-resinoids exhibited a more pronounced effect, compared with the respective concretes. The activity against the phytopathogens of the genera *Stemphyllium* and *Alternaria* was the highest. An antifungal effect on the isolates of genus *Fusarium* was found only for *Nicotiana resinoids*. The results from the study are promising for the use of natural aromatic products from *Nicotiana* species as a basis for the development of biological products with antifungal action.

POSTER PRESENTATION 33

TITLE: INTEGRATIVE GENOMIC AND METABOLOMIC ANALYSIS REVEALS KEY LOCI FOR TOMATO FRUIT QUALITY UNDER WATER STRESS

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Abstract: Water stress disrupts critical physiological processes-such as photosynthesis, respiration, and stomatal regulation—thereby limiting plant growth and productivity. In this study, we evaluated a diverse panel of 152 tomato accessions grown under well-watered and water stress conditions to elucidate the genetic basis of fruit quality through metabolic reprogramming. Comprehensive metabolite profiling of red ripe tomato fruit revealed significant alterations in both primary and specialized metabolites, with pronounced changes in phytosterol content under water stress. Genome-wide association mapping using 122,222 single nucleotide polymorphisms identified robust associations between metabolic variation and candidate genes, notably WRKY46 and a cytochrome P450 monooxygenase. Subsequent transcript analysis demonstrated a marked upregulation of both WRKY46

and the cytochrome P450 gene in response to water stress, indicating their active involvement in modulating the sterol biosynthetic pathway during stress conditions. Further functional studies was employed to validate these findings. The resulting mutants exhibited significant alterations in phytosterol levels compared to the wild type, thereby confirming the critical role of these genes in reprogramming the sterol pathway under water stress. This integrative approach-combining association mapping, transcript profiling, and functional validation-provides a robust framework for deciphering the molecular mechanisms underlying metabolic adjustments in tomato. Our results offer promising targets for marker-assisted selection and metabolic engineering, ultimately facilitating the development of water stress-resilient tomato cultivars with enhanced fruit quality. These findings contribute valuable insights toward sustainable crop improvement strategies in challenging environmental conditions.

POSTER PRESENTATION 34

TITLE: IMPROVING THE GROWING TECHNOLOGY OF TOMATOES FOR MEDIUM EARLY PRODUCTION

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Abstract: The field experiment was conducted during the period 2019-2022 on highly leached alluvial-meadow soil, in the experimental field of the Maritsa Vegetable Crops Research Institute - Plovdiv with Kopnezh F_1 a medium early production, determinate variety. The main aim of the study was to develop a modern technology, tailored to the biological requirements of the Kopnezh F_1 variety by optimizing basic technological elements. The effect of three planting schemes and two fertilization schemes using silver and black mulch on the vegetative grown and productivity of the plants were studied. The plants grown with the 120+40/50 cm scheme have the highest vegetative mass (4.02 kg/plant), due to the larger feeding area. The plants grown with the 40 and 30 cm inter-row spacing have lower values for this indicator, 3.54 and 3.02 kg/plant, respectively. Both foil and the cultivation scheme significantly affect the productivity of the plants. The plants fertilized with combined liquid fertilizers and the use of silver mulch with an inter-row spacing of 40 cm have the highest number and mass of fruits, with an increase of 21.4% and 55.4%, respectively, compared to an open bed and conventional fertilization. A weaker effect on these indicators was established when using black foil. Compared to the control variant, the highest yields were registered when using liquid fertilizers and silver mulch in all three cultivation schemes, and this was most strongly expressed at a row spacing of 40 cm - 4875 kg/da (45.5%). The results for fruit mass are also unidirectional.

POSTER PRESENTATION 35

TITLE: ADAPTATION TO SOLAR ROOFTOP URBAN AGRICULTURE: INTER- AND INTRA-SPECIFIC DIVERSITY ON GROWTH RATE AND CROP EFFICIENCY

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Abstract: Urban agriculture (UA) in developed countries is emerging as a novel crop growth environment. Due to limited available surface, rooftops are increasingly regarded as suitable spaces, competing with photovoltaic systems. To evaluate the possibility of growing vegetables in the presence of solar panels, the BINAFET project developed two rooftop greenhouses differing in the presence/absence of roofed semitransparent solar panels. Despite extensive literature on the climatic efficiency of this type of infrastructures, less is known about plant adaptation, limiting the advancement of breeding programs intended for these innovative crop environments. This work aimed to evaluate tomato (*Solanum lycopersicum*) growth rate and crop efficiency related to photosynthetically active radiation (PAR) under solar rooftop UA, comparing it with lettuce (*Lactuca sativa*) and dry beans (*Phaseolus vulgaris*), as the three most cultivated species under UA. Three genotypes

per species were evaluated in three consecutive short experiments (42 days after transplant). Periodic destructive measurements of dry biomass partitioning (14-day intervals) and plant biometric traits allowed estimation of the contribution of species, genotype, environment, and crop cycle on crop growth parameters. Tomato showed the highest growth rates, varying between 0.51±0.29 g m^{-2} day^{-1} in winter and 10.04±1.00 g m^{-2} day^{-1} in summer, significantly exceeding during the summer the values recorded for lettuce (3.81±0.71 g m^{-2} day^{-1}) and dry bean (3.34±0.23 g m^{-2} day^{-1}). No alteration in biomass partitioning was observed between treatments. Solar panels provoked a reduction in PAR irradiance (ranging from -18.7 to -47.9%), but significant differences were only observed for tomato under the low irradiance in winter conditions (mean reduction of 62.1% in total biomass). However, such reduction could easily be compensated by supplementary light. A tomato landrace genotype showed the higher total biomass production across experiments. Correlation analysis identified key biometric traits for tomato adaptation to these cropping conditions.

Acknowledgments: The research leading to these results has received funding from Agricultural Academy project ZEMDKT 17. V. Stoeva acknowledges the support of the Bulgarian Ministry of Education and Science under the National Program “Young Scientists and Postdoctoral Students – 2”.

POSTER PRESENTATION 36

TITLE: *IN VITRO* GENOTYPE-SPECIFIC RESPONSES OF TOMATO TO ZnO NANOPARTICLES: IMPACTS ON GROWTH, NUTRIENT UPTAKE, AND PATHOGEN SUPPRESSION

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Abstract: Nanotechnology, especially the application of nanoparticles, is gaining significant attention across various scientific fields, including plant science. The potential of nanoparticles to improve nutrient uptake, enhance physiological processes, and manage plant diseases is particularly promising. This study investigates the effects of zinc oxide (ZnO) nanoparticles on growth, trace element uptake, and suppression of *Fusarium oxysporum* and *Verticillium dahliae* in tomato plants *in vitro*. Seven-day-old seedlings (2 cm in height) from two tomato varieties, Rozovo sartse and Ideal, were cultured on Murashige and Skoog (MS) medium supplemented with four concentrations (0, 0.5, 1.5, and 2.5 mg/L) of ZnO nanoparticles (18 nm in size) and inoculated with *F. oxysporum* and *V. dahliae*. After 20 days of cultivation, the application of 2.5 mg/L ZnO significantly enhanced plant height, root length, number of roots per plant, and both fresh and dry biomass in the Rozovo sartse variety. However, the same concentration resulted in a reduction in most growth parameters in the Ideal variety, indicating a genotype-specific response to ZnO treatment. The findings indicate that the highest concentration (2.5 mg/L) most effectively inhibited both fungal pathogens. ZnO nanoparticles also influenced nutrient accumulation in a concentration- and genotype-dependent

manner. Rozovo sartse demonstrated superior Zn uptake at 2.5 mg/L, along with increased root and shoot accumulation of Fe and B, while Cu and Mn uptake tended to decrease with higher ZnO concentrations in both varieties. These findings emphasize the complex interactions of nano-enabled nutrient dynamics and the importance of genotype in regulating elemental homeostasis in tomato plants.

Acknowledgments: The research leading to these results has received funding from Agricultural Academy project ZEMDKT 17. V. Stoeva acknowledges the support of the Bulgarian Ministry of Education and Science under the National Program “Young Scientists and Postdoctoral Students – 2”.

POSTER PRESENTATION 37

TITLE: ADVANCING GREENHOUSE EFFICIENCY: EFFECTS OF SMART AUTOMATION ON YIELD AND FRUIT QUALITY IN TOMATO CULTIVATION

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Abstract: With rising demands for sustainable and resource-efficient food production, the integration of smart automation technologies in greenhouse systems is gaining interest in Bulgaria. This study evaluates the agronomic and qualitative impacts of an intelligent, sensor-driven automation system on tomato cultivation, compared to traditional management with minimal automation. Multiple tomato varieties were grown under two cultivation methods: a traditional manually adjusted system and a novel automated platform utilising real-time environmental monitoring and adaptive control of irrigation, fertigation, and microclimate. Comprehensive measurements including total and marketable yield, average fruit weight, firmness, soluble solids content (°Brix), titratable acidity, and uniformity were collected and statistically analysed. Across varieties, the automated system resulted in a yield increase ranging from 7% in high-yielding cultivars to 20% in lower-yielding ones, along with significantly improved fruit uniformity and firmness, without compromising taste- and flavour-associated traits. Moreover, automation reduced water and nutrient input by 18%, demonstrating not only agronomic benefits but also enhanced resource efficiency. These findings highlight the transformative potential of intelligent greenhouse automation for high-value crop production and offer a sustainable approach toward more resilient horticultural practices.

Acknowledgments: This project has received funding from the European Union's Digital Europe Programme under Grant Agreement No. 101083473 (AgroDigiRise), co-funded by the Bulgarian Ministry of Innovation and Growth, enabling the automation of the greenhouse infrastructure. Various experiments with tomato plants were carried out with the financial support of the Bulgarian Agricultural Academy through projects ZEMDKT-13 and ZEMDKT-17.

POSTER PRESENTATION 38

TITLE: GO MHE AND GO BIOFLORA, TWO RURAL DEVELOPMENT PROJECTS FOR AGROECOLOGICAL MANAGEMENT OF TRADITIONAL 'HUEVO DE TORO' TOMATO CULTIVAR PRODUCTION IN GUADALHORCE VALLEY IN ANDALUSIA, SOUTHERN SPAIN.

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Abstract: In the Guadalhorce Valley, the 'Huevo de toro' tomato is a successful example of *in situ* conservation of a local variety. The European Union Regulation 2018/848 allows the legal use of traditional seeds under the category of "Organic Heterogeneous Material" (OHM), to which this tomato could be adapted. GO MHE project aims to complete all the necessary bases to apply this new regulatory framework to the reproductive material of the 'Huevo de toro' tomato grown in the Guadalhorce Valley. Its specific objectives are to characterize the phenotypic diversity in real cultivation plots using IPGRI criteria, to de-

fine a phenotype standard for the 'Huevo de toro' tomato grown in the Guadalhorce, to characterize the genetic diversity present in plots differentiated by farmers who conserve seeds, and to document the water infrastructures and irrigation strategies used in the crops. GO BIOFLORA project uses Biodiversity as an agroecological strategy for virus diseases control in outdoor 'Huevo de toro' tomato cultivation through the use of floral bands adapted to the environmental and productive conditions that helps to shelter predators of the main vector agents that transmit the viruses and the application of bio stimulants such as chitosan to enhance natural defense of plants against the viral diseases. Moreover, the IPGRI characterization, NGS whole-genome DNA re-sequencing of different seed batches provided by local growers is being performed in order to assess the existing inter- and intra-variability in the 'Huevo de toro' germplasm. A survey of plants showing virus diseases symptoms in the region was carried out and RNA from pooled samples was sequenced by MiSeq technology and the obtained sequence reads were subjected to a bioinformatic untargeted strategy for detecting infecting viruses. Tobamoviruses and aphid-transmitted potyviruses were the prevalent viruses infecting Guadalhorce tomatoes. Finally, an experiment combining floral bands and chitosan application was performed.

Acknowledgements: MHE GOPG-23-0006 and BIOFLORA GOPG-23-0007 are funded by the European Agricultural Fund for Rural Development (EAFRD) and the Ministry of Agriculture, Fisheries and Rural Development of the Regional Government of Andalusia in the 2020 call for the Operation of Regional Operational Groups of the European Innovation Partnership on Agricultural Productivity and Sustainability (EIPAGRI).

POSTER PRESENTATION 39

TITLE: HARNESSING BIOSTIMULANTS FOR BETTER TOMATOES: A STUDY ON YIELD, QUALITY AND MACRO/MICRO NUTRIENT ACCUMULATION IN TOMATO FRUITS

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Abstract: In the context of climate change, biostimulants appear to be a promising solution for improving plant resistance to stress, increasing yields, and enhancing quality. At the same time, the growing demand for high-quality food makes biofortification a valuable strategy for improving the nutritional value of edible plant parts. This study was conducted to evaluate the effects of different biostimulants on the crop performance and accumulation of essential nutrients in tomato fruits. Tomato plants (var. Micro-Tom) were grown in pots with a peat:perlite mixture, supplemented with nutrients at optimal levels. Commercially available biostimulants of different origins were applied as soil or foliar treatments eight times during the growing season, from vegetative development to fruit formation. Four macro- and five micronutrients were quantified in ripe fruits using inductively coupled plasma spectrometry. Leaf chlorophyll content, yield and yield components, and fruit quality were also assessed. The obtained results show an improvement in the values of the chlorophyll content index (CCI) in the plants treated with biostimulants. A significant yield increase of 30–70%, along with a higher fruit number and slightly larger fruits, was observed in all biostimulant treatments compared to the untreated control. These yield improvements were not at the expense

of fruit quality, as no effects on fruit dry matter, TSS, vitamin C, acidity, firmness, or color were recorded due to biostimulant application. In two products containing amino acids, higher Fe and Zn content was observed. This, combined with the high yield and preserved fruit quality, highlights the potential of amino acid-based biostimulants as an integral component of a broader strategy for the agronomic biofortification of tomatoes.

Acknowledgement: V. Stoeva acknowledges the support of the Bulgarian Ministry of Education and Science under the National Program“Young Scientists and Postdoctoral Students - 2". All authors are grateful for the funding from Bulgarian National Science Fund, grant No KP-06-H86/13/10.12.2024.

Session:

NEW OBJECTIVES AND TECHNOLOGIES FOR PLANT BREEDING

KEYNOTE TALK 6

TITLE: TACKLING OLD AND NEW CHALLENGES FOR TOMATO BREEDING USING GENETIC RESOURCES, GENOMIC INFO AND NEW TECHNIQUES

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Abstract: Tomato Breeders and scientists face similar challenges as always, it only seems that the extent of the challenges seem to be more profound and occurring at a faster rate. Threats like Climate Change, emerging Pests and Diseases affect yield dramatically and the demand for more environmentally friendly cultural practices (ie less pesticides, herbicides and fertilizers) have then to be combined with strategies for increase productivity in low input conditions while increasing the fruit quality, nutritional, health and onrganolpetic tomato quality despite the increase in production costs. For this breeders need to use all the materials, knowledge and techniques available. It is important to realized that breeder have increasingly use knowledge to guide the breeding process and more so with new breeding techniques. A few examples will be presented on how the use of new tools (genomes; databases), approaches and knowledge together with the use of natural diversity (genetic resources) or man-generated diversity is helping to selected for the best materials, alleles and combination of them to cope with those challenges. Examples will be presented on how new genomic techniques including genome editing, cisgenesis, intragenesis and transgenesis not only contribute to increase our understanding on gene to trait relationship but also provide us with plants with the desired traits with no linkage drag.

ORAL PRESENTATIONS

ORAL PRESENTATION 22

TITLE: BREEDING TOMATOES TO IMPROVE THEIR RESPONSIVENESS TO BIOCONTROL PRODUCTS AGAINST POWDERY MILDEW

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Abstract: Powdery mildew caused by *Oidium neolycopersici* is one of the main diseases of greenhouse tomatoes worldwide. To avoid the systematic use of fungicides, the ecological and sustainable control of this disease requires integrated pest management strategies combining for example, prophylaxis, the use of genetically resistant plants and bioprotectant, such as natural substances inducing plant defense mechanisms. However, the efficacy of these plant resistance inducers (PRIs) can vary according to various abiotic and biotic factors, including the genotype of the plant. The selection of tomato varieties genetically predisposed to maximize the efficacy of PRIs could be a valuable tool for an effective control of this disease. In this context, our research aims to identify the genomic regions responsible for the variation observed in plant responses to a PRI against *O. neolycopersici*. To this end, a PRI showing a good protective efficacy against *O. neolycopersici* was selected and sprayed onto 147 tomato accessions. The susceptibility to powdery mildew of the different tomato accessions treated or not treated with the PRI was evaluated in two greenhouses.

After assessing and confirming the high heritability of plant responses to the efficacy of the PRI against the disease, a genome-wide association study (GWAS) was conducted to identify significant SNPs associated with plant responses to the PRI. In total, 17 significant SNPs were detected. These findings will provide valuable tools for breeders, facilitating the selection of new tomato varieties that respond favorably to PRIs. This work will contribute to the sustainable improvement of biological and integrated pest management strategies in protected cropping systems.

ORAL PRESENTATION 23

TITLE: INCREASING FRUIT FIRMNESS AND PATHOGEN RESISTANCE BY KNOCKING OUT A PECTATE LYASE IN TOMATO

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Abstract: As the agricultural and food systems are facing growing challenges, the improvement of crop quality and agronomic traits has become a central objective in plant breeding, which aims to find new ways to prevent post-harvest losses and discover new genetic sources for pathogen resistance. Tomato (*Solanum lycopersicum* L.) is an important vegetable crop cultivated worldwide. The softening of its fruits has a key role in determining the post-harvest quality of the product, with a significant impact on both food waste and food safety. To elucidate the role of *SlPL* (Solyc03g111690) in softening and pathogens' susceptibility processes, CRISPR/Cas9 mutants have been generated in Micro-Tom genetic background. The two obtained mutants (carrying 1-bp insertion and 2-bp deletion in the second exon of the gene, respectively) showed a significant increase in fruit firmness at two different ripening stages (breaker+4 and red ripe) and lower weight loss during post-harvest storage. An overall reduction in the activity of six

cell wall-remodeling enzymes (pectate lyase, pectin methylesterase, polygalacturonase, α-D-galactosidase, -D-galactosidase and -D-glucosidase), and the reduced susceptibility to three different pathogens (*Botrytis cinerea*, *Phytophthora capsici* and *Pseudomonas syringae* pv. *tomato*) have also been described. The insights gained from these observations suggest an intricate regulation of the cell wall-modification pathway and that the alteration of this process may trigger a compensatory defense mechanism that activates the plant immune response. These findings open the possibility to develop resilient and competitive varieties that meet both agronomic and quality needs, while helping to clarify the complexity of the cell wall disassembly process.

POSTER PRESENTATIONS

POSTER PRESENTATION 40

TITLE: *PECTOBACTERIUM CAROTOVORUM* SUBSP. *CAROTOVORUM* AND *PSEUDOMONAS SYRINGAE* ALTER GROWTH, YIELD, CHLOROPHYL CONTENT AND CRUDE PROTEIN CONTENT OF FOUR *SOLANACEACE* CROPS

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Abstract: Studies on the effect of pathogens on plant growth are commonplace but intricacies such as pathogen effects on reproduction, chlorophyll content and production of crude proteins remain unknown. Moreover, the association between the various growth, reproduction and physiology aspects is also not known. This manuscript presents results on the effect of the two phytopathogens, *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) and *Pseudomonas syringae* (Ps), on the growth, chlorophyl content and crude protein content in four *Solanaceae* crops, namely, potato, tomato, chili pepper and eggplant. Seedlings were pressure infiltrated with Pcc and Ps. Growth, chlorophyll content and crude protein content were determined on the treated in the inoculated plants and compared to the uninoculated control. The measured parameters were also correlated with each other to determined associations. Both pathogens negatively influenced growth parameters, namely, stem diameter, plant height, number of

leaves, number of fruits per plant, biomass, the physiological aspect of chlorophyll content and crude protein content. Correlation analysis highlighted that chlorophyll content, closely linked with growth and yield, was a critical factor affected by these pathogens. The analysis also indicated that changes in one parameter due to pathogen infection could lead to corresponding changes in others, underscoring the interrelationship between growth and physiology. The study suggests that although pathogens like Pcc and Ps reduce crop performance, variations in crop responses point to potential resistance mechanisms that could inform strategies to enhance resilience.

POSTER PRESENTATION 41

TITLE: *HAIRPLUS* IS TRICHOME MUTANT ALTERED IN GENOME-WIDE METHYLATION REGULATION

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Abstract: Trichomes, specialized epidermal cells on aerial plant surfaces, work as biofactories producing complex molecules toxic to pests (glandular trichomes) or act as physical barriers preventing pest dissemination (non-glandular trichomes). In tomato (*Solanum lycopersicum* L.), the HAIRPLUS (HAP) gene is a key regulator of glandular trichome formation. Loss of HAP function leads to epigenomic alterations in mutant plants, resulting in increased type I glandular trichome density. Epitranscriptomic modifications, such as N6-methyladenosine (m6A) methylation, represent reversible post-transcriptional mRNA changes that influence RNA metabolism, including translation and degradation. In fact, plants like Arabidopsis thaliana and Populus spp., such modifications are linked to developmental changes in trichome branching and morphology. This study evaluates transcriptomic and epitranscriptomic changes caused by the hap mutation across two genetic backgrounds and organs (leaves and reproductive stems) with varying trichome densities. RNA was isolated from three biological replicates per genotype and subjected to direct RNA sequencing using Oxford Nanopore Technologies on a MinION platform. Sequencing

analyses utilized the MinKNOW software, employing a protocol tailored for direct mRNA reads. Our findings reveal that HAP loss-of-function alleles induce epitranscriptomic alterations in genes associated with multiple metabolic pathways. These insights enhance our understanding of the molecular mechanisms through which HAP regulates glandular trichome development in tomato.

Acknowledgements & Funding: This research was supported by the Spanish Ministerio de Ciencia, Innovación y Universidades (grant AGL2017-88702-C2). Funding was also received from the BRESOV project (Breeding for resilient, efficient, and sustainable organic vegetable production funded by the European Union's Horizon 2020 research and innovation program; grant agreement No. 774244) and by the PPIT-UAL, Junta de Andalucía-ERDF 2021-2027. Objective RSO1.1. Programme: 54.A. Juan D. López Fábregas and José M. Medina were funded by PhD fellowships from the FPI program of the Spanish Ministerio de Ciencia e Innovación.

POSTER PRESENTATION 42

TITLE: GENOME-WIDE ASSOCIATION ANALYSIS OF THE G2P-SOL WORLDWIDE TOMATO CORE COLLECTION HIGHLIGHTS CANDIDATE GENES FOR AGRONOMIC AND FRUIT QUALITY TRAITS

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Abstract: A core collection of 353 cultivated *S. lycopersicum* accessions including landraces and modern tomato cultivars has been constructed from 15,504 accessions derived from 124 countries. These accessions were genotyped by SPET and the genetic distance matrix was used to construct the core collection. This core collection was resequenced to 20x depth, generating millions of SNPs and tens of thousands of structural variants, and phenotyped in two locations

for agronomic and fruit quality traits, including the fruit metabolome (semi-polar, lipid, volatile compounds) and ionome. Genome-Wide Association Analysis revealed hundreds of correlations between molecular markers and agronomic/fruit traits, as well as known and novel candidate genes associated with such traits. Correlation and network analyses provide a detailed view of the genetic-physiological architecture of such traits.

Acknowledgments: We acknowledge funding from the EU (projects G2P-SOL, Grant agreement 677379 and PRO-GRACE, Grant agreement 101094738) and by the Italian Research Council, special project Tomatom.

POSTER PRESENTATION 43

TITLE: ASSESSMENT OF GENETIC DIVERSITY IN BULGARIAN TOMATO VARIETIES WITH COS II MARKERS

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Abstract: The tomato (*Solanum lycopersicum* L.) is a widely cultivated crop of significant economic and nutritional value worldwide. This study aimed to assess the genetic diversity of nine tomato accessions from the Maritsa Vegetable Crops Research Institute collection using COS II markers. Genetic diversity was analyzed using nine COS II markers located on the first chromosome of tomato, spanning from 8.0 to 115.70 cM, and located near the SlMYB12 gene, which participate in flavonoid synthesis. Mutations in this gene are associated with pink fruit pigmentation. Using six reactions localized on the first chromosome, seven Bulgarian tomato varieties were identified: "IZK Niki D" F1, "Rozovo Sartse", "Plovdivska Karotina" "IZK Alya" "Kopnezh" F1, and "Olimp" F1. These markers are localized on the sixth chromosome of the tomato genomic map (TOMATO – EXPEEN 2000), ranging from 42.50 to 89.10 cM. They are positioned near the F3H gene, which encodes flavonoid 3-hydroxylase, an enzyme involved in flavonoid biosynthesis by catalyzing the hydroxylation of flavonoid precursors. The results of the C2_At4g36530 reaction, used to differentiate the amplification profiles of four tomato genotypes, revealed monomorphic profiles in "Rozovo Sartse" and "Aleno Sartse", while "IZK Niki D" F1 and "Kopnezh" F1 exhibited polymorphic profiles. The use of COS II markers enables

the characterization of genetic diversity within the tomato collection. They serve as a reliable tool in breeding programs for identifying tomato varieties and lines, as well as accelerating the breeding process.

Acknowledgments: BUL/5/020 funded by FAO/IAEA; National Program “Young Scientists and Postdoctoral Students – 2” funded by BMES; ZEMDKT 17 funded by Agricultural Academy. The Turkish Energy, Nuclear and Mineral Research Agency founded COS II part by A2.H1.P16 project.

POSTER PRESENTATION 44

TITLE: GENOMIC SEQUENCING OF TOMATO ACCESSIONS USING ILLUMINA AND OXFORD NANOPORE TECHNOLOGIES

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Abstract: Tomato is a key crop in Bulgaria, and mutagenesis represents a valuable approach for developing improved varieties for sustainable agriculture. This study aimed to analyze the genome of the initial breeding line "Hektor" and the highest-yielding mutant line (No. 11) using Illumina and Oxford NanoPore Technologies (ONT). The obtained Illumina sequencing data comparing the initial and mutant tomato lines to the reference genome consisted of 294,860 variants, including 244,439 single nucleotide polymorphisms (SNPs) and 50,421 insertions/deletions (INDELs). After filtering the data using higher thresholds for quality and depth, the results consisted of 5,019 polymorphisms, including 4,411 SNPs and 608 INDELs. The FastQC report for tomato accessions showed total bases of 13.2 Gbp for the initial genotype "Hektor" and 14.4 Gbp for mutant line No. 11, with sequence lengths of 75–151 bp and 35% GC content. The genomic DNA of the initial line was sequenced using the ONT (Promethion 2 Solo), generating a total of 6,017,804 high-quality reads with a mean read length of 8,121.6 bp. The result of the polished contigs for "Hektor" is 301.

The BUSCO analysis results indicated a genome coverage of 98.6%, a strong indicator of high-quality assembly, with 1,614 complete genes identified. The assembly statistics show a well-assembled genome with a relatively low number of contigs and a high N50 value of 11 Mb, indicating long contiguous sequences and strong structural integrity. These results will aid in understanding the genetic basis of enhanced productivity and provide a foundation for future genotyping, QTL mapping, and functional genomic studies.

Acknowledgments: This study was funded by the IAEA under the TC project BUL5020; Authors acknowledge TC project BUL5020, IAEA; National Program "Young Scientists and Postdoctoral Students – 2" funded by BMES.

PRESENTATION 45

TITLE: TARGETING SUSCEPTIBILITY GENES: A NOVEL STRATEGY TO COMBAT NECROTROPHIC FUNGI IN TOMATO

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Abstract: The absence of fully resistant tomato varieties against necrotrophic fungi threatens food security and causes substantial economic losses. This results from the lack of dominant resistance (R) genes in crops to combat necrotrophic fungi such as *Botrytis cinerea* and *Alternaria solani*. Thus, a novel approach to achieve resistance is now being taken by disabling plant susceptibility (S) genes that facilitate pathogen infection. S gene refers to any gene that plays a role in enabling infection and ensuring compatibility between a pathogen and its host. Their inactivation or downregulation is predicted to result in plant resistance. The expected durable and broad-spectrum resistance resulting from the loss-of-function mutations in S genes is proposed to arise because the pathogen must develop new functions to reestablish compatibility. Some studied S genes are *PUB17*, *MYB46*, and *WRKY57*. The first was identified from a MicroTom EMS population, and the last two were reported in Arabidopsis to confer susceptibility to *B. cinerea*. Tomato mutants of these genes obtained by EMS treatment and/or CRISPR editing showed a decreased susceptibility to the necrotrophic fungi *B. cinerea* and *A. solani*. These genes are involved

in different pathways and contribute in diverse ways to reduced susceptibility. *PUB17* is a Ubiquitin E3 ligase involved in plant cell death, *MYB46* is a regulator of secondary cell wall formation in Arabidopsis, and *WRKY57* is a negative regulator of jasmonic acid-induced senescence. Research is being performed on these pathways to identify new mutations or new S genes that can maintain resistance without affecting normal plant growth.

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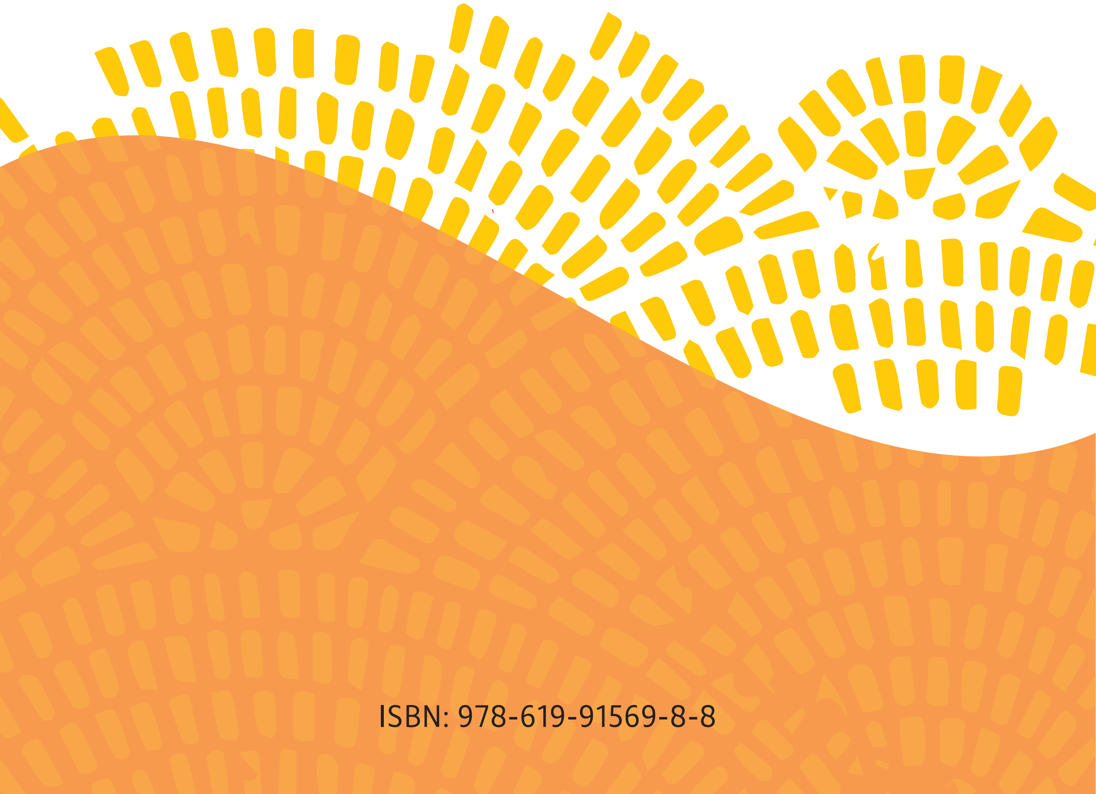

ISBN: 978-619-91569-8-8