

EUCARPIA '26

Pepper & Eggplant

19th EUCARPIA Meeting on Genetics and Breeding
of Capsicum and Eggplant

Suzan Gabriëls, Yury Tikunov, Lotte Caarls, Arnaud Bovy, Yuling Bai



WAGENINGEN
UNIVERSITY & RESEARCH

Book of Abstracts

EUCARPIA '26 Pepper & Eggplant

**19th EUCARPIA Meeting on Genetics and Breeding
of Capsicum and Eggplant**

Editors:

Suzan Gabriëls

Yury Tikunov

Lotte Caarls

Arnaud Bovy

Yuling Bai

20 - 23 September 2026

Wageningen

The Netherlands

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19th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant
20-23 September 2026
Wageningen, The Netherlands

Editors:

Suzan Gabriëls¹, Yury Tikunov¹, Lotte Caarls¹, Arnaud Bovy¹, Yuling Bai¹

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Quotation from or reference to any part of this book should be made with full reference to the above data.

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- Yury Tikunov, Wageningen University & Research, The Netherlands
- Yuling Bai, Wageningen University & Research, The Netherlands

Foreword

Foreword

Welcome to our 19th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant in Wageningen! The event, with over 150 participants, brings together scientists and professionals from all over the world to stimulate knowledge exchange, collaboration and innovation. Apart from the scientific program, you will get the opportunity to visit our state-of-the-art research facilities regarding phenotyping, physiology, technology, vision and robotics at the Wageningen University & Research campus. This booklet contains brief practical information about the venue, the scientific and social programme, poster viewing and presentations, excursions and the conference dinner. On the Eucarpia 2026 website you will find a digital version of the program and all oral- and poster abstracts that are scheduled in the programme.

May we ask you to also pay attention to our highly valued sponsors that helped to make this congress possible. For all your questions or comments, please contact the Eucarpia pepper & eggplant registration desk at hotel Wander.

We hope you will have a pleasant stay in Wageningen and fruitful scientific interactions.

On behalf of the organising committee

Dr.ir. Suzan Gabriëls

Scientist Plant Breeding, Wageningen University & Research

Programme

Programme

Sunday, 20 September 2026

Welcome & Networking at WANDER

18.00 **Pre-registration**

19.00 **Keynote:** Hidde Boersma, Dutch freelance science journalist, public speaker, and documentary filmmaker

20.00 **Social Drinks & Networking Reception**

Monday, 21 September 2026

Presentations at WANDER

08.00 **Registration and hang up of posters**

09.30 **Welcome:** Yuling Bai, Wageningen University and representing EUCARPIA

09.35 **Keynote:** Richard Visser, Wageningen University and research - A short history of and outlook into the future of pepper and eggplant breeding and research

09.55 **Practical announcements:** Suzan Gabriëls, chair

Topic 1: Plant Genetic Resource Management

chair: Lorenzo Barchi and Luciana Gaccione

10.00 **Keynote:** Lorenzo Barchi, University of Turin- Unlocking Crop Diversity in the Genomic Era: Lessons from Eggplant Diversity

10.30 Group Picture, Coffee Break & Poster Viewing

11.30 Luciana Gaccione, University of Turin - Painting Eggplant Purple: How the Genome Shaped Anthocyanin Patterns

11.45 Christina Papastolopoulou, Wageningen University & Research - Pangenome-enabled association analysis for capturing genetic diversity in Capsicum

12.00 Kritika Adhikari, INRAE Avignon - Assessing the diversity of the Espelette pepper landrace in the French Basque country

12.15 Edoardo Vergnano, University of Turin - Large scale characterization of Capsicum germplasm clarifies diversity, domestication, and taxonomic misclassification

12.30 **Lunch & Poster viewing**

Topic 2: Gene Editing

chair: Arnaud Bovy and Yuri Tikunov

- 14.00 Keynote:** Byoung-Cheorl Kang, Seoul National University - Genetic and functional characterization of capsaicinoid biosynthesis and analog composition in pepper
- 14.30** Laura Toppino, CREA, Montanaso Lombardo - CRISPR/Cas9-Mediated Editing of the HQT Gene Prevents Browning in Eggplant Fruit Flesh
- 14.45** Martina Ferrero, University of Turin - Editing Eggplant without a trace: CRISPR/Cas9 RNP-mediated Genome Editing in Protoplasts
- 15.00 Coffee Break & Poster Viewing**

Topic 3: Abiotic stress and resilience

chair Ivo Rieu and Arnaud Bovy

- 15.45 Keynote:** Ivo Rieu, Radboud University - Breeding for reproductive heat tolerance in Solanaceae: it takes two to tango
- 16.15** Derek W. Barchenger, World Vegetable Center, Taiwan - Convergent evidence for a major locus controlling fruit set under heat stress in Capsicum
- 16.30** Andrea Arrones, Polytechnic University of Valencia - Independent mutations in SP5G antiflorigen gene channeled eggplant adaptation
- 16.45** Francesco Paladini, University of Turin - Unlocking drought stress tolerance in eggplant through SmDMR6-1 genome editing
- 17.00** Bryan Anthony Diamante, Universitat Politècnica de València, Spain. Genetic dissection of trichome density in eggplant through gwas and bsa-seq
- 17.15** Akira Yamazaki, Kindai University, Japan - Detection and characterization of a quantitative trait locus associated with high-temperature tolerant fruit set in Capsicum annum
- 17.30 Informal drinks**

Tuesday, 22 September 2026

Presentations at WANDER, Excursions & Conference Dinner

Topic 4: Fruit Quality & Metabolomics,
chair Arnaud Bovy and Peter Poláček

- 09.00** **Keynote:** Yury Tikunov, Wageningen University & Research - Pepper fruit flavor: usual and unusual suspects
- 09.30** Yoshiyuki Tanaka, Kyoto University, Japan - Discovery and characterization of a novel leaky vanillin aminotransferase (VAMT) allele with an amino acid substitution, affecting capsaicinoid composition in chili pepper
- 09.45** Hagai Cohen, Institute of Plant Science, Israel - Microscopic and metabolic investigations reveal cuticle thickness and epidermal cell density as critical factors in chili pepper skin cracking
- 10.00** Lalida Sangpong, Nara Institute of Science and Technology, Japan - Analysis of specialized metabolisms in response to uv-b exposure in the Solanaceae family
- 10.15** Paolo Cerruti, University of Turin - Development of a novel magic population in pepper: phenotyping and genotyping analyses for multiple desired traits
- 10.30** **Coffee Break & Poster Viewing**

Topic 5: New Phenotyping Tools & Data Analysis
chair: Pasquale Tripodi and Laura Toppino

- 11.15** **Keynote:** Pasquale Tripodi, CREA - New Phenotyping Tools and Data Analysis
- 11.45** Jose Luna Ramos, Polytechnic University of Valencia - Morphoagronomic phenotyping and GWAS of agronomic traits in an eggplant magic population
- 12.00** Peter Poláček, Wageningen University & Research - Genome-wide association analysis of a re-sequenced Capsicum core collection through a unified, user-friendly GWAS toolbox
- 12.15** Greg Vogel, Cornell University, USA - Variation in fruit morphology characteristics, maturity, and prickly incidence in the USDA National Plant Germplasm System eggplant collection
- 12.30** Mireia Romans Escrivà, Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana - Phenotypic diversity and germination kinetics in a magic population of Capsicum spp.
- 12.45** **Lunch & Poster Viewing**
- 14.00** Walk, cycle, or public transport to excursions (all in Wageningen)
- 15.00** Visiting research facilities at WUR (NPEC, Genebank) / City Tour / Wageningen Campus tour
- 18.00** **Welcome drinks** at Hotel De Wageningsche Berg
- 19.00** **Conference dinner** followed by party with DJ and dancing at Hotel De Wageningsche Berg

Wednesday, 23 September 2026

Presentations & Closing at WANDER

Topic 6: Disease Resistance

chair: Véronique Lefebvre and chair tba

- 09.00** **Keynote:** Véronique Lefebvre, INRAE - Designing durable plant resistance in a changing world: insights from pepper pathosystems
- 09.30** Sota Koeda, Kindai University, Japan - Begomovirus resistance gene Ey-1 encodes a DEDDh exonuclease in eggplant
- 09.45** Guillermo Merino Martin, Wageningen University & Research - Identification of novel Phytophthora capsici resistance loci in Capsicum spp. through integrated QTL mapping and effecteromics
- 10.00** Angeles Ramos Peregrina, Wageningen University & Research - Validation of the S-gene concept in pepper using VIGS
- 10.15** Sunanya Das, Centurion University of Technology and Management, India - Dissecting the genetic architecture of bacterial wilt resistance in Indian eggplant: QTL-seq uncovers novel resistance loci in local germplasm
- 10.30** **Coffee Break & Poster Viewing**

Topic 7: Insect Resistance

chair: Lotte Caarls and Karen Kloth

- 11.15** **Keynote:** Lotte Caarls, Wageningen University & Research - Breeding for insect resistance in pepper: insights from studies of genetics and mechanisms of resistance against aphids
- 11.45** Erin Puspita Rini, Wageningen University & Research - Genetic mapping and metabolic profiling reveal candidates for whitefly resistance in pepper (Capsicum spp.)
- 12.00** Kietsuda Luengwilai, Kasetsart University, Thailand - Early Defense Mechanisms in Juvenile Stage of Eggplant Against Infestation by Leucinodes orbonalis Larvae
- 12.15** Tee Havananda, Kasetsart University, Bangkok - Harnessing phenotypic diversity in Thai eggplant germplasm for multi-pest resistance breeding
- 12.30** Karen Kloth, Wageningen University & Research - Purple corolla Capsicums and their resistance to the green peach aphid
- 12.45** **Lunch & Poster Lunch**

- 14.15 Round Table discussion** “The use of AI in (pepper and eggplant) breeding”, chaired by Richard Visser with representatives from Plant Breeding companies and from WUR department “Artificial Intelligence” and “Plant Breeding”
- Representatives/Participants
1. Prof Agnieszka Golicz, Professor Plant Breeding, Wageningen University and Research
 2. Aike Potze, PhD student working on Artificial Intelligence, Wageningen University and Research
 3. Nicolas Bardol , Applied genetics scientist lead, Limagrain
 4. Geo Velikkakam James, Research Leader in Computational Biology, Rijk Zwaan
 5. Robin Huibers, Breeding Principal Pepper, ENZA Zaden
 6. Peter Polacek, PhD student at Plant Breeding, Wageningen University and Research
- 15.30 Best Presentation and Best Poster Awards** – Arnaud Bovy
Conference Summary and Closing Remarks – Suzan Gabriëls
Announcement of the Host for the 2029 Eucarpia Capsicum–Eggplant Conference – Yuling Bai
- 16:00 Farewell drinks**

A SHORT HISTORY OF AND OUTLOOK INTO THE FUTURE OF PEPPER AND EGGPLANT BREEDING AND RESEARCH

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Breeding in many different crops has been done for a long time based on phenotypic improvement of offspring performance. That is now changing dramatically due to the availability of genomics in many crops including ornamentals which makes breeding more efficient and effective. It now has also become possible to breed for specific niche markets in these crops thanks to the availability of pangenomes and highly reliable phenotypic data of many different genotypes and wild accessions.

Breeding for resistance to biotic stress -and increasingly also for tolerance or resistance to abiotic stress- is still the most important target in breeding, however also other breeding goals are receiving more attention. The challenges (climate change) and demands (feeding nine billion people) are plentiful making a faster and more precise breeding of these crops necessary. Especially since these crops contain many useful secondary metabolites which also play a role in preventing and maybe even combatting certain human diseases. With the recent availability of pangenomes, high throughput phenotyping methods and large scale mapping of important traits the efficiency and efficacy of breeding these two important vegetable crops will most certainly improve dramatically.

Topic 1

Plant Genetic Resource Management

UNLOCKING CROP DIVERSITY IN THE GENOMIC ERA: LESSONS FROM EGGPLANT DIVERSITY

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Genetic resource management is entering a new era in which conservation, characterization, and use of crop diversity can be guided by genome-wide information. Eggplant (*Solanum melongena* L.), one of the world's most important solanaceous vegetables, provides a powerful example of how genomics can transform our understanding and management of plant genetic resources. Eggplant is characterized by a remarkable phenotypic and genetic diversity, together with a rich wild gene pool that represents an precious reservoir for future breeding.

By genotyping more than 3,400 accessions of cultivated eggplant and wild species, we reconstructed the crop's domestication and diversification history, revealing independent domestication centers in India and Southeast Asia and complex migration routes across continents. These data also demonstrate the practical value of genomics for germplasm management, enabling the identification of misclassified materials, duplicates, and gaps in collections, thereby improving conservation strategies.

Beyond describing diversity, genomic analyses uncovered signatures of human and environmental selection, highlighting candidate loci associated with adaptation to different agroecological conditions and potential climate resilience. Furthermore, a representative core collection was developed within the G2P-SOL project to capture global diversity in a manageable set of accessions, supporting more efficient research and breeding. Finally, graph-based pangenomes, including cultivated eggplant and its allied species (*S. insanum* and *S. incanum*), was built to provide a richer framework than a single linear reference genome for exploring structural diversity and linking genotype to phenotype.

Together, these results show how genomic tools can convert germplasm collections from static repositories into dynamic resources for crop improvement, resilience, and sustainable agriculture.

Acknowledgements: This work has been funded by the European Commission, Horizon 2020 G2P-SOL project (grant no. 677379) and by the Horizon Europe 'Promoting a Plant Genetic Resource Community for Europe (PRO-GRACE)' project (grant agreement no. 101094738)

Keywords: genome-wide association analysis, SPET, pangenome, germplasm

PAINTING EGGPLANT PURPLE: HOW THE GENOME SHAPED ANTHOCYANIN PATTERNS

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Eggplant (*Solanum melongena* L.) displays striking diversity in pigmentation across fruit and plant tissues, yet the genetic mechanisms underlying anthocyanin accumulation and its evolution during domestication remain incompletely understood. Here, we performed pangenome-wide association (Pan-GWA) analyses on a core collection of 368 accessions representing global diversity, integrating phenotypic data with graph-based pangenomes. We leveraged two complementary resources: the PG-SM graph, capturing variation within cultivated eggplant, and the PG-SMA graph, which incorporates chromosome-level assemblies from 40 cultivated accessions and wild relatives (<https://www.nature.com/articles/s41467-025-64866-1>).

Using this approach, we uncovered a multilayered architecture underlying anthocyanin accumulation that revises current paradigms on the control of pigment distribution in eggplant. Our analyses highlighted the impact of large-scale genomic rearrangements, on top of regulatory diversity affecting gene expression.

In parallel, we identified additional genetic factors that influence the activity of the core regulatory machinery controlling anthocyanin biosynthesis, with effects that vary across plant tissues (leaves, stem and fruit peel and calyx) and developmental contexts. Notably, patterns of allelic variation at these loci suggest signatures of selection and functional interactions that have contributed to phenotypic diversification.

Together, our results provide a new framework for understanding how eggplants transitioned from green wild forms to the diverse purple phenotypes observed in cultivated varieties, revealing that this process was driven by the coordinated evolution of regulatory networks rather than by simple genetic switches.

Acknowledgements: This work has been funded by the European Commission, Horizon 2020 G2P-SOL project (grant no. 677379) and by the Horizon Europe 'Promoting a Plant Genetic Resource Community for Europe (PRO-GRACE)' project (grant no. 101094738)

Keywords: pangenome graph, pangenome-wide association analysis, large scale genomic rearrangements, regulatory network

PANGENOME-ENABLED ASSOCIATION ANALYSIS FOR CAPTURING GENETIC DIVERSITY IN CAPSICUM

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The extensive genetic and phenotypic diversity within the *Capsicum* genus presents both opportunities and challenges for crop improvement. Traditional GWAS approaches suffer from reference bias. While k-mer-based GWAS provides a reference-free alternative, downstream interpretation typically relies on mapping to a single reference genome, effectively reintroducing bias. This motivates a pangenomic, reference-free interpretation of k-mers. Here, we present a graph-based *Capsicum* pangenome constructed from 12 high-quality genome assemblies across nine species, including multiple *C. annuum* accessions. Using this resource, we developed an integrative framework to associate genetic variation with fruit metabolite diversity across 343 *C. annuum* accessions. By mapping the identified significant k-mers per trait directly onto the pangenome graph, we localize them across multiple genomes simultaneously and identify the associated haplotypes, capturing diverse forms of variation including sequence polymorphisms, copy number changes, and structural variants. We demonstrate this approach through several use cases, revealing different genetic patterns underlying metabolite variation and how association signals can be translated into meaningful haplotypes in both cultivated pepper and its wild relatives. Although demonstrated in *Capsicum*, this framework is broadly applicable to other species and traits, providing a general strategy for linking genetic diversity to phenotypic variation using pangenome-based analyses. These results warrant further development of k-mer-based approaches, improving efficiency through reduced representations, to fully utilize genomic diversity in *Capsicum* breeding programs.

Keywords: *Capsicum*; pangenome; structural variation; association analysis; k-mer GWAS; plant breeding; *Capsicum* (pan)genomics

ASSESSING THE DIVERSITY OF THE ESPELETTE PEPPER LANDRACE IN THE FRENCH BASQUE COUNTRY

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Understanding genetic and phenotypic diversity of crop landraces is crucial for maintaining adaptive potential in changing environments. Landraces persist as reservoirs of variation for crop performance, shaped by long-term farmer practices and local agroecological conditions. The Espelette pepper (*Capsicum annuum* L.) is a traditional pepper landrace cultivated in the Basque region of France under a Protected Designation of Origin (PDO) representing a unique geographically and culturally defined genetic resource. A comprehensive study of the phenotypic, metabolomic and genetic diversity in this landrace remains lacking to this date. Hence, this study aims to study the extent and structure of genetic and phenotypic diversity within the Espelette population. Genomic and phenotypic data from 575 and 708 plants, respectively, sampled across 23 farmers within the PDO cultivation area (~299 ha), were analysed. Genetic diversity and population structure were evaluated using 15K genome-wide SNP markers genotyped through single primer enrichment technology (SPET) while phenotypic variation was assessed through field-based measurements of various plant developmental, disease and fruit traits as well as metabolomic patterns. Our results show a small subgrouping of Espelette genetically diverging from a largely maintained population exhibiting important genetic differentiation. Our phenotypic analyses show that there is significant variability across plant height and fruit traits within farmer as well as between farmers indicating intra-landrace diversity. As a part of the ANR COMBINE project, these research insights contribute to understanding how diversity within landraces may support future sustainable management strategies such as varietal mixtures to improve resilience and durability. Moreover, the findings may encourage integrating genomic and phenotypic information for effective maintenance, management and in-situ conservation of traditional varieties like Espelette pepper.

Keywords: *Capsicum annuum*, landrace, SPET, genotype, phenotype, diversity

LARGE SCALE CHARACTERIZATION OF *CAPSICUM* GERMPLASM CLARIFIES DIVERSITY, DOMESTICATION, AND TAXONOMIC MISCLASSIFICATION

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Understanding and exploiting genomic diversity in plant genetic resources is essential for crop improvement, germplasm conservation, and sustainable agriculture. In *Capsicum* spp., high-resolution genomic analyses are particularly valuable for clarifying relationships between cultivated forms and their wild relatives and for improving the taxonomic curation of germplasm collections. A total of 844 *Capsicum* accessions, representing the genetic diversity of pepper and belonging to the G2P-SOL and INRAE core collections, were whole-genome resequenced. For the first time, 46 accessions of *Capsicum annuum* var. *glabriusculum*, the wild progenitor of cultivated *C. annuum*, were studied. Population structure and phylogenetic analyses revealed clear genomic differentiation among and within species. The inclusion of *C. annuum* var. *glabriusculum* helped to clarify the transition from wild to domesticated forms and to identify accessions whose original classification was inconsistent with their genomic background. Selective sweep analyses highlighted candidate regions associated with domestication-related traits, including loci involved in fruit abscission, fruit size and fruit shape. The resequencing data were further exploited to carry out genome-wide association studies (GWAS) of agronomically relevant traits, performed on the G2P-SOL core collection of 350 *C. annuum* accessions with available phenotypic data. GWAS identified significant associations for fruit shape and fruit length on chromosomes P3 and P10, and for flowering time, on chromosome P12. Overall, large-scale resequencing proved effective for identifying genes underlying traits, as well as for germplasm characterization and management across *Capsicum* spp. Furthermore, the inclusion of *C. annuum* var. *glabriusculum* helped to distinguish them in respect to cultivated *C. annuum* var. *annuum* and admixed accessions between the two botanical varieties, resulting in a better understanding of *C. annuum* var. *annuum* domestication.

Acknowledgment: This work was supported by project ANR AGRODIV (Grant agreement 22-PEAE-0005) from the France 2030 program 'PEPR Agroécologie et Numérique', and the projects G2P-SOL (Grant agreement 677379) and PRO-GRACE (Grant agreement 101094738) from European Commission Horizon 2020.

Keywords: *Capsicum* spp; *Capsicum annuum* var. *glabriusculum*; genomic diversity; domestication; GWAS

Topic 2

Gene editing

GENETIC AND FUNCTIONAL CHARACTERIZATION OF CAPSAICINOID BIOSYNTHESIS AND ANALOG COMPOSITION IN PEPPER

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To elucidate the genetic regulation of capsaicinoid biosynthesis and composition in pepper, we combined EMS mutagenesis, transcriptomic analysis, metabolite profiling, and virus-induced gene editing. CRISPR/Cas9-mediated deletion mutagenesis of the *Pun1* promoter is being used to define regulatory elements controlling pungency-related gene expression. Screening of EMS-mutagenized populations revealed that mutations in BCKDH component genes significantly reduce capsaicin and dihydrocapsaicin accumulation and generate capsaicinoid analogs. Genetic analysis of the low-pungency mutant 1505-5-6i identified CaKAS I-2 and CaALDH1 as key determinants of altered capsaicinoid content and analog composition, including changes in dihydrocapsaicin ratio and the production of additional capsaicinoid-related compounds. In parallel, transcriptome-guided candidate gene discovery followed by VIGS and CRISPR/Cas9-mediated validation identified additional novel regulators of capsaicinoid accumulation. Together, these results provide new insight into the biosynthesis and diversification of capsaicinoids and suggest promising genetic targets for breeding peppers with customized pungency profiles.

CRISPR/CAS9-MEDIATED EDITING OF THE HQT GENE PREVENTS BROWNING IN EGGPLANT FRUIT FLESH

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Eggplant is a rich source of health-promoting compounds, particularly phenolics, located at high concentrations in both peel (anthocyanins) and flesh (chlorogenic_acid-CGA). Nevertheless, elevated levels of CGA are associated with a browning attitude affecting the flesh after cutting or mechanical damage, considered a major drawback impacting product quality and consumers acceptance.

The hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase(HQT) catalyzes CGA biosynthesis in eggplant and major Solanaceae. Through a CRISPR/Cas9-based approach, two edited lines carrying different frameshift homozygous mutations in smHQT were obtained. Their morphometric evaluations under greenhouse and phytotron conditions revealed normal development and production of fruits indistinguishable from wild-type. LC-MS and HPLC analysis of leaves and fruits revealed that both mutants produced significantly reduced amounts of CGA and showed a redistribution of other hydroxycinnamic acid intermediates, confirming that HQT is the primary pathway for CGA accumulation in eggplant. Notably, fruits from edited plants did not darken even several hours after the cut, demonstrating the direct role of CGA in this trait. RNA-seq analysis highlighted a significant downregulation of HQT, consistent with the induction of a Nonsense-Mediated mRNA Decay mechanism. Global expression profiles revealed a striking asymmetry in the distribution of transcript abundance in the edited lines, and a skew towards downregulated genes. GO enrichment analysis of the latter highlighted a significant overrepresentation of terms related to oxidoreductase activity, heme binding, and iron ion binding. The presence of terms such as "lignin catabolic process" and "response to wounding" suggests that the knock-out of HQT and the subsequent alteration of the phenylpropanoid pathway may trigger secondary metabolic rearrangements and stress-related signaling in the edited eggplant tissues. This work highlights the effectiveness of metabolic engineering of the CGA biosynthetic pathway to prevent flesh browning in eggplant, offering promising opportunities for incorporation into future eggplant breeding programs.

Keywords: Solanum melongena; Chlorogenic acid; CRISPR/Cas; HQT, polyphenols, flesh browning

EDITING EGGPLANT WITHOUT A TRACE: CRISPR/CAS9 RNP-MEDIATED GENOME EDITING IN PROTOPLASTS

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CRISPR/Cas9 reagents are commonly introduced into plants using conventional genetic transformation methods based on *Agrobacterium tumefaciens*, which lead to the stable integration of the gene construct into the recipient genome. To meet the requirements of the new regulatory framework proposed at a European level for plants obtained through New Genomic Techniques (NGTs), the development of new protocols generating transgene-free genome-edited plants (NGT category 1) is essential. Since CRISPR/Cas9 reagents can be delivered as ribonucleoproteins (RNPs), protoplasts represent a suitable system for genome editing. This approach induces genetic modifications transiently, leaving only the desired targeted mutations without any additional traces in the recipient DNA.

With the objective of establishing transgene-free genome editing in eggplant (*Solanum melongena* L.), we adapted and optimized a protocol previously developed for potato and tomato to isolate protoplasts from cotyledons and deliver CRISPR/Cas9 reagents. In vitro regeneration of non-transfected protoplasts was carried out, and the first wild-type shoots were obtained from calli approximately 4-5 months after isolation. In parallel, we delivered RNPs into protoplasts using two transfection approaches: one based on polyethylene glycol (PEG) at two different concentrations (25% and 40%), and the other using two lipofectamine formulations (CRISPRMAX™ and Lipofectamine™ 3000), all targeting SmCHL_H gene, whose inactivation results in a chlorotic phenotype. After optimization of the delivery procedure, edited calli were regenerated and editing efficiency was assessed by Sanger sequencing. The percentage of edited calli resulted definitely higher for PEG-mediated transfection, particularly with 25% PEG, compared with lipofection. Fully edited biallelic calli were obtained, and the chlorotic phenotype was already clearly visible at the callus stage. Although the efficiency of in vitro plant regeneration from calli still requires improvement, edited plants were successfully regenerated, representing the first report of RNP-mediated genome editing in eggplant protoplasts.

Keywords: ribonucleoproteins; protoplasts; CRISPR/Cas9; eggplant; lipofectamines

Topic 3

Abiotic stress and resilience

BREEDING FOR REPRODUCTIVE HEAT TOLERANCE IN SOLANACEAE: IT TAKES TWO TO TANGO

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Heatwaves are increasingly compromising crop fertility, threatening yield stability under climate change. In Solanaceae, reproductive heat tolerance depends on both sides of fertilization: male and female reproductive traits can each become bottlenecks under high temperature. Using tomato RIL populations cultivated in climate chambers, greenhouses, and production fields, we identified conserved and environment-specific QTLs for reproductive traits under heat stress. Fruit-set QTLs co-located with loci associated with pollen viability, pollen number, and stigma exertion, highlighting these sub-traits as key drivers of fertility in hot conditions. The discovery of high-impact loci for reproductive heat tolerance suggests that breeding for improved fertility under heat is a realistic path toward greater climate resilience in tomato and other Solanaceae.

Keywords: abiotic stress, high temperature, sexual reproduction, Solanaceae, tomato, genetics

CONVERGENT EVIDENCE FOR A MAJOR LOCUS CONTROLLING FRUIT SET UNDER HEAT STRESS IN CAPSICUM

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Heat stress reduces yield in pepper by disrupting reproductive processes, with fruit set emerging as the most consistent field-level indicator of tolerance. Genetic dissection of this trait remains limited due to strong genotype × environment effects and the difficulty of phenotyping reproductive responses under stress. A recombinant inbred line population derived from AVPP1346 and AVPP9905 was evaluated under high-temperature conditions across multiple environments in Asia. Fruit set percentage was used to quantify reproductive performance. Lines differed consistently in fruit set across locations, indicating stable genetic variation for heat tolerance despite environmental heterogeneity. Bulk segregant analysis identified a major genomic region on chromosome 3 associated with fruit set under heat stress. Transcriptomic analysis of contrasting lines revealed clusters of differentially expressed genes within the same region on chromosome 3, including candidates associated with stress response and reproductive development. The effect of this locus was further supported by its association with reduced loss of pollen viability under heat stress in both the mapping population and an independent mini-core collection. The consistency of the signal on chromosome 3 across environments and diverse genetic backgrounds supports its relevance for breeding. This study reinforces the value of field-based phenotyping for resolving complex traits and provides a clear target for improving heat resilience in Capsicum.

Keywords: heat stress tolerance, multi-location, transcriptomics, bulk segregant analysis

INDEPENDENT MUTATIONS IN SP5G ANTIFLORIGEN GENE CHANNELLED EGGPLANT ADAPTATION

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Flowering time is a crucial trait for crop domestication and adaptation to different environments. The SELF-PRUNING 5G (SP5G) gene, a member of the florigen gene family, evolved into an opposing role acting as an antiflorigen and repressing flowering. A comparative analysis across different Solanaceae species revealed that SP5G has been repeatedly selected during evolution, domestication, and adaptation. Independent mutations in both the coding sequence and regulatory regions of SP5G reduce its activity, leading to earlier flowering. To investigate the genetic basis of early flowering in eggplant (*S. melongena*), a genome-wide association study (GWAS) was conducted on a MAGIC population of 283 lines, including as founders cultivated eggplant accessions and its wild relative *S. incanum*. The analysis identified an association peak on chromosome 5 near the *SmSP5G* gene, along with a 2.15 kb coding deletion that removes nearly half of the gene body, which was associated with earlier flowering in domesticated lines. The effect of the deletion allele was further validated through co-segregation analysis using an advanced backcross population derived from a cross between a domesticated eggplant accession (MEL3) and the wild relative *S. elaeagnifolium*, which carry the deletion and wild-type alleles, respectively. Eggplant underwent two independent domestications in Southeast Asia and India, with European accessions proposed to originate from the Indian domestication lineage. By aligning SP5G gene sequences of 48 wild and domesticated accessions, an independent 13.9 kb deletion allele that eliminates the entire gene body was also uncovered, along with a 31% enrichment of the 2.15 kb deletion allele in European and Indian genotypes. These findings support the dual domestication of eggplant and demonstrate that at least two independent loss-of-function mutations in SP5G contributed to flowering-time adaptations.

Keywords: flowering time; domestication; florigen-antiflorigen; pan-genomics

UNLOCKING DROUGHT STRESS TOLERANCE IN EGGPLANT THROUGH *SMDMR6-1* GENOME EDITING

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Climate change is making drought a constraint for horticultural crops, highlighting the need for gene targets that improve plant performance under water deficit. Downy Mildew Resistance 6 (DMR6), encoding an enzyme involved in salicylic acid catabolism, is a promising candidate gene because its inactivation in tomato has been associated with enhanced pathogen tolerance and drought-avoidance strategy. A CRISPR/Cas9-mediated knockout of *SmDMR6_1* was previously carried out in eggplant and knocked-out T1/T2 lines showed improved tolerance to *Phytophthora infestans* and *P. capsici*. Here, we focused on the drought-response of edited *Smdmr6_1* plants under high-throughput phenotyping conditions. The experiment was conducted in the Phenoplant platform at the University of Turin, using 48 plants: 24 non-edited and 24 edited *Smdmr6_1*. Within each genotype, plants were assigned either to full irrigation or to 10 days of complete water deprivation, to induce severe drought stress and enable a direct comparison of drought-response dynamics. Plant responses were monitored through an integrated phenotyping approach combining ecophysiological and imaging-based measurements. Gas exchange parameters and stem water potential were collected, evapotranspiration was calculated from weight, and multispectral sensors provided spectral indices and morphological traits. The edited *Smdmr6_1* lines showed lower stomatal conductance and evapotranspiration compared to the non-edited plants under both conditions. Gravimetric evapotranspiration further supported a more conservative water-use strategy in the edited plants, consistent with anisohydric behaviour. Morphological parameters (biomass index and leaf angle) and multispectral indexes (GLI and NDVI) showed a slower decline in *Smdmr6_1* lines compared to wild type, suggesting a possible avoidance response to drought stress. These results suggest that *SmDMR6_1* inactivation may contribute to drought avoidance in eggplant, in addition to its previously demonstrated role in disease tolerance. Ongoing RNA-seq analyses will clarify the molecular basis of this physiological response.

Keywords: eggplant; multi-stress tolerance; DMR6; drought stress; high throughput plant phenotyping; drought avoidance

GENETIC DISSECTION OF TRICHOME DENSITY IN EGGPLANT THROUGH GWAS AND BSA-SEQ

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Trichomes are epidermal outgrowths that play important roles in plant defense against herbivores and environmental stress. In eggplant (*Solanum melongena*) and its wild relatives, trichomes show wide variation in density and morphology, with domesticated forms generally exhibiting reduced pubescence relative to wild relatives. However, the genetic basis of trichome variation remains poorly understood, as does their potential developmental relationship with prickles. Using a multi-parent advanced generation inter-cross (MAGIC) population comprising 325 lines evaluated in Almería and Valencia (Spain), we conducted a genome-wide association study (GWAS) for trichome density. A genomic region on chromosome 12 (94.5–97.0 Mb) was identified as significantly associated with the trait, harboring several candidate genes with putative roles in trichome development. For prickliness, GWAS confirmed SmLOG3 on chromosome 6 as a major gene controlling prickle formation, consistent with previous studies in *Solanum*. To further resolve the genomic region associated with trichome density, bulk segregant analysis coupled with sequencing (BSA-seq) is being conducted using an F₂ population derived from an interspecific cross between *S. macrocarpon* × *S. dasyphyllum*, which shows clear segregation for trichome and prickle traits. Observations in this population suggest that elongation and thickening of stellate trichome stalks may contribute to prickle development. Future work will focus on integrating GWAS and BSA-seq results to identify high-confidence candidate genes for functional characterization using CRISPR/Cas-mediated gene editing. This work will advance understanding of the genetic control of epidermal traits in eggplant and support breeding strategies aimed at improving crop manageability while maintaining desirable plant characteristics.

Keywords: eggplant; trichomes; prickles; GWAS; BSA-seq

DETECTION AND CHARACTERIZATION OF A QUANTITATIVE TRAIT LOCUS ASSOCIATED WITH HIGH-TEMPERATURE TOLERANT FRUIT SET IN *CAPSICUM ANNUUM*

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High temperature severely limits fruit set in chili pepper (*Capsicum annuum*), resulting in substantial yield losses under greenhouse and field conditions. Although genetic variation for high-temperature tolerance exists, the physiological mechanisms underlying stable fruit set remain poorly understood. In our previous study, we identified a major quantitative trait locus (QTL) on chromosome 6 associated with improved fruit set under high-temperature conditions in greenhouses. However, the physiological basis of this QTL has not been clarified. In the present study, we further characterized this QTL to examine its physiological relevance. F₂ and F₃ segregating populations were evaluated under controlled high-temperature conditions, and fruit set was quantified. Based on our previous QTL analysis using genome-wide SNP markers generated by dpMIG-seq, we focused on the identified locus for further characterization. In addition, pollen germination rate, leaf temperature, stomatal conductance, and transpiration rate were assessed to investigate physiological traits associated with the QTL. Consistent with our previous findings, the QTL was significantly associated with fruit set under high-temperature conditions, and genotypes carrying the favorable allele exhibited higher fruit set than those carrying the alternative allele. In contrast, no clear effect of the QTL genotype on pollen germination was detected. Genotypes carrying the favorable allele also tended to maintain lower leaf temperatures, together with higher stomatal conductance and transpiration rates under high-temperature conditions. These results suggest that this QTL may contribute to stable fruit set under high temperature through physiological processes related to transpiration rather than pollen fertility. Overall, our findings extend the characterization of this QTL and provide a basis for integrating genetic and physiological traits in breeding programs aimed at improving high-temperature tolerance in pepper.

Keywords: *Capsicum*; fruit set; high-temperature tolerance; quantitative trait locus (QTL)

Topic 4

Fruit Quality & Metabolomics

PEPPER FRUIT FLAVOR: USUAL AND UNUSUAL SUSPECTS

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In the nightshade family (Solanaceae), tomato is undoubtedly the “big brother” in economic terms. Pepper (*Capsicum*), however, comprising approximately 35 species, five of which are widely cultivated, exhibits larger diversity of many fruit quality traits, including a remarkably diverse composition of flavor-related metabolites.

A genome-wide association study (GWAS) based on whole-genome resequencing of genetic variation in *Capsicum annuum* identified multiple genomic regions associated with numerous compounds known to contribute to pepper fruit flavor.

Metabolomic analyses of other cultivated *Capsicum* species have revealed distinct profiles of flavor-related compounds, which are likely to translate into unique sensory characteristics. This diversity makes interspecific hybridization a powerful strategy for improving and diversifying the flavor of commercial pepper cultivars. In some cases, such hybridization may not only facilitate the introgression of existing flavor traits but also generate entirely novel flavor profiles.

One striking example is the “tropical” fruit aroma that was unexpectedly discovered about a decade ago in an introgression population derived from *C. baccatum* × *C. annuum* (Eggink *et al.*, 2014, *Theoretical and Applied Genetics*). This discovery has since led to the development of the commercial F1cultivar Tatayoyo™ (Rijk Zwaan). However, the biochemical and genetic basis of this distinctive aroma remained unknown until recently. By combining sensory evaluation, metabolomic profiling, and molecular genetic analyses, we identified the volatile compound responsible for the novel aroma, as well as a key gene that enables the biosynthesis of this unusual volatile in pepper fruit.

The research presented here has been supported by TKI project “From omics data to novel breeding targets in pepper” (LWV 23099), EU Horizon 2020 G2P-SOL and PRO-GRACE projects and Rijk Zwaan.

Keywords: *Capsicum*, fruit flavor, quality, GWAS

DISCOVERY AND CHARACTERIZATION OF A NOVEL LEAKY VANILLIN AMINOTRANSFERASE (VAMT) ALLELE WITH AN AMINO ACID SUBSTITUTION, AFFECTING CAPSAICINOID COMPOSITION IN CHILI PEPPER

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Capsaicinoids and their low pungent analogs are the important compounds that determine the quality of chili pepper fruit in food industry and medical applications. In previous screening for chili pepper bio-resources, we found that the pungent accession 'Charapita' (CH) (*Capsicum chinense*) has a unique composition with a high capsinoid content. This study aimed to identify the mutation responsible for the high capsinoid content of CH. QTL analysis using F₂ population between Red Habanero (RH) and CH revealed a major QTL on chromosome 3. Further genetic analysis narrowed the QTL to a region of approximately 400 kb containing VAMT (vanillin aminotransferase). Allelism test demonstrated that CH possesses a leaky vamt allele. The sequencing analysis revealed that CH-type vamt allele has a unique amino acid substitution (G373E) due to a SNP in exon 15. CH-type vamt decreased pungency by 50%, and increased capsinoid content about three times compared with RH-type. We designated the CH-type allele as vamt^{L3}. There were no difference in the expression levels of VAMT or other capsaicinoid biosynthetic genes between RH and CH. The vanillylamine synthesis activity was evaluated with crude extract from placental tissue. It showed that the activity in CH was 30 times less compared with RH. Given that the enzyme activity significantly decreased without transcriptional change, G373E likely reduces VAMT protein accumulation, conferring the characteristic composition of capsaicinoids and capsinoids. The novel vamt allele (vamt^{L3}) will contribute to the quantitative manipulation of pungency level and capsinoid content in chili pepper breeding.

Keywords: pungency, capsinoid, vanillin aminotransferase (VAMT), leaky mutation

MICROSCOPIC AND METABOLIC INVESTIGATIONS REVEAL CUTICLE THICKNESS AND EPIDERMAL CELL DENSITY AS CRITICAL FACTORS IN CHILI PEPPER SKIN CRACKING

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The hydrophobic cuticle encasing the fruit skin plays a critical role during fruit development and post-harvest storage. Skin failure often results in surface cracking and the subsequent formation of a wound-periderm tissue typically composed of suberin and lignin. However, the specific factors that render fruit skin susceptible to cracking are not yet fully understood. In this study, we investigated two varieties of chili pepper (*Capsicum annuum* L.): Numex Garnet, which maintains an intact skin, and Vezena Slatka, which is characterized by a cracked skin phenotype. Through microscopical observations, gas chromatography-mass spectrometry (GC-MS), and biochemical and gene expression assays, we found that Vezena Slatka fruit develop a thicker cuticle with significantly higher levels of cutin monomers and hydroxycinnamic acids. This variety also shows high expression of key cutin-related genes. Furthermore, the skin of Vezena Slatka exhibited a lower epidermal cell density due to the presence of cells with exceptionally large perimeters, alongside the high expression of genes involved in epidermal cell differentiation. We demonstrate that skin cracking in Vezena Slatka is accompanied by the spatial accumulation of lignin-like polyphenolic compounds, notably occurring without the formation of typical suberized wound-periderm tissues. Finally, we establish that skin cracking in chili-type peppers significantly diminishes fruit quality during post-harvest storage in a temperature-dependent manner. In conclusion, our data highlight cuticle thickness and epidermal cell density as two critical factors determining the susceptibility of chili pepper fruit to skin cracking.

Keywords: Fruit skin, Cuticle, Lignin; *Capsicum annuum*, Post-harvest storage

ANALYSIS OF SPECIALIZED METABOLISMS IN RESPONSE TO UV-B EXPOSURE IN THE SOLANACEAE FAMILY

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Plants, as photoautotrophic sessile organisms, are inevitably exposed to UV-B radiation, which potentially damages nucleic acids, increases ROS and disrupts metabolic processes. Specialized metabolites, particularly hydroxycinnamates and flavonoids, function as key antioxidants and UV-B absorbents. These compounds exhibit structural diversity across plant species; for example, sinapoyl esters in *Arabidopsis thaliana* (Brassicaceae) and chlorogenic acids in *Solanum lycopersicum* (Solanaceae). Similarly, accumulation of flavonoid subclasses differs among species, with flavonols in *Arabidopsis* and tomato and flavones in *Oryza sativa* (Poaceae). The Solanaceae family contains several economically important crops exhibiting diverse specialized metabolite accumulation; however, UV-B responses of these metabolites and their potential roles as UV-B protectants remain underexplored. In this work, we performed comparative metabolomic analysis of 16 species across six Solanaceae genera (*Solanum*, *Capsicum*, *Nicotiana*, *Withania*, *Alkekengi*, *Petunia*) using a photomorphogenic UV-B dose. UV-B treatment induced HY5 upregulation in most species, suggesting a conserved response of the UVR8/COP1/HY5 signaling pathway. Using liquid chromatography–mass spectrometry (LC-MS), approximately 190 annotated specialized metabolites were identified from leaf tissues. Principal component analysis (PCA) revealed species-specific accumulation of specialized metabolites, consistent with phylogenetic relationships. Among phenylpropanoids, chlorogenic acids exhibited a conserved UV-B response across species, although the predicted biosynthetic routes differed. In contrast, changes in phenolamide pathways, despite conserved responses of some putrescine derivatives, were largely species-specific. Notably, flavonoid pathways displayed lineage-specific responses between flavonol- and flavone-accumulating species. These findings provide insights into UV-B-responsive metabolism and variation across Solanaceae species, which may be useful for improving their abiotic stress adaptation in the future.

Keywords: comparative metabolomics, metabolic responses, phenylpropanoids, UV-B

DEVELOPMENT OF A NOVEL MAGIC POPULATION IN PEPPER: PHENOTYPING AND GENOTYPING ANALYSES FOR MULTIPLE DESIRED TRAITS

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Sustainable progress in agriculture requires breeding strategies able to exploit genetic diversity and support the development of cultivars with improved agronomic performance and quality traits. Multi-parent Advanced Generation Inter-Cross (MAGIC) populations represent a valuable resource for dissecting complex traits because they combine high recombination levels with broad phenotypic variability. In this study, we report the development and characterization of a novel pepper (*Capsicum annuum* L.) MAGIC population derived from eight founder accessions, selected for contrasting phenotypic among a selected pool of breeding material. The population was advanced through inter-crossing followed by eight repeated self-pollination cycles, aiming at generating recombinant inbred lines with increasing homozygosity. Over two consecutive growing seasons (2025-2026), the population was phenotypically characterized for several agronomic and fruit quality traits, including fruit colour, morphology, pulp thickness, cracking, glossiness, soluble solids content and acidity. Moreover, a phenotype-based core collection of 200 lines was selected to capture the diversity harboured by the population. This core set was further characterized for aroma-related traits through GC-MS analysis of volatile organic compounds, with particular attention to grassy, fruity and beany aromatic descriptors. Genotyping-by-Sequencing was used to assess the genetic structure of the core collection, providing a framework for identifying genomic regions associated with traits of interest. Overall, the two-year phenotypic characterization, combined with genomic and metabolomic data, provides a comprehensive overview of the variability available in this novel pepper MAGIC population. These results support its use as a valuable pre-breeding resource for the selection of lines with improved fruit quality, aroma profile and agronomic performance.

Keywords: *Capsicum annuum*; MAGIC population; multi-parent population; pre-breeding; fruit quality; volatile organic compounds; genotyping-by-sequencing

Topic 5

New Phenotyping Tools & Data Analysis

NEW PHENOTYPING TOOLS AND DATA ANALYSIS

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In the face of intensifying global challenges, including rapid population growth, climate instability, and need for food security, crop improvement has emerged as a cornerstone for sustainable agriculture. Traditionally, plant phenotyping relied on manual, labor-intensive measurements that were inherently slow, prone to human error, and difficult to scale. The transition into the “modern” phenotyping era has introduced transformative tools that revolutionize the systematic assessment of complex traits, effectively shifting the paradigm from traditional methods to modern phenomics. Central to this shift is the expansion of high-throughput cutting-edge technologies, such as 3D imaging, multi- and hyperspectral sensors, which capture high-dimensional data with unprecedented speed and non-invasive precision. These platforms utilize advanced robotic systems to enable the evaluation of physiological responses, plant architecture, and yield components at multiple scales. This facilitates the real-time monitoring of critical environmental variables and dynamic stress responses across multiple temporal and spatial scales, providing a holistic view of the plant’s interaction with its environment. The core of this revolution is the integration of Artificial Intelligence (AI) and machine learning (ML) algorithms. These tools are indispensable for managing and interpreting the massive datasets produced by HTP platforms, allowing researchers to uncover intricate patterns and correlations between phenotypic expressions and genetic factors. By leveraging predictive ML models, breeders can optimize trait selection processes, significantly reducing the duration and financial burden of variety development. This technological advancement is especially vital for controlled environment agriculture, where breeding specialized cultivars for vertical farms and high-tech greenhouses demands precise control and rapid selection cycles. Furthermore, AI-driven phenotyping is essential for bridging the “phenomics-genomics gap”. By fostering multidisciplinary collaborations across biology, engineering, and bioinformatics, these new tools establish a robust foundation for next-generation breeding, ensuring the resilience of the global food system.

Keywords: high throughput phenotyping, spectral imaging, machine learning, artificial intelligence

MORPHOAGRONOMIC PHENOTYPING AND GWAS OF AGRONOMIC TRAITS IN AN EGGPLANT MAGIC POPULATION

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Eggplant (*Solanum melongena* L.) displays remarkable phenotypic diversity, yet the extent to which agronomic and morphological traits are organized into coordinated phenotypic and genetic modules remains poorly understood. In this study, we used the MEGGIC multiparent advanced generation intercross (MAGIC) population to investigate the architecture of complex traits across contrasting open-field and greenhouse environments. A total of 46 agronomic, morphological and physiological traits were evaluated in 302 inbred lines, revealing broad phenotypic variation and frequent transgressive segregation across both environments. Genome-wide association analyses based on 144,719 filtered SNPs identified significant marker-trait associations for 35 traits and a total of 111 QTLs. Broad-sense heritability was moderate to high for most traits, with pigmentation- and prickles-related descriptors showing particularly stable and strong genetic control across environments. Correlation analyses, principal component analysis and genetic correlation networks consistently supported a modular organization of trait variation. Four major modules were identified, corresponding to anthocyanin pigmentation, chlorophyll-related traits, prickles, and plant and fruit size. Although the overall modular structure was largely conserved between environments, differences in network connectivity suggested a more interconnected architecture under open-field conditions and a more compartmentalized organization in the greenhouse. In addition, QTL co-localization patterns mirrored these phenotypic modules, highlighting discrete genomic hotspots associated with coordinated trait expression. These results provide new insights into the modular basis of phenotypic variation in eggplant and demonstrate the value of MAGIC populations for the high-resolution dissection of complex agronomic traits, with direct implications for marker-assisted breeding and multi-trait improvement.

Keywords: Eggplant, *Solanum melongena*, Traits modularity, GWAS, MAGIC Population, QTLs

GENOME-WIDE ASSOCIATION ANALYSIS OF A RE-SEQUENCED CAPSICUM CORE COLLECTION THROUGH A UNIFIED, USER-FRIENDLY GWAS TOOLBOX

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The cultivated pepper (*Capsicum annuum*) shows a notably broad metabolite diversity – vitamins, antioxidants, aroma volatiles, flavour compounds and other specialised metabolites. Yet, compared to the closely related tomato, the genetic basis of specialised metabolite production is poorly understood. Our project aims to identify novel QTLs and genes involved in pepper specialised metabolite biosynthesis. The basis of our research is a *Capsicum annuum* dataset of 423 pepper accessions, re-sequenced and profiled for over 2,000 specialised metabolites. To harness the full potential of this dataset in a reliable and statistically powerful way, we have assembled our analysis pipeline into a unified web application. This application facilitates GWAS and candidate gene discovery from raw data processing, through basic and advanced GWAS methodologies, to identifying genes of interest based on annotation, allelic variation and gene expression. Combining this comprehensive pepper dataset with our high-throughput GWAS pipeline has yielded a number of novel QTLs and candidate genes, which are being functionally characterized using *in vivo* gene silencing. The knowledge generated will enable breeding of improved pepper cultivars with optimised levels of specialised metabolites.

Keywords: GWAS, specialized metabolites, software, allele mining

VARIATION IN FRUIT MORPHOLOGY CHARACTERISTICS, MATURITY, AND PRICKLE INCIDENCE IN THE USDA NATIONAL PLANT GERMPLASM SYSTEM EGGPLANT COLLECTION

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Germplasm collections are critical reservoirs of trait diversity needed for crop improvement. The Cornell University eggplant breeding program is particularly interested in fruit morphological variation to support the development of diverse *Solanum melongena* market classes. To advance this goal, we phenotypically characterized 755 *S. melongena* accessions from the USDA National Plant Germplasm System in unreplicated field trials conducted in Freeville, NY, USA, during 2024 and 2025. Fruits were harvested at commercial maturity and imaged under controlled lighting conditions. Computer vision approaches were used to extract quantitative morphological traits, including epidermis color, calyx color, fruit length, width, curvature, and solidity. Accessions were also evaluated for key production traits, including days to flowering, days to harvest maturity, and the presence or absence of prickles on calyces, stems, and adaxial leaf surfaces. Accession geographic origin was associated with individual phenotypic traits, such as fruit size, epidermis color, and calyx color, as well as with multivariate phenotypes from principal components analysis. Ongoing work to genotype the collection with genome-wide markers via genotyping-by-sequencing will also be presented. Our ultimate goals with combined genotypic and phenotypic datasets are to improve the utility of the USDA germplasm collection by curating a phenotypically and genotypically diverse core collection, correcting erroneous species assignments, and identifying marker-trait associations both with historical data in the germplasm database and with newly collected phenotypes from our lab group.

Keywords: germplasm resources, fruit morphology, phenotyping

PHENOTYPIC DIVERSITY AND GERMINATION KINETICS IN A MAGIC POPULATION OF *CAPSICUM* SPP.

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Seed germination and early seedling establishment are key agronomic traits in pepper production, as they ensure rapid and uniform emergence and robust seedling development. Multi-parent advanced generation Inter-Cross (MAGIC) populations capture high genetic diversity and facilitate the dissection of complex traits through QTL identification. The Capsicum Breeding team of COMAV has developed the first MAGIC population in this genus, currently reaching the S₅ generation. The aim of this study was to evaluate the radicle and cotyledon emergence within the S₅ generation of the Capsicum MAGIC population. A total of 150 MAGIC S₅ lines and their 8 parents were evaluated. For each genotype, 50 seeds were sown under controlled conditions. Radicle emergence was recorded up to 15 days after sowing (DAS), and cotyledon emergence was monitored weekly up to 5 weeks after sowing (WAS). Germination curves were constructed by plotting cumulative germination over time. Several vigour indices were calculated: final germination percentage (FGP), mean germination time (MGT), time to 50% of germination (T50), germination index (GI), coefficient of uniformity of germination (CUG) and synchrony index (Z). Substantial phenotypic variation was observed for all germination and vigour traits. High coefficients of variation across indices indicated great diversity in germination speed, uniformity and synchrony, particularly for CUG (112.52%). Several lines outperformed the parental lines, indicating transgressive segregation. For instance, the parent 'Chile Serrano' showed low MGT (5.3 days) and high GI, yet some MAGIC lines exhibited even faster germination and higher vigour. Similarly, cotyledon emergence displayed variability, with lines surpassing the parental values. These results highlight the potential of MAGIC populations to generate new allelic combinations associated with improved germination and seed vigour. The integration of multiple vigour indices provides a comprehensive approach for the characterization of traits related to crop establishment and seed quality.

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Keywords: Capsicum spp., cotyledon emergence, germination, radicle emergence, seed vigour, MAGIC population

Topic 6

Disease Resistance

DESIGNING DURABLE PLANT RESISTANCE IN A CHANGING WORLD: INSIGHTS FROM PEPPER PATHOSYSTEMS

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Understanding plant–pathogen–environment interactions is crucial to developing durable crop protection strategies in the context of global change and food security. Plant resistance is no longer viewed as a fixed barrier, but as a dynamic system shaped by host genetic architecture, pathogen adaptation, and environmental context. This keynote synthesizes recent advances in pepper pathosystems to explore key concepts including durability and robustness of resistance (plant immunity) under multi-stress conditions. It draws on an integrative framework combining quantitative genetics (QTL mapping, pyramiding, GWAS), transcriptomics of host-pathogen interactions, experimental evolution, and controlled environment studies assessing temperature effects on immunity. Genome-wide analyses reveal widespread pyramiding of resistance QTLs, contributing to quantitative and potentially more durable resistance. Plant–pathogen interaction matrices often follow a simple nested structure: specialist pathogens infect subsets of hosts that generalists can infect. This structure limits pathogen evolution and helps explain why quantitative resistance often remains effective across diverse pathogen populations. At the interaction level, transcriptomic analyses of *Phytophthora capsici* in partially resistant pepper support a “strive or thrive” model, where partial resistance drives moderated pathogen activity, fostering coexistence rather than elimination. Experimental evolution reveals that virus fitness and virulence follow distinct trajectories depending on host resistance and tolerance, highlighting trade-offs shaping durability. Environmental studies show that immunity responses vary under temperature fluctuations, with immunity robustness depending on both plant genetics and pathogen type. Together, these findings highlight that durable crop protection strategies emerge from the integration of quantitative resistance, genetic diversity, and environmental robustness. They support a paradigm shift from single-gene resistance toward designing crop immunity systems that remain effective under evolving pathogens and changing agroecosystems.

Keywords: *Capsicum* L.; plant immunity; pathogen adaptation; robustness; temperature stress; *Phytophthora capsici*; PVY; *Leveillula taurica*

BEGOMOVIRUS RESISTANCE GENE *EY-1* ENCODES A DEDDH EXONUCLEASE IN EGGPLANT

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Whitefly-transmitted begomoviruses severely affect *Solanaceae* crops worldwide. Although introgression of begomovirus resistance is highly desirable, only resistance genes from tomato and pepper have been cloned. Here, we sought to identify a begomovirus resistance gene in eggplant (*Solanum melongena*). Accession No.820 was identified as resistant to tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) in our previous study. Genetic mapping using F₂ and F_{2:3} populations derived from a cross between resistant No.820 and susceptible No.47 identified a dominant resistance locus, *Eggplant yellow leaf curl disease virus resistance 1* (*Ey-1*), on chromosome 1. Whole-genome and transcriptome sequencing narrowed the candidate region to 113 kb containing ten genes, of which five were selected for functional analysis. Virus-induced gene silencing (VIGS) revealed that silencing *SmNEN3*, encoding a DEDDh family exonuclease, abolished resistance. Sequence comparison between No.820 and No.47 identified a single amino acid deletion and nonsynonymous mutations in *SmNEN3*, likely responsible for resistance. These results identify No.820 as a valuable dominant resistance source and provide DNA markers to support marker-assisted breeding in eggplant.

Keywords: DNA marker-assisted breeding; Geminivirus; NAC45/86-dependent exonuclease domain proteins (NENs); *Solanum melongena*; Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV)

IDENTIFICATION OF NOVEL *PHYTOPHTHORA CAPSICI* RESISTANCE LOCI IN *CAPSICUM* SPP. THROUGH INTEGRATED QTL MAPPING AND EFFECTOROMICS

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Phytophthora capsici is a primary constraint to global pepper (*Capsicum* spp.) production, necessitating the identification of novel, durable resistance sources. In this study, 496 *Capsicum* accessions were screened against an aggressive Mexican *P. capsici* isolate, identifying 12 resistant *C. annuum* accessions. These genotypes were further characterized against 12 genetically diverse isolates to differentiate broad-spectrum from isolate-specific resistance, and whole-genome resequencing revealed high genetic diversity relative to the standard resistance donor CM334. To identify resistance determinants, four accessions were used to develop mapping populations for Bulk Segregant Analysis (BSA), resulting in the identification of quantitative trait loci (QTL) associated with resistance. In parallel, an effectoromics pipeline was implemented to characterize host-pathogen interactions. Candidate effectors were identified via RNA-seq of *P. capsici* during infection, cloned into expression vectors, and screened against the resistant germplasm to identify specific effector-accession interactions. This integrated approach identified novel resistance loci and characterized specific effector-accession interactions, establishing the foundation for future identification of cognate resistance receptors and providing essential genetic resources for breeding improved pepper cultivars.

Keywords: *Capsicum annuum*; *Phytophthora capsici*; Effectoromics; QTL mapping; Resistance

VALIDATION OF THE S-GENE CONCEPT IN PEPPER USING VIGS

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Plant susceptibility (S) genes are often shown to be conserved for their functions across plant species. Previously, PUB17 and PUB21 were identified as S-genes in tomato for the necrotrophic pathogen *Botrytis cinerea*. Orthologous genes were subsequently identified in pepper. To investigate whether these S-genes are functionally conserved, the pepper orthologs were silenced using virus-induced gene silencing (VIGS) in different pepper genotypes. Following gene silencing, susceptibility to *B. cinerea* was evaluated through infection assays. The results suggest that the identified tomato S-genes may also contribute to susceptibility in pepper, supporting the conservation of S-gene function across species.

Keywords: PUB17, PUB21, *Botrytis cinerea*, susceptibility

DISSECTING THE GENETIC ARCHITECTURE OF BACTERIAL WILT RESISTANCE IN INDIAN EGGPLANT: QTL-SEQ UNCOVERS NOVEL RESISTANCE LOCI IN LOCAL GERMPLASM

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Bacterial wilt caused by *Ralstonia solanacearum* remains one of the most destructive soil-borne constraints in eggplant (*Solanum melongena* L.) cultivation across tropical production systems. Despite its economic impact, resistance loci in breeding-relevant Indian germplasm remain under characterized. We applied QTL-seq to an F₂ population derived from a cross between a resistant accession CARI-1 and susceptible cultivar Pusa Purple Long to rapidly identify resistance associated genomic regions. Ten F₂ individuals each with extreme phenotypes were pooled together as resistant and susceptible bulks. Whole genome resequencing of the parental and bulk samples resulted in 40 Gb clean data with 20x coverage. QTL detection employed five parallel statistical frameworks: ΔSNP-index, ED⁴, Ridit score, G'-value, and distance-based metrics. A major QTL was consistently identified on chromosome 9 across all methods, representing a locus not previously reported in Indian eggplant germplasm for bacterial wilt resistance. Secondary signals with sustained significance were detected on chromosomes 5, 6, and 10, indicating partial polygenic architecture. Variant annotation within the chromosome 9 interval revealed predominantly intergenic polymorphisms, missense substitutions and in-frame insertions, in genes with predicted roles in immunity-related signalling. Key SNPs from the chromosome 9 interval are being converted to KASP markers for validation across independent populations for deployment in marker-assisted breeding programs. This study advances the molecular understanding of bacterial wilt resistance in eggplant and demonstrates the utility of QTL-seq for rapid, cost-effective locus discovery in agriculturally important crops.

Keywords: *Solanum melongena*; *Ralstonia solanacearum*; QTL-seq; SNP-index; ED⁴; KASP markers; bacterial wilt

Topic 7

Insect Resistance

BREEDING FOR INSECT RESISTANCE IN PEPPER: INSIGHTS FROM STUDIES OF GENETICS AND MECHANISMS OF RESISTANCE AGAINST APHIDS

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Insect pests are a major challenge in pepper production, causing considerable yield and quality losses through direct feeding damage and transmission of plant viruses. Key pests such as aphids, thrips, whiteflies, and spider mites reduce crop productivity and increase reliance on chemical control. However, intensive pesticide use can promote resistance development in insect populations, negatively affect beneficial organisms, and increase environmental concerns. Breeding for insect resistance offers a sustainable alternative. Our research focuses on identifying and characterizing resistance sources in *Capsicum* germplasm, with particular emphasis on antibiosis-type resistance that reduces insect survival and reproduction. To facilitate efficient screening, we are developing a mobile-based phenotyping platform that combines smartphone imaging and deep learning for rapid counting of insects. In recent years, we screened a *Capsicum* collection for resistance to the foxglove aphid, *Aulacorthum solani*, using no-choice assays. Multiple accessions consistently showed reduced aphid reproduction relative to susceptible controls, and several also exhibited resistance to the green peach aphid indicating the potential for broad-spectrum aphid resistance. Mechanisms that interfere with feeding on the phloem may confer such resistance. To investigate the inheritance and underlying mechanisms of resistance, we developed and phenotyped an F2 population from resistant and susceptible plants. Electrical Penetration Graph analyses demonstrated that aphids feeding on resistant plants displayed altered feeding behavior, including shorter probing events and delayed phloem ingestion. Because no evidence of callose deposition was observed, we hypothesize that phloem-associated specialized metabolites contribute to resistance. Untargeted metabolite profiling identified candidate metabolites enriched in resistant plants, several of which were confirmed in phloem sap. Their biological activity is currently being validated through artificial diet assays. The identification of resistance-associated metabolites and genetic loci will support the development of molecular markers and provide valuable resources for breeding pepper cultivars with durable aphid resistance.

Keywords: Insect resistance, antibiosis, aphids, specialized metabolites, phenotyping

GENETIC MAPPING AND METABOLIC PROFILING REVEAL CANDIDATES FOR WHITEFLY RESISTANCE IN PEPPER (*CAPSICUM* SPP.)

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The increasing prevalence of insecticide resistance and stricter pesticide regulations necessitate the exploitation of natural resistance in crops. In pepper (*Capsicum* spp.), the whitefly, *Bemisia tabaci*, is a major production constraint, reducing fruit quality and transmitting viruses. Despite its importance, the genetic and molecular mechanisms underlying whitefly resistance in pepper are not well characterized, and resistant varieties are urgently needed. Previously, we identified *Capsicum annuum* accession CA9 as exhibiting broad-spectrum resistance to *B. tabaci* MEAM1 and Asia I, providing an ideal system to investigate the genetic architecture and metabolic basis of resistance. We developed an F₂ mapping population (CA9 × CA40) and found that adult survival exhibited recessive inheritance while segregated as a quantitative trait. We then integrated bulk segregant analysis (BSA) and untargeted metabolomics to dissect the genetic and biochemical mechanisms underlying resistance. BSA identified a major QTL region on chromosome 7 and a minor QTL on chromosome 9 controlling adult survival, while multiple QTL regions on chromosome 1 were associated with reduced oviposition. Critically, untargeted metabolomic analysis of extreme F₂ phenotypes identified chlorogenic acid and apigenin-related compounds as strong resistance-associated candidates. These metabolic signatures were validated through consistent enrichment patterns in F₃ progeny, suggesting their functional role in defense. This study establishes foundational genetic maps and metabolite biomarkers for whitefly resistance in pepper. The QTL are being refined through fine mapping, and functional assays with chlorogenic acid and apigenin are underway to validate their defensive roles. These findings provide a framework for marker-assisted breeding and metabolic engineering of whitefly resistance.

Keywords: whitefly resistance, bulk segregant analysis, untargeted metabolomics, *Capsicum annuum*, QTL mapping

EARLY DEFENSE MECHANISMS IN JUVENILE STAGE OF EGGPLANT AGAINST INFESTATION BY LEUCINODES ORBONALIS LARVAE

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The eggplant shoot and fruit borer (*Leucinodes orbonalis*) is a major constraint to eggplant (*Solanum melongena*) production in Asia. Reliance on insecticides raises concerns about resistance and environmental impacts, emphasizing the need for host plant resistance within integrated pest management (IPM). However, resistance mechanisms at the vegetative stage remain poorly understood. This study aimed to identify key traits associated with early-stage resistance. Under controlled infestation, SM265 showed no wilting and high early larval mortality, T18_102 (2X) wilted within 2 h with higher larval survival and adult emergence, and T14_25 showed intermediate responses. Damage severity was positively correlated with non-glandular trichome density and negatively correlated with petiole width. Resistant genotypes had higher constitutive phenolics, particularly catechol, scopoletin, and 4-hydroxybenzoic acid. In contrast, the susceptible genotype showed low basal phenolics but strong induction of polyphenol oxidase (PPO) activity and monoterpenes after infestation. Constitutive phenolics were negatively associated with damage, while induced volatiles were positively associated with susceptibility. Life table analysis confirmed reduced population growth on SM265 due to increased early larval mortality. Resistance is primarily driven by constitutive phenolic defenses that disrupt early larval establishment. These findings provide targets for resistance breeding and support sustainable IPM strategies.

Keywords: eggplant shoot and fruit borer, *Solanum melongena*, volatile profiles, catechol

HARNESSING PHENOTYPIC DIVERSITY IN THAI EGGPLANT GERMPLASM FOR MULTI-PEST RESISTANCE BREEDING

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Eggplant (*Solanum melongena* L.) is an important vegetable crop in Southeast Asia, yet the effective utilization of genetic resources for insect resistance breeding remains limited. Thailand possesses a diverse eggplant germplasm collection conserved at the Tropical Vegetable Research and Development Center (TVRC), Kasetsart University; however, systematic characterization of this diversity for multi-pest resistance is still lacking. This study aimed to characterize phenotypic diversity and identify resistance-associated traits within Thai eggplant germplasm for potential use in breeding programs. A total of 60 accessions representing different cultivar groups were evaluated for resistance to fruit and shoot borer (FSB), whiteflies (WF), and cotton leafhopper (LH). Insect responses were assessed at the vegetative stage to ensure uniform plant development and to target traits relevant for early-stage selection. Resistance was assessed based on oviposition (FSB and WF), nymphal density (LH), and damage severity (FSB and LH). In parallel, 33 morphological and seven chemical traits were analyzed. Principal component analysis and hierarchical clustering revealed substantial phenotypic diversity and enabled classification according to morphological traits. Significant variation in resistance was observed, including accessions exhibiting contrasting responses across pest groups, indicating opportunities for multi-pest resistance selection. Correlation analysis between plant traits and insect responses revealed that leaf thickness, leaf width, and POD activity were related to FSB oviposition and damage severity. Whitefly oviposition was associated with plant branching and leaf margin lobing, while LH infestation and damage were associated with branching, trichome size, and leaf morphology. These findings demonstrate the untapped potential of Thai eggplant germplasm as a source of adaptive traits, supporting germplasm utilization and early-stage, trait-based selection of resistant genotypes in breeding programs.

Keywords: aubergine; brinjal; genetic resources; germplasm characterization; pest resistance; *Solanum melongena* L.; trait-based selection

PURPLE COROLLA CAPSICUMS AND THEIR RESISTANCE TO THE GREEN PEACH APHID

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The green peach aphid, *Myzus persicae*, is one of the most threatening pests of *Capsicum annuum*. In recent years, the *M. persicae* genotype MLG-R emerged and colonized the majority of Dutch pepper greenhouses. This aphid genotype has low sensitivity to pymetrozine and flonicamid and forms a new urge for the development of insecticide-free pest control measures. As *C. annuum* is a relatively susceptible crop for aphids, one of our objectives was to explore the basis of natural plant defence mechanisms in the purple corolla *Capsicum* species *C. pubescens*, *C. eximium*, and *C. cardenasii*. On several accessions of these species, we studied aphid feeding behaviour with automated videotracking and Electrical Penetration Graph (EPG) recording and analyzed aphid fitness via reproduction assays. Trichome types and densities were characterized with Scanning Electron Microscopy. Overall, we found a large variation in aphid resistance, with higher levels of resistance in many purple corolla accessions compared to *C. annuum*. Furthermore, resistance to aphids was associated with several types of glandular trichomes that are absent on *C. annuum*. Metabolome analyses will further indicate the basis of these resistance mechanisms. Overall, we can conclude that purple corolla *Capsicum* species present a rich resource for plant breeding programmes that aim to integrate new traits in *C. annuum* crops.

Keywords: aphids; plant resistance; wild and cultivated *Capsicum* species

Abstracts
poster presentations
(alphabetical order,
presenting author)

SPICING UP INSECT DEFENSE: LINKING PEPPER CHEMOTYPES TO RESISTANCE AGAINST AN INVASIVE THRIPS SPECIES

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Thrips parvispinus, a major pepper pest in South-East Asia, has recently spread across Europe and is now the dominant thrips species in pepper (*Capsicum* spp.) greenhouses in Spain, posing a growing threat to European cultivation. In contrast to the well-studied Western flower thrips (*Frankliniella occidentalis*), resistance mechanisms against *T. parvispinus* in pepper remain largely unexplored. Due to their cell-content feeding style, resistance against thrips is thought to depend on specialized metabolites and *Capsicum* species are known for their chemical diversity. This project uses existing metabolomics data to define chemotypes within the G2P-Sol *Capsicum* core collection, select representative accessions, and identify candidate metabolites associated with thrips resistance. LC-MS profiles from 350 accessions were used to define chemotypes, from which 40 representative accessions were selected for greenhouse and laboratory assays. A *T. parvispinus* colony was established, and resistance was assessed using leaf disc assays (measuring larval development and oviposition) and whole-plant assays (measuring thrips population development). Resistance screening revealed differences in thrips performance between accessions. Metabolites correlating to thrips resistance are currently being identified and tested in artificial diets to assess detrimental effect on thrips. This metabolomics-driven approach added knowledge on the G2P-Sol *Capsicum* collection and allowed a more efficient screening of *Capsicum* accessions for thrips resistance. Linking metabolic variation to thrips resistance has the potential to identify both candidate resistance metabolites and accessions that can be used to further study genetics of thrips resistance, supporting the development of pepper varieties resilient to *T. parvispinus*.

Keywords: Thrips parvispinus, thrips resistance, chemotype, *Capsicum*, specialized metabolites

TRANSCRIPTOMIC DISSECTION OF FUNCTIONAL MALE STERILITY IN EGGPLANT REVEALS EARLY DEFECTS IN PROTEIN SYNTHESIS, POTASSIUM TRANSPORT AND ANTHER DEHISCENCE

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Functional male sterility is a valuable trait for hybrid seed production, but its molecular basis remains poorly characterized in eggplant (*Solanum melongena* L.). We investigated the transcriptomic mechanisms underlying anther indehiscence in the functional male sterile line UGA 1-MS, derived from a spontaneous mutant of cv. 'Florida Highbush', using the male fertile cultivar Florida Highbush as wild-type control. Anthers were collected at three developmental stages corresponding to gametogenesis, anthesis and post-anthesis, with three biological replicates per genotype and stage. RNA-seq analysis showed that genotype was the main source of transcriptomic variation, with principal component analysis clearly separating fertile and sterile plants. Differential expression and gene set enrichment analyses revealed that the earliest differences occurred during gametogenesis, when the sterile line showed altered expression of genes involved in ribosome biogenesis, translation, rRNA processing and ribonucleoprotein complex assembly, suggesting an early impairment of the proteosynthetic machinery during microsporogenesis. At the same stage, genes associated with potassium transport and transmembrane activity were strongly downregulated in the sterile line. Among the most relevant candidates, two HAK5-like potassium transporters showed marked downregulation, together with a MYB15-like transcription factor potentially involved in lignin biosynthesis and two polyol/monosaccharide transporters related to cell wall formation. These genes provide a mechanistic link between early defects in pollen development, altered osmotic regulation and impaired secondary wall thickening of the endothecium. Temporal analyses further indicated that the sterile line failed to activate developmental and signalling processes normally associated with late anther maturation and dehiscence. Overall, these results support a model in which male sterility in UGA 1-MS originates from early disruption of protein synthesis and ion transport, ultimately leading to defective anther wall maturation and failure of dehiscence. This study provides new insights into the molecular control of male fertility in eggplant.

Keywords: *Solanum melongena*; male sterility; anther indehiscence; RNA-seq; potassium transport; lignin biosynthesis; hybrid breeding

PEPPER ISOLATED MICROSPORE REGENERATION: HIGH EFFICIENCY AND SPONTANEOUS CHROMOSOME DOUBLING

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Androgenesis in plants can be used to produce doubled haploid plants which are pure homozygous and valuable material for breeding and breeding marker development. Protocols for androgenesis for different crops, including *Capsicum* spp. are known. However, these protocols often show low efficiency, large differences in success rate for different varieties and, little spontaneous chromosome doubling resulting in haploid plants requiring the application of toxic compounds like colchicine. In our work we aimed at very high spontaneous chromosome doubling in pepper androgenesis and compared the results between pepper and tomato.

In the tissue culture process of microspore regeneration, the haploid microspores are treated by different stresses, culture media and hormones to develop into diploid (doubled haploid) plants. The whole process requires, the induction of cell divisions, embryo formation and root-shoot induction. Early in this chain of events, a doubling of the chromosomes should occur. Spontaneous chromosome doubling is highly desired, however, in practise there can be strong variation in the occurrence of spontaneous chromosome doubling for the Solanaceae crops. In this presentation we discuss the very high (>80%) spontaneous haploid genome duplication (SHGD), ploidy reduction, and induced ploidy doubling in pepper (in comparison with other Solanaceae crops) and its implications for application in breeding. In addition, the different possibilities for using haploid plants in advanced breeding technologies, such as protoplast fusion and gene-editing, will be illustrated.

Keywords: androgenesis, ploidy, *Capsicum annuum*, doubled haploids

EVALUATION OF BRILLANT LEAVED PEPPER (*CAPSICUM ANNUUM* L.) MUTANTS CHLOROPHYLL FLOURESCENCE AND SPAD READINGS

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Climate change and global warming increasingly challenge crop productivity, emphasizing the need of identifying genotypes with improved performance and stress tolerance. In our mutant collections identified a brilliant and also visible greener leaved pepper mutant (bil) that probably has a higher stress tolerance compared to the control. In our study two types of brilliant leaved mutants, one in green and one in white fruited background has been studied compared to their corresponding controls. The plants were kept under the same greenhouse conditions, fertigated in the same manner. For a period of 10 weeks every second week the SPAD and Flourpen data were recorded at three different levels of the plants' foliage. Four different biological replicates were applied. Data were analysed using mixed-effects models, testing the effects of genotype, time point, leaf position, and their interactions, with plant individual included as a random factor. The SPAD results showed a consistently higher chlorophyll index in the bil mutants compared with the corresponding controls, in the case of the green bil 67.72 ± 0.93 versus 52.23 ± 0.88 of the green control. The bil mutants in the white background although exhibiting lower values still differed significantly from the control at $p < 0.05$ (61.18 ± 0.97 vs. 46.40 ± 0.74). The highest values were recorded at the middle level of the foliage. In contrast, FlourPen values showed only minor genotype-dependent differences. Leaf position significantly affected fluorescence parameters, lowest values were recorded at the highest level of foliage tested, while the genotype effect was generally weaker and more background-dependent. This indicates that the mutant may have altered photosynthetic characteristics, which could contribute to improved physiological performance under stress conditions, but further measurements across the full 10-week period are needed to confirm its potential stress tolerance.

Keywords: chlorophyll fluorescence, chlorophyll activity, SPAD, FlourPen, Capsicum, mutant

ALTERED GRAVITROPISM IN A PROCUMBENT CAPSICUM ANNUUM MUTANT IS ASSOCIATED WITH REDUCED PIN GENE EXPRESSION

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This study investigates the physiological and molecular background of the procumbent growth phenotype in a *Capsicum annuum* Pcx (procumbent plant) mutant breeding line. The research focuses on gravitropic responsiveness and the expression of PIN-FORMED (PIN) genes, which are key regulators of auxin transport and plant directional growth. In *Arabidopsis thaliana*, loss of PIN3 function has been associated with an anti-gravitropic phenotype. Its closest homologs, PIN4 and PIN7, alone do not induce a prostrate habit; however, in combination with PIN3 deficiency, they enhance this phenotype.

The Pcx mutant displays a horizontal, creeping growth habit, suggesting impaired gravitropic response. To explore its molecular basis, qPCR analysis was performed on stem tissues. The results revealed downregulated expression of PIN genes compared to the control genotype. These findings suggest that reduced expression of key PIN genes may contribute to the altered auxin distribution and weakened gravitropic response observed in the Pcx mutant. This study provides new insights into the genetic regulation of plant architecture in *Capsicum annuum* and highlights the potential role of PIN-mediated auxin transport in determining growth habit.

Keywords: pepper, gravitropism, procumbent, PIN gene

MULTIPLE FLOWERS PER NODE IN PEPPER, BREAKING BARRIERS, BOOSTING YIELD.

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Improving yield remains a primary objective in pepper (*Capsicum annuum*) breeding, yet it continues to be a complex challenge. The multiple-flower trait represents a promising strategy for increasing productivity in pepper.

Although the genetic basis of this trait has been well characterized in *C. chinense*, its effective transfer into cultivated pepper (*C. annuum*) has been limited by interspecific breeding barriers.

In this study, QTL mapping was used to identify major genomic regions controlling the formation of multiple flowers per node. These loci were successfully introgressed into elite pepper germplasm using targeted breeding approaches.

Major constraints, including genome incompatibility, segregation distortion, hybrid sterility, and linkage with pungency, were systematically overcome, leading to the development of improved elite parental lines in pepper.

The resulting near-isogenic F1 hybrids exhibited a 15–25% increase in total fruit yield, demonstrating the potential of this strategy for enhancing yield in commercial pepper breeding programs.

Keywords: Yield components, QTL mapping, interspecific crosses, epistatic interactions, double recombinant

INTEGRATING PEPPER GENETICS AND GENOME EDITING TO IMPROVE POSTHARVEST CHILLING TOLERANCE IN CAPSICUM

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Pepper (*Capsicum spp.*) is a major vegetable crop cultivated worldwide and particularly important in the EMEA region. Because pepper fruits are consumed year-round in western markets, long-distance transport and prolonged storage are essential components of the supply chain. However, postharvest storage is strongly limited by fruit susceptibility to chilling injury under low-temperature conditions. To address this challenge, a multidisciplinary consortium of three research teams at the ARO Volcani Institute has established an integrated platform combining pepper genetics, phenotyping, and genome editing approaches to identify sustainable genetic solutions for improved postharvest chilling tolerance. As part of this effort, we utilized the recently developed G2P-SOL pepper collection to identify genetic variation associated with chilling injury resilience following four weeks of storage at 4°C. First, we developed a phenotypic chilling injury scale enabling quantitative ranking of fruit susceptibility. Using this system, we initially screened a representative subset of 50 G2P-SOL accessions and identified substantial phenotypic diversity for chilling tolerance within the collection. Building on these results, during the last growing season, we expanded the screen to 200 G2P-SOL lines grown in three replicated commercial net-house trials and sampled throughout the November–March production season. The expanded screen confirmed the broad diversity observed in the initial panel and now provides a foundation for GWAS-based identification of haplotypes and loci associated with chilling injury resilience.

In parallel, we have established a CRISPR/Cas9-based genome editing pipeline in pepper, which will be used in future functional validation of candidate loci and for facilitating recombination and introgression of favorable QTL regions into elite breeding material.

FUNCTIONAL VALIDATION OF *SMAPRR2* AND *SMGLK2* BY CRISPR/CAS12A REVEALS THE GENETIC CONTROL OF CHLOROPHYLL PATTERNING IN EGGPLANT FRUIT PEEL

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Fruit peel chlorophyll patterning is a major determinant of visual quality in eggplant (*Solanum melongena*) and contributes to fruit marketability and nutritional value. Previous genome-wide association analysis (GWAS) in the first eggplant MAGIC (Multi-parent Advanced Generation Inter-Cross) population identified *SmAPRR2* and *SmGLK2* as candidate genes controlling uniform chlorophyll pigmentation and irregular chlorophyll distribution (netting), respectively. In the present study, these candidates were functionally validated through CRISPR/Cas12a-mediated genome editing. Three crRNAs were designed for each gene and assembled in a GoldenBraid binary vector carrying a thermotolerant Cas12a nuclease, the selectable marker *nptII*, and the reporter gene *DsRed*. *Agrobacterium*-mediated transformation was performed in the cultivated eggplant accession MEL3, which displays green fruit peel with netting.

Because eggplant is highly recalcitrant to genetic transformation, several rounds of transformation and regeneration were conducted to optimize the recovery of edited plants by evaluating the effects of multiple experimental factors, including explant type, bacterial strain, hormone composition, antibiotics, antioxidants, bacterial growth conditions, and tissue culture handling. This work represents, to our knowledge, the first report of stable Cas12a-mediated genome editing in eggplant.

Stable transgene-free homozygous edited lines have now been obtained in the T1 and T2 generations, as well as in segregating progenies derived from WT × T1 crosses, allowing the evaluation of all genotypic classes. These include WT plants with green peel and netting, double-edited lines with white peel, *SmGLK2*-edited lines with green peel, and *SmAPRR2*-edited lines with pale green peel and netting. The phenotypic segregation observed across these edited backgrounds demonstrates that *SmAPRR2* is required for green chlorophyll pigmentation in the fruit peel, whereas *SmGLK2* regulates the netted distribution pattern. These findings provide functional evidence for the genetic control of eggplant peel chlorophyll traits and establish a framework for breeding novel fruit color combinations.

Keywords: *Solanum melongena*; chlorophyll; CRISPR/Cas12a; *SmAPRR2*; *SmGLK2*

GENETIC MAPPING OF TRAITS CORRELATED WITH MECHANICAL HARVEST OF HOT PEPPER

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The majority of peppers in the US are handpicked, and harvesting can account for 20 - 50% of production costs with decreasing access to labor. Innovation in mechanical harvesting would increase availability, lower costs, and expand markets. An easy-destemming trait derived from the landrace UCD-14 facilitates machine harvest of green chiles. The inheritance and expression of easy-destemming was determined to be controlled by a major destemming QTL on chromosome 10 across populations and environments. Chromosome 10 QTL markers were used to introgress destemming into jalapeño-type peppers which enabled mechanical harvest of destemmed fruit at a rate of 41% versus 2% with a commercial jalapeño hybrid. In addition to low destemming force, our mechanical harvest trials have shown strong correlations between percent total harvested fruits (with and without the pedicel attached) and plant architecture. Detachment force (the force required to remove the fruit with the pedicel attached from the plant) and high detachment force to destemming force ratio was strongly correlated with the proportion of mechanically harvested destemmed fruits. Mapping populations consisting of 192 - S2 families derived from four related BCS1 populations were evaluated in the field using a randomized complete block design with two replications. Architectural traits and detachment force were recorded, and genotypes were determined with the AgriSeq Pepper 5k genotyping array. Significant markers associated with architecture and detachment force were identified. These markers may be useful for screening large populations to select superior lines to evaluate further in field trials. The above significantly advances mechanical harvesting in pepper.

Keywords: mechanical harvest; plant architecture; pepper genotyping array; fruit detachment force

QUANTIFICATION AND GENOMIC PREDICTION OF EGGPLANT FRUIT SHAPE

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Eggplant is an important fruit vegetable with a cultivation history of over 1,000 years in Japan. However, in recent years, its production has declined significantly compared to tomatoes and cucumbers. Although eggplant fruit shapes vary—including round, elongated, and oval—they are often evaluated using simple metrics such as aspect ratio. Furthermore, while genomic prediction (GP) shows promise for fruit vegetables, there is little research on eggplant fruit shape due to the complexity. In this study, I analyzed World Eggplant Core Collection using elliptical Fourier descriptors (EFDs) and GP. Visualization results combining EFDs with principal component analysis suggested that while the diversity of eggplant fruit contours is primarily determined by the aspect ratio, the second and third principal components contribute to oval and pouch-shaped shapes. In fact, fruit shapes can broadly be classified into round, oval, and elongated types. In GP, prediction accuracy varied significantly depending on the accession. While prediction accuracy for the elongated type was relatively low, high accuracy was achieved for some accessions of the round and oval types. These results indicate that EFD and GP hold promise for the quantification of eggplant fruit shape and for breeding.

Keywords: Fruit shape, Genomic Prediction, Elliptic Fourier Descriptors, Mini core collection, Genetic Diversity

20 YEARS EXPERIENCES ON PEPPER (CAPSICUM ANNUUM L.) DOUBLED HAPLOID PRODUCTION

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The production of doubled haploid plants has become a key tool in advanced plant breeding. Plant breeders are increasingly using this system in their mainstream pure-line programs to reduce the number of years needed from crosses to commercial variety registration.

In laboratory of Medimat Ltd. over the past twenty years have been tested more than 3000 different genotypes originating from sweet pepper types (Cecei – Hungarian wax, Kapia, tomato-shaped, apple-shaped, white blocky, light green and dark green blocky, green spice), Hungarian spice pepper genotypes, Dutch blocky types, Spanish types (Dulce Italiano, Lamuyo, red and yellow blocky), Chinese types (hot lamuyo and different spice types) and Turkish types (e.g. Dolma, Charleston, Sivri) and produced high amount homozygote breeding lines using in vitro anther and microspore culture. When doubled haploid (DH) technology has been combined with marker-based pre-selection it provided an exceptionally efficient solution for hybrid pepper resistance breeding (e.g. TMV, TSWV, Powdery Mildew, Xanthomonas, nematodes, CMV).

Medimat Ltd. can offer reliable services and strong expertise in the field of pepper and eggplant doubled haploid production.

Keywords: pepper and eggplant doubled haploid production, marker-based selection, resistance breeding

MARKER-ASSISTED EVALUATION OF VIRUS RESISTANCE GENES IN PEPPER (CAPSICUM SPP.) GENOTYPES IN AARI GENEPOOL

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Pepper (*Capsicum* spp.) is one of the most important horticultural crops worldwide and exhibits high genetic and phenotypic diversity. Although originating from South America, Türkiye represents an important center of diversity for cultivated pepper types. In pepper breeding, resistance to viral diseases is a major objective alongside yield and fruit quality. Marker-assisted selection (MAS) is widely used to identify resistance genes independent of environmental conditions. Among these, the *Tsw* gene confers resistance to Tomato spotted wilt virus (TSWV), while the *L4* gene provides resistance against several Tobamoviruses, including TMV, ToMV, BePMV, TMGMV, and PMMV. The Aegean Agricultural Research Institute (AARI) has conducted pepper breeding programs since the 1970s. To date, nine open-pollinated pepper varieties with different fruit types, shapes, and colors have been registered. In this study, a total of 96 pepper genotypes were evaluated, comprising 10 landraces, 10 open-pollinated varieties, 6 hybrid varieties, 60 F₅₋₆ inbred lines, and 10 genotypes belonging to *Capsicum chinense* and *Capsicum baccatum*. The plant material was screened to determine the presence and distribution of major virus resistance genes relevant to pepper breeding. The results provide valuable information for the effective utilization of resistance genes in the development of virus-resistant pepper varieties.

Keywords: *Capsicum*; virus resistance; germplasm screening; marker-assisted selection; Tobamovirus

PYRAMIDING *PEPY-1* AND *PEPY-2* IN PEPPER ENHANCES BEGOMOVIRUS RESISTANCE

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Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) are major pathogens causing severe yield losses in peppers (*Capsicum* spp.). Breeding for host resistance is considered the most effective and sustainable approach for disease management, and extensive screening has identified several resistance sources. However, achieving broad resistance to multiple begomovirus species and mixed infections remains a major challenge. We previously cloned two begomovirus resistance genes: the recessive *pepy-1*, encoding Pelota, and the dominant *Pepy-2*, encoding an RNA-dependent RNA polymerase. To assess their effectiveness against complex infections, pepper plants were inoculated with either a single begomovirus species or combinations of two Old World or two New World begomoviruses. Plants homozygous for both *pepy-1* and *Pepy-2* displayed durable resistance even under coinfection by two highly virulent begomoviruses. These findings provide a theoretical framework for accelerating DNA marker-assisted breeding for begomovirus resistance in pepper.

Keywords: *Capsicum annuum*; DNA marker-assisted breeding; Geminivirus; Mixed infection; Pelota; Resistance gene; RNA-dependent RNA polymerase (RDR)

STACKS ANALYSIS BASED ON RAD-SEQ DATA REVEALED THE ALLOTETRAPLOID ORIGIN OF THE NEPALESE LANDRACE CHILI PEPPER DALLE KHURSANI (*CAPSICUM* SP.)

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The genus *Capsicum* includes five domesticated diploid species (*C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens*). However, the Nepalese landrace Dalle Khursani (*Capsicum* sp.) stands out as a rare allotetraploid ($2n = 4x = 48$). Its genetic background remains largely unknown, so we aimed to elucidate it via Restriction site-associated DNA sequencing (RAD-seq)-based genetic data analysis. First, we confirmed the tetraploid nature of Dalle Khursani using flow cytometry and chromosome observation. RAD-seq was then performed on 54 accessions, including Dalle Khursani and representatives of the five domesticated species, and short genomic contigs, termed 'stacks', were generated using the Stacks 2 pipeline. The genetic background of Dalle Khursani was analyzed by characterizing the origin of stacks based on their co-presence across the five species. Most stacks in Dalle Khursani were derived from *C. annuum* and *C. chinense*, indicating its allopolyploid origin from these two species. Additionally, analysis of partial chloroplast genome sequences suggested that *C. annuum* likely served as the maternal parent. To our knowledge, this is the first report of a cultivated tetraploid *Capsicum* landrace derived from two domesticated species.

Key words: Nepal, Dalle Khursani, allotetraploid, genetic background, RAD-seq

EARLY DEFENSE MECHANISMS IN JUVENILE STAGE OF EGGPLANT AGAINST INFESTATION BY LEUCINODES ORBONALIS LARVAE

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The eggplant shoot and fruit borer (*Leucinodes orbonalis*) is a major constraint to eggplant (*Solanum melongena*) production in Asia. Reliance on insecticides raises concerns about resistance and environmental impacts, emphasizing the need for host plant resistance within integrated pest management (IPM). However, resistance mechanisms at the vegetative stage remain poorly understood. This study aimed to identify key traits associated with early-stage resistance. Under controlled infestation, SM265 showed no wilting and high early larval mortality, T18_102 (2X) wilted within 2 h with higher larval survival and adult emergence, and T14_25 showed intermediate responses. Damage severity was positively correlated with non-glandular trichome density and negatively correlated with petiole width. Resistant genotypes had higher constitutive phenolics, particularly catechol, scopoletin, and 4-hydroxybenzoic acid. In contrast, the susceptible genotype showed low basal phenolics but strong induction of polyphenol oxidase (PPO) activity and monoterpenes after infestation. Constitutive phenolics were negatively associated with damage, while induced volatiles were positively associated with susceptibility. Life table analysis confirmed reduced population growth on SM265 due to increased early larval mortality. Resistance is primarily driven by constitutive phenolic defenses that disrupt early larval establishment. These findings provide targets for resistance breeding and support sustainable IPM strategies.

Keywords: eggplant shoot and fruit borer, *Solanum melongena*, volatile profiles, catechol

IDENTIFICATION OF QTL THAT ENHANCES THE SIZE OF SEEDLESS FRUIT IN CHILI PEPPER (*CAPSICUM ANNUUM*)

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Chili pepper (*Capsicum annuum*) is an important Solanaceae crop widely consumed as both a spice and a vegetable. Seedless fruits are desirable because they are easy to cook and suitable for processing. However, the absence of seeds often results in reduced fruit size, and it can be obstacle to developing seedless cultivar. This study aimed to identify genetic loci controlling fruit size in seedless chili pepper. BIN map-based quantitative trait locus (QTL) analysis was conducted using seedless populations derived from a cross between the seedless mutant tane nashi-1 and the large-fruited cultivar 'Tongari Power'. A major QTL on chromosome 7, designated qFS7, was identified. qFS7 affected the length, width, and weight of seedless fruit, explaining up to 35.5% of the phenotypic variation. Remarkably, fruit weight increased more than twofold on average. This locus was also detected by the analysis using a seedy population derived from the same cross, indicating that qFS7 functions in both seedy-fruit and seedless-fruit. In addition, its stable effect was confirmed in progeny generations. Histological analyses of ovary and mature fruit were conducted to characterize qFS7 function. As a result, it was suggested that qFS7 contributes to enlarged ovary size by increasing the number of cell layers without affecting cell size, leading to larger fruit. Further analysis of qFS7 will help understanding the molecular mechanisms of fruit development in *Capsicum* and be useful for developing seedless pepper cultivars with improved fruit size.

Keywords: fruit size, number of cell layers, QTL analysis, seedless fruit, sweet pepper

GREAT ATLAS: A WEB-BASED PLATFORM FOR COMPARATIVE FLORAL TRANSCRIPTOMICS IN EGGPLANT AND OTHER CROP SPECIES

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The increasing availability of RNA-seq datasets in crop species offers new opportunities to explore the molecular regulation of reproductive development, but comparative analysis across species remains limited by differences in genome annotation, experimental design and data accessibility. We developed GREAT Atlas, a web-accessible platform for the integration, exploration, and comparison of floral transcriptomic data across vegetable crop species. The atlas currently includes four species from two major plant families: *Solanum melongena* and *S. lycopersicum* in Solanaceae, and *Cichorium intybus* and *Cynara cardunculus* in Asteraceae. RNA-seq data from anther, ovary and stigma/style, were processed through a standardized bioinformatic workflow based on Salmon and DESeq2. The resulting resource integrates gene expression profiles, differential expression statistics, functional annotations, Gene Ontology terms, genomic coordinates and cross-species orthology information into a unified searchable interface. A key feature of GREAT Atlas is the inclusion of a comparative orthology layer based on 3,394 one-to-one orthologous groups identified across the four species. This framework enables users to explore whether genes showing developmental regulation in one species are also transcriptionally conserved across distant crop lineages. The atlas identified 882 orthologous groups with conserved expression changes across Solanaceae and Asteraceae, including tissue-associated gene sets related to male gametogenesis, and ovary development. The platform also highlights poorly characterized conserved genes, providing candidate targets for future functional studies. GREAT Atlas is implemented as a fully client-side web application, allowing free access without server-side computation or user authentication. The interface supports gene search, species and tissue filtering, visualization of expression patterns, inspection of differential expression results and exploration of conserved regulation across species. GREAT Atlas provides a reusable resource for reproductive biology, comparative genomics and pre-breeding research in eggplant and related crop species.

Keywords: *Solanum melongena*; GREAT Atlas; floral transcriptomics; web tool; comparative genomics; orthology; reproductive development

INTEGRATED PHYSIOLOGICAL AND TRANSCRIPTOMIC PROFILING REVEALS GENOTYPE-DEPENDENT HEAT STRESS RESPONSES IN PEPPER (*CAPSICUM ANNUUM* L.)

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Heat stress is a major constraint for pepper (*Capsicum annuum* L.) production, affecting photosynthetic performance, plant water relations and reproductive stability. Understanding the genetic and molecular basis of heat tolerance is therefore essential to support breeding for climate-resilient cultivars. In this study, three genetically diverse pepper genotypes from the G2P-SOL (www.g2p-sol.eu) collection, GPC003240, GPC010350 and GPC014930, were evaluated under controlled heat stress using an integrated physiological and transcriptomic approach. Physiological profiling revealed distinct response trajectories among genotypes. GPC010350 showed the most stable genotype, maintaining an efficient photosynthetic system. In contrast, GPC014930 displayed a stronger late-stage impairment, characterized by reduced photochemical performance, ending in a less efficient and damaged photosynthetic system, while GPC003240 showed an intermediate response. Leaf water potential confirmed a clear treatment effect across genotypes, but genotype-dependent differences in water status were comparatively limited. RNA-seq analysis was performed on leaf samples on three time points. Principal component analysis showed a clear dataset structure, with transcriptome-wide variation shaped by both genotype and heat exposure time. Differential expression analysis identified both shared and genotype-specific heat-responsive genes. The common transcriptional response was limited, with 59 and 62 DEGs shared by all genotypes at early and intermediate stages, respectively, and no common DEG detected at the late stage. At this final stage, GPC010350 maintained a broad transcriptional response enriched for RNA processing, ribosome biogenesis, translation and protein-folding pathways, whereas GPC014930 showed a sharp reduction in DEGs and negative enrichment of photosynthesis-, thylakoid- and plastid-associated processes. These results suggest that heat tolerance in pepper is associated with the maintenance of photosynthetic stability and sustained late-stage molecular reprogramming.

Keywords: *Capsicum annuum*; heat stress; RNA-seq; photosynthesis; genotype-by-environment response; GSEA; climate resilience

GENOMIC CHARACTERIZATION OF HISTORICAL ITALIAN SWEET PEPPER LANDRACES FOR GERMPLASM CONSERVATION AND BREEDING

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The conservation and characterization of local crop genetic resources are essential to preserve agrobiodiversity and to broaden the genetic base available for breeding. In this study, we present the first results of the Pepper GeneBank Project (funded by the Piedmont Region under the framework of CSR 2023-2027 - Call SRA16/1/2024), focused on historical Italian sweet pepper (*Capsicum annuum* L.) landraces maintained at the Germplasm Bank of the Department of Agricultural, Forest and Food Sciences, University of Turin. The collection includes local ecotypes mainly collected in North-Western Italy between the 1970s and 1980s, many of which had undergone limited regeneration since their original acquisition. Seed viability assessment and controlled regeneration under isolation conditions were combined with molecular characterization to support both conservation and future use of these resources. Accessions were genotyped using genotyping-by-sequencing (GBS), yielding a final filtered dataset of 8,682 high-quality SNPs. This marker set provides a first genome-wide overview of the genetic diversity preserved in the collection and represents a useful tool to assess relationships among accessions, detect putative redundancies or misclassified materials, and support the genetic monitoring of regenerated seed lots. These preliminary results highlight the value of integrating regeneration, phenotypic documentation and SNP-based characterization in the management of local pepper genetic resources. The generated molecular information will contribute to the rational conservation of Italian sweet pepper landraces and to the identification of distinct materials of potential interest for pre-breeding and the development of heterogeneous populations with a broad genetic base.

Keywords: *Capsicum annuum*; landraces; plant genetic resources; genotyping-by-sequencing; SNP markers; germplasm conservation; pre-breeding

ADVANCING TARGETED GENOME EDITING IN BELL PEPPER FOR SUSCEPTIBILITY GENE KNOCKOUT

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Capsicum is among the most important genera within the Solanaceae family, with high agronomic relevance worldwide and considerable potential for biotechnological applications. Conventional breeding programs have primarily focused on the development of pepper varieties with desirable taste and nutritional quality, high yield and disease resistance. Despite ongoing scientific interest, the efficiency of tissue culture techniques in bell pepper accessions remains limited because of the strong recalcitrance to in vitro morphogenesis, which results in low or even null regeneration rates. This difficulty has been partially overcome through an empirical approach by optimising key factors such as media composition, plant growth regulators and incubation conditions and recently a new transformation and regeneration protocol has been published.

In this work, we aim at knocking out, either separately or simultaneously, CaDMR6 and CaMLO2, well-known susceptibility genes whose knockout leads to broad-spectrum tolerance to pathogens, as demonstrated in other Solanaceae such as tomato, potato and eggplant. For the regeneration, we applied the new published transformation and regeneration protocol to three bell pepper varieties, one relevant for local agriculture (i.e. Cuneo Giallo from the Italian region Piedmont) and two commercially available accessions (i.e. California Wonder and Cayenne). In parallel, we want to evaluate whether the introduction of the morphogenic regulator GRF-GIF can improve regeneration efficiency in pepper.

The results of this editing system could contribute to the development of improved bell pepper genotypes.

Keywords: bell pepper; recalcitrance; genome editing; CRISPR/Cas9; CaDMR6; CaMLO2

IDENTIFICATION AND GENETIC CHARACTERIZATION OF NEMATODE RESISTANCE SOURCES IN CAPSICUM ANNUUM

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The identification of genetic resistance to root-knot nematodes (*Meloidogyne* spp.) represents a major objective in pepper breeding programmes, particularly in the context of increasing restrictions on chemical control methods and the need for more sustainable crop protection strategies. In this study, a combined phenotypic and molecular approach was applied to characterise nematode response and to identify resistance sources within a diverse *Capsicum annuum* germplasm collection with potential relevance for breeding. Phenotypic evaluations were conducted under controlled infestation conditions, allowing accessions to be classified according to their level of susceptibility or partial resistance. Molecular characterisation was performed using highly informative microsatellite markers to assess genetic diversity and population structure, together with markers associated with nematode resistance loci to detect putative alleles of interest. Phenotypic screening revealed a wide range of responses among accessions, while molecular analyses enabled the identification of genetically distinct materials and contributed to reducing redundancy within the collection. The integration of phenotypic and molecular information revealed consistent associations between specific genetic backgrounds and reduced susceptibility to nematode infestation. Overall, this integrated approach provides a robust genetic basis for the identification of promising resistance sources and supports informed decision-making in pepper breeding programmes aimed at improving resistance to root-knot nematodes.

Keywords: *Capsicum annuum*, *Meloidogyne* spp., disease resistance, genetic diversity, molecular markers, germplasm

DEVELOPMENT, CHARACTERIZATION, AND FUTURE PROSPECTS OF AN INTERSPECIFIC MAGIC POPULATION IN CAPSICUM SPP.

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Capsicum peppers are among the most widely cultivated vegetables worldwide, exhibiting extensive diversity in morphological, agronomic, and quality-related traits. Multi-parent Advanced Generation Inter-Cross (MAGIC) populations represent powerful resources for plant breeding and genetic studies, as they gather valuable phenotypic and genetic variation from multiple founder lines, thereby enhancing recombination frequency, mapping resolution and QTL discovery. In this work, we report the development since 2015 of the first interspecific MAGIC population in *Capsicum* spp., generated within the pepper breeding program at the COMAV Institute (UPV). The population was derived from eight founder lines belonging to *C. annuum* and *C. chinense* and has been advanced through successive generations up to the F6 stage via self-pollination. Throughout its development, the population has been systematically evaluated for general agronomic performance and phenotypic variability. Preliminary observations reveal a broad range of variation for most agronomical traits. Moreover, in its F3 generation, a successful genome-wide association study was performed with highly inheritable traits, recovering some genomic regions with candidate genes. In conclusion, the results observed within the development of this population highlight its potential as a valuable resource for genetic mapping, trait dissection, and breeding applications.

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Keywords: *Capsicum chinense*, *C. annuum*, diversity, genetic resources, pepper breeding, phenotypic evaluation

WHOLE-GENOME SEQUENCING AND GENETIC CHARACTERIZATION OF THE EIGHT FOUNDER LINES OF AN INTERSPECIFIC MAGIC POPULATION IN *CAPSICUM* SPP.

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Multi-parent Advanced Generation Inter-Cross (MAGIC) populations are valuable tools for genetic analysis and crop improvement due to their ability to capture wide genetic and allelic diversity from multiple founder lines, which leads to an increased mapping resolution and improved identification of qualitative trait loci (QTLs). Capsicum peppers represent an economically important crop worldwide with remarkably morphological and genetic diversity. This study presents the results of the whole-genome sequencing of the eight founder lines of the first interspecific MAGIC population in *Capsicum* spp. This population has been advanced to the F₆ generation and phenotypically evaluated throughout the generations for key agronomic and developmental traits. The results present a comprehensive genomic characterization of these founders, including the identification of single nucleotide polymorphisms (SNPs), structural variants, and overall patterns of genetic variation. Comparative analyses among the founder lines revealed substantial diversity, providing insights into the genetic bases underlying phenotypic differences observed within the MAGIC population. Furthermore, putative candidate genes associated with relevant agronomic traits were identified by linking sequence variation to known functional annotations. As a conclusion, these results prove the potential of this MAGIC population for enhancing genomic knowledge for peppers breeding.

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Keywords: genetic variation, genome assembly, MAGIC population, pepper resources, SNP markers, structural variation, whole-genome sequencing

THE EGGPLANT RESISTANCE GENE ATLAS: PHYSICAL MAPPING OF RESISTANCE GENES AND DEVELOPMENT OF R-GENE-SPECIFIC SSR MARKERS

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Abstract Eggplant (*Solanum melongena* L.) is an economically important vegetable crop; however, its production is significantly constrained by various biotic stress factors. The most effective strategy for managing these diseases is the utilization of natural resistance genes in cultivar development. To successfully use these genes in eggplant breeding, molecular markers linked to the resistance loci must be integrated into Marker-Assisted Selection (MAS) applications. Accurate knowledge of the physical positions of resistance genes is essential for improving mapping efficiency and enabling high-resolution genotyping in target genomic regions. In this study, functional annotation of eggplant genes was performed, and 812 disease resistance-related genes were identified. Among these, 754 resistance (R) genes were physically mapped onto the complete eggplant T2T reference genome. Mapping results revealed that major resistance gene hotspots were located on Chromosome 11 (95.0–99.0 Mb, containing 84 R-genes), Chromosome 10 (13.0–16.0 Mb, 58 R-genes), Chromosome 6 (3.0–7.0 Mb, 46 R-genes), Chromosome 1 (96.0–102.0 Mb, 42 R-genes), and Chromosome 3 (12.0–16.0 Mb, 31 R-genes). A total of 3,314 intragenic SSR markers were developed from these genes to facilitate genetic mapping. Furthermore, major historical disease resistance QTLs were anchored to the eggplant resistance gene atlas. The FomCH11 locus for Fusarium wilt was mapped on Chromosome 11, the EBWR9 locus for Bacterial wilt was mapped on Chromosome 9, and the Vd40E05.1 locus for Verticillium wilt was mapped on Chromosome 5. The mapping results showed that these major functionally validated disease resistance loci strictly co-localized with the identified R-gene clusters. To our knowledge, this is the first comprehensive study to map resistance genes and construct a resistance gene atlas in eggplant. The identified resistance loci and the developed SSR markers in the present study will be a valuable resource for developing allele-specific markers to control biotic stress factors in eggplant breeding.

Keywords: Disease resistance, Physical mapping, Resistance Genes, *Solanum melongena*

GENETIC DISSECTION OF WATER-DEFICIT TOLERANCE IN AN EGGPLANT MAGIC POPULATION

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Climate change demands the development of drought-tolerant eggplant varieties. This study integrates early-stage genomic screening with yield stability validation to identify resilient genetic resources. A subset of a multiparent advanced generation intercross (MAGIC) population (184 lines) was first evaluated under water stress (30% field capacity) at the vegetative stage. A genome-wide association study (GWAS) using 293,783 SNPs identified genomic regions linked to water-deficit tolerance. A genomic selection index based on growth traits, leaf water content, and proline concentration was developed to classify lines according to tolerance.

Twelve lines showing contrasting phenotypes (tolerant vs. susceptible, including model-predicted lines) were subsequently selected for a full-cycle trial under intermittent water-deficit conditions. Yield, geometric mean productivity (GMP), and stress tolerance index (STI) were calculated, and the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) was applied to identify superior genotypes at the adult stage. Physiological and biochemical responses were also analysed.

GWAS revealed three major genomic regions associated with total dry weight, leaf water content, and flavonol index. The genomic selection model successfully distinguished tolerant and susceptible genotypes at the vegetative stage and predicted the performance of an additional 141 lines. In the full-cycle validation, lines L13 and L179 showed superior yield stability and physiological robustness, confirming the value of early-stage selection. Tolerance in L179 was linked to the preservation of photosynthetic pigments, whereas L13 exhibited enhanced osmotic adjustment through sugar accumulation.

Although vegetative-stage screening identified four of the five highest-yielding lines, adult-stage evaluation was essential to detect genotypes such as L78, whose tolerance emerged only during reproductive development. Overall, genomic selection is a powerful early-selection strategy, but adult-stage validation remains necessary to capture tolerance mechanisms specific to reproductive growth.

Keywords: eggplant; water deficit; MAGIC population; genome-wide association study (GWAS); genomic selection; yield stability

MICRO-MEL AND MINI-MEL: NOVEL DWARF, SHORT-CYCLE MODEL LINES TO ACCELERATE EGGPLANT BREEDING

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Eggplant (*Solanum melongena*) breeding is constrained by the lack of compact, rapid-cycling model lines that could accelerate both research and breeding progress. Here, we present Micro-Mel and Mini-Mel, two novel dwarf eggplant lines developed from a BC2 plant displaying a dwarf phenotype derived from a cross between the wild relative *S. anguivi* and a cultivated white-fruited eggplant. Their potential as tools for phenotyping, genetic analysis, and accelerated breeding was assessed by comparison with their parental lines and the tomato model Micro-Tom through the evaluation of 30 phenotypic descriptors, together with whole-genome analysis. Both lines exhibited an extremely compact growth habit, reaching heights of 6.83 cm and 7.93 cm at 60 days for Micro-Mel and Mini-Mel, respectively, together with faster development than conventional eggplant materials. Micro-Mel showed determinate growth and earlier development, whereas Mini-Mel displayed a plant architecture closer to that of standard eggplant, thus expanding its experimental applicability. Under controlled conditions, Micro-Mel enabled approximately three crop cycles per year, while Mini-Mel allowed around two and a half cycles. Whole-genome sequencing identified 179,653 SNPs between the parental lines, low levels of heterozygosity, and *S. anguivi* introgression percentages of 8.50% in Micro-Mel and 10.79% in Mini-Mel, as well as shared introgressed regions containing orthologs of tomato genes previously associated with dwarfism. Overall, Micro-Mel and Mini-Mel represent valuable new resources for eggplant breeding by reducing space requirements and crop duration, facilitating phenotypic and genetic analyses under controlled conditions, and providing a platform for functional studies and speed-breeding approaches. Their compact habit and abundant flowering and fruiting also highlight their ornamental potential for urban and decorative horticulture.

Keywords: Micro-Mel; Mini-Mel; dwarfism; model plant; *Solanum melongena*; *Solanum anguivi*

PARTICIPATORY BREEDING AND VALORISATION OF THE PIMIENTO BLANCO DE VILLENA (*CAPSICUM ANNUUM* L.) IN ORGANIC FARMING

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Spain, diversification centre for peppers (*Capsicum annuum* L.), encompasses a plethora of ecotypes along its territories. In this context, the "Pimiento Blanco de Villena", a wax pepper ecotype, has been grown for decades in rural areas of Villena (Alicante) and bred as conservation populations. However, in last years, old farmers were abandoning fields and the crop was threatened. To solve this problem and contribute to more sustainable and resilient farming systems, we started in 2018 to breed and valorise this pepper under organic management, using participatory networks. Starting with >30 accessions (farmers' seeds, UPV seedbank and commercial controls) we evaluated agronomic performance, yield, fruit appearance, weight and quality under participatory strategy. After previous on-farm selection (2018-2023), six lines achieved final evaluations, when we added culinary breeding to assess their value for different uses. Here we detail 2024-2025 results. All lines showed satisfactory yields for open field: 2.0-5.2 kg·m⁻², compared to commercial controls <2 kg·m⁻². Fruit weight ranged 42-146 g. Moreover, despite harvested when unripe, they showed remarkable antioxidant levels. Thus, total flavonoids ranged 432-944 mg·kg⁻¹ fw and total vitamin C achieved 770-1600 mg·kg⁻¹. Culinary events enabled i) valorising this crop with hundreds of consumers and ii) identifying the best use(s) for each line: raw in salads, roasted, pickled, fried, or coca de Villena (i.e. pizza with peppers, bacon, sardines). This initiative enabled selecting several productive and healthy lines, covering different culinary uses, and will contribute to conserve this ecotype in the future.

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Keywords: wax peppers; fruit quality; participatory breeding; valorisation of ecotypes; culinary breeding

DEVELOPING ORGANIC HETEROGENEOUS MATERIALS IN PEPPERS BY SHUTTLE BREEDING BETWEEN IN SPAIN AND PORTUGAL: SPECIFIC ADAPTATIONS AND OPPORTUNITIES FOR WIDE ADAPTATION

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Climate change is driving producers and breeders to find more diverse and resilient farming sector. Particularly peppers, sensitive to heat, water scarcity or salinity. In this context, organic heterogeneous materials (OHMs) are populations bred for organic farming, homogeneous for many traits, although keeping some underlying genetic variation, which can provide a resilient response to a climate in evolution. OHMs are usually obtained by crossing several varieties and the resulting offsprings are then bred by mass selection under cross pollination for several cycles. Here, a composite cross population of four landraces, was first evaluated and mass selected in 2023 spring-summer season open field, under Mediterranean organic farming (Valencia). The offspring was shared in 2024 for evaluation in two different organic farms: i) Valencia (repeating 2023 conditions) and ii) Coimbra (Portugal, agroforestry conditions). The best plants from each trial were selected and mass selected. Finally, two populations (3rd cycle) were evaluated in Valencia in 2025: i) the locally bred OHM population (repeating 2023 and 2024 conditions) and ii) the offspring from Agroforestry trials to continue shuttle breeding. Here we compare yield, fruit weight and soluble solids of both OHMs. Locally adapted OHM showed higher yield and fruit weight than shuttle breeding-derived OHM (on average, 2.05 kg/plant vs 1.15 kg plant and 114 g vs. 71 g). On-farm evaluations suggest that agroforestry selection, under more shady conditions of covering trees, might favour smaller fruits than open field mediterranean Valencia conditions. However, heterogeneity still present in both populations suggest opportunities for both strategies: i) locally-adapted OHM with bigger fruits and high yield and ii) OHMs adapted to both conditions by selecting productive but small fruited types.

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Keywords: bell peppers; shuttle breeding; resilient populations; climate change; yield parameters

MULTI-YEAR EVALUATION OF SPANISH TRADITIONAL PEPPER VARIETIES (*CAPSICUM ANNUUM* L.) UNDER ORGANIC FARMING AND WATER DEFICIT

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In Mediterranean region, drought is a challenge for agriculture and highlights the need to identify water-use efficient cultivars. Landraces represent a valuable source of variation for sustainable farming systems. Spain is a diversification centre for peppers (*Capsicum annuum* L.), with a range of cultivars. Here, five landraces ("Bola", "Najerano", "Padrón", "Piquillo" and "Blanco Villena" types) were evaluated under organic conditions in Valencia, Spain, over seasons 2024 and 2025. Plants were additionally grown under three irrigation regimes: 100% water dose (T100), 75% (T75, moderate deficit) and 60% (T60, severe deficit). Ripe fruits were harvested to assess fruit weight, fructose, glucose, and red and yellow-orange carotenoids. Fruit weight ranged 15-163 g among varieties, treatments and seasons. Generally, T75 did not affect fruit weight, apart from Piquillo, which increased 51% in 2024, whereas T60 reduced in most genotypes in 2025 (-52% average). Total sugars (fructose + glucose) ranged from 31 to 63 g kg⁻¹FW, and increased under water deficit, especially under T75, with average increases 19% in both seasons, particularly, "Piquillo" (45% under T75 in 2025). Total carotenoids (red + yellow-orange) ranged 503-4181 mg kg⁻¹DW. On average, T75 slightly increased carotenoids (+10%), while T60 reduced it by up to 26% in 2024, with "Blanco Villena" showing the highest decrease (-65%) under T60 in 2025, while "Najerano" increased 56% under T75 in 2024. Overall, many of these ecotypes showed good response to moderate water deficit, and genotype x environment interaction will also enable types like "Piquillo" showing an excellent response.

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Keywords: fruit weight; internal fruit quality; traditional pepper germplasm; water deficit; sustainability

PHENOTYPIC EVALUATION OF THE FIRST *CAPSICUM* SPP. MAGIC POPULATION: DIVERSITY AND SEGREGATION IN THE S4 GENERATION

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Pepper (*Capsicum* spp.) is one of the most important horticultural crops worldwide. Traditionally, breeding efforts have focused on selection of varieties or hybridization between two parentals, which limits genetic diversity and recombination. Multiparent Advanced Generation Inter-cross (MAGIC) populations overcome these constraints by incorporating multiple founders, add successive cycles of intercrossing, thus introducing more diversity and enhancing the power to detect Quantitative Trait Loci (QTLs). The COMAV *Capsicum* breeding team has developed the first MAGIC population in *Capsicum*. The objective of this study was to evaluate the distribution of phenotypic diversity within the S₄ generation. A total of 254 lines, plus the 8 founder parents as controls, were characterized for 30 traits related to plant, leaf, flower, fruit and pollen. Phenotypic distribution was analyzed for each trait, in the MAGIC lines and in the parentals. A Principal Component Analysis (PCA) was performed to assess the overall population structure. The S₄ generation displayed parental phenotypes (e.g., shoot pubescence, foliar density), alongside new phenotypes on some traits such as variations in fruit wall consistency and mature fruit color. Furthermore, transgressive segregation was observed in some quantitative traits like plant height and fruit weight, with several S₄ lines surpassing the parental range. PCA revealed that the first two components explain a low percentage of the total variation, with S₄ lines clustering centrally, indicating a balanced integration of the founders' genomes. The phenotypic diversity of the founders is effectively expressed and expanded in the MAGIC population. Genetic reorganization led to new phenotypes and transgressive inheritance, demonstrating the potential of this experimental population for high-resolution genetic mapping and the development of superior pepper cultivars.

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Keywords: *Capsicum* spp., phenotypic diversity, MAGIC population, transgressive segregation

GENOMICS-ASSISTED BREEDING FOR DURABLE RESISTANCE TO *MELOIDOGYNE INCOGNITA* IN PEPPER: INTEGRATING PHENOTYPIC AND GBS-BASED SELECTION

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Root-knot nematodes, particularly *Meloidogyne incognita*, are a major threat to pepper (*Capsicum annuum* L.) production in intensive greenhouse systems of Southeastern Spain, where high inoculum pressure accelerates the emergence of virulent populations capable of overcoming resistance genes such as Me1 and Me3. Developing durable resistance is therefore a key breeding objective. This study aims to introgress these genes into locally adapted genetic backgrounds while improving selection efficiency through the integration of phenotypic and genomic tools. Within the IMMiPeBra project, advanced backcross populations were screened under controlled conditions using artificial inoculation with *M. incognita*, assessing gall formation and nematode reproduction, while marker-assisted selection enabled the identification of homozygous resistant individuals. A total of 16 BC3 A-Me1, 11 BC3 A-Me3, 10 BC3 C-Me3, and 17 BC2 C-Me1 plants were selected, all confirmed as homozygous for Me1 and/or Me3. Their DNA is currently being analyzed by Genotyping-by-Sequencing (GBS) to quantify recurrent genome recovery and identify elite lines with minimal donor introgressions. The study focuses on Americano (A) and Alcos (C), two local genotypes native to the Campo de Cartagena and well adapted to greenhouse conditions. Based on GBS results, the five best individuals per genotype will be selected as parental lines for targeted pyramiding of Me1 and Me3. This integrated approach accelerates the identification of superior genetic combinations and provide further insight into the influence of genetic background on resistance expression, providing a robust framework for the development of more stable and durable resistance to *Meloidogyne* spp. in pepper breeding programs

Keywords: *Capsicum annuum*; root-knot nematode; breeding programs, Me1-Me3 genes

EXPERIMENTAL EVIDENCE OF VIRULENCE SELECTION IN MELOIDOGYNE INCOGNITA AGAINST THE ME1 GENE IN PEPPER

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Resistance to root-knot nematodes (*Meloidogyne* spp.) in pepper (*Capsicum annuum* L.) is mainly based on major genes such as Me1 and Me3, widely used in commercial cultivars and rootstocks. However, their durability may be compromised by the selection of virulent nematode populations under continuous cropping. The aim of this study was to evaluate the stability of Me1-mediated resistance under greenhouse conditions with a nematode population previously adapted to Me3. A three-year experiment was conducted in naturally infested soil with a population of *Meloidogyne incognita* selected for virulence to Me3 through repeated cultivation of a resistant rootstock. The plant material included two Me1 cultivars (Katmandú and Nelto), two Me3 rootstocks (Bedrock and Oscos grafted onto Maestral), a genotype with unknown resistance (Alcos), and a susceptible control (Maestral). Disease severity (galling index), percentage of infected plants, and soil nematode density (J2/100 cc) were evaluated. In the first season, Me1 genotypes showed low infection levels ($IA \leq 1.2$) and relatively low nematode densities (217.7–392.3 J2/100 cc), similar to Alcos (121.7 J2/100 cc) and significantly lower than susceptible and Me3 materials (>567 J2/100 cc). However, infection and nematode densities increased progressively over time. By the third season, Katmandú and Nelto reached high galling indices (5.79 and 6.37) and 100% infection, with nematode densities of 945.0 and 969.0 J2/100 cc, respectively, approaching susceptible levels. While virulence to the Me3 resistance gene has been widely reported, no clear evidence of virulence selection against Me1 has been previously documented. These results provide experimental evidence of progressive adaptation of *M. incognita* to Me1 under continuous cropping, highlighting potential limitations in the durability of this resistance and the need for integrated nematode management strategies.

Keywords: root-knot nematodes, resistance breakdown, greenhouse experiment, *Capsicum annuum*

INVESTIGATION OF BULGARIAN-ORIGIN *VERTICILLIUM* SP. RESISTANCE SOURCES IN PEPPER (*CAPSICUM ANNUUM*) AGAINST HUNGARIAN ISOLATES

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Verticillium wilt, primarily caused by *V. dahliae* and *V. albo-atrum*, is an increasing problem in open-field and greenhouse pepper production, leading to substantial economic losses. The long-term survival of their vegetative structures in soil makes these pathogens hazardous. Due to increasingly frequent climatic extremes, the withdrawal of soil fumigants, and the lack of curative control methods, resistance breeding appears to be the only sustainable protection strategy. While *Ve* resistance gene in tomato is well characterized, no homolog has been identified in pepper, and no resistant cultivars are officially recognized.

This study aimed to evaluate the tolerance of Bulgarian-origin pepper genotypes, previously classified as resistant or tolerant, using artificial inoculation with a Hungarian *Verticillium* isolate. The experiment was conducted under controlled conditions with five tolerant source genotypes (represented by 10 selected lines) and two sensitive genotypes, with seven replicates per line, including one non-inoculated control replicate. Plants were exposed to an inoculum dose substantially exceeding typical field infection pressure (three applications of 350 µl inoculum containing 2.5×10^6 conidia ml⁻¹ per plant). Disease severity was scored on a 0–4 scale, alongside biomass measurements and pathogen re-isolation. Wilting was observed in all genotypes, likely reflecting the extremely high inoculum pressure. Two previously tolerant genotypes exhibited wilt incidence comparable to the sensitive controls. *Verticillium* was re-isolated from most infected plants of the sensitive genotypes and from the two tolerant genotypes. The remaining tolerant lines showed markedly reduced symptom frequency, and the fungus was recovered from only a single plant.

These patterns suggested substantial variation in resistance expression and distinct resistance or tolerance mechanisms. Based on the results, F₁ and F₂ populations with different resistance backgrounds are being developed, providing a foundation for breeding commercially viable *Verticillium*-resistant pepper cultivars and investigating resistance inheritance.

Keywords: *Verticillium* resistance, pepper breeding, pathogenic fungus, disease severity

'SULTESSA F1': A NEW KAPYA HYBRID WITH PMR1 POWDERY MILDEW RESISTANCE, EXCELLENT YIELD AND QUALITY

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Leveillula taurica has emerged as one of the most destructive fungal pathogens in *Capsicum* cultivation worldwide. The pathogen can cause yield losses through severe defoliation, sunscald, and reduced fruit quality.

Resistant sources have been identified in both cultivated and wild *Capsicum* species (*C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, *C. pubescens*, *C. chacoense*, and *C. rhomboideum*). In modern breeding programs, the *pmr1* gene from lines such as 'VK515R' and 'PM-Singang' has largely replaced earlier recessive polygenic sources like HV-12, IHR-703. Molecular markers linked to *pmr1* now enable efficient marker-assisted selection.

Since 2015, Orosco Ltd. has conducted targeted powdery mildew resistance breeding in collaboration with PepGen Ltd. Initial efforts utilized the HV-12 source, while later selections incorporated derivatives of IIHR-1238 as the key resistance donor. This polygenic resistance shows dominant effects along with additive and epistatic interactions. Through more than a decade of rigorous selection under high disease pressure, superior resistant lines were developed, leading to several commercial hybrids including 'Altendos' (cecei), 'Shelton' (cecei), 'Fulton' (hot charleston) and 'Sultessa' (kappa).

'Sultessa F1' is the first kappa-type hybrid with high-level powdery mildew tolerance and wide shoulders. In addition to the *pmr1* gene, it provides resistance to TMV (L³), TSWV (tsw), and nematodes (Me1). Parental lines were developed through classical crossing and individual selection. Its resistance was confirmed by molecular markers, bioassays, and multi-location trials.

In small-plot trials, 'Sultessa' showed outstanding performance. Average fruit weight exceeded 130 g in greenhouse trials (3 locations, 22 harvests) and 100 g in open-field conditions. Greenhouse yields surpassed 2 kg/plant and outperformed relevant controls by at least 15%. The hybrid also achieved over 95% first-class marketable fruit in greenhouse trials.

By combining high yield potential, multiple disease resistance, and excellent quality traits, 'Sultessa' offers a valuable option for kappa growers.

Keywords: *Leveillula taurica*, *Capsicum annuum*, powdery mildew, *pmr1* gene, marker-assisted selection, kappa pepper, hybrid breeding, disease resistance, Sultessa F1

EVALUATION OF THE GENETIC LINKAGE BETWEEN THE L⁴ RESISTANCE GENE AND DIFFERENT POLLEN QUALITY TRAITS IN VARIOUS CAPSICUM ANNUUM TYPOLOGIES

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Tobamoviruses, particularly Pepper mild mottle virus (PMMoV), are among the most destructive pathogens in pepper production. Due to its high stability and mechanical transmission, PMMoV can rapidly infect most plants in a greenhouse, causing yield losses of 75–95%. As chemical control is largely ineffective, breeding for genetic resistance is the most sustainable strategy.

The L-gene allelic series (L¹ to L⁴) provides hypersensitive response mediated resistance to tobamoviruses. Among these, the L⁴ allele is a monogenic dominant gene that confers the broadest resistance against multiple PMMoV pathotypes, including P^{1.2.3}. However, since it was introgressed from *C. chacoense* (Boukema, 1984), its deployment is often limited by linkage drag. Negative effects on vegetative and generative traits have been observed in L⁴-carrying lines, including the segregating non-fertile dwarf (SNFD) phenotype.

To reduce the impact of lower fertility on seed production, breeders commonly use L⁴ lines as male parents. Therefore, pollen traits such as quantity, viability, germination rate, size, and morphology have become key criteria for assessing the breeding value of L⁴ lines, as they directly affect fertilization success, hybrid seed yield, and fruit uniformity.

Previous work demonstrated that shortening the introgressed segment can break the linkage between stable PMMoV resistance and the SNFD phenotype (Allersma *et al.*, 2007). Based on this, we hypothesized that variation in L⁴ introgression size may show correlation with pollen quality.

To test this, we selected six L⁴-carrying lines (three Cecei and three Kapya types) representing weak, medium, and good pollen donors based on prior experience as male parents. Controls included the L⁴-free cultivar (OP) 'Fehér özön' and one high-performing Corno-type L⁴ line.

Molecular genotyping of the L⁴ locus was combined with pollen viability and morphology assessments using image analysis. The analysis yielded promising results: clear differences observed among L⁴-resistant lines, and the L⁴-free control showed high pollen quantity and quality.

These findings support strategies to combine durable tobamovirus resistance with optimal reproductive performance. In the short term, they will help select the best L⁴ donors for backcrossing in each typology; in the mid-term, they will enhance hybrid seed production efficiency and agronomic performance of new L⁴-resistant varieties.

Keywords: *Capsicum annuum*, L⁴ gene, PMMoV resistance, linkage drag, pollen quality, image analysis, marker-assisted breeding

OCCURRENCE OF COPPER RESISTANT *XANTHOMONAS EUVESICATORIA* PV. *EUVESICATORIA* STRAINS INFECTING OPEN-FIELD SPICE PEPPER IN HUNGARY

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Xanthomonas leaf spot is one of the most serious diseases affecting open-field spice peppers. Of the many Eppo A2-listed. Xanthomonas species involved in the development of the disease, the primary pathogen is Xanthomonas euvesicatoria pv. euvesicatoria (XANTEU). The bacterium causes small, light-coloured, blister-like leaf spots that develop into irregular brown necrotic spots surrounded by weak halo. Affected leaves and flowers fall off, and the fruits developed brown scabby lesions. Control measures are primarily limited to prevention and the use of copper-based pesticides. However, the use of copper raises several issues, including the risk of the emergence of copper-resistant strains. Therefore, we aimed to investigate the copper resistance of XANTEU strains in Hungary.

During 2025, open-field plant samples exhibiting symptoms of bacterial spot were collected from three different growing regions and six pepper varieties. Nutrient agar medium containing cycloheximide (100 mg/kg) was used for isolation. Hypersensitive response (HR)-inducing ability of isolates with colonies typical of Xanthomonas was tested on Nicotiana tabacum 'Xanthi' plants. Species-level identification of HR-positive isolates was performed using PCR with a XANTEU-specific primer pair. For the copper resistance, the poisoned agar plate method was used on CYE (casamino acid-yeast extract) medium containing copper sulfate at concentrations of 0, 100, and 200 mg/kg. A copper-resistant strain (E-3) was included as a control. Copper resistance was confirmed in 2 cases out of the 65 XANTEU isolates tested. Both isolates originated from Zákányszék, from the 'Szeged 178' variety, but from two different plants. Interestingly, another 4-4 isolates from the same plants proved to be copper-sensitive suggesting that symptoms within an area may be caused by a mixed population in terms of copper resistance.

Keywords: Capsicum annuum; Xanthomonas euvesicatoria pv. euvesicatoria; copper resistance; bacterial spot of pepper

INTERACTION BETWEEN GENES ENCODING TISSUE-DESTRUCTIVE (HR) AND TISSUE-PRESERVING (GDR) REACTIONS IN PEPPER RESISTANCE BREEDING

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In 1995 we reported two tissue-preserving reactions in pepper. One of these responses shows monogenic, recessive inheritance and, based on the tissue alterations observed in compatible and incompatible interactions, it was designated gds gen. The other reaction, due to its widespread occurrence in the plant kingdom and its role in defence, was termed the General Defence Reaction (GDR). The recognition of these tissue-preserving reactions in pepper revealed a previously unknown relationship in plant–pathogen interactions, which can be effectively applied in resistance breeding. Pepper lines with high levels of tissue-preserving (GDR) were crossed with lines showing strong tissue destruction (HR). In the F₂ generation, a 12:3:1 segregation ratio corresponding to dominant epistasis was obtained. It was established that the high level of tissue-preserving is significantly controlled by recessive genes (gdr1+2). In incompatible relationships, the homozygous presence of one of the recessive genes is sufficient. In compatible host plant–pathogen relationships, however, recessive genes must be present in the homozygous state to ensure high level of tissue preservation. In pepper, the gds, gdr1, gdr2 genes, characterised by low stimulus threshold and rapid response, should replace the HR genes, which they keep in an inactive state and whose presence represents a genetic burden for the plant. In the plant kingdom, including pepper, GDR, which functions as the plant's "immune system," provides the most effective protection against biotic stresses in both compatible host–pathogen relationships and incompatible plant–microbe interactions, through a non-specific, general response manifested in cell enlargement and cell division. Since the beginning of resistance breeding, this GDR capacity has been progressively weakened by HR-based selection. It is not the pathogens that have developed more aggressive strains; rather, the GDR capacity of plants has been increasingly degraded through selection for HR.

Keywords: tissue-preserving, general resistance, recessive resistance genes, epistasis

REPRODUCTIVE ANSWER OF BULGARIAN PEPPER CULTIVARS TO DROUGHT STRESS

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The climate change and the reduction of water resources limit the productive and reproductive capabilities of pepper. Water deficit is a vital problem for the development of plants and does not allow the disclosure of their biological potential. For the purposes of seed production, the study of the impact of drought on productivity and quality of the seeds is extremely important. Disorders establishing in the development mechanisms as a result of water deficiency, provides greater clarity for solving of the problem. The resistant genotypes identification allows the initiation of targeted selection of lines and cultivars, tolerant to drought stress.

The study purpose was to assess the reproductive capabilities of pepper cultivars under the drought conditions.

The experiment was conducted at the Maritsa VCRI in polyethylene greenhouses with 15 cultivars with different fruit shape, way of production and consumption. The trail was set up in two cultivation systems: i/ optimal irrigation and ii/ with 50% water deficit in 3 replications for each system with 10 plants per each variety in replication. The fruits harvesting and seed extraction were conducted during period September-October. After one-month storage, the obtained seeds were analyzed according to ISTA requirements. The data were processed using ANOVA, Duncan and correlation analyses. The cultivation system had a proven effect on all studied traits, while the interaction genotype x environment, only on seed productivity. No proven genotypic differences were established only in the weight of 1000 seeds. No strong correlations were found between the studied traits. Genotypes with more tolerant response to drought according to the reproductive traits were identified, and will be included in the future drought breeding program.

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Key words: Capsicum, water deficit, seed productivity, seed quality, germination

GENETIC AND AGRONOMIC EVALUATION OF A RECOMBINANT INBRED LINE (RIL) POPULATION OF EGGPLANT SUBJECTED TO DROUGHT STRESS

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The reduction of agricultural water usage poses serious threats to food safety on a global scale. Plant breeding is an important technological tool for adapting crop production to the drought. In this context, the identification of drought-tolerant genotypes and of genetic determinants responsible of the better adaptability to water limitation is crucial for every crop species. In the framework of the Project PhenoGen, the impact of a prolonged water limitation stress on physiological, morphological and agronomical parameters was assessed in a RIL eggplant population of 157 lines derived from the cross between 305E40 x 67/3, two eggplant lines selected as diverging for several traits including fruit/plant feature, habitus, yield and tolerance to stresses. Plants for each RIL were grown in greenhouse conditions in big pots filled with substrate maintained either at 50% or 100% of water field capacity (control) along the entire experiment. The plants were evaluated for several traits including yield, plant and fruit features, fruit quality, physiological traits and stress indexes (including MDA, proline, transpiration and stomatal conductance). Mapping of more than 20 parameters on the resequencing low-pass (1X)-based map with more than 7200 markers led to the identification of several major QTLs associated to relevant traits as stress-related height decrease (height-rel), and transpiration (transpiration_50). Within their CI, interesting candidates involved in stress detoxification, cell division, response to stress, CO₂ assimilation and a negative regulator of abscisic acid signalling were spotted. Moreover, data evaluation allowed to select a subgroup of RILs with the best productive and growth performances under low water supply, whose high levels of proline and malondialdehyde suggest a m plant response to stress with potential use for breeding and MAS selection for the development of innovative eggplant varieties with improved tolerance to water stress.

Keywords: Solanum melongena; Drought stress; Mapping population; RIL

MULTI-TRAIT FRUIT QUALITY EVALUATION OF PEPPER HYBRIDS IN A LOCAL BREEDING PROGRAM

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Local breeding programs play a key role in developing pepper (*Capsicum annuum* L.) cultivars combining high fruit quality with adaptation to specific growing conditions and market preferences. The present study is part of an ongoing breeding effort focused on improving fruit quality traits in diverse pepper varietal types. A set of F1 hybrids and parental lines, developed within a local breeding program, was evaluated under open-field conditions at Maritsa VCRI for major quality-related attributes at two ripening stages (green and red). The hybrids, representing Conicum, Kapia, Blocky, and Pumpkin types, showed substantial variation in dry matter (DM), total soluble solids (TSS), vitamin C content, firmness, and color parameters (CIELab), highlighting the potential of local germplasm for quality improvement. Ripening strongly affected fruit composition, with increases in DM, TSS, vitamin C, and color intensity at the red stage, accompanied by reduced firmness. Quality traits showed coordinated variation, with positive associations among DM, TSS, and vitamin C, an opposite trend for firmness indicating a texture–composition trade-off. Importantly, differences among varietal types were observed not only in trait values but also in the stability of quality expression. Kapia-type hybrids combined high levels of soluble solids, vitamin C, and color intensity with more consistent quality profiles, whereas Conicum types exhibited greater variability, suggesting broader selection potential within the breeding program. An integrated multi-trait assessment enabled the identification of superior hybrids combining desirable nutritional and sensory characteristics. The results demonstrate the effectiveness of the local breeding program in generating diverse and high-quality genetic material and support the application of multi-trait selection strategies for the development of improved pepper cultivars.

Keywords: *Capsicum annuum*; local germplasm; nutritional quality, multi-trait selection

THE EUROPEPLAND PROJECT: IMPLEMENTING A TRANS-EUROPEAN PEPPER LANDRACE COLLECTION FOR RESILIENT AGRICULTURE

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In the face of global challenges to food security such as rapid population growth and climate instability, crop improvement has emerged as a cornerstone for sustainable agriculture. The EuroPepLand project supported by the ECPGR Grant scheme aims to implement a trans-European pepper (*Capsicum annuum*) landrace collection to foster resilient agriculture boosting the cooperation with other international initiatives (G2P-SOL, EVA Pepper, PRO-GRACE) aimed at evaluating and valorizing pepper genetic resources. Traditionally, landraces are locally adapted varieties that act as reservoirs of genetic diversity; however, they are increasingly being replaced by modern cultivars that often lack resilience to multiple stress. Here, we present the establishment of a core collection comprising 441 landraces, representing broad phenotypic diversity in fruit size, shape, color, pungency and adaptation across Western and Eastern European production areas. The collection was provided along with passport and main phenotypic data and was genotyped using the 10K pepper SNP array. Genomic diversity analysis using principal component analysis and ADMIXTURE revealed 12 subpopulations and identified two

primary genetic hubs in Southern Europe and the Balkans. Phylogenetic relationships further highlighted specific hotspots, revealing distinct geographical clusters that reflect the evolution occurring within Europe as a secondary center of diversification. Genome wide association mapping for pungency confirmed a significant association on chromosome 2, linked to the Pun1 gene mutation, demonstrating the population's effectiveness for candidate gene identification. To test the utility of our collection to identify potential candidates for resistance to abiotic stress, 11 selected genotypes were phenotyped for salt tolerance by implementing a 3D and multispectral platform as well as for the content primary and secondary leaf metabolites associated to stress response. By strengthening the cooperation between gene bank managers and researchers and centralizing data in public databases, we provide a tool for next-generation breeding that adds value to high-quality local genetic resources.

Keywords: Capsicum annuum, landraces high-throughput genotyping, germplasm collection, genetic resources, phenotyping, salt stress, metabolic analysis

CHARACTERIZATION AND FINE MAPPING OF A *SOLANUM ELAEAGNIFOLIUM* INTROGRESSION UNDERLYING INCREASED 4-O-CAFFEOYLQUINIC ACID LEVELS IN EGGPLANT

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Caffeoylquinic acids (CQAs) are the major phenolic compounds in eggplant (*Solanum melongena*) fruit and are important contributors to its antioxidant capacity and functional quality, with 5-O-caffeoylquinic acid (5-CQA) being the predominant isomer in cultivated materials. Wild relatives may provide additional metabolic variation for broadening these profiles. In previous work, a distinct phenolic profile characterized by a secondary chromatographic peak eluting after the main 5-CQA peak was detected in *Solanum elaeagnifolium* and in advanced backcross materials carrying introgressions from this tertiary genepool wild relative, but the identity of this compound and the associated chromosome 1 region remained unresolved. In the present study, we combined HPLC-DAD-ESI-MSn, marker-assisted screening using HRM, SPET high-throughput genotyping, fine mapping across three consecutive selfing generations, and comparative microsynteny analysis to characterize this phenotype. HPLC-MS/MS analyses identified the secondary peak as 4-O-caffeoylquinic acid (4-CQA). Fine mapping involved the screening of 612 individuals and the identification of 21 informative recombinants, allowing the candidate interval on chromosome 1 to be reduced from 2.753 Mb to 0.211 Mb. The final region contained 30 annotated genes according to the eggplant reference genome. Phenotypic evaluation showed that the 4-CQA peak was present in both homozygous and heterozygous introgression-derived materials, indicating dominant control of the trait. Comparative structural analysis revealed broad conservation between *S. melongena* and *S. elaeagnifolium* across the interval, but also uncovered a localized structurally divergent block containing acetylajmalan esterase-like/GDSL-type genes and a transcript-supported putative donor-specific gene. Overall, these results identify a novel source of phenolic diversification from *S. elaeagnifolium* and provide candidate genes and markers that may facilitate the breeding of eggplant lines with a more diverse CQA profile and potentially enhanced functional quality.

Keywords: eggplant; *Solanum elaeagnifolium*; caffeoylquinic acids; 4-O-caffeoylquinic acid; introgression; fine mapping

LEVEILLULA TAURICA RESISTANCE IN PEPPER

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Leveillula taurica (Lév.) Arnaud is a powdery mildew pathogen affecting both pepper and tomato. It initiates infection by penetrating through stomata, colonizing the apoplast, and invading individual leaf cells to establish haustoria. Infection reduces crop yield caused by various symptoms, including yellowing of the leaves, premature leaf senescence, leaf abscission, and fruit scorching. Currently, *L. taurica* is controlled through fungicide applications; however, *L. taurica* was found to be resistant to certain fungicides. Due to *L. taurica* fungicide resistance and increasing restrictions on the use of fungicides, it becomes increasingly important to use resistant varieties in pepper production. Consequently, a screening for resistant accessions was initiated, and several have been confirmed as resistant. Crosses were made between the resistant accessions and the susceptible *Capsicum annuum* cv. Maor. From these crosses, F₂ populations were produced that will be screened for resistance against *L. taurica*. In addition, effectors of *L. taurica* were predicted using RNA-seq data and genome assembly of an *L. taurica* isolate. The predicted effectors will be used to identify defense responses of resistant accessions. This combined approach aims to identify and use resistance sources for durable *L. taurica* resistance in pepper cultivars.

Keywords: *Leveillula taurica*, pepper crosses, effectors

NATURAL RESILIENCE TO PHLOEM-FEEDING INSECTS IN *CAPSICUM ANNUUM*: BROAD-SPECTRUM RESISTANCE TO APHIDS AND WHITEFLIES

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Aphids and whiteflies cause great damage to pepper (*Capsicum* spp.) cultivation. To prevent yield losses, growers still rely on insecticides, but the development of resistances in target insects, and environmental and societal concerns, have created the need for more sustainable pest control. A key alternative strategy is breeding resistant cultivars with natural resilience to insects, but plant resistance against a single species is easily broken and not durable. Phloem-feeding insects share common morphological and physiological features, such as specialized mouthparts and salivary secretions, enabling them to exploit host plants. It is hypothesized that, in turn, plants have evolved common resistance mechanisms, such as restricting phloem access or producing specialized metabolites, affecting insect feeding. Resistance mechanisms affecting multiple insect species have been identified in *Arabidopsis* and tomato, but not yet in pepper. In this project, we aim to identify and characterize broad-spectrum resistance to phloem-feeding insects in *Capsicum annuum*. We will explore natural variation in resistance to aphids (*Myzus persicae*) and whiteflies (*Bemisia tabaci* MEAM1) in the G2P-SOL *C. annuum* core collection and select broad-spectrum resistant candidates. Next we will unravel the genetic basis of broad-spectrum resistance to phloem-feeding insects using a DH mapping population and identify novel phloem metabolites associated with resistance to aphids and whitefly. Finally we will characterize the mechanism(s) underlying broad-spectrum resistance to phloem-feeding insects. Elucidating the mechanisms underlying broad-spectrum insect resistance and identifying genetic markers will support the breeding of insect-resistant pepper cultivars. Employing resistant cultivars will contribute to sustainable pest management, in line with Integrated Pest Management principles, reducing reliance on pesticides.

Keywords: *Capsicum annuum*, broad-spectrum resistance, aphids, whiteflies, specialized metabolites, metabolomics

PEPPER VEINAL MOTTLE VIRUS (PVMV; *POTYVIRUS CAPSIVENAE*) POPULATION STRUCTURE DRIVES RESISTANCE DURABILITY IN PEPPER (*CAPSICUM* SPP.) IN WEST AFRICA

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Pepper veinal mottle virus (PVMV) is one of the most devastating aphid-transmitted viruses infecting pepper in West Africa. Developing PVMV resistant pepper cultivars with high and stable yield across different environments is the most promising long-term solution to minimize losses due to the virus. The goal of this study was to assess the diversity of PVMV isolates infecting putative resistant pepper entries and to select the best performing entries in multiple environments in West Africa. Nine putative resistant pepper entries were evaluated in one environment in Benin (Abomey-Calavi= E1), two environments in Ghana (Kwadaso= E2 and Asokwa = E3) and one environment in Nigeria (Ibadan= E4). A randomized complete block design was used with three replications, each with 24 plants. Data were recorded for key horticultural traits as well as PVMV disease incidence. The presence of PVMV was diagnosed in a total of 113 leaf samples by RT-PCR and whole genome sequences were generated using next-generation sequencing. Significant variation ($p < 0.05$) was observed across environments for both horticultural and disease resistance traits. Based on partial sequences, 100% of the entries tested positive for PVMV in three environments (E1, E2, E4) while none of the entries were positive in environment E3. Similar genome sizes (~9800 bp) among PVMV isolates but significant genetic differences between isolates from West Africa were identified. The recombination analysis of eleven full-length PVMV isolates identified seven putative recombination events using RDP4. This study provides valuable insights into the genetic diversity of PVMV isolates and regional adaptation of putative resistant entries in West Africa.

Keywords: Pepper, Potyvirus, complete genome sequence, genotype-by-environment interaction

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To explore
the potential
of nature to
improve the
quality of life

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