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NATGENCROP

Employing the Natural Genetic Variation to Improve Nutritional Properties and Stress Tolerance in Vegetable Crops

PROJECT: 101087091-NATGENCROP-HORIZON-WIDERA-2022-TALENTS-01

E-NEWSLETTER

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Dr. Saleh Alseekh
ERA Chair Holder

Dr. Saleh Alseekh

Head of "Crop Quantitative Genetics" Department at CPSBB

"The NatGenCrop project will help breeders to design new crop varieties with good yield and quality even in harsh environmental conditions"

Dr. Saleh Alseekh from Germany is the ERA Chair Holder of the NatGenCrop project and Head of the Crop Quantitative Genetics department at CPSBB.

Main focuses of his researches are the metabolic and genetic regulations of plant traits, contributing to advancements in crop improvement and plant biotechnology. With numerous high-impact publications, Dr. Alseekh is widely recognized for his expertise in plant systems biology and metabolomics.

INTERVIEW

Dr. Alseekh, what was your personal motivation to get involved in the NatGenCrop project as an ERA Chair Holder?

I had been working with the Director of CPSBB, Prof. Tsanko Gechev, and other CPSBB members for several years before this project started in 2023. So, when Prof. Gechev invited me to apply for the ERA Chair call, I was very well acquainted with all the facilities and scientific expertise that CPSBB could provide. I was absolutely sure that I would join a team, professionally prepared to conduct the required world-class research and complete this large-scale scientific project.

What is the potential impact of the NatGenCrop project for sustainable agriculture?

The primary objective of this project is to investigate how plants respond to various forms of abiotic and biotic stress, with a focus on enhancing crop resilience in the context of climate change. To achieve this goal, we apply approaches such as quantitative genetics and plant systems biology. The insights gained through our research are expected to support the identification of new crop varieties with improved tolerance to drought, salinity and pathogenes.

In addition, the QTLs and candidate

genes we have identified would help breeders to design new crop varieties capable of maintaining high yield and quality under challenging environmental and climatic conditions.

What is the scope of the project?

We aim to analyze the genetic basis of responses to biotic and abiotic factors in tomato and pepper using both GWAS and QTL mapping. Specifically, we have three main tasks. First, we have to identify genetic loci and alleles associated with drought and salinity stresses, and evaluate candidate genes that are relevant to future breeding programmes. Second, we want to explore the natural variation to identify agronomic and biochemical traits associated with improved yield, quality, and tolerance. Last but not least, we aim to decipher the genetic bases related to crop resilience to fungal and bacteria diseases.

What are the future plans within the NatGenCrop project?

We have already obtained significant results and data from the research that has been done since the start of the NatGenCrop project in 2023 and we are optimistic that till the end of the project in 2027 our findings on tomato and pepper will be significant enough to be expanded to other economically important crops. ■



NatGenCrop will face global challenges by improving the nutritional properties and tolerance to abiotic and biotic stress in tomatoes and peppers



Dr. Eng. Alicja Wieteska Georgieva
Agronomist, CPSBB

By combining fundamental scientific research with practical methods for crop breeding the **NatGenCrop project** aims at coping with global challenges like climate change, food security, and human health by improving the nutritional properties and tolerance to abiotic and biotic stress in tomatoes and peppers.

Both crops are economically important as they are among the most cultivated vegetables in Europe. Enhancing their stress tolerance is crucial as more frequent droughts, heatwaves, and instances of soil salinization (from poor water quality or degraded soils) threaten crop yields nowadays.

Moreover, tomatoes and peppers are rich in vitamins (A, C, etc.),



Dr. Avanish Rai
Postdoctoral researcher, CPSBB

antioxidants, and other health-promoting compounds and enhancing their nutritional content can contribute to a better quality of the human diet.

2023

When the NatGenCrop project started in **2023**, several large-scale field and greenhouse experiments were carried out simultaneously by CPSBB researchers for studying morphological and physiological responses to water deficiency of tomato and pepper populations.

The plant material used in the project included two populations for Genome-Wide Association Studies (GWAS), one for each of the crops, and two Backcrossed Inbred Lines (BILs) panels.

What does GWAS include?



“The main goal of the GWAS analysis is to help us detect the patterns in the DNA of the plants that correlate with the expression of desired traits. We have to compare the DNA of all different plants we grow in order to see which parts of the DNA are associated with important agronomic characteristics.

In our experiments, we included 152 tomato lines from 21 countries and 180 pepper lines from 6 Balkan countries. The accessions were with a wide variety of sizes, shapes, colors, and biochemical compositions.

Once we know which genes control these traits, we can use this knowledge for selection of better crops, improve their nutritional quality and develop cultivars that survive through stressful climate and biotic conditions”, explains **Dr. Avanish Rai**, Postdoctoral researcher at CPSBB.



Analyzing GWAS data on tomato and pepper under drought stress.

Why do scientists use BILs panels in their research?

Researchers are aiming to create improved vegetable varieties with enhanced flavor, disease resistance, climate resilience or faster growth rates. For this purpose, they often cross different plants with the desired traits and study the results. This is like carefully planned breeding.

"So, consequently, alongside the GWAS panels, we incorporated in our studies two Backcrossed Inbred Lines (BILs) populations containing 56 tomato and 110 pepper varieties", outlines Dr. Eng. Alicja Wieteska Georgieva, Agronomist at CPSBB.

During their cultivation, all these accessions were carefully phenotyped for traits such as height, diameter, stem thickness, and flowering time.



During the cultivation, all the accessions were carefully phenotyped.



The phenotyping of fruits included: fasciation, blossom end rot, ripening earliness, ripening uniformity, and fruit set/truss. In order to monitor the drought experienced by the plants, the relative water content (RWC) of the leaves was also measured.



Measurement of RWC in leaves.

As a result of these trials, candidate lines potentially tolerant to drought stress were identified and selected for further analysis. Furthermore, samples from leaves and fruits were collected for high-throughput quantification of their primary and secondary metabolites, gene expression, and elemental analysis.



Preparation of samples for further analysis.

The balance of various metabolites and mineral concentrations plays a crucial role in determining vegetable and fruit flavor and nutritional quality. Specifically, the ratio of sugars to acids, the presence of specific volatile compounds, and the availability of certain mineral ions all contribute to the taste profile.



This type mass spectrometry (ICP-MS) uses inductively coupled plasma which atomizes and ionizes the sample in order to detect amounts of metals or non-metals in a the range of part per trillion.

2024

During the second year of the project the experiments with the GWAS population (both tomato and pepper) under drought stress and the subsequent data analyses were repeated in greenhouse conditions. The main goal of the research team was to confirm the results from the first year and select the most promising lines for further analysis.



Collection of fruit samples.

2025

In 2025 the researchers reinitiated the experiment with BILs for both tomato and pepper in drought conditions to compare with the results from 2023.

"In addition, we incorporated salinity stress in our research, which simulates the condition plants face when the soil or water surrounding their roots contains an excessive amount of salt. This makes it harder for plants to absorb water and nutrients, which can slow down growth, damage tissues, and reduce crop yields. We also conduct biotic stress experiments with common pathogens that are known to negatively affect agricultural yield", outlines Dr. Eng. Alicja Wieteska Georgieva.

Currently, the experiments are in progress, conducted in specialized growth rooms at CPSBB .

In summary, since the beginning of the NatGenCrop project in January 2023 till June 2025 the scientific team has completed large greenhouse experiments to test how tomatoes and peppers respond to drought.

The plants from both GWAS and BILs populations were grown under normal and low-water conditions to compare their performance.

For each plant type, researchers measured the number of fruits, the fruit weight, total yield, sweetness, and signs of fruit damage. They also studied how well the plants handled drought by using special drought-tolerance scores.



Measuring size and weight of fruits of each plant type, included in the experiments.



Blossom-end rot is an indication of stress in crops due to a calcium deficiency.



Comparing the performance of plants in normal and low-water conditions



Thousands of fruits from both tomatoes and peppers were collected for analysis of the levels of sugars, organic and amino acids, and other natural compounds. This was done using a method called liquid chromatography-mass spectrometry (LC-MS) that helps detect many different metabolites at once.

Each plant's DNA was also analyzed for the identification of genetic regions linked to useful traits using GWAS and quantitative trait loci (QTL) mapping.



LC-MS analysis of metabolites in samples.

“We started studying gene activity both in drought-tolerant and sensitive accessions using RNA-seq analysis. Finally, we began building gene-editing tools like CRISPR-Cas9 to test the function of key genes related to drought tolerance”, highlights Dr. Avanish Rai.

The research has already identified several promising gene candidates from both GWAS and BIL populations, which demonstrate enhanced yields and nutritional value of the fruits even when exposed to water-deficient environments.

Furthermore, certain accessions have been chosen for in-depth analysis due to their salinity tolerance in terms of root elongation and ability to decrease the sodium accumulation in the root system of tomatoes from the GWAS panel.

WHAT IS NEXT?

Until the end of the project in 2027 the NatGenCrop team will finish analyzing how the selected genes behave in the plants, including which of them turn on or off under stress and how they work together.

The gene activity data will be combined with the biochemical data from the fruits to better understand how plants respond to drought and salinity stress.

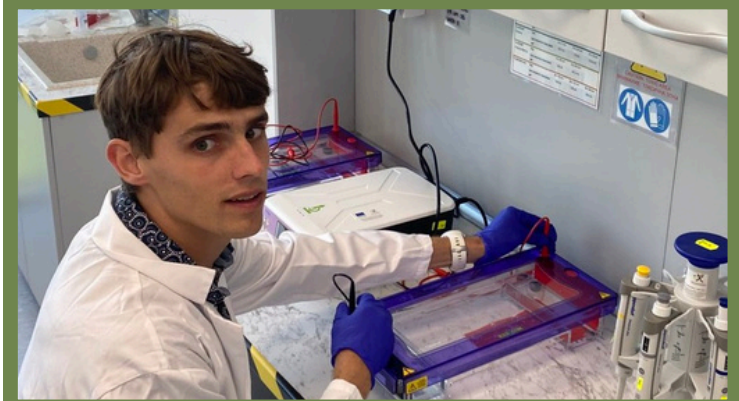
To test the function of the selected genes in tomatoes, the researchers will use the CRISPR-Cas9 technology to knock them down and see how that affects the plant under drought conditions.

With peppers, they will use a fast method (called virus-induced gene silencing, VIGS) to check if specific genes are involved in fruit quality and yield during water stress.

Regarding the salinity stress studies, the team will conduct more experiments to see how tomatoes and peppers handle high salt concentrations, following the same steps as the drought stress trials. These salinity experiments will help them identify plants that grow better when the soil has more salt. ■

PhD students from South Africa and Italy joined the NatGenCrop project

The PhD students **Gabriele Adornato (Italy)** and **Michael Wittenberg (South Africa)** joined the Crop Quantitative Genetics department at CPSBB, which was established within the NatGenCrop project. The department is headed by Dr. Saleh Alseekh, the ERA Chair Holder of the project.



Gabriele Adornato has graduated in Molecular and Medical Biotechnology at the University of Verona. Gabriele joined the NatGenCrop team and the Crop Quantitative Genetics department of CPSBB in October 2024.

Within the project he investigates tomato and pepper's genetics, molecular and physiological responses to biotic stress, caused by the pathogens *Botrytis cinerea* and *Clavibacter michiganensis*.

Michael Wittenberg has completed Bachelor's degrees in Genetics, Biochemistry and Applied Statistics at the University of Cape Town. He has a Master's degree in Molecular Biology with a research, focused on metabolomics and metagenomics in resurrection plants to study their drought and desiccation tolerance. Michael joined the project on January 2025 and he investigates salinity tolerance in tomato and pepper.



A documentary film about the NatGenCrop project was produced by CPSBB

CPSBB produced a documentary film presenting the NatGenCrop project and the scientific team of the Crop Quantitative Genetics department, involved in the implementation of the large-scale scientific research on pepper and tomato crops.

The film was directed by Mr. George Tornev and the cast included the ERA Chair Holder of the project, researchers, students, as well as the Management and Coordination Board's members.

The main objectives of the research, the scientific approaches, and the potential impact of the project on food security and sustainable agriculture are main highlights of the material.



[**WATCH HERE**](#)



The NatGenCrop Project Was Presented during the 21st International EUCARPIA Group Meeting

The CPSBB's researchers, Dr. Eng. Alicja Wieteska Georgieva, Dr. Avanish Rai, and Dr. Nikolay Anachkov presented different aspects from the scientific research within the NatGenCrop project during the 21st EUCARPIA Meeting of the Tomato Work Group.

The event took place on 10-13 June 2025 in Plovdiv, gathering over 200 researchers worldwide, devoted to the investigation of tomato crops - genetics, breeding techniques, abiotic and biotic stress resistance, sustainable production practices, etc.



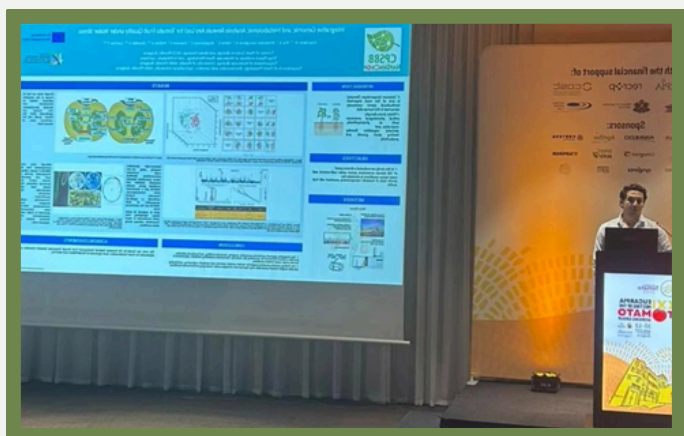
Dr. Eng. Alicja Wieteska Georgieva presented preliminary data on salinity stress from experiments with the GWAS tomato panel within the NatGenCrop project.

The presentation was entitled **"Comprehensive Morphological and Ionomic Characterization of a Tomato Association Panel Reveals Mechanisms of Salinity Stress Tolerance"**.



Dr. Avanish Rai presented preliminary data on drought stress from GWAS tomato panel within the NatGenCrop project.

The presentation was entitled: **"Deciphering Water Stress responses in Tomato through Genetic and Metabolic Variation for Improved Fruit Yield and Quality"**.



Dr. Nikolay Anachkov presented preliminary data on identification of locations on tomato chromosomes, related to fruit quality under water stress.

The presentation was entitled **"Integrative Genomic and Metabolic Analysis Reveals Key Loci for Tomato Fruit Quality under Water Stress"**

A photo exhibition in the center of Plovdiv showcased the NatGenCrop project

CPSBB organised a public photo exhibition, presenting some of the large-scale research projects conducted by the researchers at the Center, including NatGenCrop.

Main highlights of the project's billboard were the objectives of the research, the scientific approaches for their achievement, as well as the potential impact of the project on food security and sustainable agriculture.

The exhibition was available to hundreds of thousands of citizens and tourists for the period 28 February- 31 March 2025.



The NatGenCrop was presented on the National Private Bulgarian Television bTV



[WATCH HERE](#)

The Bulgarian Telegraph Agency presented the NatGenCrop research as a successful Bulgarian project funded by the EU



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