PhD THESIS

Conservation of the Genetic Potential and Biodiversity of Local Tomato Landraces from Areas Affected by Water and Salinity Stress

SUMMARY OF THE DOCTORAL THESIS

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INTRODUCTION

Climate change is seriously affecting tomato cultivation due to high average temperatures, droughts, floods, and the proliferation of diseases and pests. These conditions reduce yields and put pressure on pesticide use. Fungal and bacterial diseases, such as late blight and fusarium wilt, are intensifying due to increased humidity and milder winters, which reduces the effectiveness of crop rotation.

Dependence on pesticides raises environmental and food safety concerns. Proposed solutions include the development of resistant varieties through biotechnology and the application of sustainable farming practices. In Romania, local tomato populations from areas such as Salonta commune, Bihor county, have proven promising under difficult conditions, contributing to biodiversity and food security.

The results presented in this thesis highlight valuable genotypes, suitable both for household cultivation and breeding programs. Promoting these local populations supports biodiversity, food security, and the sustainability of agriculture in the context of current climate change.

STRUCTURE OF THE DOCTORAL THESIS

The doctoral thesis is structured in two main parts containing eight chapters, in which there are 4 tables, 42 figures and 159 bibliographic sources, totaling a number of 95 pages. In the first part of the thesis, the current state of knowledge on the subject studied is presented and is structured in two chapters, which total 23 pages (29,5%). The second part of the thesis includes the personal contribution made on the basis of the research obtained from the experiments which sums up a number of 55 pages (70,5%), structured in six chapters, to which the bibliography is added.

CURRENT STUDY OF KNOWLEDGE

PART I: CURRENT STATE OF KNOWLEDGE

The first part of the thesis is dedicated to the current state of knowledge, presented in two chapters that include up-to-date information from the specialized literature, aligned with the objectives set for the research conducted.

Chapter 1, titled **Tomatoes – Importance and Impact in the Context of Climate Change**, includes four subchapters and three sub-subchapters, presenting information on the origin of the species *Solanum lycopersicum*, the global significance of tomato cultivation, and the impact of climate change on tomatoes. It discusses the current climate context, the past, present, and projected future of tomato cultivation, as well as international concerns regarding abiotic stress factors, which are the focus of the studies included in the thesis.

Chapter 2, titled **The Use of Local Populations to Enhance the Resilience of Tomato Cultivation to Current Climate Change**, contains six subchapters referring to the genetic and adaptive diversity of local tomato populations, drought tolerance and fruit quality, biodiversity and pest resistance, water stress adaptation strategies of different tomato genotypes, plant adaptation strategies to saline stress, and data on the nutritional and culinary value of tomatoes.

PART II: PERSONAL CONTRIBUTION

The second part presents the personal contribution and research conducted, structured into four chapters that reflect the objectives formulated in the thesis.

Chapter 3, titled **Purpose and Research Objectives**, outlines the aim of the doctoral thesis and the proposed objectives for achieving it.

Chapter 4, titled **Phenotypic and Genotypic Characterization of the Tomato Populations Studied**, includes the experiments through which the phenotypic and genotypic characterization of the local tomato populations was carried out.

Chapter 5, **titled Results of Drought and Salinity Tolerance Screening of Local Tomato Populations Selected from Bihor County**, presents the morpho-physiological parameters of the local populations studied under water and saline stress conditions, as well as the analysis of gene expression changes in the populations subjected to these stress factors.

Chapter 6, titled **Study of Chlorophyll Fluorescence Kinetics in Local Tomato Populations as a Response to the Stress Factors Studied**, characterizes the local tomato populations using chlorophyll fluorescence kinetics, a methodology that enables early detection of photosynthetic dysfunctions.

Chapter 7 synthesizes the **Conclusions and Recommendations**, presenting the research findings in a well-argued manner, along with recommendations based on the results obtained.

Chapter 8, titled **Originality and Innovative Contributions of the Thesis,** includes the novel elements and personal input brought to the research field addressed.

RESEARCH PURPOSE AND OBJECTIVES

The purpose of this doctoral thesis is to analyze local tomato populations from saline areas located in the northwestern region of Romania, with the aim of identifying germplasm sources resistant to salinity and drought, which can later be used in tomato breeding programs.

To achieve this goal, the following objectives have been established:

1. Phenotypic and genotypic characterization of local tomato populations from saline areas in Northwestern Romania

A saline soil area was identified, specifically the Salonta commune in Bihor County,

where local tomato populations have been cultivated in traditional households for over 10 years, showing potential resistance to high salinity. For genotypic characterization, genes involved in molecular protection mechanisms against saline and drought stress will be selected.

2. Screening for drought and salinity tolerance of local tomato populations selected from Bihor County

The identified and selected local populations, along with a control variety, will be used in experiments for phenotypic and genotypic characterization under water and saline stress conditions.

3. Study of chlorophyll fluorescence kinetics in local tomato populations as a response to the stress factors analyzed

By analyzing specific fluorescence parameters, the local tomato populations will be characterized, enabling early detection of photosynthetic dysfunctions and contributing to a deeper understanding of the adaptation mechanisms of local genotypes to abiotic stress conditions.

CURRENT STATE OF KNOWLEDGE

Climate crisis is a present reality, not a future threat, and demands profound changes in agriculture, industry, and lifestyle. Ethical and practical questions arise regarding who should act first—individuals or governments—and whether technology can replace behavioral change.

The agriculture of the future must adapt to climate stress (drought, salinity, extreme temperatures) through research and the development of resilient crops. International initiatives support the genetic and phenotypic characterization of crops to protect biodiversity and ensure food security.

Tomatoes, the second most cultivated vegetable globally, are essential for both nutrition and the economy. Local populations exhibit valuable genetic diversity, with natural tolerance to water and heat stress, and are being studied for breeding and climate adaptation.

In Romania, soil salinization increasingly affects agricultural land, including in Bihor County, where over 9% of the mapped soils show varying degrees of salinity.

1st STUDY – PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF THE TOMATO POPULATIONS UNDER STUDY

The aim of this study is to perform phenotypic and genotypic characterization of local tomato populations originating from the saline area of Bihor County, as well as a control variety, under standard cultivation conditions.

Materials and Methods. To assess the morphological traits of the tomato plants under study, observations and measurements were conducted regarding plant habit, leaf type, fruit shape, color, and weight.

Table 1 presents the morphological characteristics of the three local tomato populations studied, alongside the control variety 'Marmande'. As observed, the main distinguishing feature is fruit weight, which is significantly higher in the local populations compared to the 'Marmande' variety.

Table 1

Phenotypic characteristics of the studied tomato populations and the control variety under standard growth conditions

Local landrace/variety	Plant height (cm)	Leaf shape	Friut shape	Friut colour	Fruit weight (g)
'Ateaș 136'	150-180	Pinnate, entire margine	heart shape	red	600-700
'Ateaș 37'	150-180	Pinnate, serrated margine	heart shape	pink	600-700
'Cefa 7'	150-180	Pinnate, entire margine	flattened round	red	700-800
Marmande Mt.	150-180	Pinnate, entire margine	rounded	red	150-250

For genetic characterization, plant tissue samples were collected and total RNA was extracted. Complementary DNA (cDNA) synthesis was then performed and used as a template for polymerase chain reaction (PCR), employing specific primers for each target gene. The relative abundance of transcripts was mathematically calculated from the amplification curves of each gene.

Genotypic characterization of the studied populations and the control variety was essential to determine the expression levels of genes involved in molecular protection mechanisms against saline and drought stress. Selected genes included those related to oxidative stress (SOD), the DREB group (Dehydration Responsive Element Binding), which are highly expressed under osmotic stress, as well as HSP-type genes (HSP70) and photosynthesis-related genes (PSII, PSIID2).

Comparative analysis of gene expression levels was performed for each gene across the studied populations.

Results and Conclusions

Phenotypic characterization revealed that the three local tomato populations are very similar in terms of fruit weight and plant height. Leaf morphology is comparable between the local population 'Ateaș 37' and the control variety 'Marmande', both having lanceolate leaflets with serrated margins and smooth surfaces. The local populations 'Ateaș 136' and 'Cefa 7' have similar leaves with entire margins. Despite

differences in fruit shape, all local populations produced fruits weighing between 600–700 g under standard cultivation conditions.

Genetic characterization under standard growth conditions showed varying levels of gene expression among the studied populations and compared to the control variety 'Marmande'. These differences may indicate distinct responses of local populations to various stress factors.

This study serves as a reference point for the next phase, in which the plants will be subjected to drought, salinity, and UV-B stress conditions.

2nd STUDY – RESULTS OF DROUGHT AND SALINITY TOLERANCE SCREENING OF LOCAL TOMATO POPULATIONS SELECTED FROM BIHOR COUNTY

The aim of the study is to describe the morpho-physiological parameters of local tomato populations under drought and salinity stress conditions. Additionally, the expression of target genes was analyzed in plants subjected to these stress factors.

Experimental Design and Treatments for Phenotyping

Three local tomato populations were used in this study, named after the locations in Bihor County from which the seeds were collected: 'Ateaş 136', 'Ateaş 37', and 'Cefa 7'. The control variety used was 'Marmande'. The experimental variants are summarized in Table 2.

Experimental Design and Treatments for Genotyping

To obtain data on the transcriptional responses of the studied tomato plants to salinity and UV-B stress, four treatment types were applied to groups of eight plants: control plants (Mt.), plants exposed to salinity stress at two salt concentrations—300 mM (V1) and 450 mM (V3)—and a combined treatment of 300 mM salinity and UV-B radiation (V2). The UV-B radiation applied ranged from 0.45 to 0.55 mW/cm^2, representing an increase of approximately 30% compared to natural outdoor levels measured on a sunny day at noon.

Table 2

Experimental variants used in the phenotyping experiment

Experimental variants	Soil moisture		Salinity	
	60%	20%	0,2%	0,3%
i (Mt.)	**x**	-	-	-
ii	**x**	-	**x**	-
iii	-	**x**	**x**	-
iv	**x**	-	-	**x**
v	-	**x**	-	**x**

Plants were irrigated with saline solution to maintain 60% soil moisture, and UV-B radiation was applied for three consecutive days, two hours per group, totaling three treatment rounds.

Results and Conclusions

Phenotyping experiments revealed the following:

Leaf area in local populations decreased slightly under 0.2% NaCl and 20% drought stress, but significantly under 0.3% NaCl and 20% drought stress compared to the control. 'Cefa 7' and 'Ateaş 37' showed approximately 30% larger leaf area than the control variety 'Marmande', indicating enhanced adaptability to adverse environmental conditions and potential for breeding programs.

Water uptake was significantly limited under 0.3% NaCl and 20% drought stress. Among the local populations, 'Ateaş 136' was the least affected.

When correlating leaf area, water consumption, and root density under combined drought (20%) and salinity (0.3%) stress, 'Cefa 7' had the largest leaf area, 'Ateaş 136' showed the highest water consumption, and 'Ateaş 37' had the highest root density.

These results suggest that the studied local populations are capable of adapting to saline and drought-affected soils and could be used in breeding programs aimed at improving tomato crop resilience. Moreover, their use may contribute to sustainable agricultural practices and food security in regions exposed to abiotic stress factors.

Gene Expression Analysis

Comparative analysis of target gene expression (SOD, DREB, HSP70, PSIID2) showed increased expression levels in response to stress:

SOD gene expression was generally repressed across treatments. The highest expression was observed in control plants of 'Ateaş 136'. This population, when treated with 300 mM saline solution, showed positive expression levels similar to those of 'Ateaş 37' treated with 450 mM saline solution.

DREB gene expression was repressed in control plants of 'Ateaş 37' and 'Ateaş 136', as well as in all local populations treated with 300 mM saline solution, 300 mM saline + UV-B, and 450 mM saline. Low but positive values were observed in control plants of 'Marmande' and 'Cefa 7'. After treatment with 300 mM saline solution, 'Marmande' showed positive gene expression. The combined treatment (300 mM saline + UV-B) induced DREB expression in both 'Marmande' and 'Cefa 7', with values similar to those obtained under 450 mM saline treatment.

The control variety 'Marmande' generally showed higher induction of stress-response genes compared to local populations. This is likely correlated with the greater tolerance of local populations to salinity, UV-B stress, and their combination.

Local tomato populations perceived the applied stress as less intense and activated molecular protection mechanisms at lower levels than the control variety 'Marmande'.

3rd STUDY – ANALYSIS OF CHLOROPHYLL FLUORESCENCE KINETICS IN LOCAL TOMATO POPULATIONS AS A RESPONSE TO STUDIED STRESS FACTORS

The aim of the study is to characterize local tomato populations through chlorophyll fluorescence kinetics, offering insight into the functional health of tomato plants and enabling the assessment of the physiological state of the photosynthetic apparatus under abiotic stress conditions. By analyzing specific fluorescence parameters, this methodology allows early detection of photosynthetic dysfunctions and contributes to a deeper understanding of the adaptation mechanisms of local genotypes.

Materials and methods

Three local populations—'Ateaș 37', 'Cefa 7', and 'Ateaș 136'—were sourced from seed stocks belonging to farmers in Bihor County. The commercial variety 'Marmande' was used for comparison. Tomato plants were grown in a greenhouse under controlled conditions (25°C day / 18°C night, 16/8 hour photoperiod, and 60% relative humidity) in pots containing a 50% sandy soil (Maros) and 50% Terra peat mix. Fertilizer was added in equal amounts to each pot (SUBSTRAL®, Osmocote Plus®, Wals-Siezenheim, Austria). Plants were maintained under optimal watering and fertilization until the first true leaves developed, at which point stress treatments were applied.

Experimental Design

The experiment followed a design with eight replicates per treatment/genotype. Plants were exposed to the following climate-relevant stress treatments:

V1 Control (normal irrigation): plants were watered daily to maintain 60% field capacity.

V2 Normal irrigation + NaCl: plants were watered to maintain 60% field capacity using a 0.2% NaCl solution.

V3 Drought stress: irrigation was gradually reduced over 7 days to reach 20% field capacity, simulating severe drought conditions.

V4 Combined drought and salinity stress: irrigation was reduced to 20% field capacity and a 0.2% NaCl solution was applied.

Chlorophyll Fluorescence Measurements

Chlorophyll a fluorescence transients were measured using a portable modulated fluorimeter (Pocket PEA, Hansatech). Leaves were dark-adapted for 15 minutes using special clips before measurement. A saturated pulse of actinic light (3000–3500 μmol photons m^{-2} s^{-1}) was applied for 1 second, and the rapid rise in fluorescence (OJIP curve) was recorded with a time resolution between 10 μs and 1 s. Measurements were taken on the first fully developed leaf from the top of the plant, early in the morning to minimize circadian variation.

The following fluorescence parameters were extracted:

- Maximum quantum efficiency of PSII photochemistry (Fv/Fm)

- Total performance index (PI total)
- Oxygen-evolving complex integrity (OEC)
- Density of active reaction centers per absorbed energy unit (RC/ABS)
- Fluorescence rise curve (Sm)
- Energy dissipation per reaction center (DI_o/RC)

Results and conclusions

Local tomato populations showed significant changes in chlorophyll fluorescence kinetics in response to environmental signals associated with climate change.

Normalized values of Fv/Fm, representing the maximum quantum efficiency of PSII photochemistry, were higher in all three local genotypes compared to the commercial control 'Marmande' across all treatments. Notably, 'Ateaş 37' exhibited the highest Fv/Fm values under both individual and combined stress conditions.

Among the local populations, 'Ateaş 37' also showed the highest normalized values of the total photosynthetic performance index (PI total) under all stress treatments, indicating superior tolerance likely due to more efficient photoprotective mechanisms and intrinsic genetic traits supporting resilience in adverse conditions.

Combined salinity and drought stress caused the most pronounced reduction in OEC integrity in the control variety 'Marmande', while all local tomato populations demonstrated greater resistance, with 'Ateaş 136' standing out as the most resilient genotype under this treatment.

Regarding energy dissipation per reaction center (DI_o/RC), 'Marmande' showed a marked increase under V2, V3, and especially V4, indicating significant photoinhibition or stress under simultaneous salinity and drought, resulting in greater energy loss as heat. Local populations 'Ateaş 136' and 'Ateaş 37' demonstrated stress tolerance, particularly by limiting energy loss.

RECOMMENDATIONS

1. The study data indicate that the local populations are suitable for inclusion in breeding programs due to their native genetic resistance to drought and salinity stress.
2. These local populations can be cultivated on water-unstable soils, and their integration into breeding programs could lead to the development of new, more resilient tomato varieties, improving food independence in regions affected by such climatic conditions.
3. The studied local populations are promising candidates for organic farming due to their robustness.
4. To ensure technology transfer to farmers, cultivation guides can be developed and demonstration sessions organized for farmers in vulnerable areas.

5. The chlorophyll fluorescence study revealed valuable traits in local populations based on photosynthetic parameters, indicating that chlorophyll fluorescence is a high-throughput and accurate screening method for identifying stress-tolerant local populations.
6. Further results on local population resistance require monitoring of PSII efficiency under combined stress conditions (salinity + UVB + drought) and its post-stress recovery capacity.
7. Integration of fluorescence imaging in early selection using OJIP and NPQ parameters as physiological indicators for rapid screening of stress-tolerant genotypes.
8. Creation of a comparative database with fluorescence profiles for both local and commercial varieties.
9. Elucidation of genetic and molecular mechanisms through transcriptomic analyses to identify genes involved in NPQ regulation and photosynthetic stress response.
10. Correlation of gene expression with changes in fluorescence kinetics to understand the genotype–phenotype relationship.
11. Involvement of specialists in bioinformatics, plant physiology, and agrochemistry for an integrated approach.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

1. The experimental design of the thesis is original, combining both classical phenotypic studies and those assisted by a semi-automated phenotyping platform. The phenotyping studies are complemented by gene expression analysis and chlorophyll fluorescence kinetics, aiming to achieve a highly accurate characterization of the local tomato populations under study, while also offering an innovative approach that integrates modern and traditional methods.
2. The local tomato populations analyzed in this study add a distinct element of originality, as no similar research has been conducted in Romania on such local tomato genotypes.
3. In the current global context, where increasing vegetable production is essential to support a growing population and climate change poses major challenges to agriculture, this study offers a series of potential solutions. It characterizes local tomato populations that demonstrate resilience to environmental factors similar to those caused by climate change and show resistance to growth on affected soils.
4. Therefore, the introduction of these local populations into breeding programs could lead to the development of tomato varieties resistant to various adverse environmental conditions, as well as their cultivation on degraded soils. This could help ensure food independence for communities living in areas affected by such soil conditions.