SUMMARY OF PhD THESIS

Studies on the physiological reactions to water and heat stress conditions of potato – *Solanum tubersoum* L.

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INTRODUCTION

Contemporary agriculture faces multiple challenges related to the intensification of extreme weather events, which negatively affect crop yields and quality (Popescu et al., 2023). The potato (Solanum tuberosum L.), one of the most important food crops in Romania and globally, is particularly sensitive to water and heat stress, factors that are becoming increasingly frequent and severe in the context of climate change (Bărăscu et al., 2014).

Under these conditions, this thesis highlights the physiological and biochemical mechanisms through which potatoes react and adapt to restrictive soil and climate conditions. These reactions are investigated through a comparative approach between four potato varieties grown in two locations with distinct soil and climate conditions. By monitoring morphological, physiological, and biochemical parameters, both in the field and under controlled conditions, the paper contributes to the identification of major physiological reactions following thermal and water stress and genotypes with increased stress tolerance, providing a scientific basis for practical recommendations in breeding, cultivation technology, and the adaptation of agricultural systems to new climatic realities.

STRUCTURE OF THE DOCTORAL THESIS

The doctoral thesis comprises a total of 149 pages and is structured in two parts:

Current state of knowledge – four chapters, 31 pages (30.4%);

Personal contribution – six chapters, 71 pages (69.6%).

The research reviewed and analyzed 198 bibliographic references in the field in order to report the results obtained in the most realistic manner possible and to highlight the originality and innovative potential of the doctoral thesis. The paper contains 29 tables, of which 11 are original, and 77 figures, of which 42 are original and 29 are graphs. Of the total number of figures and tables, 31 are statistically verified (21 figures and 10 tables).

RESEARCH PURPOSE AND OBJECTIVES

The overall aim of this study is to analyse the physiological, morphological and biochemical responses of potato varieties grown in Romania under conditions of water and heat stress, both in the field and in controlled environments, in order to highlight their ability to adapt with the lowest possible response to abiotic stress.

To achieve this goal, the research was structured around the following objectives:

O1 - Detailed characterization of the pedoclimatic conditions in the two experimental locations (Mândra and Rusciori) in the agricultural years 2023 and 2024, to outline the context of abiotic stress;
O2 - Evaluation of the biometric traits of the bush and tubers of the four potato varieties studied (`*Alouette*`, `*Paradiso*`, `*Productiv*`, and `*Salad Blue*`);
O3 - Determination of anthocyanin and glycoalkaloid accumulation in tubers as biochemical indicators of stress and as potential markers of adaptation or vulnerability;
O4 – Evaluation of biomass, water content, and roots under controlled water stress conditions;
O5 – Correlation of biometric, biochemical, and physiological results with climate data to identify relevant relationships between abiotic stress and the behavior of the tested genotypes;

PERSONAL CONTRIBUTIONS

The first stage of the research consisted of a detailed characterization of the pedoclimatic conditions in the two localities, Mândra (Brașov County) and Rusciori (Sibiu County), where the experimental fields were located. The aim of this stage was to outline a realistic soil and climate framework in order to understand how water and heat stress influence the physiological reactions of potato plants.

Soil analyses were carried out and climate data were collected at the two locations over two agricultural seasons, 2023 and 2024. To this end, soil samples were taken from each experimental plot, followed by agrochemical and pedological determinations. These samples were analyzed in accordance with national standards and the methodology in the specialized literature, in the specialized laboratories of the Faculty of Agricultural Sciences, Food Industry, and Environmental Protection in Sibiu and the Office of Pedological and Agrochemical Studies in Sibiu.

At the same time, climate data were recorded daily using an automatic weather station (Vantage Pro2 - Davis Instruments) in Rusciori and a manual rain gauge and the metoeblue weather forecast website in Mândra. The parameters monitored included maximum and minimum temperatures, total precipitation, and its distribution across vegetation phenophases. The data were centralized and processed to highlight the frequency of exceeding critical heat stress thresholds (above 25 °C) and precipitation levels below the optimal threshold of 500 mm, corresponding to potato cultivation.

This stage formed the basis for the entire subsequent experimental process and allowed the identification of relationships between edaphic and climatic factors and the manifestation of abiotic stress on the tested varieties.

In the second stage of the research, attention was focused on conducting systematic field observations in order to capture and characterize the behavior of the four potato varieties studied. The activities were carried out in the two locations

mentioned above, providing a representative framework for analyzing plant responses to abiotic stress.

Within each experimental field, a randomized block design was implemented, adapted to local conditions, following uniform agrotechnical practices. Planting was done manually, respecting the cultivation distances recommended by the suppliers of the planting material, and during the growing season, phytosanitary treatments were applied against the main diseases and pests, without the use of chemical fertilizers or irrigation.

Throughout the growing seasons, regular biometric observations were made on the bush and tubers, including detailed measurements such as plant height, number of stems, foliage development, and tuber characteristics such as length, circumference, and weight. After each harvest, samples of plant material were collected for subsequent biochemical analysis.

In parallel with the field observations, part of the research was extended to controlled environmental conditions, carried out in the protected space of the specialized laboratory at the Rusciori Educational Farm. The *'Productiv'* and *'Salad Blue'* varieties were subjected to different soil moisture treatments to simulate water stress conditions. This experiment was made possible by the development and installation of an automation system based on sensors and modules controlled by microcontrollers. The system was configured to monitor and regulate soil moisture levels through automatic irrigation control, thus providing a precise and replicable experimental framework for testing the physiological responses of plants. In addition, light, temperature, and relative air humidity were also monitored and controlled.

In the third stage of the research, the focus shifted to the processing and detailed analysis of plant samples and tubers collected during the experiments. This stage involved a complex, multidisciplinary approach aimed at thoroughly investigating the physiological and biochemical responses of the studied varieties depending on the environmental conditions and treatments applied.

Thus, after completing the experiments in a controlled environment, quantitative determinations were made regarding the total biomass and water content of the leaves and stems, using standardised procedures mentioned in the literature, adapted to the specific conditions of the experiment.

For the experiments conducted in situ, the harvested tubers were subjected to advanced biochemical analyses in the laboratories of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, aimed at identifying and quantifying the level of bioactive compounds in tubers with nutritional and toxicological importance, such as anthocyanins and glycoalkaloids, using high-precision chromatographic methods (HPLC with UV-Vis and MS detection), as well as comparing the profiles obtained with certified standards and data from the literature.

The final stage of the research consisted of detailed morphological investigations of the roots of plants grown under controlled environmental conditions,

using scanning electron microscopy (SEM) in collaboration with colleagues from the Faculty of Engineering in Sibiu. This approach provided a microstructural perspective on the effects of water stress, allowing the observation of subtle changes in root tissues.

RESULTS AND DISCUSSION

The research highlighted significant differences between the varieties studied and between the experimental locations, Mandra and Rusciori, under the influence of pedoclimatic conditions. These differences were evident both at the **bush** level and at the **tuber** level, with **biometric parameters** being directly correlated with the adaptability of each genotype.

Of the four varieties, *'Alouette'* showed the best vegetative development, with an average stem height of 51.74 cm and an SPAD index of 38.05, reflecting a favorable physiological state. This robust development was accompanied by a good total tuber yield with an average quantity of approximately 475 grams per nest, which is statistically the second highest average per nest among the four varieties. These aspects indicate a very good response to water and heat stress conditions during the experiments, with good efficiency in converting above-ground biomass into underground reserves. Finally, this variety had the most balanced development between leaf mass and tubers compared to the other varieties.

On the other hand, the *'Salad Blue'* variety had the lowest values for stem height with an average of 31.54 cm and a SPAD index of 31.06, and this vegetative limitation was also reflected in the lowest tuber yield, with an average total nest weight of approximately 170 grams, considerably lower than the other varieties. In addition, *'Salad Blue'* had the lowest average number of edible tubers (less than 2 edible tubers/nest), making it more vulnerable to stress and more dependent on controlled or optimal environments.

The *'Paradiso'* variety ranked third out of four in terms of plant height, with an average of 40.4 cm, but excelled in terms of tuber production, achieving the highest mass/nest and the largest tubers with an average of almost 150 g/tuber. These aspects suggest an effective adaptation to the growing environment, favoring the accumulation of reserve substances in the tuber, to the detriment of leaf development.

`*Productiv*` stood out with the highest production of small tubers, with an average of about 13 tubers/nest, compared to `*Paradiso*` with 5 tubers/nest or `*Alouette*` with 9 tubers/nest.

Correlating these results with the pedoclimatic and agrochemical conditions in the experimental locations of Mândra and Rusciori, distinct **physiological strategies** for adaptation to thermal and water stress were highlighted. The 2023–2024 agricultural years were marked by temperatures above the optimal threshold for

potatoes and precipitation below the recommended values, affecting the process of plant development and tuberization.

`*Alouette*` stood out with well-developed foliage and a high SPAD index, suggesting increased nitrogen use efficiency and high photosynthetic potential. The high yields in Mândra confirm this efficiency under unfavorable climatic conditions, while the poorer results in Rusciori are probably due to theories such as reduced Rubisco enzyme activity and ABA accumulation where the same influences of water and heat stress exist, but accentuated by low soil fertility (Handayani & Watanabe, 2020).

`*Paradiso*` demonstrated a strategy of preferential resource allocation to tubers, a reaction that can be associated with the StSUT2 transporter theory, favoring the transport of nutrients from the aerial part of the plant to the underground part (Gong et al., 2023) and an efficient correlation between nutrients (P and K) and energy metabolism. Even under stress conditions, it maintained a high individual tuber mass, suggesting stable hormonal regulation.

`*Productiv*` exhibited a conservation strategy based on the formation of a large number of tubers, theoretically supported by a balance between auxins and cytokinins, with a uniform distribution of carbohydrates in the tubers and high SPAD index levels indicating effective adaptation in both locations, with some stress tolerance and productive potential in a more favorable environment.

`Salad Blue`, on the other hand, showed poor performance, with limited vegetative development and low yield, theoretically due to deficient hormonal regulation, characterized by excessive ABA accumulation and poor ability to produce osmoprotectants (Guillemette et al., 2024).

Overall, the results suggest that the adaptability of potato varieties is determined not only by their genetic background but also by the plants' ability to activate effective physiological mechanisms in response to pedoclimatic stress. Each variety followed its own adaptive strategy: *'Alouette'* capitalized on aerial biomass, *'Paradiso'* prioritized tuber quality, *'Productiv'* maximized tuber number, and *'Salad Blue'* invested in protective mechanisms, with an obvious metabolic cost on production.

The analysis of **anthocyanin compounds** in the tubers of the *'Salad Blue'* variety revealed an inversely proportional relationship between the intensity of abiotic stress (thermal and water) and the total accumulation of anthocyanins. In the four experiments conducted in the 2023–2024 agricultural years, the highest amount of anthocyanins was recorded in Rusciori in 2023 (over 800 mg/100g of substance), where climatic conditions had a lower intensity of water and thermal stress compared to the other experiments. In contrast, the lowest accumulation was observed in Mândra in 2024 (below 400 mg/100 g), in the context of the greatest water deficit.

There are statistically significant differences in the amounts of **glycoalkaloids** accumulated depending on the year, location, and variety. The highest average concentration was recorded in the experiment conducted in Rusciori, 2024 (18.25 mg/100g), followed by Rusciori, 2023 (16.11 mg/100g), and the lowest values were in

Mândra, 2023 (11.48 mg/100g) and Mândra, 2024 (12.94 mg/100g), but unlike anthocyanin accumulation, glycoalkaloid accumulation was influenced more by the fertility of the experimental fields and less by climatic stress (Liebig's law).

The *Paradiso* variety had the highest average glycoalkaloid accumulation (20.13 mg/100g), exceeding the food safety threshold of 20 mg/100g, followed by *'Productiv'* (15.96 mg/100g), *'Alouette'* (12.13 mg/100g), and *'Salad Blue'* had the lowest value (10.87 mg/100g).

Separate analysis of the accumulation areas indicated a significantly higher concentration in the skin than in the pulp, which is also supported by the literature. Comparison between the main glycoalkaloid compounds showed a prevalence of α-chaconine over α-solanine in all varieties, with an average difference of approximately 20 mg/100g. The highest α-chaconine values were also recorded in the *'Paradiso'* and *'Productiv'* varieties, while α-solanine was below the 10 mg/100g threshold for all varieties, with *'Paradiso'* still leading the way.

The analysis of the influence of irrigation on the **biomass** and **water content** of the aerial part of potato plants showed a significant impact of the water regime on physiological development. The statistical results obtained by ANOVA analysis show that both the irrigation variable and the irrigation * variety interaction had statistically significant effects ($p = 0.003$ and $p = 0.001$), while the "variety" variable alone did not generate significant differences ($p = 0.213$).

This situation suggests that the response of varieties is not uniform to different irrigation treatments and that physiological performance is influenced more by interaction with environmental factors than by the genotype itself. The *'Productiv'* variety showed small differences between the irrigated and non-irrigated variants, indicating better tolerance to water stress, while *'Salad Blue'* showed large fluctuations in biomass and water content between treatments, signaling increased sensitivity to water deficiencies.

Microscopic analyses of the roots of the `*Productiv*` and `*Salad Blue*` varieties revealed significant anatomical changes induced by water stress. Under optimal irrigation conditions, both varieties showed clear and well-differentiated tissue organization, with thin cell walls, porous structures in the bark area, and precise boundaries between the exoderm, endoderm, and conducting vessels, signs of normal and efficient metabolic functioning.

In contrast, under water stress conditions, thickening of cell walls, reduced bark porosity and elasticity, a decrease in the number of conducting vessels, and a loss of structural clarity between tissue layers were observed. These changes probably reflect the activation of adaptive mechanisms, such as increased synthesis of lignin and suberin, which strengthen tissues and reduce water loss through transpiration.

The epidermal layer showed notable changes between treatments. In the *'Productiv'* variety, the epidermal cells remained well organized under optimal conditions, but became collapsed and cracked under stress conditions. In *'Salad Blue'*,

the degradation was more severe, the epidermal cells completely lost their turgidity, and the presence of cracks and irregular formations indicates damage to structural integrity and an increased risk of pathogenic infections (Barber & Silberbush, 2015).

CONCLUSIONS

The study conducted in the agricultural years 2023 and 2024 demonstrated a significant impact of thermal and water stress on potato plant development in all four field experiments. Frequent temperatures above the critical threshold of 25°C and below-optimal rainfall created unfavorable conditions, especially in Mândra in 2024. These climatic conditions coincided with sensitive phenological stages such as flowering and tuber formation, amplifying the negative effects on yield.

Soil analysis showed higher fertility in Mândra, with a more aerated texture and reduced compaction. Rusciori offered a lower nutrient regime, with a higher potential for soil compaction. These edaphic differences influenced the plants' ability to cope with abiotic stress.

Morphological evaluation of the four varieties revealed distinct genetic traits. The `***Alouette***` variety stood out for its vigorous vegetative development and intense photosynthetic activity. ***'Paradiso'*** had the largest tuber masses, while ***'Productiv'*** produced a large number of tubers, but of small size. ***'Salad* Blue'**, on the other hand, showed the poorest morphological performance, especially under water and heat stress conditions.

From a biochemical perspective, the `*Salad Blue*` variety accumulated the highest amounts of **anthocyanins** under less stressful conditions, demonstrating a non-specific antioxidant response to stress.

The `*Paradiso*` and `*Productiv*` varieties recorded the highest **glycoalkaloid** values in the less fertile soils of Rusciori, suggesting an activation of mechanisms reactive to abiotic stress. However, the glycoalkaloid content in the *'Paradiso'* variety exceeded the food safety threshold (20 mg/100g), which requires additional attention.

Experiments conducted in a **controlled environment** confirmed that the irrigation regime significantly influences **biomass** and **water content**. The `*Productiv*` variety showed better adaptability, maintaining stable values, while `*Salad Blue*` was severely affected by water stress. These results were supported by morphological **analysis of the roots under a microscope (SEM).** Under stress conditions, the following aspects were observed: cell collapse, wall thickening, loss of turgidity, and the appearance of microcracks, indicating structural damage and an increased risk of infection.

In conclusion, the study demonstrates that **physiological responses to water and heat stress are the result of a complex interaction between climatic, edaphic, and genetic factors.**

RECOMMENDATIONS

The research results support a series of practical recommendations for adapting potato cultivation technologies in the context of climate change and intensified abiotic stress:

1. Continuing experiments and including new relevant indicators in potato physiology, considering the theoretical hypotheses formulated in this thesis regarding the synthesis of ABA, auxins, cytokinins, StSUT2 transporters, or HSP proteins under abiotic stress conditions.
2. The adoption of irrigation technologies adapted to the critical phenophases of potatoes (flowering and tuber formation) is essential for stabilizing production under water stress conditions and using irrigation water efficiently in terms of economics and freshwater resources. Especially in areas prone to rainfall deficits, such as Mândra and Rusciori, the implementation of controlled deficit irrigation using drip irrigation could ensure a more stable moisture regime.
3. The selection of cultivated varieties should take into account their adaptive capacity to thermohydric stress, not just their productive potential under optimal conditions. The *'Alouette'* and *'Paradiso'* varieties have demonstrated better phenotypic plasticity and superior agronomic performance under variable soil and climate conditions and may be viable options for expansion in areas affected by climate change, with the caveat that *'Paradiso'* has a high toxicity potential.
4. Monitoring of bioactive compounds, especially glycolcaloids, is necessary in the context of intensified abiotic stress to avoid exceeding food safety limits (20 mg/100 g fresh matter). Potentially toxic varieties, such as *'Paradiso'*, require increased attention in dry years and on soils with low fertility.
5. The integration of controlled environment cultivation into research and breeding provides an effective tool for rapid testing of responses to water stress. These conditions allow for the isolation of variables and accurate testing of genotypes. It is recommended that this type of experimentation be extended to other abiotic stresses (salinity, photoperiod, extreme temperatures).
6. The use of the SPAD index and other physiological parameters (water content, root morphology) as rapid screening tools can contribute to the early selection of genotypes tolerant to drought and high temperatures in breeding programs and precision agriculture.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The doctoral thesis stands out for its integrative, complex, and interdisciplinary approach to how abiotic stress, especially thermal and water stress, influences the morphological, physiological, and biochemical development of four potato varieties grown in different soil and climate conditions.

The originality of the work lies in the simultaneous investigation, both in the field and in a controlled environment, of the combined effects of high temperatures and water deficit, an approach that is rarely documented in the literature.

An essential contribution of the paper is the integration of biometric (bush and tuber parameters), physiological (SPAD index, water content, above-ground biomass, root cell structure) and biochemical (anthocyanins and glycolaloids) analyses in direct correlation with local pedoclimatic factors. This unified analytical framework has made it possible to highlight differences in response between varieties and to identify genotypes with high adaptive value under stress conditions.

The thesis also draws attention to the risks associated with the high glycolaloid content of some varieties (e.g., *Paradiso*), highlighting the implications for food safety and processing.

An important methodological innovation is the use of scanning electron microscopy (SEM) for the detailed characterization of structural changes in the root under the influence of water stress. This technique is rarely applied in specialized studies on potatoes. Supplementing field experiments with tests under controlled irrigation conditions provides a valuable application framework for the selection of genotypes in breeding programs.

Through the results obtained, the thesis validates and complements existing data in the international literature and contributes new perspectives on the physiological and biochemical behavior of potato varieties in agroecosystems specific to Romania. The paper is in line with contemporary research directions in sustainable agriculture, having significant scientific value both from a fundamental and an applied point of view, in the context of the need to develop resilient and climate-adapted agricultural production systems.

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