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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

STUDY OF RESPONSES OF TOMATO VARIETIES (*Solanum lycopersicum* L.) TO DROUGHT STRESS, REHYDRATION, AND THE EFFECT OF POTENTIAL BIOSTIMULATORS

Specialty: 2406.02 – Biochemistry

Field of Science: Biology

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The dissertation work was carried out at the Bioadaptation Laboratory of the Institute of Molecular Biology and Biotechnologies of the Ministry of Science and Education of the Republic of Azerbaijan and at the Institute for Sustainable Plant Protection of the National Research Council of Italy.

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GENERAL CHARACTERISTICS OF THE RESEARCH

Relevance of the Topic. In the modern era, the increasing global climate changes have led to the emergence and intensification of numerous interconnected problems affecting society, the economy, and ecosystems. Global climate changes cause a decrease in precipitation and the increase in temperature which have led to the intensification of drought in most regions of world, as well as our country. Drought is considered one of the main threats that could cause a sharp decline in agricultural production worldwide and lead to food scarcity for the global population. Currently, the growing crop loss due to intensification of drought is a significant concern for ensuring food security.

Tomato is one of the most widely cultivated and economically significant vegetable crops in agriculture, due to its global food importance, high nutritional value and the presence of various organic acids, vitamins, and antioxidant compounds[1]. According to the Food and Agriculture Organization (FAO) of the United Nations, its annual global production amounts to 189.1 million tons[2]. This plant also holds great significance in the field of horticulture in our country due to its agricultural production. In this regard, it is significant to increase the tomato plant's tolerance to environmental stress factors such as phytopathogen virus, bacterial and fungal infection, drought, salinity, heavy metals and to minimize crop loss for both for ensuring food security for the population and preventing economic loss. The tomato plant is affected by the negative impacts of drought stress as an environmental stress factor at all stages of their ontogenesis. Drought stress leads to negative impact on the growth and development of tomato plants and causes significant reduction of its productivity, as well as, the decrease in the quality of tomato fruits[3]. The changes at the

[1] Liu W. *Solanum lycopersicum*, a model plant for the studies in developmental biology, stress biology and food science. / W. Liu, K. Liu, D. Chen, Z. Zhang, B. Li, M.M. El-Mogy [et al.] // Foods - 2022. 11 (16), 2402.

[2] FAO. 2022. Agricultural production statistics. 2000–2021. FAOSTAT Analytical Brief Series No. 60. Rome. https://doi.org/10.4060/cc3751en

[3] Kuo C.E. Early detection of drought stress in tomato from spectroscopic data: A

molecular, biochemical and physiological levels in plant organism under water deficite conditions result in wilting of plant leaves, reduction in the diameter of stem, and significant decrease in photosynthetic productivity[4]4. Therefore, investigation of the biochemical changes resulting from drought stress and the recovery processes after stress in tomato plants is of great importance.

In the last few decades, the use of various chemical agents such as various chemical fertilizers, fungicides, as well as synthetic growth stimulants and protective agents against to the effects of various stress factors has increased in traditional agricultural practice in order to increase the growth and development of agricultural plants, as well as their productivity under the abiotic and biotic stress conditions[5,6] Although the application of such agrochemicals in agriculture increases the productivity of agricultural plants under stress conditions, they cause detrimental environmental pollution, disruption of the natural ecosystem, reduction of biodiversity, and accumulate in soil, water and plant organisms, leading to significant negative effects on human health.[7]

As a result of these negative consequences, there has been an increasing need for the development of eco-friendly natural alternatives to replace chemical fertilizers and pesticides in the agricultural sector.

A new approach and promising tool in this field is the development of biostimulants derived from various biological

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novel convolutional neural network with feature selection. / C.E. Kuo, Y.K. Tu, S.L. Fang, Y.R. Huang, H.W. Chen, M.H. Yao, B.J. Kuo // Chemometrics and Intelligent Laboratory Systems - 2023. 239. art. 104869

[4] Seleiman M.F. Drought stress impacts on plants and different approaches to alleviate its adverse effects. / M.F. Seleiman, N. Al-Suhaibani, N. Ali, M. Akmal, M. Alotaibi, Y. Refay [et al.] // Plants-Basel - 2021. 10 (2), 259.

[5] Parmar P. Microalgae as next generation plant growth additives: Functions, applications, challenges and circular bioeconomy based solutions. / P. Parmar, R. Kumar, Y. Neha, V. Srivatsan // Frontiers in Plant Science - 2023. 14. art.1073546

[6] Gonçalves A.L. The use of microalgae and cyanobacteria in the improvement of agricultural practices: a review on their biofertilising, biostimulating and biopesticide roles. / A.L. Gonçalves // Applied Sciences - 2021. 11 (2), 871.

[7] Gitau M.M. Evaluation of the biostimulant effects of two Chlorophyta microalgae on tomato (Solanum lycopersicum). / M.M. Gitau, A. Farkas, V. Ördög, G. Maróti // Journal of Cleaner Production. 2022. 364. 132689.

sources. In recent years, researchers are conducting intensive research on the application of organic-based biostimulants to enhance plant growth and strengthen their defense mechanisms to different abiotic and biotic stress factors of the environment. In this regard, the study of phyto-stimulating and phyto-activating properties of various extracts obtained from macro- and microalgae can be considered a promising step in the production of eco-friendly tools in agriculture[8].

Object and Subject of the Research. The research involves the use of ten local tomato (Solanum lycopersicum L.) varieties obtained from the seed bank of the Vegetable Science Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan (Ilyas, Elnur, Nuru, Khazar, Shakar, Zerrabi, Mirvari, Azerbaijan-94, Elim, and Yubiley-60), as well as two foreign tomato varieties (Marmande and Potato-Tomato) obtained from the National Research Council of Italy's Institute of Sustainable Plant Protection.

Aims and Objectives of the Research. The main aim of this dissertation is to study the responses of local and European-origin tomato varieties to drought stress, rehydration, and the effect of potential biostimulants. To achieve this, the following objectives have been set:

During drought and rehydration in tomato genotypes

- Study of the relative water content, hydrogen peroxide, and malondialdehyde levels;
- Analysis of the antioxidant defense system’s enzymatic and non-enzymatic components.

In tomato genotypes treated with cyanobacteria and macroalgae extract during drought stress and fungal infection:

- Study of water exchange parameters and photosynthetic characteristics;
- Investigation of catalase enzyme activity and the levels of small molecular weight metabolites;
- Analysis of the expression of the dehydrin gene;
- Examination of the inhibitory effect of the cyanobacteria extract

[8] Ali O. Biostimulatory activities of *Ascophyllum nodosum* extract in tomato and sweet pepper crops in a tropical environment. / O. Ali, A. Ramsubhag, J. Jayaraman // PLoS One. 2019. 14 (5). e0216710.

on seed germination rate and the *Botrytis cinerea* fungus.

Main points of the dissertation for defense:

- Tomato varieties respond to drought stress by activating the antioxidant defense system.
- Drought stress causes significant physiological and biochemical changes in tomato plants, but after rehydration, the plant can recover to some extent.
- Cyanobacteria and macroalgae extracts have biostimulatory properties that increase the drought tolerance of tomato plants.
- Application of cyanobacteria and macroalgae extracts enhances the expression of dehydrins.
- Extract from *Nostoc piscinale* cyanobacteria inhibits the development of the Botrytis cinerea fungus.

Scientific novelty of the study. For the first time in Azerbaijan, the biostimulatory and antifungal effects of extracts obtained from *N. piscinale* cyanobacteria and macroalgae on tomato plants were investigated, and the optimal concentration (10%) of the cyanobacteria extract that inhibits the development of *Botrytis cinerea* fungus was determined. Among the studied local varieties, Elnur, Azerbaijan-94, and Mirvari varieties exhibited more dynamic self-recovery abilities after rehydration. During drought and rehydration, 8 isoforms of ascorbate peroxidase, 2 isoforms of guaiacol peroxidase, and 5 isoforms of benzidine peroxidase were detected in the local tomato varieties. Under water deficit conditions, the increased activity of antioxidant enzymes was accompanied by an increase in the intensity of the APX5, APX7, and APX8 isoforms in Elnur, Khazar, Nuru, Zerrabi, Mirvari, and Azerbaijan-94 varieties, QPO2 isoform in Elnur, Khazar, Nuru, Zerrabi, Mirvari, and Azerbaijan-94 varieties, and BPO2 and BPO3 isoforms in all the studied local tomato varieties, leading to the conclusion that these isoforms play a significant role in the drought tolerance of the plants. It was shown that in the local Ilyas and foreign Potato-Tomato varieties, the expression of the dehydrin gene was enhanced under drought conditions, but after the application of cyanobacteria and macroalgae extracts, the expression significantly decreased in both local Ilyas and European-origin Potato-Tomato varieties. Based on the obtained results, it was concluded that different tomato varieties demonstrate variety-specific responses to drought and rehydration processes as well as the application of potential biostimulyators.

Theoretical and Practical Significance. The results obtained from the research provide a better understanding of the stress response mechanisms of plants during drought stress and recovery processes, and can contribute significantly to the development of strategies to increase the resilience of tomato genotypes to unfavorable ecological conditions. The study of the biostimulatory and antifungal effects of cyanobacteria and macroalgae extracts on plants opens up new opportunities for the use of these extracts in biotechnology as well as creates an important foundation for the development of eco-friendly and effective biostimulants in agriculture. The findings encourage the use of natural biostimulants as an alternative to various chemical agents, such as, chemical fertilizers, and fungicides, which are widely used in traditional agriculture. This can contribute to the implementation of environmentally sustainable agricultural practices, the protection of ecosystems, and the reduction of environmental pollution. The application of cyanobacteria extracts due to its potential antifungal effects against phytopathogenic fungus may reduce the need for chemical agents in plant disease control in the agricultural sector, thereby promoting the cultivation of healthier crops.

Approbation. The main scientific results of the dissertation were presented and discussed at several conferences, including the II International Conference of Students and Young Researchers on "Sustainable Development in Chemistry and Chemical Engineering," dedicated to the 98th anniversary of National Leader Heydar Aliyev (Baku, 2021), the IV International Conference of Students and Young Researchers on "Towards Chemistry and Chemical Engineering: Innovations to Build a Better World," dedicated to the 100th anniversary of National Leader Heydar Aliyev (Baku, 2023), the XVIII International Scientific and Practical Conference "Unconventional Methods of Scientific and Thought Development" (Madrid, 2023), the International Conference "Heydar Aliyev and the Development of Modern Biology Science: Achievements and Challenges" (Baku, 2023), and the XVI International Scientific and Practical Conference "Scientific Trends: History, Development, and Current Problems" (Krakow, 2025), as well as at laboratory meetings and seminars of the Molecular Biology and Biotechnology Institute of the National Academy of Sciences of Azerbaijan (ANAS).

Institution Where the Dissertation Was Carried Out. The dissertation was conducted at the Institute of Molecular Biology and Biotechnology of the Ministry of Science and Education of the Republic of Azerbaijan and at the Institute for Sustainable Plant Protection of the National Research Council of Italy.

Publications. Based on the materials of the dissertation, a total of 11 scientific works, including 5 articles and 6 abstracts, have been published in local and international journals.

Structure and Volume of the Dissertation. The total volume of the dissertation consists of the introduction, six chapters, the conclusion, findings, a list of references, a list of abbreviations, and appendices. The structure of the dissertation is as follows: the introduction comprises 6 pages with 12,161 characters; the first chapter comprises 48 pages with 91,314 characters; the second chapter comprises 15 pages with 24,479 characters; the third chapter comprises 25 pages with 34,014 characters; the fourth chapter comprises 21 pages with 34,198 characters; the fifth chapter comprises 3 pages with 3,149 characters; and the sixth chapter comprises 4 pages with 5,817 characters. The conclusion and findings section spans 11 pages with 21,302 characters. The list of references includes 314 sources across 32 pages with 62,484 characters. In total, the dissertation consists of 175 pages of computer-typed text, amounting to 222,924 characters.

CHAPTER I. MAIN CONTENT OF THE STUDY

This chapter provides extensive analyses of recent literature related to the physiological, biochemical, and molecular responses of plants to stress factors, including oxidative stress and the functioning of the enzymatic and non-enzymatic components of antioxidant defense system. A comprehensive review of the scientific literature concerning tomato plants is presented. In addition, the biostimulant properties of cyanobacteria and macroalgae, along with their fields of application, are examined.

CHAPTER II. MATERIALS AND METHODS

The study focused on 10 local tomato varieties — Elim, Azerbaijan-94, Nuru, Ilyas, Jubilee-60, Mirvari, Zarrabi, Elnur, Khazar, and Shakar — obtained from the Vegetable Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan, as well as two European varieties — Potato-Tomato and Marmande. The research examined plants subjected to drought, rehydration, and treatments with cyanobacterial and macroalgal extracts. The following parameters were analyzed: relative water content[9], the content of hydrogen peroxide[10], malondialdehyde level[11], photosynthetic pigments concentration[12], soluble proteins content[12] [13], proline level[14], the concentration of the soluble sugars[15], phenolic compounds content[16], ascorbic acid levels[17]. The activity of antioxidant enzymes such as catalase,

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[9] Turner N.C. Techniques and experimental approaches for the measurement of plant water status. / N.C. Turner // Plant Soil. 1981. 58 (1). 339–66.

[10] Bellincampi, D. Extracellular H2O2 induced by oligogalacturonides is not involved in the inhibition of the auxin-regulated rolB gene expression in tobacco leaf explants/ Bellincampi, D., Dipierro, N., Salvi G. [et al.] // Plant physiology, –2000, 122 (4), –p. 1379-1386.

[11] Hodges D.M. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. / D.M. Hodges, J.M. DeLong, C.F. Forney, R.K. Prange // Planta. 1999. 207. 604–611.

[12] Sumanta N. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. / N. Sumanta, C.I. Haque, J. Nishika, R. Suprakash // Res J Chem Sci. 2014. 2231. 606X.

[13] Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // Analytical bio- chemistry, – 1976, 72 (1-2), – p. 248-254..

[14] Bates, L.S., Waldren, R.A., Teare, I.D. Rapid determination of free proline for water-stress studies // Plant and soil, – 1973. 39, – p. 205-207.

[15] DuBois M. Colorimetric method for determination of sugars and related substances. / M. DuBois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith // Analytical Chemistry. 1956. 28(3). 350–356.

[16] Odabasoglu F. Comparison of antioxidant activity and phenolic content of three lichen species. / F. Odabasoglu, A. Aslan, A. Cakir, H. Suleyman, Y.Karagoz, M. Halici, Y. Bayir // Phytother Res. 2004. 18 (11). 938–941.

[17] Matei, N., Dobrinas, S., Radu, G.L. Spectrophotometric determination of ascorbic

ascorbate peroxidase, guaiacol peroxidase, and benzidine peroxidase was determined spectrophotometrically, and their isoenzyme composition[18] were analyzed using native polyacrylamide gel electrophoresis, as well as, the expression level of the dehydrin *tas14* gene was determined using molecular methods. Additionally, the antifungal and biostimulant properties of the cyanobacterial extract were studied. The biostimulatory effect of the extract obtained from cyanobacteria on tomato seed germination was determined by the percentage of seed germination.

Statistical analyses were performed using the Tukey HSD post-hoc test. In the results, $p < 0.05$ (*) and $p < 0.01$ (**) indicate statistically significant differences between the mean values, while ns denotes statistically insignificant differences.

CHAPTER III. RESULTS AND DISCUSSION

3.1. Study of Relative Water Content in Tomato Plants under Drought and Re-Irrigation

The relative water content of tomato varieties subjected to drought and repeated irrigation was determined. Under drought conditions, a decrease in the relative water content (RWC) was observed in all varieties. After re-irrigation, the minimal recovery was recorded in the Khazar genotype, while the maximal recovery was observed in the Zarrabi genotype.

3.2. Study of Hydrogen Peroxide and Malondialdehyde Levels in Tomato Varieties under Drought and Re-Irrigation Conditions

Drought stress leads to increased generation of reactive oxygen species in plants, which results in disruption of metabolic processes in plant cells, accumulation of high amounts of H_2O_2 in the cell, and peroxidation of membrane lipids. The levels of H_2O_2 and MDA, which are indicators of stress, were analyzed in local tomato varieties. Under

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acid in grapes with the Prussian Blue reaction // Ovidius University Annals of Chemistry, – 2012. 23 (2), – p. 174-179.

[18] Davis, B.J. Disc electrophoresis. II. Method and application to human serum proteins // Annals of the New York Academy of Sciences, – 1964, 121(2), – p. 404- 427.

drought conditions, a significant increase in these indicators was observed. It was shown that, three days after rehydration, the H_2O_2 levels were fully restored in the Elnur, Khazar, Zarrabi, and Mirvari varieties, while the partial recovery was observed in the Azerbaijan-94, Shakar, and Elim varieties (Figure 1). Seven days after rehydration, the H_2O_2 levels were fully restored in all samples except for the Nuru and Ilyas varieties.

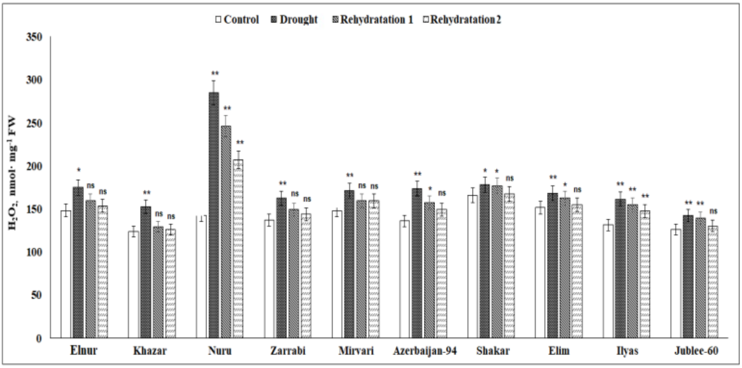

Figure 1. Hydrogen Peroxide Levels in the Leaves of Local Tomato Varieties.

A greater increase in MDA levels during drought was observed in the Khazar, Nuru, and Elnur varieties. After rehydration, full recovery was noted in the Azerbaijan-94 and Shakar varieties, while partial recovery was observed in the Elnur, Zarrabi, and Jubilee-60 genotypes. The variability observed in the recovery dynamics of MDA levels after repeated irrigation can be explained by the variety-specific differences of these genotypes.

3.3. Study of the Enzymatic Components of the Antioxidant Defense System in Tomato Leaves under Drought and Re-Irrigation Conditions

Plants use various defense mechanisms based on the activation of enzymatic and non-enzymatic antioxidants to neutralize toxic levels of reactive oxygen species. The enzymatic components of the antioxidant defense system in tomato leaves were comparatively studied under drought and rehydration conditions. It was determined that drought led to a significant increase in the activity of ascorbate peroxidase (APX) in all varieties compared to the control (Figure 2). This increase was more pronounced in the Elnur, Nuru, Zarrabi, Ilyas, and Elim varieties. Three and seven days after re-irrigation, the APX activity significantly decreased in all genotypes.

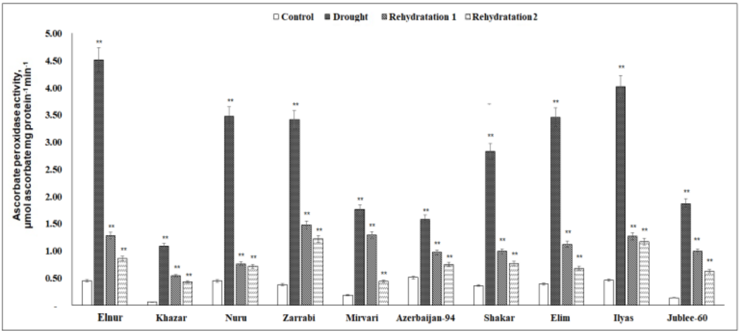

Figure 2. Ascorbate Peroxidase Activity in the Leaves of Tomato Varieties.

Electrophoretic analysis revealed 8 isoenzymes of APX with different mobility in the tomato leaves of all variants (Figure 3).

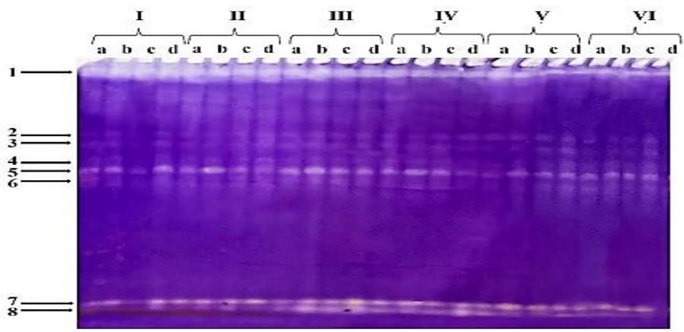

Figure 3. Analysis of the APX Isoenzyme Composition in the Leaves of Tomato Varieties. a – irrigated; b – drought; c – 3 days after re-irrigation; d – 7 days after re-irrigation. I - Elnur, II - Khazar, III - Nuru, IV - Zarrabi, V - Mirvari, VI - Azerbaijan-94.

An increase in the intensity of the 5th, 7th, and 8th isoforms was observed in the Elnur, Khazar, Nuru, Zarrabi, Mirvari, and

Azerbaijan-94 varieties subjected to drought, while a decrease in intensity was noted after rehydration. The results obtained provide evidence that the expression of the genes encoding these isoforms is regulated by drought stress.

Under drought conditions, the activity of benzidine peroxidase (BPX) significantly increased in all varieties compared to the control, with the increase being more pronounced in the Mirvari, Elnur, Khazar, Azerbaijan-94, and Ilyas varieties (Figure 4). Three days after re-irrigation, the BPX activity was partially restored in the Khazar, Shakar, Elim, and Jubilee-60 varieties, and fully restored in the Zarrabi and Azerbaijan-94 varieties. Seven days after rehydration, the activity of benzidine peroxidase was fully restored in all varieties except for Nuru and Ilyas, with partial recovery in Elim, bringing the activity closer to the control level.

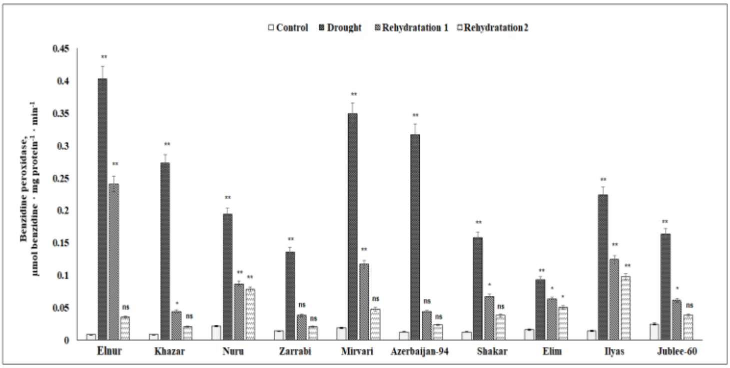

Figure 4. Benzidine Peroxidase Activity in the Leaves of Tomato Varieties.

Based on the results of the electrophoretic analyses, 5 isoforms of benzidine peroxidase were detected in the Khazar, Elnur, Nuru, Mirvari, Ilyas, and Jubilee-60 varieties, 4 isoforms in Elim, and 3 isoforms in the Azerbaijan-94, Shakar, and Zarrabi varieties (Figure 5). Under drought conditions, the intensity of the BPX2 and BPX3 isoforms increased, and decreased after rehydration.

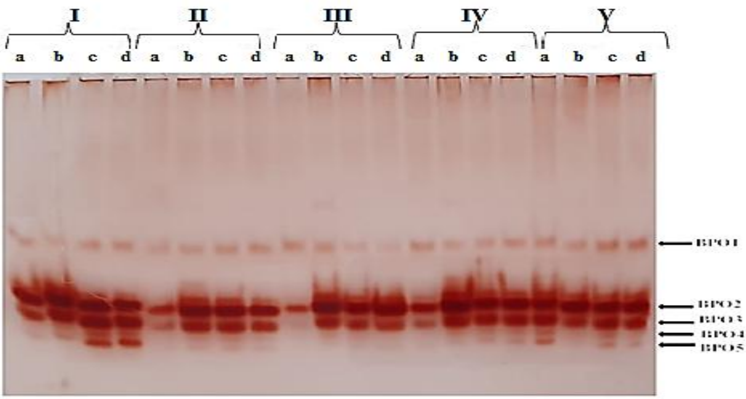

Figure 5. Analysis of the BPO Isoenzyme Composition in the Leaves of Tomato Varieties. a - control; b - drought; c - 3 days after rehydration; d - 7 days after rehydration. I - Nuru, II - Mirvari, III - Azerbaijan-94, IV - Ilyas, V - Jubilee-60.

In the Elim and Nuru varieties, the intensity of the BPX4 and BPX5 isoforms decreased under drought conditions and was restored after re-irrigation.

Under drought conditions, the activity of guaiacol peroxidase increased to varying degrees across all tomato varieties, with a more pronounced increase observed in the Elnur, Zarrabi, Azerbaijan-94, Shakar, and Ilyas varieties (Figure 7).

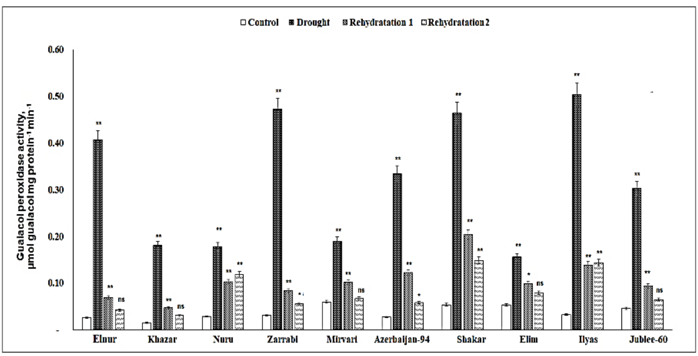

Figure 6. Guaiacol Peroxidase Activity in the Leaves of Tomato Varieties.

After seven days of rehydration, the enzyme activity was fully restored in the Elnur, Khazar, Mirvari, Elim, and Jubilee-60 varieties. Electrophoretic analysis revealed the presence of two isoforms of guaiacol peroxidase (Figure 7).

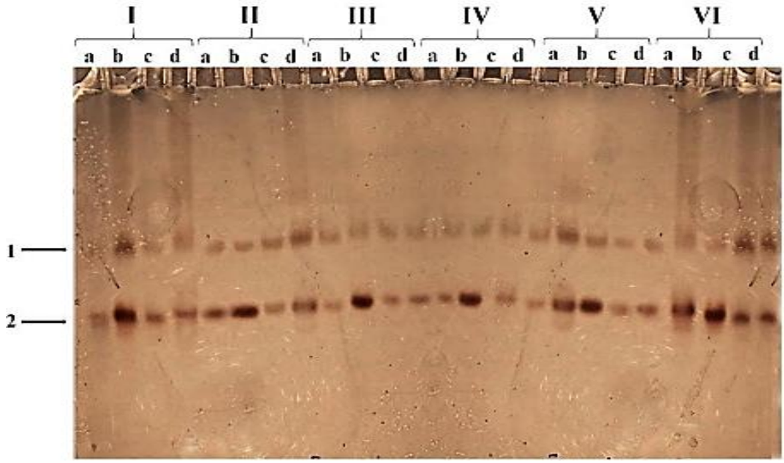

Figure 7. Analysis of Guaiacol Peroxidase (GPO) Isoenzyme Composition in the Leaves of Tomato Varieties. a – control; b – drought; c – after 3 days of rehydration; d – after 7 days of rehydration. I – Elnur, II – Khazar, III – Nuru, IV – Zarrabi, V – Mirvari, VI – Azerbaijan-94.

A significant increase in the intensity of the second isoform of guaiacol peroxidase (GPX) was observed in the drought-stressed Elnur, Khazar, Nuru, Zarrabi, Mirvari, and Azerbaijan-94 tomato varieties. The presence of isoforms with different electrophoretic mobility suggests that they are likely encoded by distinct genes located on different chromosomes.

3.4. Study of Ascorbic Acid Content in Tomato Genotypes under Drought and Re-Irrigation Conditions

Ascorbic acid (AsA) plays a vital role in the growth and development of plants as a non-enzymatic component of antioxidant defence system and it induces the activity of enzymatic components of the antioxidant defense system, especially APX, under drought stress condition. Our research revealed that, compared to the control variants, the content of AsA significantly increased in all tomato cultivars subjected to drought stress (Figure 8). Under drought conditions, the highest rate of AsA increase was observed in the Mirvari cultivar (67%), while the Elim and Jubilee-60 cultivars showed the lowest increase. Three days after rehydration, the AsA content decreased in the Elnur, Khazar, Nuru, Mirvari, Azerbaijan-94, Shakar, and Ilyas cultivars, while it remained unchanged in the Jubilee-60 cultivar.

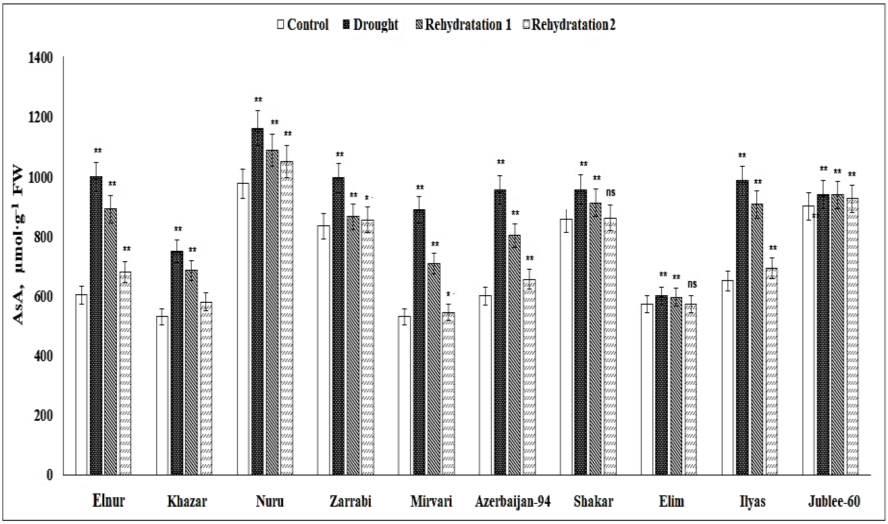

Figure 8. The amount of ascorbic acid in the leaves of tomato varieties.

Seven days after rehydration, the amount of AST in the Zarrabi and Mirvari varieties was partially restored, while in the Shakar and Elim varieties, it was completely restored.

3.5. Study of the amount of phenolic compounds under drought and re-irrigation conditions in tomato genotypes

Drought stress leads to changes in many metabolic pathways, including the synthesis of secondary metabolites. In our studies, drought caused a significant reduction in the amount of phenolic compounds in all varieties, except for the Khazar and Shakar varieties (Figure 9).

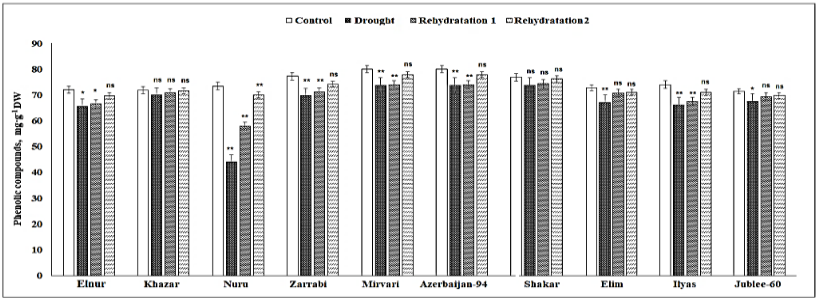

Figure 9. The amount of phenolic compounds in the leaves of tomato varieties.

A more pronounced decrease in the amount of phenolic compounds was observed in the Nuru variety (40%). Seven days after rehydration, complete recovery was observed in all varieties (except for the Nuru variety). The decrease in the amount of phenolic compounds under water deficit conditions can be explained by the oxidative inactivation of enzymes involved in the synthesis of these compounds.

3.6. Study of the amount of proline under drought and re-irrigation conditions in tomato genotypes

The accumulation of osmolytes under stress conditions in plant cell ensure to increase plant stress tolerance. Proline is an important osmolyte involved in regulating osmotic balance in plants during water scarcity. Water deficit caused a sharp increase in the amount of proline in the leaves of all studied tomato varieties. which plays a crucial role in regulating osmotic balance (Figure 10).

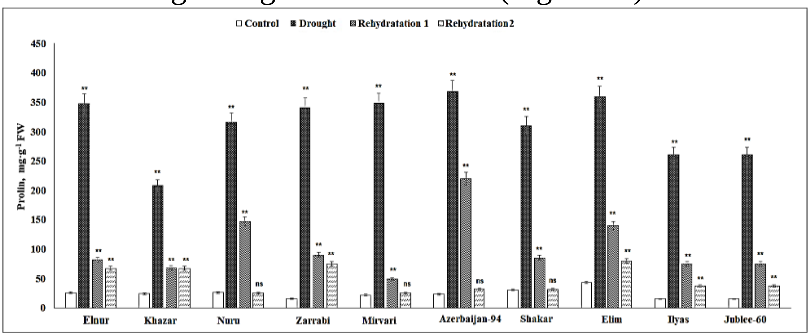

Figure 10. The amount of proline in the leaves of tomato varieties.

The maximum amount of proline was observed in the Azərbaycan-94 variety, which decreased by 40% and 91.3% after 3 and 7 days of rehydration, respectively, and nearly completely recovered. Seven days after rehydration, complete recovery was recorded in samples taken from the Nuru, Mirvari, Azerbaijan-94, and Shakar varieties. These results demonstrate the different responses of various tomato varieties to water stress and rehydration, as well as the varying dynamics of the recovery processes.

4. Study of the potential biostimulant effect of cyanobacteria and macroalgae extracts on tomato varieties exposed to drought stress

4.1 Study of water status in tomato varieties treated with cyanobacteria and macroalgae extracts

The changes occurring due to the effect of potential biostimulants were studied in Italian-origin Potato-Tomato (moderately drought-sensitive) and local Ilyas (moderately drought-tolerant) tomato varieties exposed to drought stress. It was found that under irrigated conditions, the amount of NST in these varieties was 67% and 68%, respectively, while under drought conditions, it decreased to 40% in both varieties (Figure 11).

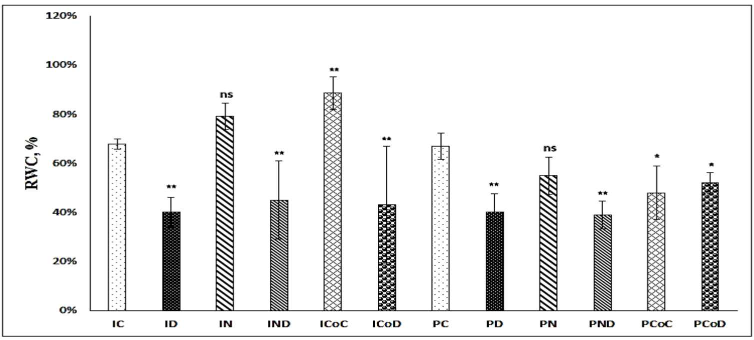

Figure 11. Relative water content in tomato leaves treated with cyanobacteria and macroalgae extracts. I - Ilyas, P - Potato-Tomato, C - control, D - drought, N – *N. piscinale* extract applied (irrigated), ND – *N. piscinale* extract applied (drought), CoC - macroalgae extract applied (irrigated), CoD - macroalgae extract applied (drought).

After the application of *N. piscinale* extract, the amount of NST in the drought-exposed Potato-Tomato variety was 39%, while in the İlyas variety, it was 45%. As seen, the *N. piscinale* extract did not cause significant changes in the foreign variety under drought conditions, but it was able to maintain the plant's water status at a slightly higher level in the local variety. Similar results were observed with the effect of the macroalgae extract. This fact demonstrates the positive effect of the macroalgae extract in maintaining the plant's water status during drought.

4.2. Study of photosynthetic parameters in tomato varieties treated with cyanobacteria and macroalgae extracts under drought conditions

One of the main mechanisms plants use to prevent water loss is the closure of stomata. Research showed that during drought, the stomatal conductance significantly decreased in both the İlyas and Potato-Tomato varieties (Table).

Table

Photosynthetic parameters in the leaves of tomato varieties.

Variants	Stomatal conductance (mol $m^{-2}s^{-1}$)	ENS (μmol $m^{-2}s^{-1}$)	ΦPS2
IC	0.67±0.08	206±7.5	0.7±0.08
ID	0.010±0.006**	101±6.8**	0.38±0.02**
IN	0.65±0.05 ^{ns}	203±6.9^{ns}	0.60±0.06 ns
IND	0.010±0.005**	101±5.9**	0.39±0.04**
ICoC	0.71±0.03*	208±5.2^{ns}	0.65±0.01^{ns}
ICoD	0.02±0.006**	102±8.9**	0.41±0.09**
PC	0.53±0.04	203.5±8.4	0.64±0.04
PD	0.009±0.002**	99.9±7.7**	0.30±0.02**
PN	0.45±0.16*	231.5±5.06**	0.69±0.01^{ns}
PND	0.004±0.002 **	89.3±14.6 **	0.27±0.04 **
PCoC	0.65±0.096 *	169.7±7.95 *	0.66±0.03 ns
PCoD	0.031±0.015 **	152.2±41.9 *	0.46±0.12 **

The effect of *N. piscinale* extract did not significantly influence the stomatal conductivity in the Ilyas and Potato-Tomato varieties.

However, the application of macroalgae extract led to a significant increase in stomatal conductivity in both irrigated and drought variants (respectively, 1.06 times and 2 times in the irrigated variant; 1.2 times and 3.4 times in the drought variant). Water deficit significantly reduces the electron transport rate in both tomato varieties (~2 times). In the drought conditions, no significant changes were observed in the varieties treated with cyanobacterial extract, while in the irrigated variant, slight stimulation was noted only in the Potato-Tomato variety (~1.13 times). The application of macroalgae extract did not affect ENS in the Ilyas variety, while it significantly increased it in the Potato-Tomato variety (~1.5 times).

The quantum efficiency of photosystem II (~2 times) was significantly reduced in both varieties exposed to drought. As a result of the macroalgae extract, the activity of ΦPS2 increased under drought conditions in both varieties, with a statistically significant increase observed in the Potato-Tomato variety. It is assumed that the macroalgae extract increases the stomatal conductivity in the leaves of tomato plants under drought conditions, leading to higher photosynthetic activity.

Photosynthetic pigments play a crucial role in the energy metabolism of plants. Under drought conditions, an increase in chlorophyll content was observed in the Ilyas variety compared to the control, while a statistically insignificant decrease was found in the Potato-Tomato variety (Figure 12).

Plants have adapted to reduce the transpiration area by decreasing the leaf surface area in response to water scarcity, thereby minimizing water loss. Therefore, the increase in chlorophyll content per unit leaf area can be explained as a mechanism for maintaining photosynthetic activity under drought conditions through the induction of chlorophyll synthesis in the Ilyas variety.

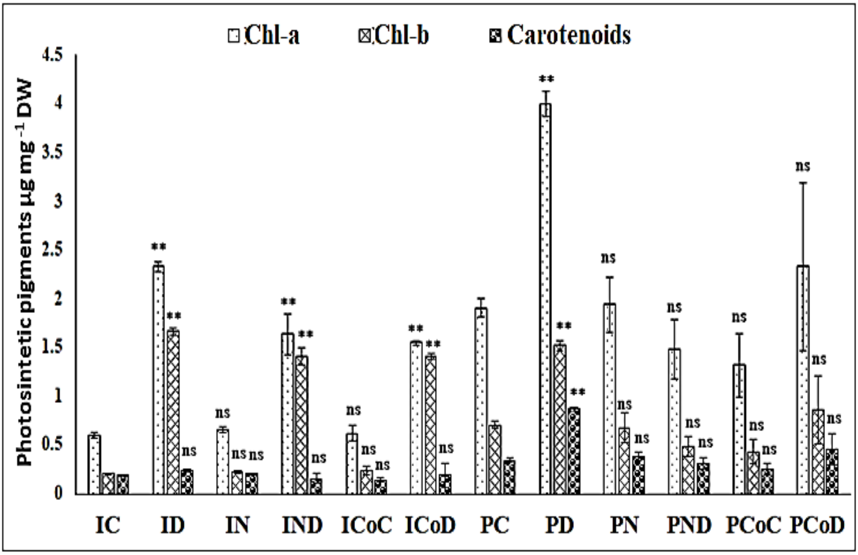

Figure 12. The amount of photosynthetic pigments in the leaves of tomato varieties (Abbreviations are given on page 16).

4.3. Study of proline content in tomato varieties treated with cyanobacterial and macroalgae extracts.

When the water level in the soil decreases, the de novo synthesis of osmolytes occurs in plant cells to ensure osmotic adaptation, one of which is proline, a marker of the plant's stress response. Under drought conditions, the proline content in the leaves of the Ilyas variety sharply increased to 229 $mg \cdot g^{-1}$ fresh weight, while in the Potato-Tomato variety, it reached 118 $mg \cdot g^{-1}$ fresh weight (Figure 13). In the drought variant of the Ilyas variety, the proline content in the leaves decreased by 39% due to the cyanobacterial extract and by 44% due to the macroalgae extract. In the drought-exposed Potato-Tomato variety, the proline content continued to increase after the application of both cyanobacterial and macroalgae extracts. The decrease in proline content under water deficit conditions in the Ilyas variety following the exogenous application of cyanobacterial and macroalgae extracts is considered a positive effect of these extracts in reducing the impact of stress.

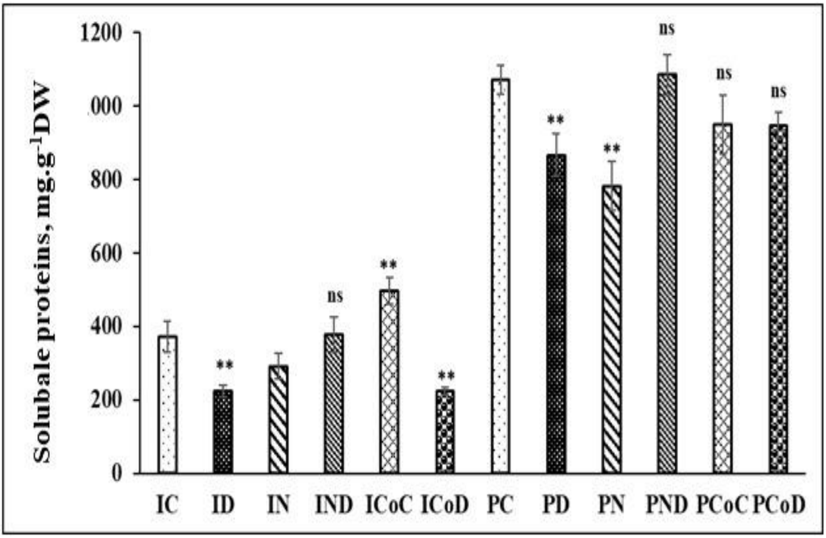

Figure 14. The amount of soluble proteins in the leaves of tomato varieties (Abbreviations are given on page 16

4.4. Study of the amounts of soluble proteins, phenolic compounds, and soluble sugars in tomato varieties treated with cyanobacterial and macroalgae extracts.

Under drought conditions, the amount of soluble proteins in the Ilyas and Potato-Tomato varieties decreased by approximately 1.7 and 1.2 times, respectively, compared to the control variant. After cyanobacterial treatment, the protein content in both varieties increased, approaching the control variant, with values of 378 and 1085 mg·g^{-1} dry weight, respectively (Figure 15). In the Ilyas variety treated with macroalgae extract, a significant increase in protein content was observed under irrigated conditions, while under drought conditions, it decreased and approached the drought variant. In the Potato-Tomato variety treated with macroalgae extract, a statistically insignificant decrease in protein content was observed compared to the control variant, both under irrigated and drought conditions. The increase in protein content observed after cyanobacterial extract application could be the result of these extracts' positive effect in minimizing the impact of stress.

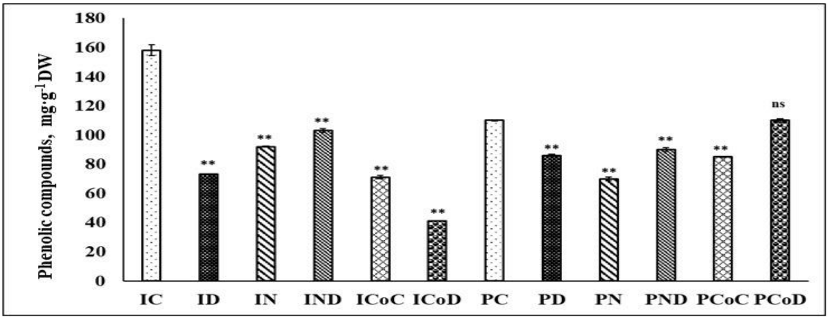

Figure 15. The amount of phenolic compounds in the leaves of tomato varieties (Abbreviations are given on page 16).

Phenolic compounds (PC) are antioxidant compounds that play a significant role in mitigating the effects of oxidative stress (OFF) and preventing cell damage. Analyses show that the amount of phenolic compounds in the drought-exposed Ilyas and Potato-Tomato varieties significantly decreased compared to the control variant, reaching 73 and 86 mg·g^{-1} dry weight respectively (Figure 15). The application of cyanobacterial extract increased the phenolic compound content during drought in the local variety. The application of the extract to the Italian-origin variety had a slight effect on the rate of phenolic compound increase during drought. During the application of macroalgae extract, a decrease in phenolic compound content was observed in the Ilyas variety under drought conditions, while an increase was noted in the Potato-Tomato variety.

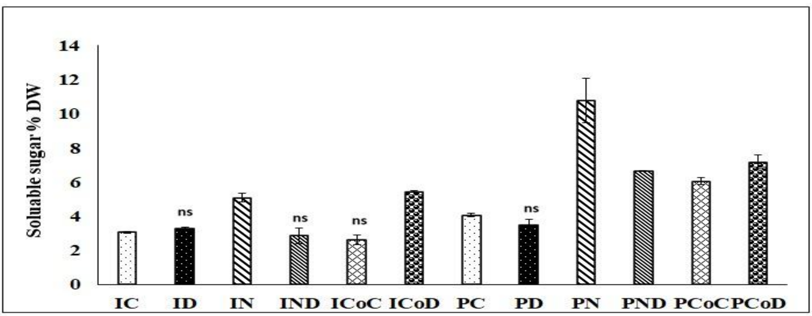

Figure 16. The amount of soluble sugars in the leaves of tomato varieties (Abbreviations are given on page 16).

The results of the conducted analyses show that the use of cyanobacteria as a biostimulant for plant stress protection can be considered one of the promising approaches.

Sugars play an important role in maintaining the intracellular osmotic balance under water scarcity conditions, as well as serving as substrates for the synthesis of defense proteins and chemical compounds essential for the protection of cell membranes. The analyses revealed that under drought conditions, the amount of soluble sugars in the Ilyas variety remained almost unchanged, while in the Potato-Tomato variety, it decreased slightly, although not statistically significant (Figure 16). After the application of cyanobacterial extract, there was no statistically significant difference in the Ilyas variety compared to drought conditions, but in the Potato-Tomato variety, the amount of sugars significantly increased. Under drought conditions, the application of macroalgae extract resulted in a significant increase in the amount of these compounds in both varieties compared to the control variant.

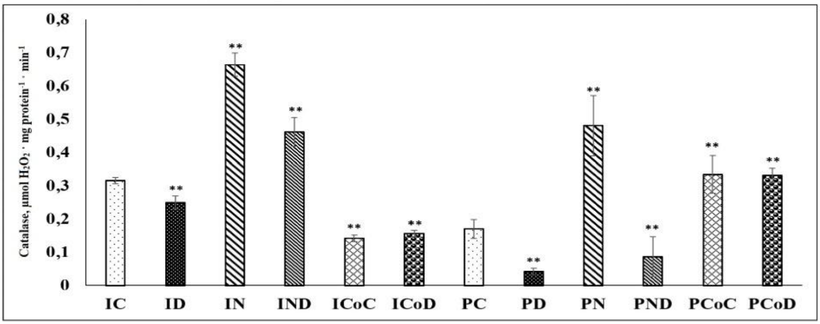

Figure 17. Catalase enzyme activity in the leaves of tomato varieties (Abbreviations are given on page 16)

Overall, based on the obtained results, it can be concluded that the application of the extracts led to an increase in the amount of soluble sugars in the leaves of the tomato plant.

4.5. Study of catalase enzyme activity in tomato varieties treated with cyanobacterial and macroalgae extracts.

Catalase (CAT) is a heme-containing tetrameric enzyme that catalyzes the decomposition of H_2O_2. Analyses revealed that under water deficit conditions, the activity of the enzyme significantly decreased in both the Ilyas and Potato-Tomato varieties (by approximately 1.3 and 4.3 times, respectively) (Figure 17). In the leaves of the Ilyas variety treated with *N. piscinale* extract under drought conditions, CAT activity increased compared to the drought variant, reaching 0.46 µmol·mg protein^{-1}·min^{-1} and under irrigated conditions, it significantly increased compared to the control variant, reaching 0.66 µmol·mg protein^{-1}·min^{-1}.

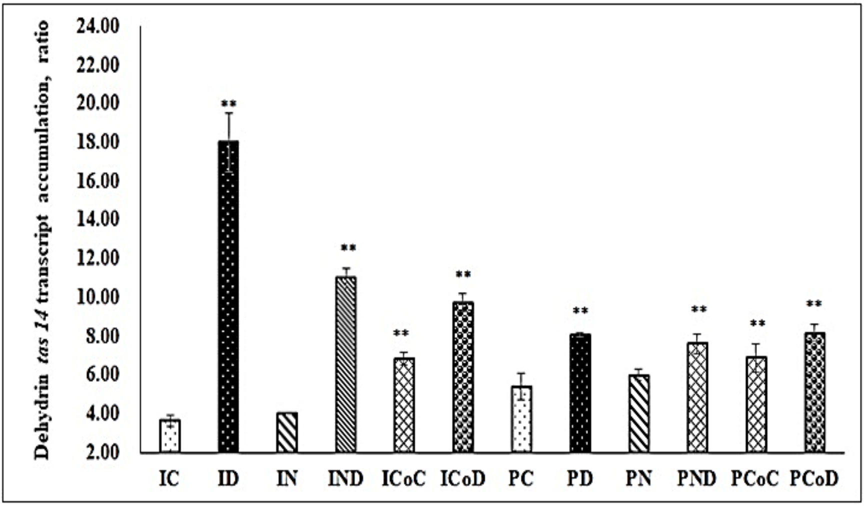

Figure 18. Accumulation of tas14 transcript in the leaves of tomato varieties (Abbreviations are given on page 16).

The application of macroalgae extract to the drought-exposed Ilyas variety led to a statistically significant decrease in catalase enzyme activity. Under irrigated conditions, the application of *N. piscinale* extract significantly stimulated CAT enzyme activity in the Potato-Tomato variety compared to the control variant. Interestingly, the application of macroalgae extract to the Potato-Tomato variety increased enzyme activity by approximately 1.96 times compared to the control under both normal irrigation and drought conditions. It can be concluded that the application of macroalgae extract helps maintain antioxidant enzyme activity at a stable level.

4.6. Study of the accumulation of dehydrin tas14 transcript under drought stress in tomato varieties treated with cyanobacterial and macroalgae extracts.

Abiotic stress activates the expression of dehydrin genes in plant cells. Drought stress enhanced the accumulation of the tas14 transcript by 4.95 times in the Ilyas variety and by 1.49 times in the Potato-Tomato variety compared to the control variant (Figure 19). After the application of *N. piscinale* extract, the accumulation of the tas14 transcript significantly decreased under drought conditions in the Ilyas variety (value of 11). A similar trend was observed after the application of macroalgae extracts to drought-exposed Ilyas plants (value of 9.75). Based on the results of dehydrin gene expression analysis in response to drought, it can be concluded that the application of *N. piscinale* and macroalgae extracts may play a significant role in enhancing drought tolerance in the Ilyas variety.

In the Potato-Tomato variety treated with *N. piscinale* and macroalgae extracts, the accumulation of the tas14 transcript significantly increased compared to the control variant under both drought and normal irrigation conditions.

V. Study of the Inhibitory Effect of Nostoc piscinale Extract and Culture Medium on the Development of the Phytopathogenic Fungus Botrytis cinerea

Cyanobacteria synthesize chemical substances known as antibiotics, which inhibit the growth of pathogenic fungi and can even lead to their destruction. The analysis revealed that various concentrations of the extract and culture medium obtained from the cyanobacterium *N. piscinale* significantly reduced the development of B. cinerea after 48 hours of incubation. This can be considered as the inhibitory effect of the extract on the growth and development of the phytopathogenic fungus (Figure 20). The maximum inhibition of fungal infection, compared to the control, was observed at a 10% concentration of the cyanobacterial extract.

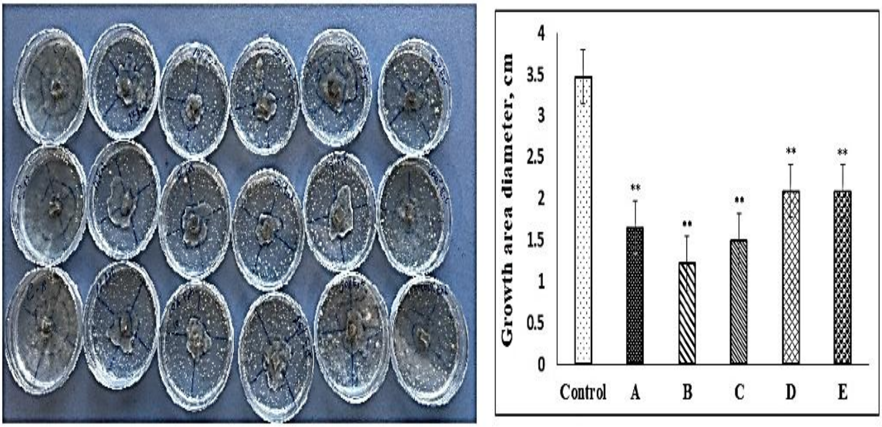

Figure 19. Inhibitory effect of different concentrations of N. piscinale extract on the development of B. cinerea fungal infection. A - 1% extract, B - 10% extract, C - 25% extract, D - 50% extract, and E - 100% extract

The application of the medium obtained from the *N. piscinale* culture resulted in a significant reduction in the development of the phytopathogenic fungus at all applied concentrations of the medium, compared to the control variant.

VI. Study of the Potential Biostimulatory Effect of Nostoc piscinale Extract and Medium with Different Concentrations on Tomato Seed Germination

There is information available about the positive effects of extracts obtained from cyanobacteria on seed germination, mineral nutrient uptake, seedling development, and flowering. During our research, the effect of various concentrations of *N. piscinale* extract and medium on the germination dynamics of tomato seeds was studied. It was determined that as a result of the application of *N. piscinale* extract, the Marmande variety showed a high germination rate within the first 3 days, while the Ilyas variety demonstrated high germination within the first 4 days, with both varieties reaching their maximum rate in the subsequent days.

CONCLUSIONS

1. Physiological and biochemical parameters during drought stress and rehydration in local tomato (Solanum lycopersicum L.) varieties were comparatively studied. It was found that relative water content (RWC), one of the key indicators of plant water balance, significantly decreased in all varieties subjected to drought stress. The most pronounced changes were observed in the Jubilee-60 and Zarrabi varieties (30.9% and 35.4%, respectively), while relatively lower decreases were recorded in the İlyas and Nuru varieties (24.7% and 24%, respectively). After 3 and 7 days of rehydration, the Jubilee-60 variety showed a slower recovery process, while in other varieties, RWC was partially restored. This phenomenon can be explained by certain varieties' ability to absorb water more quickly and possess higher membrane permeability [2, 4].

2. A significant increase in the levels of hydrogen peroxide and malondialdehyde, which are considered indicators of stress, was observed in all varieties subjected to drought stress. After rehydration, among the varieties studied, the Elnur, Khazar, Azərbaijan-94, Zarrabi, and Mirvari varieties showed near complete recovery of these indicators, which reflects the more dynamic self-repair capacity of these varieties [8, 9].

3. It has been shown that during water scarcity, the activity of ascorbate peroxidase (APX), guaiacol peroxidase (GPO), and benzidine peroxidase (BPO) enzymes increases sharply, while after rehydration, a statistically significant decrease is observed in all varieties. During irrigation, eight APX, two GPO, and five BPO constitutive isoforms were identified in the studied varieties. Under drought conditions, the intensity of the APX5, APX7, and APX8 isoforms increased in the Elnur, Khazar, Nuru, Zarrabi, Mirvari, and Azərbaijan-94 varieties, the GPO2 isoform increased in Elnur, Khazar, Nuru, Zarrabi, Mirvari, and Azərbaijan-94 varieties, and the BPO2 and BPO3 isoforms increased in all varieties. Based on the results, it is concluded that these isoforms play an important role in the drought tolerance of the plant [4, 6, 9].

4. Compared to the irrigated variant, an increase in the levels of ascorbic acid (AsA) (1.04-1.67 times) and proline (8.3-21.9 times) was

observed due to the drought stress, while a decrease in the amount of phenolic compounds was noted in all tomato varieties, except for the Khazar and Shakar varieties. This decrease can be explained by the oxidative inactivation or degradation of enzymes involved in the synthesis of small-molecule metabolites during stress. After rehydration, the levels of AsA were fully restored in the Elim and Shakar varieties, the levels of proline were restored in the Nuru, Mirvari, Azərbaijan-94, and Shakar varieties, and the levels of phenolic compounds were restored in the Elnur, Zarrabi, Mirvari, Azərbaijan-94, and Ilyas varieties [3, 9].

5. For the first time in Azerbaijan, the potential biostimulatory effects of extracts obtained from cyanobacteria (*N. piscinale*) and macroalgae on local (Ilyas) and Italian (Potato Tomato) tomato plants were investigated. It was found that in both varieties, the stomatal conductivity and electron transport rate (ETR) decreased under drought conditions. After the application of the macroalgae extract, these parameters were restored in the Potato-Tomato variety but remained unchanged or slightly increased in the Ilyas variety. Due to drought stress, the levels of photosynthetic pigments (chl a, chl b) significantly increased in the Ilyas variety, but after the application of the extract, these levels decreased and partially recovered. In the Potato-Tomato variety, the levels of photosynthetic pigments (chl a, chl b) slightly decreased under drought stress, although the change was not statistically significant [5].

6. In both varieties exposed to drought, the CAT activity decreased, but it increased again in the variants where the extract was applied. In the Potato-Tomato variety, the application of macroalgae extract resulted in a significant increase in CAT activity. After the application of cyanobacteria extract, CAT activity sharply increased in both varieties under irrigated conditions. During drought, a decrease in the amount of soluble proteins was observed in both varieties. However, after the application of cyanobacteria extract, an increase in the amount of soluble proteins was recorded [11].

7. During drought, the amount of proline increased in both varieties. After the application of cyanobacteria and macroalgae extracts, proline levels significantly decreased in the Ilyas variety, while they continued to increase in the Potato-Tomato variety. The amount of

phenolic compounds significantly decreased in both varieties due to the effects of drought. The exogenous application of cyanobacteria extract caused a slight increase in the amount of these compounds in both varieties. The application of macroalgae extract led to an increase in phenolic compounds in the leaves of the Potato-Tomato variety, bringing them closer to the control variant. During drought, the amount of soluble sugars in the leaves of the Ilyas variety, treated with cyanobacteria extract, showed no significant change compared to the control variant. However, in the leaves of the Potato-Tomato variety, it increased by approximately 1.6 times, while in the Ilyas variety treated with macroalgae extract, it increased by 1.7 times, and in the Potato-Tomato variety, it increased by 1.8 times [5]. In the Ilyas variety, the amount of tas 14 transcript increased under stress, but after the application of extracts, it significantly decreased [11].

8. For the first time, the optimal concentration (10%) of the extract obtained from *N. piscinale* cyanobacteria that inhibits the development of Botrytis cinerea fungus has been determined. The application of the cyanobacteria extract also significantly increased the germination rate of the seeds. These results show that *N. piscinale* extract both stimulates plant growth and has antifungal effects [11].

RECOMMENDATIONS

1. The tomato varieties Elnur, Mirvari, and Azerbaijan-94, which demonstrate dynamic self-restoration ability, are recommended to be used as initial material in practical selection programs for the creation of drought-resistant tomato varieties.
2. The use of cyanobacteria and macroalgae extracts in tomato plant cultivation as an alternative to various chemical agents such as fungicides and synthetic fertilizers is advisable.

List of published scientific works on the topic of the dissertation

1. Niyazova N.N., Əliyeva D.R. The effect of drought stress on antioxidant enzymes and lipid peroxidation in two tomato genotypes. //Transactions of the Institute of Molecular Biology and Biotechnologies of ANAS, Baku -2020, vol 4, -p.73-76.
2. Niyazova N.N. Quraqlıq stresinə məruz qalmış yerli tomat sortunda bəzi fizioloji parametrlərin tədqiqi. //"Kimya və kimya mühəndisliyində dayanıqlı İnkişaf" mövzusunda Tələbə və Gənc Tədqiqatçıların II Beynəlxalq Elmi Konfransının Materiallar Toplusu. Baku, Higher Oil School, 13-28 April, Baku, -2021, -p. 255.
3. Niyazova N.N.. The effect of drought stress on ascorbate peroxidase enzyme activity and content of ascorbic acid in leaves of tomato (*Solanum lycopersicum* L.) genotypes. //Transactions of The Institute of Molecular Biology and Biotechnologies of ANAS, Baku -2021, vol 5, -p.47-50.
4. Niyazova N.N. The study of benzidine peroxidase activity of tomato (*Solanum lycopersicum* L.) varieties under drought conditions followed by recovery. //Transactions of The Institute of Molecular Biology and Biotechnologies ANAS, Baku, - 2022, vol 6, № 2, -p.17-23.
5. Niyazova N.N., Maserti B. The studyıng of the potentıal effects of macroalgal and cyanobacterıal extracts as biostımulants of tolerance on tomato plants in drought conditions. //Proceedings of XXVIII International Scientific and Practical Conference "Unusual methods of development of science and thoughts", July, 17-19, -2023, Madrid, Spain, -p.15-16.
6. Niyazova N.N. Quraqlıq və təkrar suvarmaya məruz qalmış tomat sortlarının yarpaqlarında bəzi biokimyəvi parametrlərin tədqiqi. //Ümummilli lider Heydər Əliyevin 100 illik yubileyinə həsr olunmuş "Dayanıqlı kimya və kimya mühəndisliyinə doğru: daha yaxşı bir dünya qurmaq üçün yeniliklər" Tələbə və Gənc Tədqiqatçıların IV Beynəlxalq Elmi Konfransının Materiallar Toplusu. Baku, Higher Oil School, 12 Aprel-3 May, Baku, - 2023, - s. 441-443.

7. Niyazova N.N., Aliyeva D.R., Huseynova I.M.. Assessment of long-term drought on tomato leaves based on antıoxıdatıve response.//International Scientific Conference "The development of modern bıology: achıevements and challenges", 19-20 December, Baku, -2023, -p.3-8
8. N.N.Niyazova. Lipid peroxidation dynamics in tomato varieties under drought stress and post-recovery period. //Annali d'Italia, № 59, -2024, -p.3-8
9. N.N.Niyazova, İ.M.Huseynova. The antioxidant defense system of tomato (*Solanum lycopersicum* L.) varieties under drought stress and upon post-drought recovery. Biochemistry (Moscow), -2024, vol. 89, № 6, -p.1146-1157
10. Niyazova N.N., Aliyeva D.R., Huseynova I.M. The study of antioxidant enzyme activity and proline accumulation in local tomato varieties under drought and post-drought recovery. //Zəfər gününə həsr olunmuş "İnnovativ ekosistemlərin inkişafında universitetlərin rolu" mövzusunda Respublika Elmi-Praktik Konfransının materialları, Gəncə, -2024, -səh. 90-92
11. Niyazova N.N. Investigating the biostimulant and antifungal effects of cyanobacterial extract in tomato (Solanum lycopersicum l.) plant. //Abstracts of XVI International Scientific and Practical Conference "Scıentıfıc Trends: Hıstory, Development And Existing Problems", Krakow, Poland, -2025, -p. 9-11.

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