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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

STUDY OF ANTIOXIDANT DEFENSE SYSTEM AND PHOTOSYNTHETIC ACTIVITY IN WHEAT GENOTYPES DURING DROUGHT STRESS AND REHYDRATION

Specialty:	2411.02 - "Plant Physiology"
Science field:	Biology
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GENERAL DESCRIPTION OF THE RESEARCH

Relevance of the topic: Under abiotic stress factors, metabolic processes in the plant cells are disrupted, plant growth and development are delayed, resulting in a sharp decline in productivity[1]. Among the abiotic factors, drought is the most dangerous and long-lasting factor that directly affects crop yield and quality. Wheat, which is strategically important in terms of meeting the needs of the world population for food, is exposed to a number of stressors, including drought, during development. The growth of the population in our country, the improvement of the financial situation, the growing demand of the population for food increase the domestic demand for bread, especially high-quality wheat bread. The loss of productivity of bread wheat varieties grown in Azerbaijan due to climate-based water deficiency is one of the important problems of agriculture, and this issue is becoming more destructive because of global warming. When developing new elite wheat varieties that can meet current challenges, the main research area is not only productivity but also tolerance to changing environmental factors. In this regard, the development of wheat varieties tolerant to abiotic and biotic stresses is always in the focus of researchers.

Under abiotic stress, the plant undergoes various morphological, cellular, physiological, biochemical, and molecular adaptations to defend itself. Under drought conditions, the direction of biochemical processes in traditional metabolic networks changes. The effect of any stressor is known to cause the formation of various reactive oxygen species O_2^-, $OH^\cdot$, $^1O_2$, H_2O_2, which also damage the vital macromolecules (DNA, RNA, proteins, lipids, etc.) of the cell[2].

The antioxidant defense system, which eliminates the reactive oxygen species is the most important among the defense mechanisms

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[1] Aliyev J.A. Peculiarities of photosynthesis of wheat genotypes contrast in grain yield and their use in breeding programs. In: Crop yields: production, management practices and impact of climate change (eds. by L. Huang and Q. Zhao). – New York: Nova Science Publishers, – 2013, – Chapter 1, – p. 1-59.

[2] Huseynova, I.M. Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought // Biochimica et Biophysica Acta (BBA)-Bioenergetics, – 2012, 1817(8), – p. 1516-1523.

operating in plants. This system includes antioxidant enzymes and small molecule antioxidants (ascorbic acid, glutathione, α-tocopherol, carotenoids, flavonoids)[3]. Genotypes that demonstrate sustainable productivity under stress have superior physiological and molecular mechanisms to combat stress. To maintain water status in the cell, osmotic regulation occurs through the biosynthesis of dynamic molecules such as glycine betaine, proline, soluble sugars, etc.

Selection of germplasm based on both high productivity and secondary physiological-biochemical properties that are significantly related to crop potential under drought stress conditions can provide genetic gain in a stressful environment.

Purpose and tasks of the research. The main purpose was to study the physiological and biochemical changes in contrasting bread wheat genotypes (*Triticum aestivum* L.) under drought stress and during recovery processes after re-watering. To achieve this goal, the following tasks have been set:

- Study of water exchange indices;
- Study of gas exchange parameters;
- Study of secondary metabolites – phenolic compounds and tocopherols;
- Determination of osmolites - the amount of free proline and glycine-betaine;
- Comparative analysis of antioxidant enzyme activities and izoenzyme content;
- Clarification of the role of low-molecular-weight metabolites-ascorbate and glutathione;
- Determination of the amount of photosynthetic pigments;
- Determination of the electron transport chain and photochemical efficiency of PSII in chloroplasts of bread wheat genotypes during drought and re-watering.

Main points presented to the defense of the dissertation:

– The plant ability to recover after re-watering depends on the

[3] Huseynova, I.M. Hydrogen peroxide generation and antioxidant enzyme activities in the leaves and roots of wheat cultivars subjected to long-term soil drought stress / I.M.Huseynova, D.R.Aliyeva, A.Ch.Mammadov [et al.] // Photosynthesis Research, – 2015, 125(1), – p. 54-60.

water exchange parameters of the leaves;

- Drought-tolerant genotypes were found to have a more dynamic self-recovery ability than sensitive genotypes;
- Wheat plants respond to drought and rehydration by regulating the activity of antioxidant enzymes and isoenzyme content;
- There is a positive correlation between the ability of wheat genotypes to recover under drought conditions and after re-watering and the dynamics of accumulation of antioxidant metabolites.

Scientific novelty of the research. For the first time in Azerbaijan, the processes of recovery after drought and re-watering of local bread wheat varieties have been studied in detail. Comparative analysis of the enzymatic and non-enzymatic components of the antioxidant defense system in wheat leaves has been performed, and changes in photosynthetic parameters have been studied. Although drought resulted in reduced stomatal conductance, rates of photosynthesis, and transpiration in bread wheat genotypes, after re-watering, these parameters were recovered in the tolerant genotype, contrary to the sensitive Tale-38. During water deficiency, the inhibition of the synthesis of photosynthetic pigments was accompanied by a sharp increase in the content of malondialdehyde (MDA), superoxide anion radicals (O^{2-}) and hydrogen peroxide (H_2O_2) in the leaves of both genotypes, which are considered to be indicators of stress. During rehydration, the levels of MDA and H_2O_2 changed differently. Although the formation of free radicals weakened in the tolerant genotype, this process continued in the sensitive genotype. The activity of antioxidant enzymes and isoenzyme content in the studied wheat genotypes responded to drought and re-watering at various levels. It has been found that a new isoform of benzidine peroxidase (BPO2) is formed in the sensitive Tale-38 genotype during drought, indicating its important role in the drought tolerance of the plant. Under drought conditions, low molecular weight metabolites were more concentrated in the tolerant genotype than in the sensitive genotype. Different amounts of osmolites were accumulated in the leaves of Gobustan and Tale-38 genotypes due to drought, which is considered one of the factors ensuring the drought tolerance of wheat.

Although electron transfer rate and PS II efficiency decreased during water deficiency in both genotypes, after re-watering, this indicator increased in the Gobustan genotype and recovered, but no recovery was observed in the sensitive genotype. The results show the genetic plasticity of the Gobustan genotype.

Theoretical and practical significance of the research. The study of changes in morphophysiological, biochemical, and photosynthetic properties of the plant after drought and re-watering is important for understanding the mechanisms of post-stress repair and can be a successful approach in developing drought-tolerant wheat genotypes.

The results obtained are of fundamental importance for a deeper understanding of the plant adaptation mechanisms to abiotic stressors. Determination of activities and isoenzyme content of antioxidant enzymes can be used as a test system in the screening of drought-tolerant genotypes. The Gobustan variety, which demonstrates high physiological parameters during drought and re-watering, can be used as a starting material for the creation of stress-tolerant wheat varieties in practical breeding programs.

Approbation of the work. The main scientific results of the dissertation work were presented and discusssed at the International Scientific Conference "Innovative Problems of Modern Biology" dedicated to the 94th anniversary of National Leader Heydar Aliyev (Baku, 2017), the First International Conference of Young Researchers (Baku, 2017), the 8th International Conference "Photosynthesis and Hydrogen Energy Research for Sustainability" (Hyderabad, 2017), the Second International Conference of Young Researchers (Baku, 2018), the Scientific-Practical Conference of Young Researchers dedicated to the 90th anniversary of Academician Jalal Aliyev (Ganja, 2018), the proceedings of the 1st International Conference "Air-Land-Water Interaction" (Baku, 2018), the 10th International Conference "Photosynthesis and Hydrogen Energy Research for Sustainability" (Saint Petersburg, 2019), the International Symposium of the Society for Plant Breeding (GPZ) (Tulln, 2020), the Second International Scientific Conference of Young Scientists and Specialists on "Multidisciplinary approaches in solving modern problems of fundamental and applied sciences (Natural sciences)" (Baku, 2020), the I International Scientific Conference of Students and Young Researchers on "Sustainable Development in Chemistry and Chemical Engineering" (Baku, 2020), as well as in the laboratories and seminars of the Institute of Molecular Biology and Biotechnologies.

The organization where the dissertation was performed. Experimental analyses in the dissertation were carried out in the Bioadaptation Laboratory of the Institute of Molecular Biology and Biotechnologies of ANAS, and morpho-physiological researches in the field were carried out in the Research Institute of Crop Husbandry of the Ministry of Agriculture of the Republic of Azerbaijan.

Structure and volume of the dissertation work. The dissertation consists of an introduction and 3 chapters, a final analysis of the research, conclusions, recommendations, a list of references, and a list of abbreviations used in the dissertation. The dissertation includes 18 figures, 20 diagrams, 3 graphs, and 1 table; 321 literature sources were used and 50% of them are foreign literature. The dissertation consists of 168 pages, including tables and figures, as well as a list of references.

CONTENT OF THE WORK

Chapter I. Literature Review

This section provides a summary of literature sources on the topic of the dissertation. The mechanisms of morphological, physiological, biochemical, and molecular tolerance of plants to oxidative stress caused by global warming have been studied, and an extensive analysis of the enzymatic and non-enzymatic components of the antioxidant defense system has been provided. The effects of water deficiency on the dark phase of photosynthesis, the electronic transport chain, the activity of PSI and PSII, the stability of the photosynthetic apparatus, and photoassimilation have been clarified. The scientific classification and biological properties of the bread wheat (scientific name *Triticum aestivum* L.) plant, which is the object of the research, have been given in detail.

Chapter II. Objects and Methods of the Research

The objects of the research were local bread wheat varieties Gobustan and Tale 38 with contrasting tolerance and productivity obtained from the Genbank of the Research Institute of Crop Husbandry of the Ministry of Agriculture of the Azerbaijan Republic.

The research was carried out in 2017-2019 in the experimental field of the Department of Plant Physiology and Biotechnology of the Absheron Auxiliary Experimental Farm of the Ministry of Agriculture of the Azerbaijan Republic. In parallel, the experiments were carried out with bread wheat varieties grown under drought, well-watered and re-watered conditions, in an artificial climate chamber in the laboratory. Relative water content in leaves, root length, dry biomass, leaf blade area were measured by morphometric methods. The amounts of hydrogen peroxide[4], malondialdehyde, ascorbic acid, phenolic compounds, tocopherols, proline, glycine betaine were determined spectrophotometrically, and the amount of GSH was measured using a ready-made kit (Sigma-Aldrich).

The activity of antioxidant enzymes was determined spectrophotometrically with corresponding reactions, isoenzyme content was studied using the PAAG electrophoresis method at 3°C for 3 hours at a constant electric current (30 mA) using the Davis method[5,6]. Detection of isoenzymes of APO, CAT, GPO, SOD, BPO, GR enzymes in the gel was performed using appropriate staining buffers.

The amount of free radicals accumulated in the leaves was determined by the histochemical method, photosynthetic parameters

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[4] Bellincampi, D. Extracellular H_2O_2 induced by oligogalacturonides is not involved in the inhibition of the auxin-regulated rolB gene expression in tobacco leaf explants / D. Bellincampi, N.Dipierro, G.Salvi [et al.] // Plant Physiology, – 2000, 122(4), – p. 1379-1386.

[5] Heath, R.L., Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation // Archives of biochemistry and biophysics, – 1968, 125(1), – p. 189-198.

[6] 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.

by the spectrophotometric method[7,8,9]. Gas exchange parameters in leaf samples (photosynthesis rate-Pn, stomatal conductivity-g_s, CO_2 concentration in intercellular areas-C_i, transpiration rate-E) were determined using the LI-COR 6400 XT Portable Photosynthesis System (LI-COR Biosciences, Nebraska, USA).

Statistical analysis: All experiments were performed in 3 repetitions and errors were calculated using the Student's t-test statistical analysis program. When the value of P was <0.01, <0.05, the differences between the mean values were considered significant.

Chapter III. Results and Discussion

3.1. The study of morphophysiological characteristics of the wheat plant under drought and during recovery

Parameters of water and gas exchange were studied in leaves of the wheat plant under drought and during recovery.

It was found that during drought, the length of the root and the amount of dry biomass in the leaves of the plant increased in both Gobustan and Tale 38 varieties. Under drought, the relative water content of the leaves, and the area of the leaf blade decreased. The drought-tolerant Gobustan genotype demonstrated more dynamic recovery than the sensitive variety during re-watering.

Determination of transpiration rate plays an important role in assessing the drought tolerance of plants. Genotypes having a low rate of transpiration and thereby limiting the evaporation of absorbed water are considered more tolerant. Drought stress resulted in a 2-fold decrease in stomatal conductance of the Gobustan genotype and a 1.4-fold decrease in the Tale 38 genotype. While stomatal conductance

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[7] Mahalingam, R. Analysis of oxidative signalling induced by ozone in *Arabidopsis thaliana* / R.Mahalingam, N.Jambunathan, S.K.Gunjan [et al.] / Plant, Cell & Environment, – 2006, 29(7), – p. 1357-1371.

[8] Sims, D.A., Gamon, J.A. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages / //Remote sensing of environment, – 2002, 81(2-3), – p. 337-354.

[9] Huseynova, I.M. Suleymanov S.Y., Aliyev J.A. Structural-functional state of thylakoid membranes of wheat genotypes under water stress // Biochimica et Biophysica Acta, – 2007. vol. 1767, – p 869-875.

increased in the tolerant genotype after re-watering, the sensitive genotype could not recover (Figure 1A).

As the amount of water entering the plant decreases sharply during drought, the stomata are closed to prevent water evaporation (Figure 1C), and the amount of carbon dioxide accumulated in the intercellular space is reduced accordingly (Figure 1D). In both genotypes, the CO_2 concentration decrease in the intercellular space can be attributed to the closure of most stomata and inhibition of photosynthesis during drought (Figure 1B).

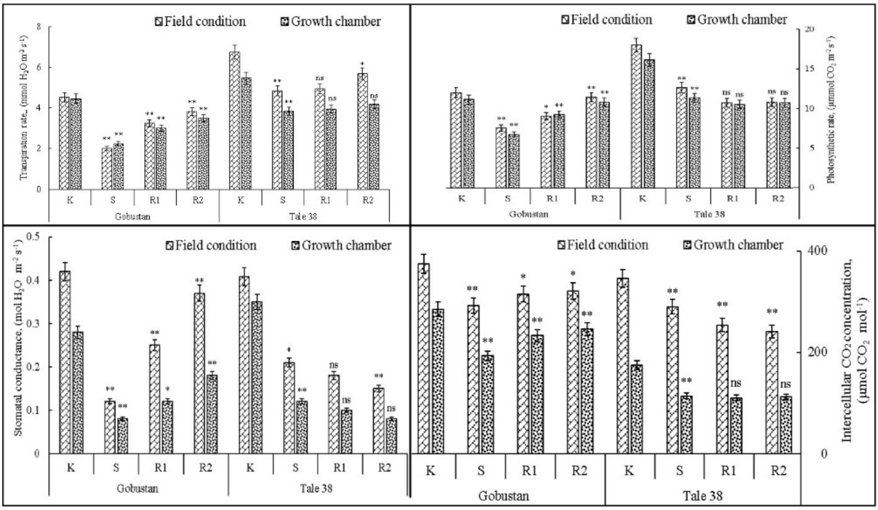

Fig. 1. Results from the measurement of gas exchange parameters in the leaves of bread wheat genotypes (Gobustan and Tale 38) grown under field and artificial climate conditions: K - watered, S - drought, R1 - 3 days after re-watering, R2 - 7 days after re-watering.

3.2. The amount of secondary metabolites - free proline and glycine betaine in the plant under drought and during recovery

The results of the experiments performed with plants grown in the field and under controlled conditions showed a 2-fold increase in the proline amount in the Gobustan genotype and ~1.4-fold increase in the Tale 38 genotype. Although the recovery process in the tolerant genotype after re-watering was fast, the sensitive genotype was unable to recover (Figure 2A).

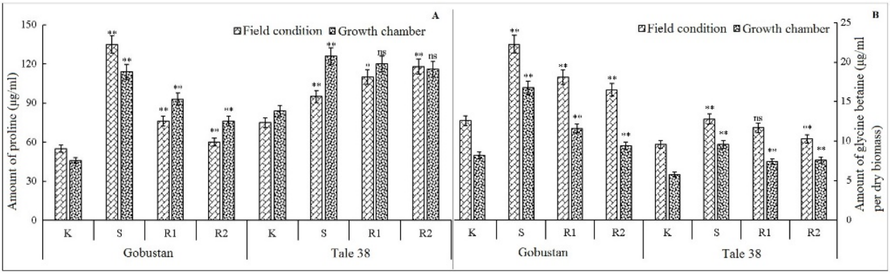

Fig. 2. Osmolites in the leaves of bread wheat (Gobustan and Tale 38) genotypes grown under field and artificial climatic conditions. K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering.

Histochemical analysis revealed the accumulation of ROS in the leaves of bread wheat genotypes damaged by drought stress. Compared to watered plants, in drought variants, a small concentration of superoxide radicals was observed in the leaves of the Gobustan genotype, and a high concentration was observed in the leaves of the Tale 38 genotype. Three and seven days after re-watering, the amount of radicals in the Gobustan variety decreased, and for the Tale 38 genotype, the situation remained almost unchanged (Figure 3A).

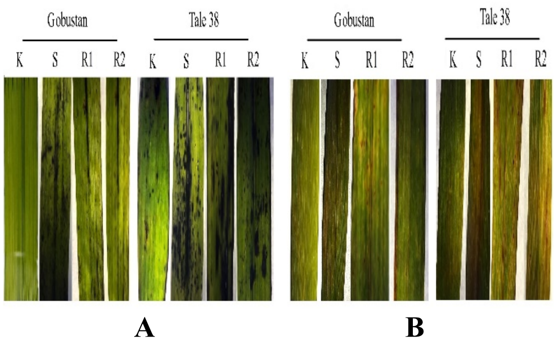

Fig. 3. Accumulation of superoxide and (A) and hydrogen peroxide (B) in leaves of bread wheat genotypes (Gobustan and Tale 38) grown under field conditions: K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering.

3.3. The amount of hydrogen peroxide and intensity of lipid peroxidation in leaves of wheat plants under drought and during re-watering

In both genotypes grown under natural and artificial climatic conditions, a sharp increase in the amount of MDA and H_2O_2, which are indicators of stress in the leaves, was observed due to the effects of drought. Thus, the amount of H_2O_2 in the leaves increased 1.6 and 5 times, respectively. After re-watering, the Gobustan genotype recovered and approached the watered variant, while in the Tale 38 genotype, the plant defense system could not completely eliminate the accumulated H_2O_2 (Figure 4A). The same trend was reflected in the dynamics of the accumulation of MDA, which is considered an indicator of stress. In the drought-tolerant wheat genotype, during rehydration, these indicators are almost completely restored, which indicates a more dynamic recovery ability. From this point of view, based on the MDA accumulation dynamics in the leaves of wheat under drought, this indicator can be used in the screening of tolerant varieties (Figure 4B).

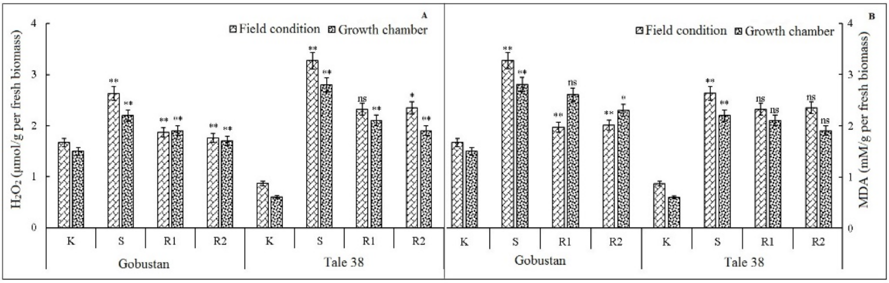

Fig. 4. Amounts of hydrogen peroxide and malondialdehyde in leaves of bread wheat genotypes (Gobustan and Tale 38) grown under field and artificial climatic conditions: K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering

3.4.1. The study of changes in activities and izoenzyme content of antioxidant enzymes during drought and recovery

Activities and izoenzyme content of antioxidant enzymes have been studied in leaves of wheat genotypes during drought and recovery. Under the influence of stress, an increase in the activity of the enzyme ascorbate peroxidase (APO) was observed in the leaves of both genotypes. Although the enzyme activity began to decline in Gobustan after re-watering, there was no statistically significant difference between drought and re-watered variants of the Tale 38 genotype. On the electropherogram, 7 isoforms stained with various intensities were observed in both genotypes. Genotypes have been found to respond to drought and rehydration with qualitative changes in isoenzymes rather than quantities (Figure 5B).

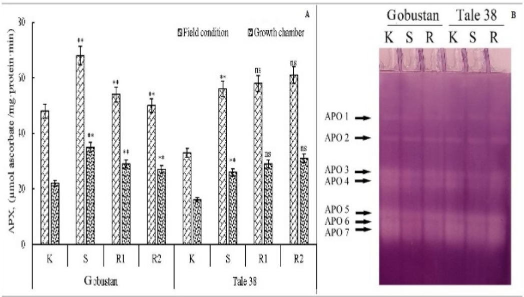

Fig. 5. Activity (A) and izoenzyme content (B) of APO in leaves of bread wheat genotypes (Gobustan and Tale 38) grown under field and artificial climatic conditions: K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering.

According to the results of the experiments, the activity of the enzyme catalase (CAT) under natural drought and artificial drought created in phytotron increased by ~ 32% in the Gobustan genotype and 30% in the Tale 38 genotype compared to the watered variant. Three days after re-watering, the enzyme activity began to decrease, and after seven days it continued to decrease and approached the watered variant. In the Tale 38 genotype, there was no statistically significant change in enzyme activity after re-watering compared to that under drought. The same trend was observed in experiments conducted under artificial climatic conditions (Figure 6A). Only one isoform of the catalase enzyme was observed in the enzyme extract of both genotypes. The enzyme activity was higher in the drought and re-watered variants compared to watered plants and this difference was more pronounced in the enzyme extract obtained from the leaves of the Gobustan genotype (Figure 6B).

Decreased activity of GPO and BPO, type III peroxidases, was observed in field-grown genotypes under drought. Although both enzymes recovered their activity in the Gobustan variety after re-watering, the decline continued in the Tale 38 variety (Figures 7A, 8A).

The electrophoretic study of the isoenzyme content of guaiacol peroxidase revealed high molecular weight (GPO1 and GPO2), and medium molecular weight (GPO3) isoforms of GPO in the Gobustan genotype under normal conditions. Under drought, the intensity of the high molecular weight isoform was reduced and recovered during re-watering. In the Tale 38 genotype, two high molecular weight isoforms (GPO1 and GPO2) were observed in all three variants, which differed in the intensity of staining (Figure 7B).

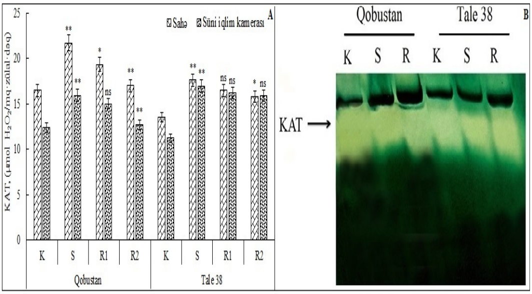

Fig. 6. Activity (A) and izoenzyme content (B) of CAT in leaves of bread wheat genotypes (Gobustan and Tale 38) grown under field and artificial climatic conditions: K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering.

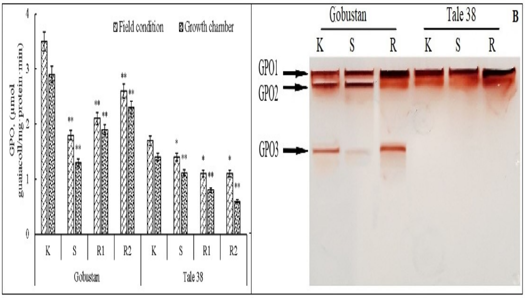

Fig. 7. Activity (A) and izoenzyme content (B) of GPO in leaves of bread wheat genotypes (Gobustan and Tale 38) grown under field and artificial climatic conditions: K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering.

According to the electropherogram, of benzidine peroxidase, four isoenzymes of benzidine peroxidase (BPO1, BPO2, BPO3, BPO4) in the Gobustan genotype and three isoenzymes (BPO1, BPO3, BPO4) in the Tale 38 genotype occurred in the control variant (Figure 8B). Under drought, the intensity of the high molecular weight BPO1 isoform decreased in both genotypes, and this decrease continued after re-watering. The intensity of the low molecular weight BPO4 isoform increased in both drought and re-watered variants. Medium molecular weight isoforms BPO2 and BPO3 lost under drought in the Gobustan genotype were almost completely recovered after re-watering. In the sensitive Tale 38 genotype, the intensity of the medium molecular weight BPO3 isoform decreased in the drought variant, and the synthesis of this protein increased again after re-watering. Interestingly, a new isoform (BPO2) is formed in the drought variant of this genotype, which is believed to play an important role in the drought tolerance of the plant.

In the field-grown Gobustan genotype, the activity of the glutathione reductase (GR) enzyme increased by 41% under stress compared to the watered variant, and by 16% under the phytotron condition, and after rehydration, the activity decreased and approached the watered variant. In the Tale 38 genotype, a sharper increase in the enzyme activity was observed under drought, and there was no marked change in enzyme activity in this genotype after re-watering.

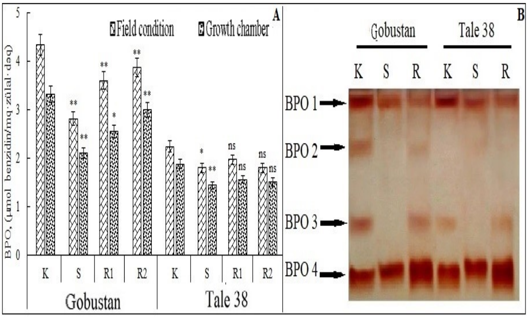

Fig. 8. Activity (A) and izoenzyme content (B) of BPO in leaves of bread wheat genotypes (Gobustan and Tale 38) grown under field and artificial climatic conditions: K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering.

Electrophoretic analysis revealed seven isoforms of GR in all variants of both genotypes. Although the intensity of high molecular weight isoforms increased under drought, the intensity decreased again after rehydration. No significant change in the intensity of medium and low molecular weight isoforms was observed during rehydration.

3.4.2. The role of low molecular weight metabolites under drought and during the subsequent recovery

Low molecular weight metabolites are key components of the cell antioxidant defense system, facilitating the plant recovery after oxidative stress with minimal damage. The results of the analysis showed that the amounts of ascorbic acid (1.6 and 1.3 times, respectively), GSH (72% and 53%, respectively), phenolic compounds (29%, and 36%, respectively), and tocopherols increased

(1.8 and 2 times, respectively) in the leaves of both tolerant and sensitive genotypes under drought, and after re-watering, they decreased relative to drought variants and approached the watered variant. Analysis of the dynamics of accumulation of small molecular weight metabolites in plant leaves during drought and recovery revealed that under drought, these compounds accumulate more in the tolerant genotype compared to the sensitive genotype. It can be concluded from the results that the Gobustan genotype is able to recover more quickly from the damaging effects of drought by responding to re-watering processes due to a strong antioxidant defense mechanism compared to the Tale 38 genotype,.

3.5. The study of photosynthetic parameters in leaves of the wheat plant during drought and the subsequent recovery

3.5.1. The amounts of photosynthetic pigments during drought and rehydration

Due to the cleavage of photosynthetic pigments and reduced gas exchange during drought, the plants become shorter and their productivity decreases. The accumulation of osmolites as a result of stress and the enhancement of the synthesis of ROS are considered to be the response of plants to drought.

Drought has been found to reduce the amount of photosynthetic pigments in plant cells. In the field-grown Gobustan genotype, the total amount of chlorophyll was reduced by 22% during the drought. Three days after re-watering, the leaf pigments were recovered by 19%, and seven days after re-watering, 3% was recovered approaching the watered variant. In the sensitive Tale 38 genotype, total chlorophyll decreased by 21% during water deficiency and recovered by 9% after 3 days of re-watering but a 7% decrease was observed again after 7 days of re-watering.

Under the controlled artificial climatic conditions, a decrease in the chlorophyll content was observed in the Gobustan genotype during drought, and pigment recovery occurred 3 days after re-watering. In the Tale 38 genotype, total chlorophyll levels decreased by 22% during water shortages, and although chlorophyll levels were relatively recovered 3 days after re-watering, no change was observed after 7 days. Thus, the recovery processes after re-watering were more intensive in the Gobustan genotype.

A decrease in the amount of carotenoids was observed in both genotypes under drought. In the field-grown Gobustan genotype, the amount of carotenoids was reduced in general, by 20% during the drought, and the amount of the pigments in the leaves was recovered 3 and 7 days after re-watering, approaching the watered variant. In the Tale 38 genotype, the amount of carotenoids decreased by a total of 29% during water deficiency, an increase in the amount of pigments was observed after re-watering, and a relative recovery occurred in the end.

Under artificial climatic conditions, the amount of carotenoids in the Gobustan genotype decreased in general during drought, and the amount of pigments was recovered after re-watering (R1 and R2). In the Tale 38 genotype, carotenoid levels were reduced by 35% during water deficiency and recovered 3 and 7 days after re-watering. Thus, the Gobustan genotype maintained the recovery ability.

3.5.2. The study of the photosynthetic efficiency in the leaves during drought and rehydration

Under field conditions, the maximum photochemical efficiency (Fv/Fm) in the Gobustan and Tale 38 genotypes was approximately the same (0.811 and 0.808, respectively). Due to drought stress, the Fv/Fm ratio in the Gobustan genotype decreased by 8% and in the Tale 38 genotype by 16%, and maximum photochemical efficiency began to recover at different levels in both genotypes 3 and 7 days after re-watering. Thus, the Gobustan genotype recovered by 6% after re-watering, and the Tale 38 genotype by 5% (Figure 9). The statistical analysis also confirmed the absence of significant changes during the drought in the Gobustan variety. In the Tale 38 variety, Fv/Fm decreased during drought whereas the recovery process was not completed. Similar results were observed with plants grown in artificial climate chambers. According to the results of the analysis, the Gobustan genotype demonstrated photochemical stability against drought, achieved less damage to the photosynthetic apparatus, and was successfully recovered.

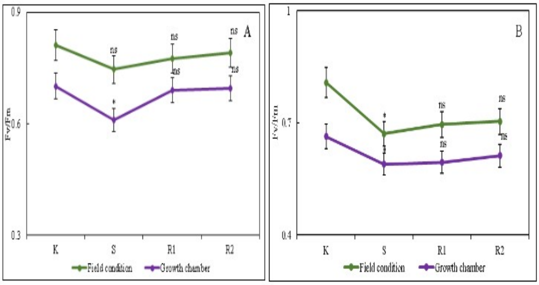

Fig. 9. Photosynthetic efficiency in leaves of Gobustan (A) and Tale 38 (B) varieties grown under field and artificial climatic conditions: K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering

3.5.3. The study of the electron transport chain in chloroplasts during drought and rehydration

During drought, the amount of CO_2 assimilated due to temperature rise, water deficiency, and stomatal closure decreases, whereas the amount of ROS formed by stress increases, which causes damage to the electron transport chain. During field experiments, the electron transport chain was reduced by 40% in the Gobustan genotype and by 57% in the Tale 38 genotype under drought. The electron flow was restored in both genotypes in the samples taken 3 days after re-watering. Although the Gobustan genotype maintained its recovery ability 7 days after re-watering, the electron transport chain was observed to weaken in the Tale 38 genotype (Figure 10). A similar trend was observed in wheat grown in artificial climate chambers. Although the electron flow, which had been severely weakened by the drought, was recovered in the samples taken 3 and 7 days after re-watering in the Gobustan variety, it increased after 3 days in the Tale 38 genotype and began to decrease after 7 days (Figure 10).

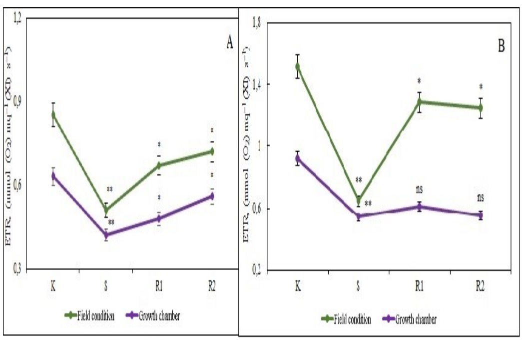

Fig. 10. Changes in electron transfer rate of the electron transport chain in leaves of bread wheat genotypes (Gobustan (A) and Tale 38 (B)) grown under field and artificial climatic conditions depending on drought and re-watering: K – watered, S – drought, R1 – 3 days after re-watering, R2 – 7 days after re-watering.

The photosynthetic apparatus can not only convert solar energy into the energy of chemical bonds needed for cell metabolism but also acts as a sensitive receptor. Therefore, it reacts immediately to changing environmental factors. The results show that in wheat genotypes during drought, the electron transport chain of PSII can restore its function at different levels after the re-watering process.

Thus, the observed changes may form the basis of defense-adaptation processes aimed at increasing the drought tolerance of wheat genotypes.

CONCLUSIONS

1. For the first time in Azerbaijan, photosynthetic gas exchange parameters have been studied in bread wheat genotypes exposed to drought stress and re-watering. Under normal watering, the stomatal conductance, the concentration of carbon dioxide in the intercellular space, and the rates of photosynthesis and transpiration were higher in the Tale-38 genotype compared to the Gobustan genotype. Although these rates decreased in both genotypes under drought, they approached the control level in the drought-tolerant Gobustan genotype after re-watering and did not change or continued to decrease in the drought-sensitive Tale-38.
2. Under stress, the relative water content of the flag leaf decreased in both genotypes (79 and 80% in the control variants and 54 and 51% in the stress variants, respectively). The Gobustan genotype recovered after re-watering (78%), and in the Tale-38 genotype, the self-recovering process was found to be relatively weak (70%). This is attributed to the fact that the root system in the Gobustan genotype extends deeper and is less exposed to osmotic effects.
3. Under the drought in the leaves of Gobustan and Tale-38 genotypes, a sharp increase in the amount of MDA (2 and 3 times, respectively) and H2O2 (1.6 and 4 times, respectively), which are indicators of stress was accompanied by the inhibition of the synthesis of carotenoids, chlorophyll a and b. During rehydration, MDA and H_2O_2 levels decreased differently in both genotypes, and photosynthetic pigments were almost completely restored in the

drought-tolerant genotype.

4. Histochemical analysis of free radicals in plant leaves showed that during drought, the amount of superoxide anion (O_2^-) radicals and H_2O_2 increased in both genotypes. Although the formation of free radicals after rehydration was weakened in the tolerant genotype, this process continued in the sensitive genotype. These results can be explained by the genetic plasticity of the Gobustan genotype.
5. During water deficiency, activities of the enzymes ascorbate peroxidase (APO), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) increased in both genotypes compared to the watered variants, while activities of benzidine peroxidase (BPO) and guaiacol peroxidase (GPO) decreased. After rehydration, these enzymes were found to restore their activity more dynamically in the tolerant genotype. Besides, feedback in the action of CAT and PO enzymes involved in the utilization of H2O2 was observed, which can be explained by the distribution of H_2O_2 in different areas [15-17].
6. In the control variants, 4 constitutive isoforms of BPO and 3 of GPO were found in the tolerant Gobustan genotype, and 3 constitutive isoforms of BPO and 2 of GPO were detected in the sensitive Tale-38 genotype. These genotypes responded to drought and re-watering with quantitative and qualitative changes in their isoforms. During water deficiency, medium molecular weight BPO2, BPO3, and GPO3 in the Gobustan genotype and medium molecular weight BPO3 isoform in the Tale-38 genotype disappeared and were restored after re-watering. In the sensitive Tale-38 genotype, a new BPO2 isoform was formed during drought, indicating that it plays an important role in the drought tolerance of the plant [6, 10].
7. Under drought, a statistically significant increase in the content of ascorbic acid, glutathione, tocopherols, phenolic compounds, glycine-betaine, and proline occurred in the leaves of Gobustan and Tale-38 varieties. After 3 days of re-watering, the amount of proline in the tolerant variety was significantly reduced, and after 7 days it reached control levels. No self-recovering processes were observed in the sensitive genotype. Such a change in the amount of proline

can be considered one of the mechanisms that ensure the drought tolerance of wheat.

8. Electron transfer rate (ETR) and photosynthetic efficacy of PS II in chloroplasts isolated from wheat genotypes during drought and rehydration were studied. ETR decreased in both genotypes under stress and was restored during re-watering. The maximum photochemical efficacy of PS II was also reduced in both genotypes, but after re-watering, contrary to the Tale-38 genotype, this indicator increased in the Gobustan genotype and recovered. The weakening of photosynthetic efficiency is thought to be due to disruption of the electronic transport chain between PS II and PS I and/or reduced CO_2 assimilation [13, 15].

RECOMMENDATIONS

1. Use of the Gobustan genotype, which demonstrates high-speed (dynamic) recovery of physiological indicators, as a starting material in the creation of stress-tolerant wheat varieties in practical breeding programs;
2. Cultivation of the Gobustan genotype in dryland and semi-arid regions;
3. It is recommended to use the determination of the amount and photosynthetic effectiveness of proline as a test system in the assessment of drought-tolerant wheat genotypes.

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