Determination of the mechanisms of resistance of *Helianthus annuus* L. to drought using the osmopriming method

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Abstract. The purpose of the study is to assess the physiological and antioxidant parameters of sunflower seedlings under osmopriming. Two series of experiments were carried out aimed at: 1) establishing the viability of seeds; 2) assessment of physiological parameters of seedlings. Each experiment included a group of control samples grown under conditions of sufficient moisture and four impact groups exposed to varying levels of osmotic stress. The intensity of accumulation of lipid peroxidation products and the rate of accumulation of reactive oxygen species were determined based on the reaction of malondialdehyde with thiobarbituric acid. Catalase activity was determined photocolorometrically by the interaction of hydrogen peroxide with potassium iodide, the content of chlorophylls a (*Cl a*) and b (*Cl b*), carotenoids (*Car*) - spectrophotometrically in an acetone extract. It has been established that as a result of increasing moisture deficiency, the energy of seed germination decreases in proportion to the increase in the concentration of the osmotic solution. When stress increases to 3.5 atm, seed germination decreases by 29%, and to 8 atm – by 64%. A linear relationship was revealed between the inhibition of the photosynthetic system and the stress factor of moisture deficiency, expressed in a decrease in *Cl* a by 60% relative to the control with osmopriming of 8 atm. It has been established that a slight decrease in the moisture supply of the substrate causes oxidative stress of cells, as evidenced by a linear increase in malondialdehyde with increasing moisture deficiency. At the same time, antioxidant protection is provided by the enzyme catalase, the concentration of which increases with increasing drought, while low-molecular carotenoids have an indirect effect on the provision of protective antioxidant mechanisms - there is a trend of increasing *Cl a* + *Cl b* / Car with a decreasing *Cl a* / *Cl b*.

1 Introduction

Reduced yields of major food crops under abiotic stress such as drought are becoming a serious problem worldwide, affecting food security. At the same time, even short periods of lack of moisture combined with high temperatures can negatively affect the formation of yield. To cope with drought stress, plants can improve water uptake and retention by

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reducing leaf area, reducing photosynthetic activity, or accumulating osmoregulators under water-stressed conditions.

Identifying the physiological and molecular mechanisms of plant response to drought stress remains a major challenge. Plants rely on various physiological mechanisms, including photosynthesis, antioxidant systems, plant hormones, and secondary metabolism, to attempt to mitigate the adverse effects of drought on growth, development, and yield. A process such as osmotic regulation, in which the plant maintains cell turgor pressure at reduced soil water potential, is classified as a mechanism of drought tolerance. Soil moisture deficiency at this stage inhibits the ability of seeds to absorb essential water from the soil and delays germination by inhibiting enzymes responsible for hydrolyzing endosperm starch into metabolizable sugars and providing energy for plant growth and this can lead to loss of yield. As a result, this can significantly reduce the actual germination rate, resulting in uneven germination. In addition, drought stress can induce oxidative stress and increase the production of reactive oxygen species (ROS) in seeds. This causes a decrease in cell volume, causing the cellular contents to become more viscous, which ultimately leads to protein denaturation and aggregation and abnormal functioning of photosynthesis-related enzymes. When developing methods for selecting agricultural crops for drought resistance, it is advisable to preliminary simulate the limited supply of liquid into the cells of the crop in laboratory conditions, i.e. creating a high osmotic potential in the nutrient medium by introducing solutions of osmotic agents (sucrose, mannitol and polyethylene glycol).

Sunflower (*Helianthus annuus* L.) is an annual plant belonging to the Asteraceae family, which is the fourth largest source of vegetable oil in the world and has important agricultural importance. Sunflower has strong adaptability to various abiotic stresses. However, the growing shortage of water resources has seriously affected the yield and quality of sunflower seeds. Crop production is threatened by various stressors, including drought and heat. During germination, seeds are exposed to various biotic and abiotic stresses, which individually or jointly inhibit germination and seedling development. Depending on the severity of stress and genetic background, germination is either delayed or suppressed. Seeds become susceptible to oxidative stress when exposed to stress conditions, which is an imbalance between the production of reactive oxygen species (ROS) and enzymatic and non-enzymatic antioxidant mechanisms.

To date, research on drought tolerance in sunflower has mainly focused on drought tolerance identification, physiological trait analysis, and molecular markers. An increase in the osmotic pressure of the solution leads to a significant transformation of pigment systems in drought-resistant varieties. Based on this, the goal of this study was to assess the effect of osmopriming on the stress resistance of sunflower seedlings under simulated drought conditions.

2 Materials and methods

Object of study – *Helianthus annuus* L. 'Poseidon 625'. The variety is defined as a crop with a high degree of drought resistance, therefore, to assess the mechanisms of resistance to the drought factor, a study was conducted to simulate conditions of moisture deficiency using the osmopriming method.

Two series of experiments were carried out:

1. Germination of seeds in Petri dishes in a thermostat under the same conditions of temperature (25 °C), humidity (60%) and in the absence of light. The lack of moisture (drought) during seed germination in laboratory conditions can be simulated by adding a 1.4% sucrose solution to Petri dishes, which is equivalent to normal atmospheric pressure

(745 mmHg). In subsequent versions of the experiment, conditions for increasing moisture deficiency were simulated by increasing the concentration of the osmotic solution.

- Control – distilled water.
- Option 1. Osmopriming – sucrose solution 1.4% (P = 1 atm).
- Option 2. Osmopriming – sucrose solution 4.4% (P = 3 atm).
- Option 3. Osmopriming – sucrose solution 7.4% (P = 5 atm).
- Option 4. Osmopriming – sucrose solution 10.5% (P = 8 atm).

2. Growing seedlings in growing vessels.

The experiment was carried out in a JIUPO BPC500 climate chamber. The moistened soil was placed in containers, followed by sowing sunflower seeds. Simulation of drought conditions was carried out by stopping watering 5-day-old seedlings for 2 days, reducing humidity to 15% without adding an osmotic solution. In the variants of the experiment with osmopriming, the osmotic solution was added to containers with 5-day-old seedlings for 2 days.

The study of pigment composition and assessment of antioxidant protection parameters was carried out on 7-day-old seedlings. The experimental design is shown in Figure 1.

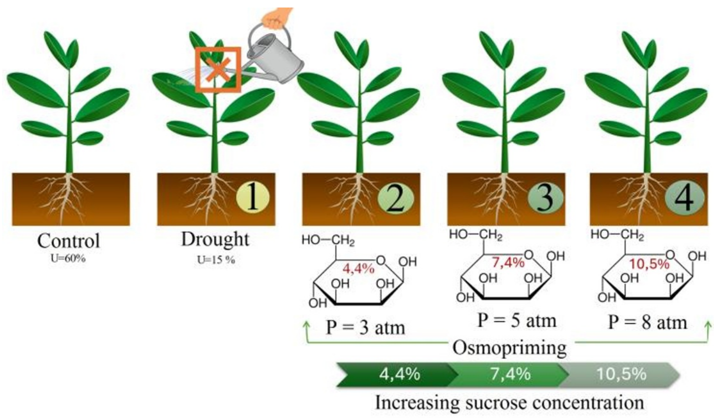

Fig. 1. Experimental scheme for growing sunflower seedlings.

- Option 1. Drought – stopping watering 5-day-old seedlings for 2 days, U = 15% (P ~ 1 atm).
- Option 2. Osmopriming – U = 15%, sucrose solution 4.4% (P = 3 atm).
- Option 3. Osmopriming – U = 15%, sucrose solution 7.4% (P = 5 atm).
- Option 4. Osmopriming – U = 15%, sucrose solution 10.5% (P = 8 atm).

Isolation and calculation of FP was carried out in acetone extracts of biomaterial - in the cotyledons of 8-day-old sunflower seedlings according to the Shlyk method, catalase (CAT) - according to the Maehly and Chance method. The optical density of the extracts was determined on a KFK 3-01 spectrophotometer at different wavelengths: for chlorophyll a (Cl a) - 662 nm, chlorophyll b (Cl b) - 644 nm, carotenoids (Car) - 440.5 nm, CAT - 440 nm in triplicate. Data analysis was performed based on 60 series of experiments.

Statistical processing of the research results was carried out using the Statistica 10.0 program.

3 Results and Discussion

Figure 3 shows the features of the formation of seedlings in a sucrose solution. The maximum germination energy was noted in the control version of the experiment and was

80%. Indicators of seed germination energy are recorded close to the control when germinated in a 1.4% sucrose solution, simulating the absence of watering. The energy of seed germination throughout the experiment shows a tendency to decrease with increasing stress factor and reaches its minimum at osmopriming of 8 atm. The germination rate of seeds is higher than the energy of their germination in each variant of the experiment. The maximum of sprouted seeds was found not only in the control, but also in a 1.4% sucrose solution and amounted to more than 90%. Despite the primary depression in the germination energy of seeds germinated in a 1.4% sucrose solution, their high germination rate remains. With an increase in osmotic stress, germination significantly decreases to 64% in 4.4 and 7.4% sucrose solutions. The minimum germination rate of 29% was observed when seeds were germinated in a 10.5% sucrose solution (Figure 2).

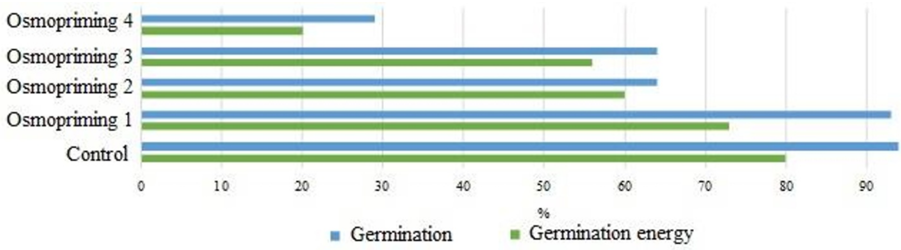

Fig. 2. Germination and germination energy of sunflower seeds when exposed to osmotic solutions of different concentrations.

An insufficient supply of soil moisture has a greater effect on germination than on the energy of seed germination. Seed germination in the control has significant differences with the experimental variants with solutions of 3, 5 and 8 atm. The difference in germination in a solution with an osmotic pressure of 1 atm is insignificant. However, it was noted that seed germination in solutions of 1, 3 and 5 atm was significantly higher than in solutions of 8 atm (Table 1).

Table 1. Determination of the degree of influence of different osmotic pressure on the germination of sunflower seeds (Tukey's HSD test).

Pressure, atm.	{1}	{2}	{3}	{4}	{5}
Control {1}	-				
1 {2}	0.8	-			
3 {3}	0.008*	0.07	-		
5 {4}	0.013	0.11	0.99	-	
8 {5}	0.0002	0.0004	0.047	0.0304	-

* - statistically significant values are highlighted

In model systems, FP activity was analyzed under soil moisture deficiency. Under normal humidification, the maximum concentration of 0.4 mg/g was noted for *Cl a* as the main FP and the minimum concentration of 0.2 mg/g for *Cl b*. The ratio coefficient (*Cl a*/*Cl b*) is 1.8. When watering of 5-day-old sunflower seedlings is stopped, a decrease in *Cl a* to 0.2 mg/g is observed, which indicates a decrease in photosynthetic activity due to moisture deficiency in the soil. There is also a significant increase, more than 2 times in comparison with the control, in the concentration of *Cl b*, which indicates the activation of the protective response of the photosynthetic system to stress. The ratio coefficient (*Cl a*/*Cl b*) is reduced to 0.5.

Car are low molecular weight compounds, one of the functions of which is to protect the plant from oxidative stress under the influence of a stress factor. However, in an experiment with no watering of sunflower seedlings, a decrease in their concentration was noted from 0.22 in the control to 0.16 mg/g. It can be assumed that antioxidant protection during hydration deficiency is provided by high molecular weight antioxidants (Figure 3).

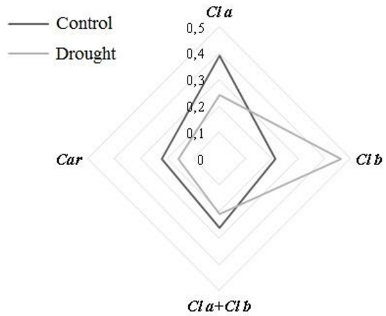

Fig. 3. FP ratio under different moisture conditions.

Further research is aimed at analyzing a set of data on the photosynthetic activity of seedlings under conditions of increasing osmotic stress, simulating an increasing moisture deficit in the soil. An increase in depression of the photosynthetic system with increasing osmotic stress was noted. An increase in the concentration of *Cl b* and a decrease in *Cl a* in comparison with the control are clearly determined, which provides grounds for stating disturbances in the functioning of the photosynthetic apparatus. Moreover, the *Cl b* content increases in proportion to the concentration of the osmotic substance solution - from 0.21 mg/g in the control to 0.52 mg/g under stress of 8 atm. Due to the activation of signal molecules *Cl b*, which contribute to the capture of rays of the short-wave part of the spectrum, the work of the main photosynthesis pigment - *Cl a* - is supported, therefore the decrease in the concentration of this pigment with increasing osmotic stress is not so significant - from 0.39 mg/g in the control to 0.25 mg /g under stress of 8 atm. Carotenoids, like *Cl a*, show a tendency to decrease and at the maximum concentration of the osmotic solution, their concentration reaches 0.17 mg/g (Figure 4).

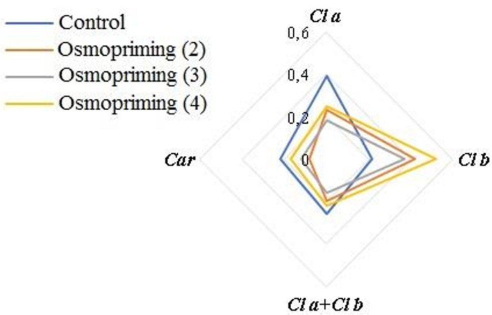

Fig. 4. Changes in FP activity during exposure (two days) to increasing concentrations of osmotic solution in the soil.

In turn, the ratio of green pigments differs markedly from the control in plants grown on sucrose medium and shows a downward trend, which indicates an increase in the stress factor. However, the ratio of green to yellow pigments follows an increasing trend compared to the control, indicating that carotenoids indirectly perform an antioxidant function (Figure 5).

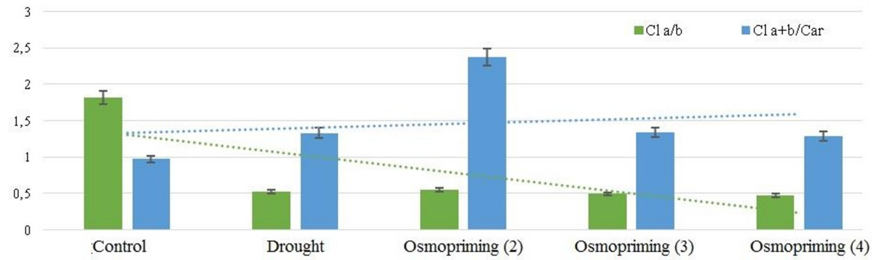

Fig. 5. Coefficients of photosynthetic activity at increasing concentrations of osmotic solution in the soil.

The content of malondialdehyde (MDA) in the biomass of seedlings indicates damage to membrane lipids as a result of their peroxidation by reactive oxygen species. The level of its content in the experiment increases in proportion to the degree of stress exposure. The minimum indicator was noted in the control and the maximum, respectively, when the highest concentration of sucrose was introduced into the experiment in option 4. Plant adaptation to stress caused by drought is closely related to CAT, as a marker antioxidant enzyme that reduces the toxic effect of reactive oxygen species and contributes to the stabilization of metabolic processes of the plant organism. Due to the increase in the concentration of lipid peroxidation (LPO) in sunflower cells under the influence of osmotic stress, there is a proportional increase in catalase synthesis. The study found that the concentration of catalase (CAT) increases with increasing soil moisture deficiency from 5 units in the control to 60 units with an osmotic stress of 5 atm. It was noted that at a maximum osmotic stress of 8 atm, the enzyme concentration decreases almost 2 times compared to the previous version of the experiment, which indicates a decrease in the pool of high-molecular defense systems and a critical disruption of the vital activity of seedlings. The only substrate of CAT is perhydrol, as evidenced by an increase in malondialdehyde and causing a multiple increase in the concentration of the enzyme. This helps to catalyze the reaction of perhydrol decomposition into water and molecular oxygen, thereby protecting cells from the damaging effects of lipid peroxidation (Figure 6). Based on previously described results, sunflower seedlings can regulate the activity of antioxidant enzymes, reactive oxygen species content under drought stress (Jie Shen et al., 2023).

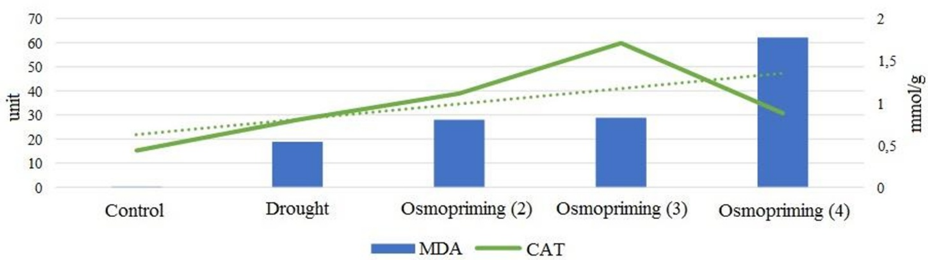

Fig. 6. The ratio of the level of TBA-reactive products and the antioxidant enzyme in the phytomass of sunflower seedlings.

Under drought conditions, plants exhibit different regulatory mechanisms. Thus, in experimental samples growing in different moisture conditions, a statistically significant (at $p < 0.05$) relationship between the concentrations of *Cl a* and *Cl b* (r = 0.9) and an inversely proportional relationship between *Cl a*, *Cl b* and CAT was proven (r = –0.6, r = –0.7), which proves the role of *Cl b* as a signaling molecule under the action of a stress factor and catalase as an enzyme that provides protection of the plant organism from the damaging effects of drought (Table 2).

Table 1. Correlation matrix for analyzed parameters (according to Spearman).

Options	*Cl a* (1)	*Cl b* (2)	*Car* (3)	CAT (4)	MDA (5)
1	1				
2	0.932	1			
3	0.004	0.071	1		
4	- 0.649	- 0.705	- 0. 015	1	
5	- 0.304	0.002	0.086	- 0.05	1

Experimental plants coped with stress by enriching in restorative signaling molecules and activating photosynthetic metabolism, which is consistent with previous studies in sunflower. The results provide a theoretical basis for elucidating the molecular mechanism underlying sunflower adaptation to drought.

4 Conclusion

Based on the results of the confession, a number of conclusions were formulated:

- The maximum value of germination energy of seeds of *Helianthus annuus* L., 'Poseidon 625' in the control version of the experiment was set to 80%. As a result of an increase in moisture deficiency, the energy of seed germination decreases by 7%, 23%, 26% and 60% in proportion to the increase in the concentration of the osomotic solution.
- It is noted that the germination rate is maximum in the control and is determined at the level of 93%. There is no difference between this parameter in the control and the variant with osmopriming of 1.4% sucrose solution. This indicates the high drought resistance of sunflower and is manifested in the maintenance of seed viability with a non-critical lack of soil moisture. When osmotic stress increases to 3 and 5 atm, seed germination decreases by 29%; when exposed to stress of 8 atm, it decreases by 64% compared to the control.
- Modeling of drought conditions using osmopriming methods showed the negative impact of soil moisture deficiency on sunflower seedlings. A linear relationship was revealed between the inhibition of the photosynthetic system and the stress factor - moisture deficiency, expressed in a decrease in the main photosynthetic pigment - *Cl a* by 60% relative to the control at maximum osmotic stress.
- It has been shown that an increase in moisture deficiency leads to a depletion of the pool of low molecular weight antioxidants (*Car*) - by 70% in contrast to normal moisture conditions. The nature of the influence of exogenous sucrose has an ambiguous manifestation: the stress effect is manifested not only for green FPs, but also negatively affects the content of intracellular low-molecular antioxidants.
- The role of *Cl b* as a marker of osmotic stress has been proven. The concentration of this pigment increases by 40% relative to the control with increasing soil moisture deficiency. The accumulation of lipid peroxidation under drought stress contributes to an increase in the content of TBA-reactive products in sunflower phytomass, which is confirmed by an increase in the concentration of malondialdehyde in the cells.
- It has been established that CAT is the main component of the antioxidant protection of sunflower seedlings. Due to the activation of its synthesis, the concentration of *Cl b* decreases, which is evidence of the activation of antioxidant defense mechanisms (r= - 0.7) from lipid peroxidation that accumulates in the plant body due to a lack of moisture.

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