Influence of low-intensity electromagnetic radiation of the millimeter range on the antioxidant system of juvenile *Glycine max* L. plants under osmotic stress

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Abstract. This study is devoted to identifying the adaptogenic effect of low-intensity electromagnetic radiation (EMR) of the millimeter (MM) range (wavelength – 7.1 mm, radiation frequency – 42.3 GHz, radiation power flux density – 0.1 mW/cm²) on the antioxidant system indicators of juvenile plants *Glycine max* L., cv. Apollo under osmotic stress caused by salinity. The stimulating effect of millimeter-wave electromagnetic radiation on the content of proline and carotenoids in *Glycine max* L. plants was revealed. The results obtained on the quantitative content of carotenoids showed that their content increased both in the normal conditions and under osmotic stress by an average of 1.2–1.8 times. An increased content of free proline in leaves was found in comparison with soybean roots under optimal conditions and under salinity.

1. Introduction

The effect of low-intensity electromagnetic radiation (EMR) of the millimeter (MM) range on plants has been studied by researchers from different countries for over 50 years [1, 2]. However, the mechanism of its action has not yet been fully elucidated, but it has already been established that during the processing of EMR, enzymes are activated in the plant cell. In plants grown from treated seeds, the energy of germination, laboratory and field germination increases, productivity and resistance to unfavorable environmental conditions, including those of a biogenic nature, increase [2]. Such a plant response to EMR treatment is associated with the triggering of stress reaction and the formation of an excess of reactive oxygen species (ROS). First of all, the antioxidant system (AOS) of plants reacts to the effect, including low- and high-molecular components, enzymatic and non-enzymatic components of defense [3].

Non-enzymatic components include carotenoids and proline (Pro). It was shown that an increase in the content of proline in plants characterizes the physiological response to salinity, deficiency of mineral nutrition, and other unfavorable factors. When salinized, plants experience a water deficit, and proline is one of the most common osmolytes that maintain the osmotic potential of the cell.

One of the promising methods, which is aimed at maintaining a balance, increasing yield and plant resistance to unfavorable environmental factors, in particular to osmotic stress, is the pre-sowing effect of EMR of the MM range [4, 5].

In connection with the abovementioned, the purpose of this study was to identify the adaptogenic effect of low-intensity EMR of the MM range on the antioxidant system of *Glycine max* L., cv. Apollo under osmotic stress.
2. Research methodology
The object of the study was the seeds and plants of cultivated soybean (*Glycine max* (L.) Merr.), cv. Apollon [6, 7].

The seeds of this plant were selected by their average size and soaked for 20 minutes in a solution of hydrogen peroxide for disinfection, after which they were exposed to a low-intensity EMR of the MM range. Untreated seeds served as control.

We used therapeutic generators “EHF RAMED-EXPERT – 04” (wavelength – 7.1 mm, radiation frequency – 42.3 GHz, radiation power flux density – 0.1 mW/cm²) for experimental irradiation. The seeds were exposed to this physical factor once with exposition of 30 minutes [4].

Then the seeds were germinated in cuvettes on moistened filter paper, 25 seeds in each cuvette, 3 replicates in a thermostat of the type (TC–80–M–2) for 3 days in the dark at + 25 °C, in accordance with the requirements of the state standard 12038–84 for agricultural crops.

To simulate osmotic stress, 150 ml of NaCl solution with a concentration of 50 mM, 100 mM, 150 mM was poured into the cuvettes.

The experiment scheme was as follows:
- Control 1 – seeds, without exposure to MM radiation;
- Control 2 – seeds exposed to MM irradiation;
- Variant 1 – seeds, with the addition of 50 mM NaCl to Petri dishes;
- Variant 2 – seeds, with the addition of 100 mM NaCl to Petri dishes;
- Variant 3 – seeds, with the addition of 150 mM NaCl to Petri dishes;
- Variant 4 – seeds exposed to MM-irradiation, with the addition of 50 mM NaCl to Petri dishes;
- Variant 5 – seeds exposed to MM-irradiation, with the addition of 100 mM NaCl to Petri dishes;
- Variant 6 – seeds exposed to MM irradiation, with the addition of 150 mM NaCl to Petri dishes.

On the 4th day, we transferred the seedlings to an aqueous culture (Knop medium) and grew under natural light in vegetation vessels with a capacity of 0.5 liters.

To determine the content of free proline, we took three samples of leaves, 1 g each. Dry plant material was ground into powder and extracted with 3% sulfosalicylic acid and transferred to a filter. Then, 2 ml of the filtrate was taken and further determination was carried out. The proline concentration was determined using a calibration graph. The calculation results were expressed in milligrams per gram of dry matter [8].

Carotenoids are classified as antioxidants of the lipid phase, which in turn protect the photosynthetic apparatus of plants from the effects of reactive oxygen species; they also perform a membrane stabilizing function. The antioxidant role of carotenoids is mainly expressed in the binding of singlet oxygen, but they can also detoxify peroxide radicals. The action of osmotic stress triggers the accumulation of carotenoids.

To determine carotenoids, a sample of 300 mg plant material was ground in a mortar with the addition of calcined Na₂SO₄ (2– 4 g) for 10 min. Then we added 4 ml gasoline to the powder, ground for another 2 minutes, then another 2 ml of gasoline was added, and ground for 1 minute, and filtered. The extract was brought to a certain volume (10 ml). Optical density was measured using photoelectrocolorimetry. To construct a calibration graph, a K₂Cr₂O₇ solution was used: 290 mg of solution dissolved in 1 l of water (1 ml of this solution corresponds in color to 2.35 μg / ml of carotene and 2.52 μg / ml of xanthophyll) [8].

Statistical processing of the data obtained was carried out by calculating the arithmetic mean and standard error of the arithmetic mean, standard deviation, as well as the Kruskal-Wallis test of reliability. All measurements and studies were carried out on equipment that passed metrological verification and expertise [9].

3. Research results

3.1. Evaluation of the influence of MM radiation on the content of free proline in *Glycine max* L. plants under chloride salinity
The study revealed a higher content of Pro in the top organs of *Glycine max* L. compared to the root system, which can be a quantitative measure of water stress, since when plants adapt to unfavorable factors, including salinity, Pro accumulates in its tissues. Thus, under favorable conditions of cultivation, the top organs of *Glycine max* L. contain 0.34 and 0.43 mg/g dry weight Pro in control 1 (H2O) and control 2 (MM), respectively (Figure 1). Pre-irradiation of seeds with EMR of the MM range increases the Pro level by 0.23 mg under conditions of exposure to NaCl with a concentration of 50 mM, relative to the experimental variants that were not exposed to irradiation. In the course of research, we found that at 100 mM NaCl, the Pro content in control plants is 1.42 mg/g dry weight, and in experimental plants – 2 mg/g dry weight, which is 0.58 g more.

![Figure 1. Influence of MM radiation on the accumulation of proline in the aerial part of 10-day-old *Glycine max* L. plants under osmotic stress.](image)

There is insufficient scientific data in the literature on the content of Pro in the root system of *Glycine max* L. plants, especially when exposed to such an unfavorable factor as salinity. However, the study of this issue is important, since the root system is most strongly susceptible to the action of damaging Na⁺ and Cl⁻ ions.

When assessing the content of free Pro in the roots of 10-day-old *Glycine max* L. plants with a simulated water deficit, the Pro content was found to be 0.10 mg and 0.34 mg/g dry weight, under normal conditions, in control 1 (seeds were not irradiated before germination) and control 2 (seeds were irradiated before germination), respectively. Under conditions of water deficit (under the action of 50 mM NaCl), the amount of proline is 0.85 mg, which is 0.75 mg more than control 1; the same dynamics is observed in the experimental plants, the seeds of which were irradiated with EMR. It should be noted that the highest Pro content in the roots of *Glycine max* L. is observed under the influence of 100 mM NaCl in the control variant and is 1.11 mg/g dry weight.

In 10-day-old seedlings of experimental plants under osmotic stress (100 mM NaCl), the Pro content in the root system exceeds the value in comparison with control 2 at normal water supply by 0.57 mg (Figure 2).

Analyzing the data obtained on the effect of MM waves on the Pro content in the top organs and the root system of cultivated soybean seedlings, we may conclude that this physical factor has a stimulating effect. The participation of Pro in increasing plant resistance to abiotic factors is associated with its polyfunctional action as a low molecular weight antioxidant and osmoprotector, as well as its ability to stabilize subcellular structures.
Figure 2. Influence of MM radiation on the accumulation of proline in the root system in 10-day-old *Glycine max* L. seedlings under osmotic stress.

3.2. Evaluation of the influence of MM-range radiation on the quantitative content of carotenoids in the leaves of *Glycine max* L.

The results obtained on the quantitative content of carotenoids in the leaves of 10-day-old seedlings of *Glycine max* L. showed that as the concentration of salts increased, the content of carotenoids increased both under normal conditions and with chloride salinization (Figure 3). It should be noted that irradiation did not have a stimulating effect on the studied parameter at a concentration of 50 mM NaCl (the analyzed parameter is 0.55 mg / g dry weight, which is 0.16 mg lower than control 1). With increasing salt concentration, the content of carotenoids increased and reached a maximum of 0.71 mg / g dry weight at 100 mM (Figure 3).

Figure 3. Influence of MM radiation on the quantitative content of carotenoids in 10-day-old *Glycine max* L. plants under osmotic stress.

In the course of the study of the influence of MM waves on the change in the amount of carotenoids in the leaves, this physical factor did not affect the studied parameter at a concentration of 50 mM NaCl. At a concentration of 100 mM sodium chloride, a decrease in their amount by 11% was found relative to control plants (seeds were not exposed to irradiation).

Thus, pre-sowing irradiation of seeds with low-intensity EMR of the MM range has a stimulating effect on the antioxidant system of *Glycine max* L., both under optimal conditions and under conditions of chloride salinity. The results obtained on the quantitative content of carotenoids showed that their content increased both under normal conditions and under osmotic stress by an average of
1.2–1.8 times. An increased content of free proline in leaves was found in comparison with soybean roots under optimal conditions and under salinity.

4. Conclusion
We have stated positive effect of low-intensity electromagnetic radiation of the millimeter range on the antioxidant system of juvenile plants Glycine max L. under osmotic stress, both in the optimal conditions and in the conditions of osmotic stress. Pre-sowing exposure to EMR EHF increases the salt tolerance of Glycine max L., cv. Apollon. The stimulating effect of millimeter-wave electromagnetic radiation on the content of proline and carotenoids in Glycine max L. plants has been revealed. The results obtained on the quantitative content of carotenoids showed that their content increases both in the normal conditions and under osmotic stress by an average of 1.2–1.8 times. An increased content of free proline in leaves was found in comparison with soybean roots in the optimal conditions and under salinity.

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