Adaptogenic effect of low-intensity millimeter-wave electromagnetic radiation on *Glycine Max* L. under osmotic stress

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Abstract. This study is devoted to identification of 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 seed germination and growth processes of *Glycine max* L., cultivar Apollo under osmotic stress. The stimulating effect of millimeter-wave electromagnetic radiation on seed germination, morphometric parameters (height of the aerial part, length of the main root, accumulation of wet and dry matter mass) in plants of *Glycine max* L., cultivar Apollo has been revealed. Thus, it has been shown that pre-sowing irradiation with this physical factor increases the salt tolerance of *Glycine max* L., cv. Apollo. Germination energy and laboratory germination increase on average by 8–12% at simulated chloride salinity in the experimental variants, compared to the control ones. The positive effect of low-intensity EMR of the MM range on the morphometric parameters of *Glycine max* L. was established, while the shoot height significantly increased by 14%, the length of the main root – by 18%, the weight of the wet matter – by 5–13% in the experimental plants compared to control conditions of chloride salinization.

1. Introduction

On the territory of the Crimea, irrigation with the waters of the North Crimean Canal and an increase of the area of irrigated land led to secondary soil salinization. The area of saline lands in the Crimea is on average more than 60 thousand hectares, and this figure is increasing every year. It is chloride salinity that most strongly inhibits both growth and development of plants [1].

A priority direction in crop production is the study of the mechanism of the damaging effect of salinity and ways to increase the salt tolerance of agricultural crops, determined by a set of properties, which are based on specific adaptation mechanisms. Their study at different levels of plant organization is of great importance for increasing the yield of agricultural plants on saline soils [2].

The study of this problem is carried out in two directions: the study of the physiological mechanisms of salt tolerance and changes occurring in the plant under the influence of salinity; study of the genetic basis of plant salt tolerance [3].

Currently, in crop production, in order to reduce the volume of artificial mineral fertilizers used, a search is being made for effective methods and technologies, aimed at maintaining a balance, increasing yields and increasing plant resistance to unfavorable environmental factors, in particular to osmotic stress. These methods include the effects of various physical factors, for example, low-
intensity electromagnetic radiation (EMR) of the millimeter (MM) range. It has been shown that the use of MM radiation is a simple, cheap (low energy consumption) and environmentally friendly method of influencing germinating seeds and can be used to regulate seed germination [4, 5], improve the growth and development of plants. For example, the MM effect on legumes (namely Phaseolus vulgaris L., Pisum sativum L.) showed a significant increase in seed germination and acceleration of their growth in comparison with unirradiated seeds [6].

The cultivated soybean plant (Glycine max L.) is one of the most widespread legumes and oilseeds. This economically profitable crop is highly environmentally friendly, since it does not require the introduction of synthetic nitrogen fertilizers, but it is not salt-tolerant [7]. At the same time, the effect of MM radiation at the early stages of ontogenesis of Glycine max L., cv. Apollo in the conditions of osmotic stress has not been studied.

In connection with the above stated, the purpose of this study was to identify the adaptogenic effect of low-intensity EMR of the MM range on seed germination and growth processes 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. Apollo [8].

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 the 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 [5].

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–М–2) for 3 days in the dark at +25 °C. According to the requirements of the state standard for agricultural crops 12038–84 for Glycine max L., the germination energy was determined on the 3rd day, and the seed germination on the 7th day.

To simulate osmotic stress, 150 ml of a solution with a NaCl, 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 EHF radiation;
Control 2 – seeds exposed to EHF-irradiation;
Option 1 – seeds, with the addition of 50 mM NaCl to Petri dishes;
Option 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 EHF irradiation, with the addition of 50 mM NaCl to Petri dishes;
Variant 5 – seeds exposed to EHF irradiation, with addition of 100 mM NaCl to Petri dishes;
Variant 6 – seeds exposed to EHF irradiation, with the addition of 150 mM NaCl to Petri dishes.

The calculated number of germinated seeds was expressed as a percentage of the total number of seeds in the variant of the experiment in each replication, and then the germination energy was found, expressed as a percentage of the total number of seeds taken for the experiment [9]. On the 4th day, the seedlings were transferred to an aqueous culture (Knop’s medium) and grown under natural light in 0.5 L pots.

The determination of morphometric parameters was carried out on the 4th, 7th and 10th days according to the methods generally accepted in plant physiology [10]. The following morphometric parameters were studied: the height of the top part of plants, the length of the main root, the mass of wet and dry matter [10].

When assessing salt tolerance, the indicator was the germination of seeds according to the method of V. N. Sinelnikova [11], the germinated seeds were counted. Depending on the germination of seeds, the plants were divided into 6 groups: I – highly resistant (seed germination more than 80%), II –
resistant (61–80%), III – medium resistant (41–60%), IV – weakly resistant (21–40 %), V – very weakly resistant (<20%), VI – unstable (there was no germination).

When evaluating the cultivated plants by standard laboratory methods, salinity tolerance criteria are usually the indicators of germination energy and laboratory seed germination. To obtain more reliable and objective research results, it is recommended, in addition to taking into account the germination or seed germination energy, to determine the number of germinal roots, the length of seedlings and roots, and the intensity of seedling growth over time [10].

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 the equipment that passed metrological verification and expertise [12].

3. Research results and discussion

The results of the study showed that chloride salinity significantly delayed germination of *Glycine max* L. seeds. Irradiation of seeds with MM radiation increased this indicator in the control variant by 12% (Figure 1).

![Figure 1](image-url) **Figure 1.** Influence of electromagnetic radiation of the millimeter range (EHF) on the germination energy of *Glycine max* L. seeds under osmotic stress.

Cultivated soybeans experience the greatest sensitivity to salt content at the first stages of ontogenesis, already starting from the moment of seed germination and emergence of seedlings. This pattern is manifested in the delay in the swelling of the seed; it is the chloride salinity that reduces the productivity of *Glycine max* L. Under the influence of Na⁺ and Cl⁻ ions, the cell ultrastructure is disturbed; which is associated with damage to membrane structures, namely the plasmalemma, on the basis of which its permeability increases and, as a result, the ability to accumulate substances selectively is lost.

It was found out that even at 50 mM NaCl, the germination energy of *Glycine max* L. decreases by an average of 8% compared to control 1. In variants with pre-sowing EMP irradiation and simulated chloride salinity (50 mM NaCl), the germination energy is 76%, which is 4% higher than control 1. Therefore, we can conclude that pre-sowing treatment of *Glycine max* L. increases the salt tolerance of the culture and has an adaptogenic effect under osmotic stress.

When exposed to 150 mM NaCl, irradiation increases the germination energy to 60%, which corresponds to the value at 100 mM sodium chloride in control plants. Based on the above, it can be concluded that low-intensity MM irradiation develops adaptogenic mechanisms of plants to the effect of increased concentrations of salinizing ions, and also significantly increases the resistance of *Glycine max*, cv. Apollo to salt exposure.

As our studies have shown, at such increased NaCl concentrations, the laboratory seed germination rates change as well as the germination energy indices. Thus, at 50 mM NaCl in experimental plants,
the percentage of seed germination is 84%, which is on average 4% more than seeds germinating in the control variant (Figure 2).

![Figure 2](image)

**Figure 2.** Effect of MM radiation on germination of *Glycine max* L. seeds under osmotic stress.

The effect of salts on *Glycine max* L. begins with their penetration into the swelling seeds; at the initial stage, the amount of Cl⁻ ions entering the seeds is not large. However, with an increase in the intensity of metabolism in seeds, and the appearance of seedlings, the rate of intake of Cl⁻ ions increases, which leads to a decrease in germination energy and laboratory germination. So, for example, at 100 mM and 150 mM NaCl, the germination energy in seeds significantly decreases by 12% and 20%, respectively. With the highest sodium chloride content, namely 150 mM, seed germination is lower than all parameters of the experimental variants and is 52%, which may be associated with the development of plasmolysis in cells, which inhibits seed germination.

In the variants with preliminary MM waves irradiation of seeds before germination, as our studies have shown, stimulation of the germination and germination energy of *Glycine max* L. is observed. For example, at 50 mM NaCl, the seed germination energy is 76%, which is on average 4% higher than in control plants grown in the normal conditions.

Thus, with an increase in the concentration of sodium chloride, the effect of MM radiation on seeds decreases. This physical factor increases the germination energy in the experimental variants with 100 and 150 mM NaCl by an average of 8% compared to the control.

This trend in the positive effect of MM radiation is also traced in the assessment of laboratory germination. In the normal conditions, this parameter is 12% higher than control 1. Under simulated salt stress, preliminary irradiation gave a positive effect – laboratory germination of experimental plants increased by an average of 11% compared to control ones.

With a 30-minute EMR with MM waves, the germination rate of *Glycine max* L. seeds is on average 12% higher relative to control 1, and this allows us to consider this treatment mode as optimal.

The irradiated seeds swelled faster, which is associated with an increase in the permeability of the membranes of the seed coat cells for water by changing the biophysical characteristics of the plasmalemma.

It is worth highlighting the fact that MM radiation did not cause visible anomalies of germination during the experiment, and promoted the acceleration of the growth of the embryonic root, which could be associated with the induction of an electric potential and activation of growth by stretching due to electroinduced ion fluxes.

It is important to note that the effect of the influence of radiation depends on the parameters of the seed treatment mode, which, in turn, have individual characteristics for a particular type of seed and depend on the geometric size of these seeds, moisture content, and a number of other physiological and radiophysical characteristics.

Figure 3 shows 3-day-old seedlings of *Glycine max* L., which demonstrate a negative correlation between the length of the main root and the concentration of sodium chloride.
The adaptogenic action of plants to osmotic stress is based on certain physiological mechanisms that trigger metabolic reactions, neutralizing the damaging effect of salts. Salinity resistance can be increased by using various methods of hardening and seed treatment, which will help to mobilize natural defense mechanisms and the appearance of physiological adaptations.

3.1. Influence of low-intensity EMR of the MM range on the change in the salt tolerance of Glycine max L.

During the experiment, it was recorded that the MM effect increases salt tolerance under chloride salinization. In the optimal germination conditions, the seeds of Glycine max L. are stable, and after pre-sowing irradiation of dry seeds with MM radiation, they are highly resistant. Thus, at 50 mM NaCl, Glycine max L. can be classified as resistant, and under the influence of the studied physical factor, highly resistant to salinity. In the conditions of 100 mM NaCl, EMR did not affect the salt tolerance index of Glycine max L. (Apollo cultivar), and these plants are considered resistant (Table 1).

Table 1. Influence of millimeter range (MM) electromagnetic radiation on the change in salt tolerance of Glycine max L. (Apollo cultivar).

| Experiment options          | Seed germination, % | Salinity tolerance groups |
|-----------------------------|---------------------|---------------------------|
| Control 1                   | 80.0 ± 2.0          | II                        |
| Control 2 (MM)              | 92.0 ± 1.0*         | I                         |
| 50 mM NaCl                  | 72.0 ± 2.0*         | II                        |
| 50 mM NaCl + MM             | 84.0 ± 2.0*         | I                         |
| 100 mM NaCl                 | 64.0 ± 4.0***       | II                        |
| 100 mM NaCl + MM            | 72.0 ± 4.0**        | II                        |
| 150 mM NaCl                 | 52.0 ± 2.0***       | III                       |
| 150 mM NaCl + EHF           | 64.0 ± 3.0***       | II                        |

Note to the table: Indicated mean ± standard error of the mean; asterisks mark significant differences compared to control at * P≤0.05; ** P≤0.01; *** P≤0.001. Groups of plants for salt tolerance: I – highly resistant, II – resistant, III – medium resistant, IV – weakly resistant, V – very weakly resistant.

When assessing the degree of salt tolerance of cultivated soybeans in the conditions of chloride salinization, at a sodium chloride concentration of 150 mM, it was found out that the studied plants are medium tolerant. Pre-irradiation of the seeds of Glycine max L., then germination in a sodium chloride concentration of 150 mM when simulating chloride salinity, increased salt tolerance.

Thus, we can draw a conclusion about the adaptogenic effect of EMR of the MM range on Glycine max L. under simulated chloride salinity by increasing the resistance.

3.2. Influence of MM radiation on morphometric parameters under the influence of osmotic stress

Studies have shown that in the normal conditions of germination of cultivated soybeans until the
seventh day, it was found out that the height of the stem is 13.0 cm, and under the action of sodium chloride concentrations of 50 mM and 100 mM, the height decreases markedly. When exposed to MM waves, a statistically significant increase in morphometric parameters is noted. For example, in control variant 2 (in normal conditions), the length of the aerial part of a 7-day-old seedling is 15.1 cm, which is 2.1 cm higher than the control variant 1.

Under the osmotic stress at the early stages of ontogenesis of Glycine max L., the morphometric parameters of seedlings significantly reduced, starting from the 4th day. This can be explained by the fact that salt stress has a suppressive effect on the processes of the mitotic cycle, which causes a slowdown in plant development. As for the top organs, the cells of the conducting system are most susceptible to the stress factor of salinity in the stem.

Thus, with artificially created salinity at a concentration of 150 mM sodium chloride, complete inhibition of the growth of soybean plants is observed. This reaction of the plant to such high salt concentrations can be explained by the fact that this crop, namely Glycine max L. (Apollo cultivar), belongs to salt-resistant agricultural plants.

When using a sodium chloride concentration of 50 mM, the investigated parameter – the shoot height – on the 7th day decreased by an average of 4 cm in comparison with the control variant of the experiment. A higher concentration of Na+ and Cl- ions (100 mM) inhibits the growth of the aerial part of the plant by an average of 6 cm compared with control 1 by 7.8 cm – with control 2. It should be noted that preliminary irradiation of seeds with EMR MM in 7-day-old seedlings do not stimulate an increase in the height of the top organs of Glycine max L., while stimulating effect is weak in 10-day-old seedlings (Figure 4).

The length of the root system on the 4th day of germination in the control variant 1 is 7.1 cm, which is 1.4 cm less than in the control variant of the seeds that were subjected to pre-sowing MM irradiation.

The study of morphometric features – the length of the main root on the 4th day – showed that pre-sowing treatment of seeds with EMR has a positive effect, stimulating this indicator by 18% relative to control 1.

The inhibitory effect of high NaCl concentrations primarily affects the development of the root system, already at the initial stages of the development of Glycine max L. So, for example, in the experimental variant with a concentration of 50 mM NaCl, the length of the top part of 4-day-old Glycine max L. seedlings is 4.4 cm, but when processing seeds, before germination, the length is 5.7 cm (Figure 4).

The effect of low-intensity EMR of the MM range has an adaptogenic effect even at a higher concentration of salts, namely, 100 mM NaCl (the length of the main root is 2.4 cm, and in the variant, the seeds of which were exposed to irradiation of the MM range, it is 3.5 cm, which is 1.5 times more).

The concentration of 150 mM NaCl completely inhibited the initial development of the root system of Glycine max L. This can be explained by the fact that the culture we have chosen as an object of study is not resistant to the influence of high NaCl concentrations. In this case, the impact of MM waves had a less pronounced effect.

Further cultivation of Glycine max L. shows that the stimulating effect of the influence of MM radiation on the length of the root system remains in the dynamics of development up to 7 and 10
days. So, for example, in the optimal cultivation conditions, on the 7th day, the length of the main root in the control variant of the experiment (under the action of MM waves) is 8.7 cm, which is 0.9 cm higher than the control variant (Figure 5).

![Figure 5](image)

**Figure 5. Influence of MM radiation on indicators of linear growth of Glycine max L. under osmotic stress.**

At higher NaCl concentrations, namely 100 mM, the growth and development of the root system inhibits to a high degree. While tracking the dynamics of the growth and development of the root system of Glycine max L. in the conditions of the damaging effect of the abiotic factor, we noted that at 100 mM NaCl the studied indicator is 3.1 cm on the 7th day. This value differs from control 1 by 4.7 cm with the same artificial salinization. Throughout the entire germination process, this regularity of the influence of the negative abiotic factor remains (on the 10th day, the analyzed parameter is 3.5 cm, which does not significantly differ from the value obtained on the 7th day of cultivation).

At high salt concentrations – 100 mM NaCl, pre-sowing irradiation of dry seeds of Glycine max L. by EMP MM range did not give an experimentally significant result.

After assessing the morphometric parameters of the seedlings of Glycine max L. cultivated under simulated chloride salinity, it can be concluded that the shoot height and the length of the main root differ significantly already from the 3rd day in comparison with control plants germinated in the normal conditions. This can be explained by the effect of salinity on the growth and division zones of root cells.

Reliably obtained experimental data on the irradiation of MM waves suggest the use of this physical factor for Glycine max L. as an environmentally friendly technology, which leads to an increase in morphometric parameters in unfavorable environmental conditions, namely, osmotic stress [13].

Growth processes that reflect general functional changes and metabolic changes in plants are most correlated with the course of biomass and dry matter accumulation. In this case, the most effective accumulation of biomass of the terrestrial part in the experimental variants occurred under the influence of MM radiation. The stimulating effect can be traced both in 7-day-old seedlings and 10-day-old plants, against the background of salinity. The mass of the raw matter of plants in both control
variants of the experiment, in normal conditions, does not differ significantly throughout the entire cultivation period (from 4 to 10 days). A sharp decrease in the biomass of the top part is observed at 100 mM NaCl relative to control 1 by 6.34 g and 5.67 g, on the 7th and 10th days, respectively.

Preliminary MM irradiation increases the adaptogenic properties of *Glycine max* L. to the effect of the stress factor – salinity. So, for example, at 50 mM NaCl, the wet weight of the top part of 7-day-old plants (with MM) increased by 1.74 g in comparison with seedlings, plants that were not exposed to irradiation. The same dependence on the accumulation of terrestrial biomass is observed on the 10th day.

Pre-irradiation of seeds with MM waves stimulated the accumulation of wet mass by the root system in the conditions of salt stress by an average of 33% in relation to control plants.

The indices of the accumulation of wet weight by roots in plants germinated at 50 mM and 100 mM NaCl approaches the control variant both on the 7th and 10th days. For example, the value of biomass accumulation by roots in control 1 is 1.06 g, and at sodium chloride concentration of 50 mM and 100 mM – 0.98 g and 0.95 g, respectively.

Pre-irradiation of soybean seeds with MM waves on the 10th day of cultivation gave an increase in the wet weight of roots relative to control 1 by 0.42 g, under the action of 100 mM NaCl. In the course of the research, it was noted that, with a simulated chloride salinity of 50 mM NaCl, there was no statistically significant difference with control 2 in terms of root biomass on days 7 and 10 in experimental plants [14].

Analyzing the data obtained, it should be noted that the highest rate of dry mass accumulation by the root system, namely 0.14 g, is noted in the control variant number one.

It was found out that the studied physical phenomenon – EMR of the MM range stimulates the accumulation of dry mass by the roots under the influence of osmotic stress throughout the entire cultivation process.

Thus, the salt tolerance of plants can be assessed by the accumulation of wet and dry mass, in comparison with seedlings germinated at optimal conditions of water supply. In the course of research, an adaptation of *Glycine max* L. was revealed to the damaging effect of salts, which is achieved by preliminary irradiation of dry seeds before cultivation. The use of low-intensity EMR of the MM range leads to an increase in the values of all the studied parameters in the experimental plants in comparison with the control ones in the salinization conditions, at the used concentrations of sodium chloride.

4. Conclusion
The positive effect of low-intensity electromagnetic radiation of the millimeter range on seed germination and growth processes of *Glycine max* L. was established both in the optimal conditions and in the conditions of osmotic stress. It is shown that pre-sowing irradiation with this physical factor increases the salt tolerance of *Glycine max* L., cv. Apollo. Germination energy and laboratory germination increase by an average of 8–12% with simulated chloride salinity in the experimental variants as compared to the control ones. The positive effect of low-intensity electromagnetic radiation of the millimeter range on the morphometric parameters of *Glycine max* L. was established, while the shoot height significantly increased by 14%, the length of the main root – by 18%, the weight of the wet matter – by 5–13% in experimental plants compared to control plants in the conditions of chloride salinization.

The positive effect of millimeter-wave electromagnetic radiation on dry seeds of the studied crop is of practical importance for the pre-sowing treatment technology because it does not require preliminary soaking of the seeds, while giving a noticeable growth advantage.

5. References
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