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Synthesis of Phenolic Compounds in Barley Seedlings Under the Influence of the Microwave Electromagnetic Field of Ultrahigh Frequency

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Abstract

Phenolic substances are involved in the processes of growth, morphogenesis, respiration and photosynthesis, are reserve and signaling substances, and have antioxidant properties. Spring barley (*Hordeum vulgare* L.) of variety Nikita was used in the experiments. In seven-day-old seedlings, phenolic substances were determined using reversed phase high-performance liquid chromatography analysis (RP HPLC) with an amperometric detector. The maximum number of phenolic compounds is registered in the chloroform extract of the endosperm of the microwave-treated grain, their number is 26 names of substances. The minimum number of individual substances is 10-11 and is found in samples of roots and sprouts of native seedlings, as well as roots of microwave-irradiated barley plants. The microwave treatment affected both the number of isolated substances and their amount in the extract. The most biochemically active anatomical part of a seven-day-old barley seedling is the endosperm: it contains significantly more substances of a phenolic nature, recorded by an amperometric detector, than in sprouts and roots. The microwave field significantly affected the amount of phenolic compounds with antioxidant properties in the endosperm. In relation to phenolic substances in the composition of barley seedlings, there is an organ-specific response, which is expressed in the difference in their quantitative and qualitative content. Individual phenolic substances are registered in all the organs of the seedling (roots, leaves, endosperm), and some have a certain localization. The total amount of phenolic substances with antioxidant properties increases after microwave treatment.

Keywords: Barley; *Hordeum vulgare*; Phenolic substances; Antioxidant properties; Ultrahigh frequency electromagnetic field; Seedlings.

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1. Introduction

Currently, there are about several tens of thousands of phenolic compounds in the plant world. Although they indirectly affect the primary metabolic processes and belong to the group of "secondary metabolites", nevertheless, they are vital for plants. Unlike other secondary metabolites, plant phenols are characterized by the versatility of their natural distribution, occurring in almost all cells. Phenolic compounds have different biological activity and key physiological significance for the plant organism depending on the structure [1]. First of all, phenolic compounds have a protective effect on the organo-tissue structure of the plant organism and neutralize the negative effects of ultraviolet radiation, can act as natural antibiotics and also determine the color and taste of grain and its products [2]. It is also noted that they are involved in the processes of growth, morphogenesis, respiration and photosynthesis, are reserve and signal substances and have antioxidant properties.

Phenolic compounds are secondary metabolites formed naturally during swelling and germination of the seed [3]. They are synthesized both during normal growth and development [4] and are markers of stressors [5], such as high temperature [6], salinity [7], heavy metal exposure [8], UV irradiation [9] and others. Various physical factors influence the content of phenolic compounds. Microwave radiation [10], magnetic fields [11] and electric fields can have a certain effect on plant tissues [12], thereby regulating the processes of germination and growth [13]. Ultrahigh frequency electromagnetic field (UHF EMF) can promote seed germination and increase the content of biologically active components [14, 15]. A plant can absorb electromagnetic energy and change the structure of cellular macromolecules by influencing their physiological and biochemical characteristics [12]. Electromagnetic energy affects the content of phenolic compounds and other secondary metabolites [16, 17], as well as the amount of flavonoids, soluble protein, sugar in the plant, free amino acids [10], has a positive effect on the activity of catalase and superoxide dismutase [13], significantly increases gamma-aminobutyric acid content. These data indicate that microwaves can increase the content of these metabolites and the nutritional value of sprouts and grains, as well as improve the quality of germinated grains [18].

On the other hand, it has been proven that microwave treatment induces the production of reactive oxygen species (ROS) in plants [19], which is accompanied by protective reactions in the form of increased synthesis or activation of antioxidant compounds. The latter also include phenolic substances, because they have properties such as absorption and inhibition of reactive oxygen species, electrophilic absorption and metal chelation. Phenolic hydroxyl groups are good hydrogen donors and can react with oxygen and nitrogen species of organic radicals. Due to their antioxidant properties, plant phenolic compounds are useful in diabetes, cardiovascular and neurodegenerative diseases, mutagenesis and carcinogenesis occurring in the human body [20].

To control the level of reactive oxygen species and protect cells under unfavorable conditions, plant tissues can produce various neutralizing substances [21], such as antioxidant enzymes and phenolic compounds. With the synergistic action of these substances, free radicals and oxidative intermediates generated during plant metabolism can be quickly eliminated [22]. Severe treatment regimes, such as high power and long exposure to a microwave field, can inactivate enzymes in seeds [23] and reduce the level of gene expression [24]. After microwave treatment of seeds, the content of rutin in seedlings increased exponentially, and antioxidant activity improved significantly [25]. Microwave radiation can enhance the ability to eliminate free radicals of wheat seedlings [26] and significantly increase the activity of antioxidant enzymes in seeds [27]. The biological effect of microwaves significantly increases the total content of phenolic substances and flavonoids, enhances the antioxidant defense mechanism of seedlings. The optimal mode of microwave treatment can increase the activity of antioxidant enzymes in plant sprouts [22]. The change in the level of phenolic compounds confirms the opinion that electromagnetic field can be a small stress factor and the reactions that develop in treated plants can be considered as phases of phytostress [28]. Microwave treatment of sorghum seeds can help improve their composition in terms of phenol profile, antioxidant activity and in vitro protein digestibility of seedlings, which makes them a valuable ingredient for inclusion in functional foods [29].

Recently, cereal sprouts are increasingly used as components of functional foods due to their health-saving composition and high nutritional value. The biochemical composition of seedlings includes amino acids, fiber, microelements, vitamins, flavonoids and phenolic substances [30, 31]. Cereal sprouts grown to 1-2 weeks of age, also referred to as microgreens [4], are increasingly being used as natural health supplements [32]. Among the microgreens of cereals, special attention is paid to barley. The term “green barley” in the literature is used to describe barley seedlings growing up to 200 hours from germination and reaching about 20-30 cm in height [33]. Other terms used are young barley, barley shoots or barley grass [34]. The influence of microwave electromagnetic radiation on plants has been studied by researchers from different countries for quite a long time. The mechanism of its action has not yet been fully studied, but it has already been established that the activation of the antioxidant system of plants, which includes low and high molecular weight components, occurs when exposed to microwave energy.

Therefore, the study of the state of this system and in particular of low molecular weight antioxidants, is important for understanding the mechanism of interaction between UHF EMF and plants. In this regard, the goal was to study the nature of quantitative and qualitative changes in the content of phenolic substances in different anatomical parts of barley seedlings under the action of an electromagnetic field of ultrahigh frequency.

2. Materials and Methods

To determine the effect of UHF EMF on the content of substances of phenolic nature with antioxidant properties in the organs of seedlings, we used spring barley (*Hordeum vulgare* L.) of Nikita variety reproduction 2019.

The experimental scheme included two modes:

1. Control, without processing;
2. UHF EMF: power 0.42 kW, magnetron frequency 2.45 MHz, exposure 11 sec.

The output power of UHF radiation was measured by the method of Zhang, *et al.* [35] using the formula:

$$Q_{abs} = \frac{m \cdot c_p \cdot \Delta T}{t}$$

where Q_{abs} – power absorbed by water per unit time (W), m is the mass of water (g); c_p – specific heat capacity of water (kJ/kg · ^{o}C), ΔT – temperature rise in the water load (°C), t – turn-on time of the magnetron (sec).

Air-dry barley seeds were processed under specified conditions, then germinated in water culture in Petri dishes on sterile filter paper discs. Germination was carried out at uncontrolled temperature (average 18°C) and relative humidity (average 60%). The room was subject to a diurnal cycle with fluctuations in temperature, humidity and light. Barley seedlings were grown in the laboratory in daylight, but without direct sunlight, for best growth. To assess the reproducibility of the results, the study was conducted in two blocks with an interval of 15 days between blocks. When the seedlings reached the age of 7 days, chromatography was carried out.

Preliminary sample preparation for analysis included a number of operations: grinding in an agate mortar a sample of 2.0 g (accurately weighed) of fresh material divided into separate anatomical parts (roots, shoots and endosperm with an unseparated shell); three times extraction with trichloromethane with a volume of 3 ml; sonication of the extract for 10 minutes for the completeness of the extraction of substances; settling for 15 minutes; filtering through a paper filter; air drying under draft; processing in 10% trichloroacetic acid with a volume of 5 ml, shaking; heating in a water bath at 90°C for 30 minutes; cooling to a temperature of 25°C; centrifugation at 5000 rpm. within 5 minutes; decanting; adding concentrated isopropyl alcohol with a volume of 5 ml. Then the organic phase was evaporated in an inert medium and quantitatively dissolved in 2 ml of the mobile phase.

RP HPLC analysis was performed on a Tsvet Yauza-04 HPLC instrument with an amperometric detector (Research and Production Organization “Khimavtomatika”, Russia). The instrument was controlled and the obtained results were processed using the MultiChrom software, version 3.1.1550 (CJSC Ampersend, Russia). We used a chromatographic column: Gemini 5 µm C18, 110A, 250 x 4.6 mm (Phenomenex, USA), guard column Security Guard with Gemini C18 cartridge 4 x 3 mm. Loop volume was 20 µl. Flow rate was 1 ml/min., pressure 45.5+1 bar. The components of the mobile phase, consisting of acetonitrile and bidistilled water were mixed in a volume ratio (70:30), after which degassing was carried out using a vacuum aspirator. The elution mode was isocratic.

Identification of individual compounds was carried out on the basis of matching the retention times and UV spectra of standard samples. The relative content of individual identified phenolic substances in the test sample was determined by the method of internal normalization. All measurements were carried out in double biological and triple analytical replicates. The tables show arithmetic means and errors of mean values. The obtained results were statistically processed. The significance of differences compared to the control was found by the F-test at a significance level of 0.05 and marked *.

3. Results and Discussion

The physical factor under study is an electromagnetic field of ultrahigh frequency. It starts a complex of biochemical processes, culminating in the formation of various phenolic compounds during the germination of seeds and the development of plants. The use of an amperometric detector leads to the selective isolation of phenolic acids with antioxidant properties [36]. The maximum fluctuations in the level of phenolic substances are noted at the initial stages of ontogenesis and are species- and variety-specific [28], which was also revealed in the work. The change in the number and amount of substances isolated during the chromatographic analysis indicates the development of a response in barley seedlings to UHF EMF.

The number of isolated substances differs depending on the localization in one or another anatomical formation of the plant (Figure 1). The maximum number of compounds was registered in the chloroform extract of caryopsis residues (hereinafter, for brevity, we will designate “caryopsis”) of microwave-treated plants, their number is 26 items. The minimum number of individual substances is 10-11 and was found in samples of roots and leaves of control plants, as well as roots of microwave-irradiated barley plants. Microwave treatment affected both the number of isolated substances and their amount in the extract.

According to the characteristics of the isolated phenolic compounds, several general trends were found. Thus, actively growing anatomical parts of barley (roots and leaves) are characterized by a relatively short retention time of the bulk of the studied substances. The time range of measurement and reaching a plateau ends at the 8th minute and at the 13th minute, respectively. For the “caryopsis”, the time for processing the results had to be increased to 18-19 minutes.

The form of the sample elution curve is also peculiar, which depends not only on the organ, but also on the processing mode. Thus, chloroform extracts of leaves and grains are characterized by a decrease in the signal value in the variants with microwave irradiation, which indicates a relative decrease in the concentration of dominant compounds compared to non-irradiated plants. The most contrasting pattern of changes is observed in the analysis of the chloroform extract of barley leaves (Figure 1). Here, the biotropic effect of EMF was fully manifested. The number of isolated phenolic components changes significantly: from 11 in the control variant to 19 in the experimental variant, after microwave treatment, as well as their quantitative content.

The Table 1 shows the calculated results on the content of individual representatives of phenolic compounds isolated from the chloroform extract of barley leaves and identified. The most significant difference between the two studied samples was recorded for syringic acid in the “caryopsis” and is manifested in an increase of 19.7 times relative to the control. For most of the identified phenolic compounds, an increase was recorded compared to untreated plants.

Table-1. Results of HPLC analysis of barley chloroform extract, %

No.	Substance	Leaves		“Caryopsis”		Roots	
		control	420 W / 11 sec.	control	420 W / 11 sec.	control	420 W / 11 sec.
1	Caffeic acid	5,21 ± 0,34	10,17* ± 0,87	33,90 ± 2,43	24,21* ± 1,93	2,83 ± 0,26	2,21 ± 0,21
2	Ferulic acid	4,97 ± 0,13	7,49* ± 0,66	4,90 ± 0,42	6,04* ± 0,57	0,72 ± 0,06	2,75* ± 0,18
3	Vanillic acid	1,40 ± 0,09	6,01* ± 0,59	0,30 ± 0,02	0,93* ± 0,08	–	–
4	Gallic acid	7,67 ± 0,54	27,68* ± 1,98	–	0,67 ± 0,06	–	–
5	Coumaric acid	–	0,17 ± 0,01	–	2,17 ± 0,19	–	–
6	Syringic acid	–	–	0,07 ± 0,01	1,38* ± 0,11	–	–
7	Salicylic acid	–	–	–	0,99 ± 0,08	–	–
	Amount	**19,25**	**51,52**	**39,17**	**36,42**	**3,55**	**4,96**

Gallic acid with a content of 7.67% makes up about half of all identified phenolic acids in the leaves of barley control plants and also caffeic acid (5.21%) makes up a significant proportion. The trend continues in microwave-treated plants, however, the content of these acids becomes equal to 27.68% and 10.17%, respectively, i.e. their content increases by 3.6 and 2.0 times.

The most biochemical active anatomical part of a seven-day-old barley plant is the "caryopsis". It contains significantly more substances of a phenolic nature recorded by an amperometric detector than leaves and roots (Figure 1). The microwave field significantly affected the amount of phenolic compounds with antioxidant properties in the grain. The total number of individual items increased from 24 to 26, identified in the control 4, in the experiment – 7. The most significant positive difference between the "caryopsis" of untreated and microwave-treated plants was recorded for syringic acid, followed by vanillic acid (3.1 times). The decrease relative to the control indicators was recorded for caffeic acid (28.6%). Under conditions of abiotic stress (drought), caffeic acid can improve the growth of roots and shoots [37] and reduce the accumulation of reactive oxygen species (ROS) [38]. In this work, we see that the content of caffeic acid has an organ-specific character. Thus, in control plants, the maximum accumulation of caffeic acid is observed in the "caryopsis", in the leaves – 6.5 times less, in the roots – 12.0 times less. The nature of the distribution of this phenolic acid changes in a quantitative ratio when exposed to a microwave field: caffeic acid is 2.4 times less in the leaves, relative to the "caryopsis", in the roots – 11.0 times. Thus, the total amount of caffeic acid in all organs of the juvenile barley plant after microwave treatment decreased by 12.8% compared with the content in the control.

Greater, compared with leaves, stability in terms of the amount of identified phenolic substances is noted in the "caryopsis": the difference is 7.0%, while for the roots this figure is 39.7%, for leaves – 2.7 times. But there are also changes provoked by exposure to UHF EMF. Three acids were found only in processed grain samples. The data obtained indicate that even in week-old barley plants, the caryopsis residue retains its biochemical activity and takes part in the regulatory and adaptive reactions of plants in response to the action of an abiotic stressor, which manifests itself both in the redistribution of some phenolic acids and in the *de novo* synthesis of others. Apparently, the decrease in the content of caffeic acid in the "caryopsis" and roots with its simultaneous growth in the leaves indicates both resynthesis and redistribution of substances between different anatomical parts of the plant: such a possibility is not ruled out in the literature. Thus, in [39] it is indicated that phenolic acids can be in free, conjugated and bound states. Caffeic acid is actively involved in plant physiology and stress resistance mechanisms, and is primarily used by plants for the synthesis of lignin. The mechanism of adaptation to stress also includes the production of ferulic acid through methylation of caffeic acid [40]. In this work, the content of ferulic acid in all organs increases under the influence of a microwave stressor.

The microwave treatment carried out led to a decrease in the number of compounds in the roots relative to native plants: the number of individual names decreased from 11 to 10. Thus, our data partially confirm the results obtained in [17, 29] – the content of individual phenolic components with antioxidant properties in the composition of certain anatomical organs of the barley seedling actually increases in comparison with native samples. However, for some substances there is a decrease and in some cases quite significant.

The synthesis of such secondary metabolites in juvenile barley plants as phenolic compounds depends on the preliminary exposure of the seeds to the electromagnetic field of ultrahigh frequency. The qualitative composition of phenolic substances with antioxidant properties after microwave treatment of barley seeds changes: in total, 55 substances were recorded for all organs instead of 46, found only in the control variant. The most active antioxidant status is possessed by the "caryopsis" containing, on average, 25 phenolic compounds.

In most scientific works, an increase in the number of phenolic compounds is stated, but the physiological and biochemical mechanism of this phenomenon is not disclosed due to the complexity and diversity of the biochemical pathways that implement it. It is possible that an increase in the level of phenolic compounds is a compensatory response to the release of ROS and is described by the following relationships: microwave treatment leads to the formation of ROS in the seed and seedling [19], which makes it necessary to activate molecular mechanisms of protection against excess free radicals and, in particular, increase the production of phenolic compounds with antioxidant activity [16].

4. Conclusion

Thus, studies of the synthesis of secondary metabolites such as phenolic compounds in barley microgreens under the influence of UHF EMF showed that there is an organ-specific response. This is expressed in a different quantitative and qualitative ratio of the content of secondary metabolites initiated by the action of electromagnetic fields. Some substances are registered in all three studied organs of the seedling and some have certain localization. The total amount of phenolic substances with antioxidant properties increases after microwave treatment. The data obtained make it possible to recommend the studied mode of microwave treatment (420 W, 11 sec.) for pre-treatment of barley seeds in order to include them in the composition of food and feed products of a functional orientation and a high content of antioxidant components.

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Figure -1. Chromatographic profile of the chloroform extract of barley seedlings: native samples (A) and after microwave treatment at 420 W / 11 sec. (B); 1 – HPLC chromatogram of chloroform leaf extract; 2 – HPLC chromatogram of endosperm chloroform extract; 3 - HPLC chromatogram of the chloroform root extract