INVESTIGATION OF THE INFLUENCE OF ACOUSTIC TREATMENT ON BIOLOGICAL AND MORPHOFUNCTIONAL PARAMETERS OF HORDÉUM VULGÁRE SEEDS

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ABSTRACT

The article presents the results of a study of the influence of infrasonic treatment on biological and morphofunctional parameters of Hordeum vulgare seeds. It was discovered that with an increase in the intensity of acoustic vibrations of the acting signal on the seeds, the amplitude of the excitation centers at the initial stage changes according to a linear law (15-30 min), then it stabilizes (45 min), and further exposure to acoustic radiation leads to the "blurring" of the excitation centers and the occurrence of more complex types of oscillations, which in turn leads to a decrease in the amplitude of oscillations. It was found that after exposure for more than 45 minutes, the metabolic process in the cell is non-linear, which can lead to a violation of biochemical processes in the cell up to the destruction of the nucleus and death of the cell. Differences in the degree of transformation of seeds with different duration of treatment are most likely associated with different thresholds and resonance properties of cells. The results obtained allowed us to conclude that infrasound-treated barley seeds show more intensive growth and development at the first stages of germination, which is especially important in dry regions.

Key words: acoustic treatment, infrasonic vibrations, seeds, productivity, germination energy

I. INTRODUCTION

The progress of any branch of agricultural production is associated with the continuous updating of technologies, the development of more modern technical solutions, the improvement of its structure [1-3, 19]. In this regard, both scientific and practical interest is aroused in the study of new methods of effective plant protection, stimulation of their growth and development that meet the requirements of environmental safety [6,7,9]. One of the directions of such research is the use of physical factors in crop production [13, 22, 23]. Today, the achievements in this area of science are quite significant. Treating seeds with physical factors, many researchers in laboratories, field and industrial experiments observed: an increase in seed vigor, germination, photosynthetic activity, plant survival, an improvement of product quality and increased yields [21, 27, 29-31]. Plants grown from seeds treated with physical factors are more resistant to diseases. Physical factors can be used to control growth speed and to stimulate plants by influencing the physiological processes of their life [8,16,17]. Research is carried out on a wide range of factors: constant and variable electric and magnetic fields, ultrasonic vibrations, ultraviolet and infrared rays, gamma rays and other methods [4,5, 10-12,14,18].
Ultrasound technology can be used to enhance the hydration process without negatively affecting seed quality [16]. Nesterenko et al. (2017) stimulated seed germination and early growth wheat by 50 Hz electromagnetic fields. High electric fields also have a positive effect on seeds germination [30] as well as magnetic fields [28]. It was shown by Bitarishvili et al. (2018), Ndiku et al. (2014), Hermelin et al. (2009) that γ-irradiation of seeds in small doses exerted a stimulating effect on the seedling development.

One of the reasons for the insufficient use of physical factors into agricultural production is the lack of mass-produced machines for pre-sowing treatment of seeds. [24-26]. In this regard, it is necessary to select physical methods for processing plants, which would require the development of devices characterized by simplicity of design, high productivity, small dimensions, low cost and high reliability.

The purpose of our research was to study the effect of infrasonic treatment of seeds of the agricultural crop Hordéum vulgáre on the rate of their growth and ripening.

II. MATERIALS AND METHODS

Experimental studies were carried out on the seeds of Hordéum vulgáre using an innovative acoustic water treatment unit "ERADA" provided by "Soil Respiration" LLC (Stavropol, Russia). This company is engaged in the acoustic treatment of seeds of cucumbers and tomatoes in hydroponic greenhouses.

To select the mode of acoustic treatment, the frequency of plant vibrations during their growth was studied using the method of holographic interferometry.

In laboratory conditions, the influence of infrasonic vibrations on the sowing quality and biological properties of seeds was studied. Germination and seed vigor, as well as morphological parameters such as the number and length of roots, and the length of sprouts were measured

Reagent grade chemicals and grade A glassware were used in present study. Conductivity of distilled water used was less than 1 µS/cm.

2.1. Measuring the natural frequency of seeds during their growth

The amplitudes of vibrations of seeds are small and it is rather difficult to measure them. In this regard, we hypothesized that seeds influence each other during their growth. The set of seeds in a spatially closed space can be represented as the sum of elementary oscillators operating in common-mode, therefore, the total signal can be represented as the sum of elementary signals. Taking this assumption into account, the determination of the natural frequency of seed germination was carried out according to the method described below. Seeds (100 pcs.) were placed on the bottom of a Petri dish with filter paper moistened with distilled water. One end of the filter paper was placed in a cup of water, providing constant moisture to the seeds. A Petri dish with seeds was placed in a soundproofing chamber; light was supplied to the soundproofing chamber through a light-fiber bundle. A receiver for infrasonic vibrations was placed above the Petri dish. Signals were recorded using an oscilloscope, spectrum analyzer, and a computer (using the Sonic Visualizer software). The seeds were germinated for 5 days.

The seeds in the Petri dish germinated and emitted low-frequency sound vibrations. These vibrations were captured by a remote sensor and transmitted to the receiver of acoustic vibrations, which is built on the principle of a real-time interferometer. Acoustic signal converted into an electrical signal was sent for analysis and further processing to an oscilloscope, spectrum analyzer, and computer.

The infrasonic vibrations of the seeds were recorded by the receiver of the infrasonic vibrations. The converted signals were fed to an oscilloscope, where the temporal structure of the signal was recorded, the spectral analysis of the signal was carried out on the spectrum analyzer, and the temporal structure of the signal was memorized on the computer and further processing and analysis of the resulting sound track was carried out.

2.2. Infrasonic treatment of seeds

Seeds (groups of 100) were placed on the bottom of a Petri dish with filter paper moistened with distilled water. A source of infrasonic vibrations with a power of 0.01 W/cm² was used. The frequency of the emitter of acoustic vibrations corresponded to the natural frequency of vibrations of the seeds in the process of their growth. The
seed treatment time was 15-30-45-60 minutes. In accordance with the duration of processing, experimental groups were allocated, which received the following designations:

- Sample A – no treatment (control),
- Sample B – 15 minutes,
- Sample C – 30 minutes,
- Sample D – 45 minutes,
- Sample E – 60 minutes.

2.3. Study of biological parameters of seeds

The experiment was performed with accordance to all the ISTA (2006) germination requirements.

Seeds in Petri dishes were germinated under constant environmental conditions: $t=+20^\circ\text{C}, W = 78\%$.

The germination energy and germination ability were evaluated according to the ISTA (2006) standard. The seed vigor was measured after 3 days, and the seed germination after 7 days from sowing. The linear dimensions of the number of roots and shoots were determined after 3 days.

Macrophotography of seeds was performed using a laboratory binocular microscope Axio Imager 2 (Carl Zeiss Microscopy GmbH, Germany) at x20 magnification.

The experiment was set in five replicates.

2.4. Statistical analysis

All parameters obtained were submitted to one-way analysis of variance (ANOVA) and Duncan’s test ($p < 0.05$) through the statistical package Statistica for Windows (Statsoft, Tulsa, USA). To construct histograms and graphs of the obtained data, Microsoft Excel 2010 and Origin software were used.

III. RESULTS AND DISCUSSION

3.1. Finding the natural frequency of vibration of seeds

The amplitude of plant oscillations during growth, caused by biochemical processes in cells, is so small that it is not possible to measure it using any known methods. At the same time, the oscillation frequency is in the range of infrasonic oscillations, presumably from 0.01 Hz to 15 Hz. Consequently, the receiver of infrasonic vibrations must have a high sensitivity (approximately from 0.01 µm in the linear displacement of the sensing element) and have a nonlinear frequency conversion as a function of the amplitude in order to transfer the signal to the sound range of vibrations.

The natural frequency of oscillations during seed germination was measured using a receiver of infrasonic oscillations. To form a nonlinear element in the receiver of infrasonic oscillations, a scheme of holographic recording and formation of an interferogram in colliding beams was used (Figure 1). The use of the holographic interferometry method provided the minimum error.

Registration of the converted signals of infrasonic waves was recorded on an oscilloscope and processed on a computer using the Sonic Visualizer software. The synthesized receiver of infrasonic vibrations provided the minimum error in assessing the natural vibration frequency of plants during growth.

The results of determining the natural vibration frequency of the seeds of Hordeum vulgare are shown in Figure 1.
With the help of an infrasonic signal receiver, the natural vibration frequencies of the central part of the seed germination and the initial growth of barley were measured. It was found that the lowest oscillation frequency occurs on the first day during seed germination and is 7.8 ± 4.5 Hz. The oscillation frequency on days 2-5 of germination does not differ significantly and is 13.9-14.3 Hz. Based on the data obtained, in order to accelerate the process of seed germination in the experiment, the frequency of acoustic treatment was chosen at 7.8 Hz.

3.2. Investigation of the effect of low-frequency acoustic treatment on the indicators of seed germination

It is known that the life form of plants can change with the variable conditions of existence - environmental factors (climatic, soil, value, etc.). According to the mode of action, environmental factors can be divided into direct acting (directly on the body) and indirectly acting (influencing other factors).

However, the same factor may be directly acting in some conditions, and indirectly acting in others. Moreover, indirectly acting factors can be of decisive importance, changing the cumulative effect of other, directly acting factors. Modern biology considers the cell of a living organism, including a plant, as a single, complex, integrated system, where individual functions are interconnected and balanced with each other. Violation and loss of individual stages of cellular metabolism leads to the activation of detour paths or to the deployment of events of a pathological nature.

According to our assumption, the effect on the germination and seed vigor can be exerted by the resonance frequency between the plant and the emitter, which arises only when the frequencies of the natural oscillations of the seeds and the spring source completely coincide. In this regard, the frequency of infrasonic radiation was determined based on the previously studied and presented data on the natural frequency of vibrations of barley seeds during germination.

The results of calculating the number and length of roots and the length of seedlings of Hordeum vulgare for convenience of perception are presented in the form of histograms in Figures 2-4.
Figure 2. Histogram of the distribution of the number of roots of *Hordéum vulgáre*

Figure 3. Histogram of the distribution of the length of the sprouts of *Hordéum vulgáre*
Mathematical analysis of the data presented in Figures 2-4 made it possible to obtain dependencies presented in the form of graphs in Figures 5 and 6.

Figure 4. Histogram of the distribution of the length of the roots of *Hordéum vulgáre*

Рисунок 5. Dependence of the average length of the sprouts of *Hordéum vulgáre* on the treatment time
The obtained dependences showed that the pre-sowing treatment of seeds with infrasonic radiation promotes the length of roots and sprouts, and the peak value was obtained after treating for 30 minutes. Seed treatment for more than 30 minutes has a less significant effect.

Another very important indicator of the rate of growth and development of seeds is the germination energy and the percentage of germination of the primary stem and roots. Figure 7 shows the trend obtained during calculation of the seed vigor and germination.

Calculations have shown that infrasonic radiation has a reliably significant effect on the increase in seed germination energy. The maximum value of the germination energy was obtained after treating for 30 minutes -
76%, which is 19% more than in the control sample. Treating for more than 30 minutes increased the value of germination energy up to 66-71%. Seed germination in the control group was 91%, which corresponds to the standard data provided by ISTA [4]. The treatment increased the percentage of seed germination in the experimental groups to 95-99% with the maximum value in the experimental samples treated for 30 minutes.

The obtained data indicates the nonlinearity of the influence of infrasound on the morphological parameters of seeds during germination.

3.3. Interpretation of the nature of biochemical processes that are activated by low-frequency acoustic treatment

Apparently, with low-frequency acoustic treatment, vessels and fibers of the xylem and sieve elements of the phloem are formed in the zones of development of structural anomalies. At the same time, differentiating cambial derivatives preserve the protoplasm and turn into cells of the storage parenchyma, which accumulate large amounts of storage substances.

Differentiation of cambium derivatives into parenchymal cells occurs due to the low activity of sucrose synthase and is accompanied by a decrease in the cellulose content per unit mass of xylem. The low activity of sucrose synthase is precisely explained by the effect of infrasonic radiation, which inhibits this enzyme (Verma, E et al, 2018). In this case, the high acceptor force of the trunk tissues is maintained due to the metabolization of the inflowing sucrose with the participation of apoplastic invertase.

It was found that during the period of cambial growth, the regulation of apoplastic invertase activity occurs not only at the level of expression of the genes encoding it, but also at the post-translational level through protein inhibitors: CIF, cell-wall inhibitor of β-fructosidase.

Based on the data presented, it was suggested that during infrasonic treatment there is a partial suppression of sucrose synthase and an increase in the activity of apoplastic invertase, since this is a compensatory mechanism for maintaining donor-acceptor relations aimed at eliminating the excess content of disaccharide in the phloem. When sucrose is metabolized by apoplastic invertase, the sucrose / hexose ratio is shifted towards hexose, which can promote the induction of cell division and, through the expression of a number of genes, lead to a change in the development program of roots and shoots of treated seeds. As a result, the synthesis of storage metabolites is enhanced, contributing to the conversion of cambial derivatives into storage parenchyma cells.

In other words, the intensive growth of the root system and seedlings of seeds treated with infrasound is a response to stress or supercompensation of physiological changes occurring in experimental samples.

Nevertheless, in the course of research, the nonlinear nature of the influence of infrasound on the morphological and physiological parameters of seeds during germination was established. Based on 100% correlation of the data obtained from different experiments, the optimal processing time for obtaining longer shoots and roots is 30 minutes. Apparently, the treatment with infrasonic radiation with the established parameters lasting more than 30 minutes has an effect on the hormonal system of the seeds.

In addition to the enzyme apoplastic invertase, the hormone auxin plays an important role in the differentiation of the structural elements of roots and shoots, which affects the processes of cell elongation.

It is believed that free (physiologically active) auxin is a signal required for vascular differentiation. In vascular plants, auxin forms a conjugate with glucose - IAA-glucose. This process is catalyzed by the enzyme IAA-glucose synthase.

Since the bound hormone is not involved in the differentiation of vessels, it was suggested that the disorientation of structural elements in experimental samples occurs as a result of the transformation of auxin from a bound form into a free one.

It is widely believed that auxin, together with reactive oxygen species (ROS), are the main regulators of plant development under various stresses. The concentration of auxin may decrease due to a decrease in the biosynthesis of the hormone with an increase in the content of ROS, conjugation of the hormone, or due to its direct oxidation in the event of oxidative stress.
The accumulation of ROS in seeds induced by stress in the form of infrasound radiation affects the metabolism of auxin by affecting the genes responsible for hormone signaling.

It is noted that prolonged exposure to stress leads to changes in growth mechanisms, reductions in cell division, and increased lateral growth. These processes are carried out through interactions between ROS and auxin. This means that when the seeds were treated for 45 minutes, the samples were subjected to more serious physical stress, coupled with the intense formation of reactive oxygen species, which led to a reorientation of the growth of the root system towards thickening. The treatment for 60 minutes apparently turned out to be excessive, which is most likely due to the partial suppression of the hormone auxin with the abundant formation of reactive oxygen species, which also contributed to the oxidation of many biosynthetic processes. Since sample E from the biological and morphological point of view did not differ significantly from the sample. And, the treatment of seeds with infrasonic radiation for 60 minutes was found to be ineffective. At the same time, further study of the mechanisms of multidirectional stimulation of the growth of seeds of agricultural crops during treatment for 30 and 45 minutes is of scientific interest.

Thus, the results of laboratory experiments indicate a positive effect of infrasound in the optimal mode on the sowing properties of barley seeds and suggests an effective effect of treatment on field germination and the density of field crops. Of practical interest is not only the field germination rate, but also germination friendliness. The earlier emergence of seedlings, as is known, promotes the intensification of the further development of the plant.

IV. CONCLUSION

It was found that with an increase in the intensity of acoustic vibrations of the acting signal on the seeds, the amplitude of the excitation centers at the initial stage changes according to a linear law (15-30 min), then it stabilizes (45 min), and further exposure to acoustic radiation leads to the “blurring” of the excitation centers and the occurrence of more complex types of oscillations, which in turn leads to a decrease in the amplitude of oscillations.

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Consequently, the use of such a physical factor as acoustic vibrations at the natural frequency of vibrations of seeds makes it possible to obtain high-quality grain, increases productivity and reduces the cost of production.

The obtained results allow us to conclude that infrasonic-treated barley seeds show more intensive growth and development at the first stages of germination. This fact is especially important in dry regions, since, first of all, the root system develops vigorously. In this regard, after fundamental research on the effect of acoustic treatment on the ultrastructure of seeds, scientific and practical interest will be associated with real field tests.

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