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OPTIMIZATION OF THE MODES OF PRE-SOWING ELECTRICAL STIMULATION OF WINTER RAPE SEEDS BASED ON THE STUDY OF THE INTENSITY OF SINGLE PHOTON EMISSION BY THEM

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ABSTRACT

The article presents the results that can be a prerequisite for the introduction of a pre-sowing electrotreatment of winter rape seeds into the production process. The results of photon emission by rape seeds after

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sowing properties

electrical stimulation are highly correlated with the data obtained during determination of its sowing qualities and field germination. Absorption and transformation of the energy of the external electric field during the electrostimulation of winter rape seeds occurs non-linearly. It was established that the main transformations take place during the first 15 s, regardless of the electric field intensity. Relaxation processes were revealed, which become dominant after 15 s of electrical stimulation. The pre-sowing electrical stimulation improved the sowing properties of winter rapeseed. The highest values of germination energy and laboratory germination (87% and 96%), which exceeded the control by 9% and 8%, were obtained under the treatment mode E=2 kV·cm^{-1}, t=30 s. Pre-sowing electrostimulation of winter rape seeds at optimal conditions helps to increase its field germination. Under the treatment mode E=2 kV·cm^{-1}, t=30 s was 90%, and with E=3 kV·cm^{-1}, t=30 s – 83% versus 79.7% in the control.

Introduction

Various physical methods of a pre-sowing treatment of the seed material of agricultural crops are used in order to increase its sowing and yield properties. The most effective and ecologically safe ones are those that are based on the use of physical factors of influence. Among them, a treatment with laser beams, a weak magnetic field, a variable electromagnetic or electrostatic field turned out to be the most popular.

The laser is one of the sources for achieving the effect of biostimulation and genetic changes in higher plants. Smaller laser doses activate plants, which leads to an increase in the bioenergetic potential of the cell and a higher activation of their biochemical and physiological processes (Hernandez et al., 2010). Higher doses affect the genetic material of the cell, causing genetic changes in plants. This is confirmed by the obtained experimental data concerning the effect of seed laser irradiation on the variability of spring barley traits (Rybiński, 2000), on the leaf area and parameters of photosynthetic activity in DH lines of spring barley (Rybiński and Garczyński, 2004). Performing laser irradiation before sowing bean seeds revealed more water absorption, more intensive germination of the treated seeds compared to the seeds without irradiation (Podleśny et al., 2001), a significant effect of biostimulation on the weight gain of the total aerial part and roots (Podleśny, 2002) as well as increase in their productivity (Podleśny and Podleśna, 2004) were established. In other works, it was established that the damage to clover and alfalfa crops obtained from the seeds treated with laser rays was significantly less compared to the control (Wilczek et al., 2004; Wilczek et al., 2005; Mierzwa-Hersztek, M. Et al., 2019). Pre-sowing stimulation of seeds with laser light in doses of R6x5 and R6x3 causes a significant increase in the content of specific protein, phosphorus and molybdenum (Ćwintal et al., 2010). The study (Muszyński and Gładyszewska, 2008) investigated the effect of He-Ne laser irradiation of radish seeds as a potential means of accelerating their germination. The pre-sowing laser treatment has a stimulating effect on amaranth seeds. Pre-sowing stimulation with a He-Ne laser resulted in increased germination, increased yield of dry matter, crude protein, crude fiber and crude ash (Sujak et al., 2009; Dziwulska-Hunek et al., 2009). Studying the effect of low-intensity laser irradiation on the field parameters of hybrid corn seeds, no significant positive effects were obtained, compared to the control. For some hybrids, a negative effect of biostimulation on the percentage of seedlings, the speed of their appearance and the content of chlorophyll in plants was observed. These results show that it is necessary to find optimal irradiation parameters to induce positive biostimulation in the corn seeds (Hernandez-Aguilar et al., 2009). Laser biostimulation has a positive effect on certain indicators of sugar beet seeds, in particular the activity of nitrate reductase, photosynthetic pigments, contributes to the accumulation of sugar concentration in certain varieties (Sacała et al., 2012), improves the yield of vegetative and reproductive organs of peas (Podleśna et al., 2015).

To improve germination and increase the yield, a seed treatment with a magnetic field is widely used. By studying the stimulation of sugar beet seeds by a magnetic field on the yield and chemical composition of roots, an increase in the yield and biological yield of sugar was established in all treatment combinations (Pietruszewski and Wójcik, 2000). The same researchers established a positive effect of such processing on the germination of winter wheat seeds (Pietruszewski et al., 2001).

Other studies confirmed the positive effect of the magnetic treatment on germination and increase in the yield of bean seeds (Podleśny et al., 2004), wheat (Pietruszewski and Kania, 2010; Rochalska et al., 2011), sugar beet (Rochalska et al., 2009), sunflower (Matwijczuk et al., 2012), potato (Marks and Szecowka, 2010), peas (Podleśny et al., 2005; Iqbal et al., 2012). Stimulated pea seeds showed faster water uptake and achieved greater mass during imbibition compared to unstimulated seeds (Podleśny et al., 2005). However, the magnetic field treatment of lentil seeds did not show significant differences between the values of germination energy and germination in variants with the treated and control seed material (Aladjadjiyan, 2010).

Individual researchers have studied the effect of magnetic fields on the activity of enzymes in wheat plants. The magnetic field caused a decrease in the activity of alpha- and beta amylases. This can be very important in breeding and seed production. Along with this, it activates the glutathione S-transferase enzyme. This has resulted in plants being more resistant to pathogen attack, oxidative stress, and heavy metal toxicity (Rochalska and Grabowska, 2007). The influence of a stationary magnetic field on the germination and initial stages of growth of tomato seeds (Lycopersicon esculentum L.) was studied. As a result, it was found that germination in each treatment variant was lower than the corresponding control values, while the germination rate of the treated seeds was higher than the control (Martínez et al., 2009).

The pre-sowing treatment of seeds with an electromagnetic field deserves special attention. In the work (Stašelis et al., 2004) the influence of electromagnetic fields on tomato plants during the entire growing season was investigated. It was established that under their influence the seedlings developed faster, forming a larger total area of leaves, and were distinguished by higher growth. In the course of other studies (Lynikiene et al., 2006) it was established that the seeds exposed to the field of the corona discharge germinate faster and the dynamics of their germination is greater. Due to the stimulating effect, the viability of carrot seeds increased by 24%, radish and beet - by 12%, barley - by 9%. The positive effect of treatment in the electromagnetic field, consisting in an increase in the energy of germination and yield, was found for the seeds of several researched hybrids of corn (Zepeda-Bautista et al., 2010; Zepeda et al., 2011), millet (Wang et al., 2012) and chickpea (Mahajan and Pandey, 2014).

The pre-sowing electrotreatment of seeds, in particular oil crops, has a positive effect on oil yield, which increases by 4.9% from the treated seeds compared to untreated ones (Sarkis et al., 2015).

Summarizing the above, it can be stated that thanks to the pre-sowing treatment of the seed material, seed germination energy and germination increases, the yield increases by 10...30%, some types of plants increase the content of dry matter, the assimilation surface of the leaves and the branching of the root system. Due to such a treatment, the efficiency of cell functioning increases and intracellular metabolic processes are activated.

However, the mentioned indicators that reflect the impact of pre-sowing seed treatment on its sowing and yielding qualities, as well as certain physiological and biochemical properties of plants, do not provide a clear answer to the main question - how and according to which indicators it is possible to choose the optimal stimulation mode, under which the expected results will be positive and sustainable. In other words, there is currently not enough theoretical and experimental data that would reveal the cause-and-effect relationship between pre-sowing seed treatment modes and their sowing and yielding qualities. Therefore, it is necessary to select and determine in laboratory conditions such indicators of treated seeds that can help determine the optimal mode of their pre-sowing treatment.

To solve the problem, it is proposed to use the methods of photoluminescence and time-correlated counting of single photons of TCSPC, which treated seeds emit, in the visible range of the spectrum and to apply them to determine the residual effect of pre-sowing treatment on the seed material of certain agricultural crops.

Pilot studies of food products using time-correlated single photon counting methods have revealed photon emission in many microscopic and macroscopic systems, including lipid systems, bacteria, yeast, leukocytes, nerve cells, mitochondria, chloroplasts, cancer cells, etc. Recent studies also indicate that ultra-weak photon emission is an effective method for analyzing the interaction of nanoparticles with various biological objects (Kiełbasa et al., 2017; Oziembłowski et al., 2017).

Due to the method of time-correlated counting of single photons TCSPC and its adaptation for the studied types of seeds, it is possible to reveal the cause-and-effect relationship of pre-sowing treatment with sowing and yield qualities, determine its optimal parameters, and confirm the effectiveness of treatment.

The purpose of the research is to increase the sowing and yield properties of the seed material of winter rapeseed, to improve the quality characteristics of the products obtained from it by adjusting and optimizing the modes of its pre-sowing electrostimulation based on the determination of the photon emission of the stimulated seed.

Mateilas and Methods

The seed material of winter rapeseed hybrid MAXIMUS PR44D06 was used for the research. Its mass of 1000 grains was 4.64 ± 0.07 g, and the initial moisture content was 7.1 ± 0.3%. The seeds used in the experiments were uniform, high-quality, without any damage.

Sampling was carried out according to a standardized method. The number of replicates was representative of conditions of random variation in controlled seed parameters.

Electrical stimulation in the constant electromagnetic field was carried out at the experimental setup 1.

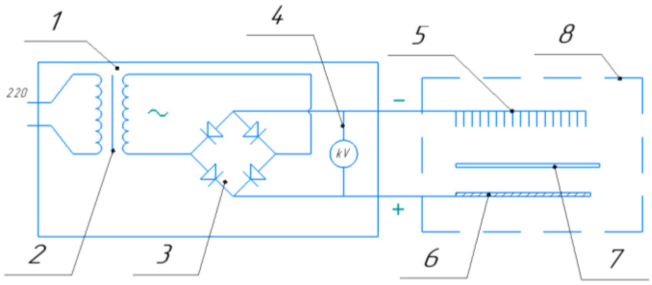

Figure 1. Experimental setup for pre-sowing electrostimulation of seeds: 1 – power element; 2 - power step-up transformer with adjustable voltage; 3 - diode full wave rectifier; 4 - 0-10 kV output voltage meter; 5 - coronating electrode; 6 – counter electrode; 7 – working surface; 8 - electrical stimulation unit

The experimental setup consists of a power element (1) and an electrical stimulation unit (8). The power supply unit contains a power step-up transformer with adjustable voltage (2), a diode full wave rectifier (3) based on 1N4007 power diodes and a 0-10 kV output voltage meter (4). This design allows smooth adjustment, rectification, and direct control of the output voltage.

The electrical stimulation unit contains a coronating electrode (5), a counter electrode (6) and a working surface (7). In its turn, the coronating electrode (5) consists of 15 metal plates with a length of 280 mm placed at the distance of 25 mm from each other. Metal needles with a length of 28 mm and a diameter of 0.8 mm are soldered to each plate, the distance between the needles is 30 mm. Through one plate along its width, needles are soldered with an offset of 15 mm. Under such conditions, their projections on the horizontal working surface form the vertices of an equilateral triangle, the side of which is equal to 30 mm. The distance from the edge of the needles to the working surface (7) made of a transparent polymer material with a thickness of 1 mm is 20 mm, and the distance from the working surface to the lower plate electrode (6) made of aluminum alloy is 10 mm.

The coronating electrode is connected to the negative pole of the power supply unit, and the counter electrode is connected to the positive pole, which ensures the supply of high regulated voltage and the formation of a negative corona discharge between them.

Pre-sowing electrostimulation was carried out at different values of the electric field strength E, $kV \cdot cm^{-1}$and duration (exposure) t, c, in particular: E=1.0, 1.5, 2.0 and 3.0 $kV \cdot cm^{-1}$; t=5, 10, 15, 20, 25 and 30 s. The incubation time - the period from the time of stimulation to the beginning of germination - was 1 day.

Before the treatment, the samples of 100 seeds were formed. Each of them was evenly distributed over the working surface 7 of the experimental setup (Fig. 1). After that, the required voltage was set for a specific time that corresponded to a certain exposure value. Processing time was monitored using a stopwatch. Subsequently, the seeds of the stimulated sample were placed in a glass Petri dish on the filter paper and left to rest for 1 day. After resting, the filter paper placed in the Petri dishes was moistened. Germination of electrotreated winter rape seeds was carried out in a climatic chamber of the RGX series. It automatically maintained a temperature of 25°C and a humidity of 60%. On the 4th day, the number of germinated seeds was recorded to determine the energy of germination, and on the 7th day, the number of germinated seeds was recorded to determine the laboratory seed germination.

To determine the field germination of stimulated seeds, small-scale field experiments were conducted. For this purpose, the treated seed material in the amount of 60 pieces was sown on the area of 1 m^2. The number of repetitions is 6 times. The plots were placed in a randomized order. The number of similar seeds in variants with stimulated seeds was determined on the 15th day after sowing and compared with the number of germinated untreated seeds in the control plots.

To determine the optimal mode of electrical stimulation, studies were conducted on the registration of photons emitted by the treated and untreated winter rape seeds in the electric field. A HAMAMATSU R4220 photomultiplier was used for this purpose. The measuring device ensured long-term thermal stabilization of the sample. The light-tight camera is equipped with a system of screens for periodically interrupting photon registration, as a result of which the noise and the useful signal were measured alternately, which made it possible to obtain reliable results even at low signal intensity. The duration of registration of photon radiation was at least 30 minutes for each sample. The result of the measurement of ultra-weak photon emission was the absolute difference between the number of photons registered by the photomultiplier in the light-tight chamber with stimulated rape seed and the number of photons registered by the photomultiplier in this chamber without seeds, according to:

$$L = A - B, \tag{1}$$

where:

L – a number of photons emitted by the studied sample,
A – a number of photons emitted by a sample placed in a light-tight chamber.
B – a number of photons created by an empty light-tight chamber.

Calibration of the sensor was carried out each time on the day of measurements and consisted in determining the ratio of the response of the system to the standard radiation dose according to the equation (2).

$$K = (A_o\text{-}B)/D \tag{2}$$

where:

K – the calibration coefficient (the measurement system was considered effective and ready for measurement if K = 0.8...1)
Ao – a number of photons in an interval of 500 s with reference forcing
B – a number of photons registered in the empty chamber
D – a known dose used for calibration (400 photons)

After starting the measuring system in the first phase, with a time interval of 120 s, stabilization of the system took place in order to prevent interference arising from temporary destabilization of standard conditions. The initial phase was followed by the main (measuring) phase with a time interval of 500 s. The frequency of recording the result was 4 Hz, that is, each recorded result represented the sum of photons counted during 0.25 s. These parameters were determined, as mentioned above, by the previous experiments, but they also included the minimum exposure time required for observations to lead to statistically significant test results. The main phase was followed by the final phase of the measurement, during which there is a stop, but no action of the measurement sequence. The entire measurement process was monitored in real time using the original program made in LabView.

Results

Based on the results of the literature review and considering the technological features of the electrostimulation process, several modes of pre-sowing electrostimulation of rapeseed were selected, which will allow to evaluate the efficiency of absorption of external energy by the seeds depending on the duration and intensity of irradiation. The experimental results of observing ultra-weak emission of photons after electrical stimulation are shown in Fig. 2.

The analysis of the obtained dependencies showed that the main accumulation of electrostatic field energy by the seeds occurs during the first 15 seconds of electrical stimulation, regardless of the field strength. With an increase in the duration of treatment, no significant increase in positive dynamics is observed.

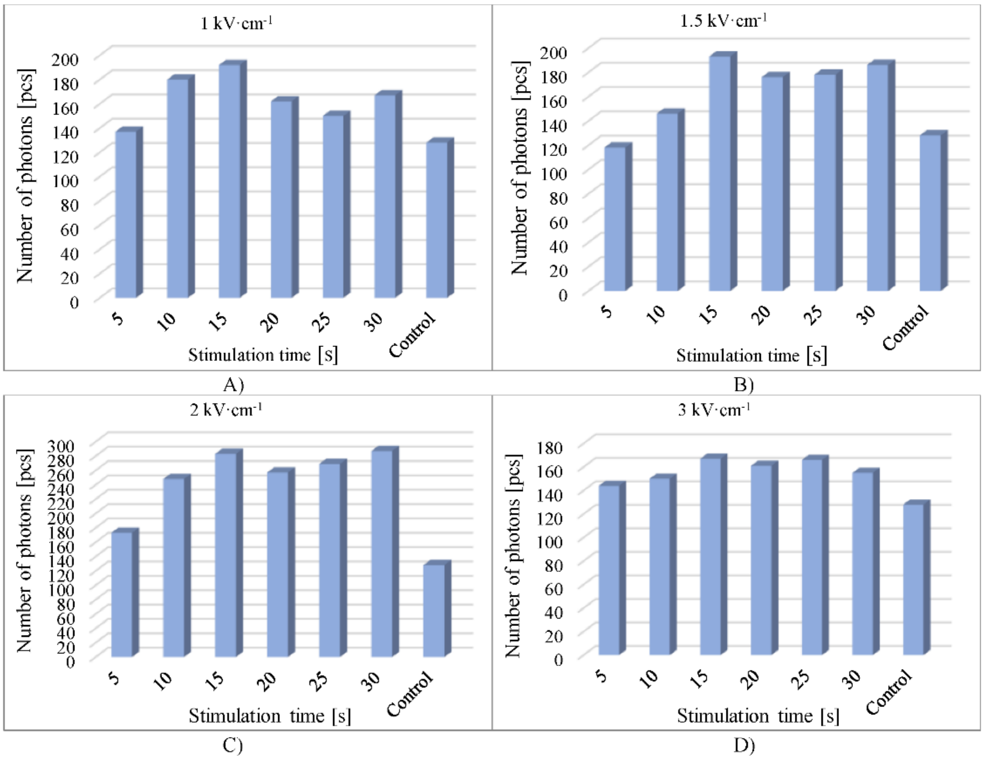

Figure 2. Histograms of photon emission by the rapeseed after electrical stimulation: 1 kV·cm^{-1} (A); 1.5 kV·cm^{-1} (B); 2 kV·cm^{-1} (C); 3 kV·cm^{-1} (D)

At the same time, the maximum increase in ultra-weak photon emission compared to the control sample was detected at the electric field intensity E = 2 kV·cm^{-1}, which, together with the previous results, indicates the presence of relaxation processes and the non-linear character of energy absorption and conversion by the rapeseed. The generalized measurement result is shown in Table 1.

Table 1.
Generalized results of observation of ultra-weak photon emission

Electric field strength, kV·cm^{-1}	Processing time [s]						Control
	5	10	15	20	25	30	
1	137	180	192	162	150	167	128
1.5	118	146	193	176	178	186	128
2	173	248	283	257	269	287	128
3	144	150	167	161	166	155	128

Since during the electrical stimulation the temporal and spatial distribution of the electric field is uniform, it is expected that the samples with the same absorbed energy should show similar dependences of photon emission. As can be seen in Table 1 for the samples: E = 2 kV·cm^{-1}, t = 5 s and E = 1 kV·cm^{-1}, t = 10 s, the number of photons is 173-180 pcs., for the samples E = 1.5 kV·cm^{-1}, t = 10 s and E = 3 kV·cm^{-1}, t = 5 s, the number of photons is 144-146, which corresponds to the proposed hypothesis. On the other hand, for the samples with a longer duration of electrical stimulation, no similar dependencies were found, for example, for the sample E = 3 kV·cm^{-1}, t = 10 s, the number of photons is 150, and for the sample E = 2 kV·cm^{-1}, t = 15 s, the number of photons increases to 193. These results additionally confirm the presence of certain relaxation processes in seeds with increasing duration of electrical stimulation.

The obtained results of photon emission by the rape seeds after electrical stimulation are largely correlated with the results obtained during the determination of its sowing qualities. Shown in Fig.3, data on the germination energy of stimulated seeds show that in all processing modes this indicator exceeds the control.

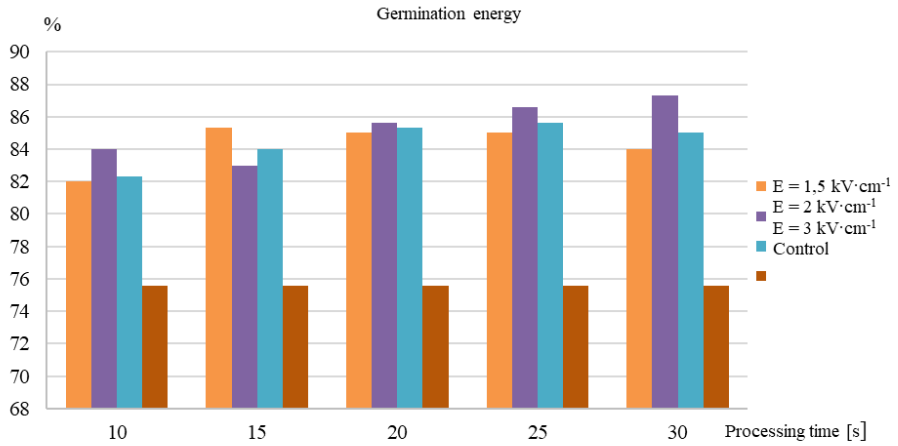

Figure 3. The effect of pre-sowing electrostimulation modes on the germination energy of winter rape seeds

The highest value of germination energy (87%), which exceeded the control by 9%, was obtained in the processing mode E = 2 kV·cm^{-1}, t = 30 s. In this situation, it should be noted that under this processing mode, the maximum increase in ultra-weak photon emission was observed compared to the control sample - 287 against 125. At the same time, it should be noted that at the field strength E = 2 kV·cm^{-1} and all investigated values of the processing duration as energy germination of treated winter rape seeds, as well as the emission of photons emitted by them was the highest at t = 30 s. Based on this, it is possible to draw preliminary conclusions that the efficiency of emitted photons can serve as one of the indicators for determining the effectiveness of pre-sowing electrostimulation of the studied seed material.

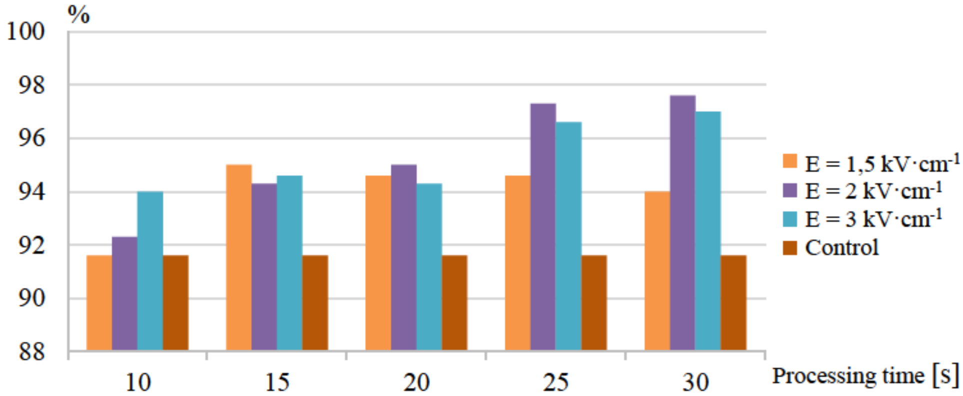

Figure 4. The effect of pre-sowing electrostimulation modes on the laboratory germination of winter rape seeds

The results of the determination of laboratory germination (Fig. 4) also testify to the positive effect of the electrotreatment on the studied seed material. The highest laboratory germination of seeds was obtained under the processing mode in which the electric field strength E = 2 kV·cm^{-1}, t = 30 s. Under such conditions, its value was about 98% against 91% in the control, exceeding its value by 7%.

Field germination was determined for the stimulated winter rape seed at the electrotreatment modes in which the highest germination energy and laboratory germination was achieved, as well as the maximum increase in ultra-weak photon emission was observed compared to the control sample. The obtained results are shown in Table 2.

Analyzing the data given in Table 2, it can be asserted that the field germination of winter rape seeds processed in both modes exceeds the control. However, the mode E = 2 kV·cm^{-1}, t = 30 s turned out to be better, during which the field similarity exceeded the control by 10.3 %.

Table 2.
The effect of pre-sowing electrostimulation modes on the field germination of winter rape seeds

Processing modes	The number of germinated seeds in replicates						Average	Field germination, (%)
	1	2	3	4	5	6		
E = 2 kV·cm^{-1} t = 30 s	54	55	53	53	54	55	54	90
E = 3 kV·cm^{-1} t = 30 s	51	50	52	49	50	48	50	83
Control	49	47	47	49	48	47	47.8	79.7

Having investigated the effect of pre-sowing treatment of winter rapeseed on its sowing qualities (germination energy and laboratory germination) and field germination, as well as the results of observing its ultra-weak photon emission, it can be seen that the investigated indicators are the highest under those processing modes in which the number of emitted photons is the largest. Based on this, it is possible to draw a preliminary conclusion that the highest efficiency of pre-sowing electrostimulation of the studied seed material is achieved in the modes in which the emission of photons of the seeds treated in the electric field is the largest. The obtained results can be a prerequisite for the introduction of pre-sowing electrotreatment of winter rape seeds into the production process.

Conclusions

1. The absorption and transformation of the energy of the external electric field during the electrostimulation of winter rape seeds occurs non-linearly. It was established that the main transformations occur during the first 15 s regardless of the electric field strength. Relaxation processes that become dominant after 15 s of electrical stimulation have been revealed.
2. Pre-sowing electrical stimulation improved the sowing properties of winter rapeseed. The highest values of germination energy and laboratory germination (87% and 96%), which exceeded the control by 9% and 8%, were obtained under the treatment mode $E = 2$ $kV \cdot cm^{-1}$, $t = 30$ s. Under this mode, the maximum increase in ultra-weak photon emission was observed compared to the control sample – 287 versus 125.
3. Pre-sowing electrostimulation of winter rape seeds at the optimal modes helps to increase its field germination. Under the processing mode $E = 2$ $kV \cdot cm^{-1}$, $t = 30$ s, it was 90%, and with $E = 3$ $kV \cdot cm^{-1}$, $t = 30$ s – 83% versus 79.7% in the control.
4. The results of photon emission by the rape seeds after electrical stimulation are highly correlated with the data obtained during the determination of its sowing qualities and field germination. On the basis of this, we can draw preliminary conclusions that the most effective mode of processing the seed material can be considered the one in which the emission of single photons emitted by it is the largest.
5. The obtained results can be a prerequisite for the introduction of pre-sowing electrotreatment of winter rape seeds into the production process.

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OPTYMALIZACJA METOD PRZEDSIEWNEJ STYMULACJI ELEKTRYCZNEJ NASION RZEPAKU OZIMEGO NA PODSTAWIE INTENSYWNOŚCI EMISJI POJEDYNCZEGO FOTONU

Streszczenie. Niniejszy artykuł przedstawia wyniki, które mogą stanowić przesłankę wprowadzenia przedsiewnego zabiegu elektrycznego nasion rzepaku ozimego w procesie produkcji. Wyniki emisji fotonu przez nasiona rzepaku po elektrycznej stymulacji są powiązane mocno z danymi otrzymanymi podczas określania ich właściwości siewnych oraz kiełkowania. Absorpcja oraz transformacja energii zewnętrznego pola elektrycznego podczas elektrostymulacji nasion rzepaku ozimego występuje nieliniowo. Stwierdzono, że główne transformacje mają miejsce w pierwszych 15 sekundach bez względu na intensywność pola elektrycznego. Odkryto procesy relaksacyjne, które staly się dominujące po 15 sekundach stymulacji elektrycznej. Przedsiewna stymulacja elektryczna poprawiła właściwości siewne rzepaku ozimego. Najwyższe wartości energii kiełkowania i kiełkowania laboratoryjnego (87% i 96%) co przekroczyło próbę o 9% i 8%, uzyskano w trybie zabiegu $E = 2$ kV·cm^{-1}, $t = 30$ s. Elektrostymulacja przedsiewna nasion rzepaku ozimego przy optymalnych warunkach pomaga zwiększyć kiełkowanie na polu. W trybie zabiegu $E =$ kV·cm^{-1}, $t = 30$ s wynosiło 90%, a przy $E = 3$ kV·cm^{-1}, $t = 30$ s – 83% versus 79.7% w próbie.

Słowa kluczowe: rzepak ozimy, nasiona, foton, stymulacja elektryczna, optymalizacja, właściwości siewne