Winter rye tolerance to low temperatures after seed treatment with surface barrier discharge

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Abstract. In this work, the experimental results of surface barrier discharge plasma products affection to winter rye seed germination at the initial stage of growth and seedling tolerance to low temperatures are shown. The treatment was carried out for 10, 60, and 180 s in the plane-parallel electrode system with sinusoidal voltage of 2.7 kV with frequency 4.4 kHz applied to the strip electrodes at a distance of 5 mm from each other. It is shown that the treatment has no effect on seed germinating ability. In 180 s exposure treatment mode the stimulation of 3-day seedling shoot and root system length occurs. The freezing tolerance response of two-stage cold hardened seedlings grown from treated seeds shows that both the exposure of seed and the freezing temperature affect on the formation of positive response.

1. Introduction
Pre-sowing treatment of crop seeds by products of gas-discharge low-temperature plasma is one of the promising methods to increase plant productivity [1]. Various applications of different atmospheric discharge types are considered—dielectric barrier discharge, discharge in inert gas flow [2–6]. Efficiency of these treatment methods is shown in field tests [2, 3]. However, field experiments are quite labor-intensive, and there are no theoretical methods of choosing and adjusting seed exposure modes for now. For seed response estimation a significant amount of studies of seedling development is most often carried out at the initial stage of ontogenesis. Various parameters indirectly related to field productivity or characterizing the induced stress are analyzed. These are morphological indicators, germinating ability, the water uptake by seeds, changes in seed surface properties, the contamination rate, the work of the antioxidant system, etc [5,6]. In recent years, researches of the efficiency of the seed treatment with discharge products have also appeared in terms of the response to simulated stresses, for example, to the drought stress [7,8].

Tolerance of seedlings grown from seeds treated with discharge products to certain types of stress can be an effective method of exposure modes determination to increase field productivity. It is also worth drawing attention to the fact that in risky farming regions plant tolerance is the primary factor of their yield potential estimation.

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In this work, the researches of morphological indicators (shoot and root system length, seed germinating ability) and tolerance of winter rye seedlings treated with surface barrier discharge plasma products to low temperatures are carried out.

2. The experimental setup
Seed treatment was carried out in the plane-parallel strip system with surface dielectric barrier discharge. Twelve parallel strip electrodes (1 mm wide and 100 mm long) made of copper foil (20 µm) were placed on the surface of dielectric barrier (corundum ceramics of 1 mm thickness) at a distance of 5 mm from each other. The reverse electrode occupied the whole opposite surface of dielectric barrier and was grounded. A sinusoidal voltage of 2.7 kV with frequency of 4.4 kHz was applied to the strips. Seeds were located in one layer on a grounded plane 10 mm distant from the surface of the dielectric barrier. Seed exposure time was 10, 60, and 180 s. Figure 1 shows sectional drawing of the electrode system.

Seedling tolerance to low-temperature effects was determined by the method [9]. The method of determining relative freeze tolerance consists in freezing sprouted to certain level seeds and defining their survival rate in various samples compared with each other and with control sample. The method is implemented in several stages. The first stage is germination and selection. Seeds of one sample have germinated in an individual container in one layer on two layers of filtering paper moistened with distilled water in the dark at 24°C for 40–45 hours. In such test, seedling freeze tolerance depends on shoot length, so seedlings with 4–8 mm shoots are selected. Stronger or weaker seedlings are not used in the experiment. The required number of selected samples with 40–50 seedlings per sample is provided. Each selected sample of seedlings is placed in a piece of gauze (moistened and squeezed), tied and marked. The second stage is seedling cold hardening. In this experiment, the selected seedlings are provided with two phases of cold hardening. The samples are placed in containers providing aeration in one layer. The first phase of cold hardening is 7 days at a temperature from 0 to +2°C. The second phase of cold hardening is that containers are transferred to temperature from −4 to −5°C for three days. The third stage is freezing and thawing. After the second phase of cold hardening, the temperature is reduced to −12, −14, −16, −18, −20°C (the climate chamber “Binder”) with 24-hours exposure at each temperature. After each 24-hours exposure, a part of samples is removed. After removal from freezing, the samples are transferred to the temperature of +2°C to thaw for a day. After the thawing stage, the containers are removed and transferred to the thermostat with the temperature of 24°C for two days. At the bottom of the thermostat there

Figure 1. Sectional drawing of the electrode system.
Figure 2. Morphological indicators of seedlings grown from seeds treated at various exposure modes: (a) shoot length; (b) total length of individual roots; (c) 3-day germinating ability.

are containers of water. After staying in a warm, moist environment, seedlings are laid out in rows groove down on two layers of paper moistened with distilled water and grown in the dark at 24 °C for 7 days. Then the number of surviving seedlings is determined. The freeze tolerance of the sample is determined as a relation of the surviving seedlings number to the laid seeds number.

After treatment with a surface discharge, a part of the seed sample was taken to assess the morphological parameters of seedlings. The treated seeds were germinated for three days in a dark place in a thermostat (24 ± 1 °C) on two layers of filtering paper moistened with distilled water. The seeds were spread out in plastic containers of 100 seeds per sample, 400 seeds per container. The paper was daily moistened with 2 ml of distilled water per sample, the containers were ventilated and rearranged in the thermostat. On the third day morphological indicators (shoot length, length of individual roots) and 3-day germinating ability (the ratio of the number of normally germinated seeds to the total number of seeds) were estimated.

As a treated object, high quality winter rye seeds of 2018 year yield were used from the collections of the core facilities center “Bioresource Center” SIPPB SB RAS. All experiments
Figure 3. Seedling relative freeze tolerance.

were carried out in 2019. On the histograms average values are shown with 95% confidence intervals.

3. Results and discussion

Figure 2 shows the results of measuring the morphological indicators of seedlings obtained after the seed treatment. Figure 3 shows the estimation results of seedling relative tolerance to low temperatures. Seed germinating ability almost does not change with exposure time changing and retains its high values, figure 2(c). At the same time, in 180 s exposure treatment mode a tendency to the increase of shoot and root system length occurs. The shoot length stimulation [see figure 2(a)], and especially the root system stimulation [see figure 2(b)], indirectly point to the increase of plant tolerance. The winter rye relative tolerance to low temperatures has quite wide confidence intervals that converge only in some cases (10 s of exposure at $-12 \degree C$ and $-20 \degree C$, 180 s of exposure at $-20 \degree C$) (see figure 3). Despite this fact, a tendency to the increase of seedling tolerance occurs at all treatment modes at the temperature of $-20 \degree C$. At the temperature of $-18 \degree C$ this tendency is only at the mode of 180 s exposure. At the temperature of $-12 \degree C$ only 10 s exposure treatment provides the increase of seedling tolerance. The other treatment modes (60 and 180 s) decrease seedling tolerance at $-12 \degree C$. At $-14 \degree C$ and $-16 \degree C$ there are no significant differences.

4. Conclusions

With the winter rye seed treatment by surface barrier discharge plasma products the comparison of seedling morphological characteristics and their freeze tolerance shows formation of different responses in seeds. The treatment effect on the freeze tolerance of hardened seedlings depends both on the exposure time and on the level of stress affect (in this case, on the freezing temperature). Thus, 180 s exposure treatment mode which provides morphological characteristics stimulation decreases the tolerance at the temperature of $-12 \degree C$ and increases it at temperatures of $-18 \degree C$ and $-20 \degree C$. 
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References
[1] Rifna E J, Ratish Ramanan K and Mahendran R 2019 Trends Food Sci. Technol. 86 95–108
[2] Zhang B, Li R and Yan J 2018 Arch. Biochem. Biophys. 655 37–42
[3] Gordeev Yu A 2007 Stimulirovanie Biologicheskikh Processov v Semenah Rasteniy Izhucheniyami Nizkotemperatura Plazmy. Monografiya (Smolensk: Campus of the Russian State University of Tourism and Services Studies)
[4] Lotfy K, Awad Al-Harbi N and Abd El-Raheem H 2019 Plasma Chem. Plasma Process. 39 897–912
[5] Zahoranová A, Henselová M, Hudcová D, Kaliňáková B, Kováčik D, Medvecká V and Černák M 2016 Plasma Chem. Plasma Process. 36 397–414
[6] Los A, Zinuzina D, Boehm D, Cullen P J and Bourke P 2019 Plasma Processes Polym. 16 1800148
[7] Guo Q, Wang Y, Zhang H, Qu G, Wang T, Sun Q and Liang D 2017 Sci. Rep. 7 16680
[8] Ling L, Jiangang L, Minchong S, Chunlei Z and Yuanhua D 2015 Sci. Rep. 5 13033
[9] Samygin G A 1967 Bystroe Opredelenie Otnositel’noy Morozostoykosti Obrazkov Pshenicy Putem Promorazhivaniya Proroshikh Semyan. Metody Opredeleniya Morozostoykosti Rasteniy (Moscow: Nauka)