

Treatment of spring wheat seeds by ozone generated from humid air and dry oxygen

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Abstract

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The paper presents an analysis based on conflicting data regarding the results of the treatment of soft spring wheat seeds by ozone generated from humid air and dry oxygen. Morphological characteristics of treated seeds (the length of a sprout, the total length of roots and the sprout-to-root ratio), 7-day germination ability along with the extent of 7-day-old seedlings contamination are considered in terms of ozone concentrations. The experiments were conducted using the wheat seeds of 2013 and 2014 yields. For the same concentrations of ozone, morphological characteristics of treated seeds and efficiency of seed surface treatment changed similarly for both ways of ozone production. However, the efficiency of seeds treatment and stimulation of seeds germination with ozone are not correlated; and the germination ability of the seeds is not changed after ozone treatment.

Keywords: ozone treatment; spring wheat; morphological characteristics; surface dielectric-barrier discharge; seed germination

High concentration of tropospheric ozone in the Earth's atmosphere is well-known to be able to cause a significant damage of cultivated plants. The data on the analysis of the ozone action on soft wheat *Triticum aestivum* L. (FENG et al. 2008) suggest that a long-term ozone exposure can (i) reduce the level of crop yield, (ii) limit the accumulation of biomass, (iii) decrease the quantity and quality of seeds, and (iv) depress the photosynthesis.

However, ozone effect on the plant material (seed grains) at the moderate ozone concentrations can lead to the improvement of morphological characteristics of seeds and stimulate seeds germination. At the same time, a biological membrane in the cells of the seeds is not damaged and antioxidant activity is not significantly changed, but the formation of stable organic radicals can be provoked (ŁABANOWSKA et al. 2006). Ozone inhibits the surface biocontami-

nations, but it does not change the capability of the seeds to germinate (MARIQUE et al. 2012) and it does not affect the physical and biochemical properties of the seeds (SAVI et al. 2014). This allows for using ozone for disinfectant treatment of the seeds (RAILA et al. 2006; MARIQUE et al. 2012).

Today, some efforts of various research groups are aimed at the development of gas discharge methods for pre-sowing seed treatment of cultivated plants (DOBRIN et al. 2015; LAZUKIN et al. 2015; ZAHORANOVÁ et al. 2016). This approach to improve the seed germination of agricultural plants is currently considered to be an alternative to the traditional seed processing technologies (FILATOVA et al. 2010; DOBRIN et al. 2015). The main question which remains unclear is which mechanisms play a dominant role in the plant growth enhancing. Ozone or oxygen radicals are likely to be the most important germination improvement factor due to their high efficiency in seed treatments (KITAZAKI et al. 2012; MASTANAIAH et al. 2013).

However, a number of conflicting data on the effect of ozone exposure duration on the germination parameters of seeds have been revealed. Some meaningful results on the stimulation of seeds growth have been obtained using a coplanar surface barrier discharge (ZAHORANOVÁ et al. 2016) and a surface discharge reactor with the barrierless electrodes (DOBRIN et al. 2015). They suggest that overexposure reached at a treatment duration of 80 sec depresses the ability of seeds to germinate and accumulate biomass, whereas other data (LAZUKIN et al. 2015) do not show any noticeable deterioration of the quality parameters of the seeds after the exposure for 20 min. The differences among these data could be caused by the seeds being located towards plasma in different ways: directly in the plasma zone (DOBRIN et al. 2015; ZAHORANOVÁ et al. 2016) and at distance of 6–8 mm from the plasma zone (LAZUKIN et al. 2015). Therefore, various compositions of agents that influence the germination ability of the seeds were created. A negative effect of the overexposure presented in (DOBRIN et al. 2015; ZAHORANOVÁ et al. 2016) is probably related to the effect of electrical fields and the high density of volume charge existing in the plasma zone rather than the ozone itself.

To verify the idea, in the paper, ozone is assumed to be a single active agent. It was generated by the surface dielectric-barrier discharge from humid air or dry oxygen. Therefore, the goal of this work is to reveal the effect that ozone generated from humid

air or dry oxygen by the surface dielectric-barrier discharge (SDBD) has on the extent of contamination, seed's germination and morphological characteristics of the sprouts of spring wheat seeds.

EXPERIMENTAL MATERIAL AND TECHNIQUE

Spring soft wheat of variety Novosibirskaya 29 harvested in 2013 and 2014 was used as a model object. All experimental data were obtained from December 2015 to April 2016. The seed yield in 2014 had a high germination ability of $98 \pm 2\%$, which was determined by examining of 4 groups 80 seeds each. The extent of contamination of the seeds was low. The seeds of 2013 yield were weakened by storage and a high extent of contamination. The check of germination ability was determined by examining of 6 groups with 80 seeds each, and it was equal to $64 \pm 9\%$. Hence, the seeds of 2013 yield were expected to give more demonstrative response in terms of the germination ability and extent of contamination, whereas the seeds of 2014 yield – in terms of morphological characteristics and the reduction of germination.

Fig. 1 shows the schematic experimental setup. The seeds (80 g, i.e. 1,800–2,200 grains) were placed into a cylindrical plastic tank. Ozone was generated from humid air (52%) or dry oxygen using a surface dielectric-barrier discharge reactor with extensive electrodes. The gas flow rate was $1 \text{ l} \cdot \text{min}^{-1}$.

Oxygen was produced with an oxygen concentrator Oxymat 6000, which provided the oxygen concentration of 96–98% with the gas humidity of less than 1%. Ozone concentration was measured with an optical gas analyser of ozone Tsiklon 5.31 (OPTEK, Russia) at the inlet and outlet of the container with the seeds. The time interval of data recoding at the inlet and outlet was 5 sec. All the joints and components were fitted to rule out the interaction with ozone. After the second gas analyser, the ozone was wasted by a deozoneator.

The extent of contamination h of 7-day-old seedlings was determined visually. Each contamination degree was given a reference number of 0 to 4. This estimation technique based on the visual inspection of separate seedlings was described in (GAGKAEVA et al. 2011): $h = 0$ – healthy seeds; $h = 1$ – healthy seeds with a mycelium thin coat; $h = 2$ – the darkening of seedlings with spots and strokes; $h = 3$ – weak seedlings with extensive ne-

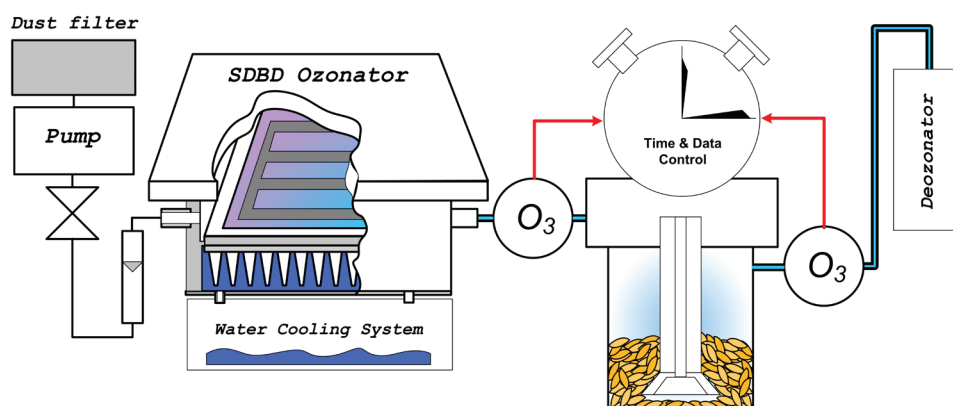
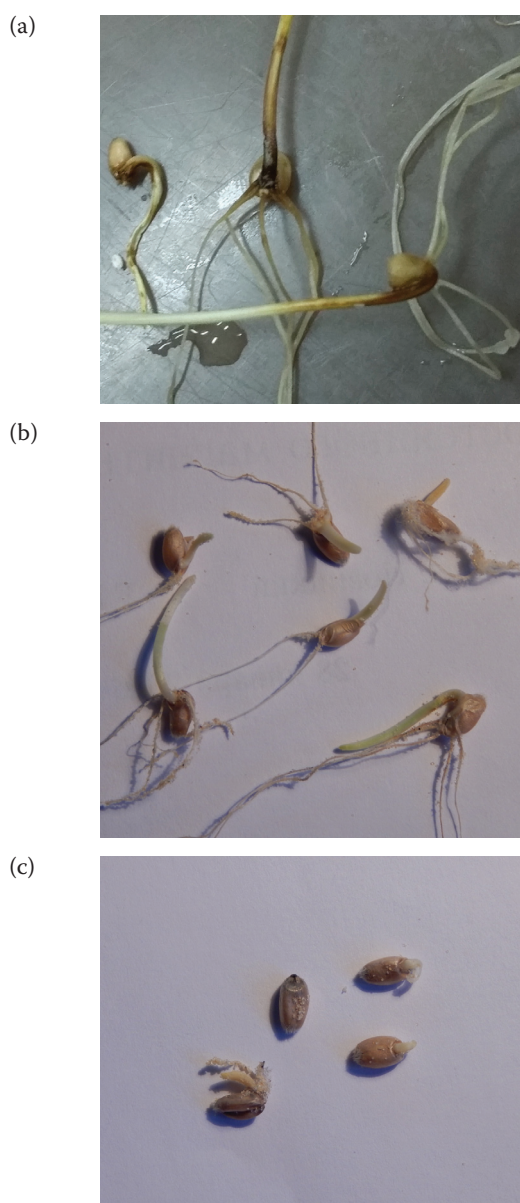


Fig. 1. Schematic experimental setup

Fig. 2. 7-day-old seedlings with the different extent of contamination: $h = 2$ (a); $h = 3$ (b); $h = 4$ (c)

crosis; $h = 4$ – decayed and dead seeds. The relevant photos of the seeds with different extent of contamination are presented in Fig. 2.

The seeds after treatment were placed into the plastic containers filled with sand, which were ignited, sifted, and wetted with distilled water with 80% of maximal moisture. The seeds were not buried into the sand and were located at a distance of 1 cm from each other. Then, the containers were put in a dark place at a temperature of $20 \pm 1^\circ\text{C}$ for 7 days. Daily wetting with 1 ml of distilled water per a container and winding for 1 min was provided.

The number of sprouts appearing on the seventh day after sowing in relation to the total number of seeds sowed for a trial was considered as the germination ability. The sprout with a length of more than a half of linear size of the seed and more than two roots was taken over the normal one.

Morphological characteristics that were estimated are the length of a sprout, the total length of roots and the sprout-to-root ratio. The results were analysed using an average magnitude obtained from three trials of 50 replications ($n = 150$). The probability P of the appearance of differences in trials for morphological characteristics was estimated by means of the Tukey's method of multiple comparisons (ZAITSEV 1984). Differences were considered to be valid at $P < 0.05$. Confidence intervals for the germination ability ($p = 0.95$) were calculated using the test of proportions (NIKOLAEVA et al. 1999). *R Statistical Software* was used for the data processing.

RESULTS AND DISCUSSION

To determine the amount of ozone that is absorbed by the seeds, ozone concentration was meas-

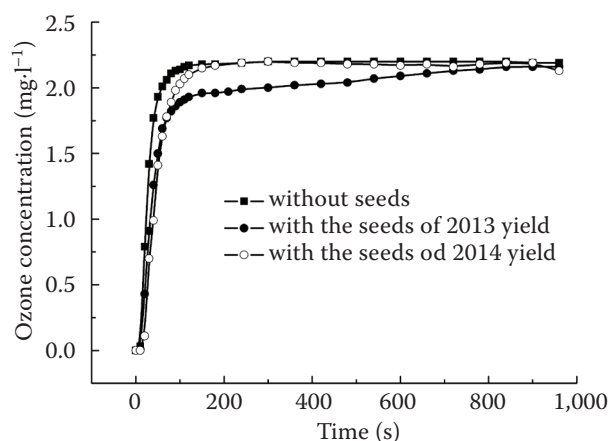


Fig. 3. The time dependence of ozone concentration at the outlet of the empty container and container filled with seeds of 2013 and 2014 yields

ured as a function of time for a container without seeds and for the container filled with seeds. The results of these measurements are shown in Fig. 3.

Ozone concentration at the outlet of the empty container quickly reached saturation, whereas the curve for the container filled with the seeds dropped behind it. The curve obtained for the seeds of 2013 yield displayed far slower than the curve for the seeds of 2014 yield did.

Analysis of the ozone concentration time dependence for the empty and full containers enables us to determine the mass of ozone that is absorbed by the seeds. It is 2.32 mg for the seeds of 2013 yield and 0.93 mg – for the seeds of 2014 yield.

Ozone inside a cell is dissolved by water. Water in biological objects is a complex heterophase system containing free water, bulk water and bound water (Krishnan et al. 2004). The amount of water contained in the seeds was measured by the comparison of the mass of the seeds before and after drying. Seed drying was conducted at 60°C up until the mass reached its stable state. Moisture content in the seeds of 2013 yield was $5.81 \pm 0.08\%$, and that in the seeds of 2014 yield – $5.55 \pm 0.05\%$. Taking into account the weight of the treated seeds (80 g) the moisture content of 4.64 and 4.44 ml of water was obtained for 2013 and 2014 seed yields, respectively. Even if the maximal water dissolubility of ozone was used at a room temperature, i.e. $13 \text{ mg} \cdot \text{l}^{-1}$, it was found that the amount of absorbed ozone does not exceed 0.06 mg. This means that ozone diffusion and ozone dissolving by water can be neglected for the purpose of analysing the changes in the ozone concentration.

Apart from the direct absorption of ozone by water, the ozone is consumed by chemical reactions with the sources of surface contamination, seed cover, and seed inner structures which ozone reaches through the pores due to diffusion. Hence, oxidative processes might be expected to be completed when the ozone absorption curve reaches the saturation. For the wheat seeds of 2013 yield this period lasts 15 min; for 2014 seed yield – 6 min. Yet to produce a similar effect on the both groups of the seeds, the seeds of 2014 yield were treated for 15 min similarly to the seeds of 2013 yield. The

Table 1. Data on the visual estimation of the seeds contamination

Treatment conditions	The extent of contamination, <i>h</i>					<i>P</i>
	0	1	2	3	4	
The yield of 2013						
Control	52.4*	14.8	1.5	14.2	17.1	—
OinA 1.5 g·m ⁻³	49.3	9.3	7.3	9.3	24.7	<0.05
OinA 2.0 g·m ⁻³	66.2	8.8	0.0	6.9	18.1	<0.01
OinA 3.5 g·m ⁻³	70.7	4.0	0.0	25.3	0.0	<0.01
OinO 4.0 g·m ⁻³	72.0	5.3	5.3	6.7	10.7	<0.01
OinO 25.0 g·m ⁻³	58.7	6.7	0.0	33.3	1.3	>0.05
The yield of 2014						
Control	61.2	22.0	7.8	7.1	1.9	—
OinA 3.5 g·m ⁻³	50.0	21.3	14.0	13.3	1.3	<0.05
OinO 25.0 g·m ⁻³	55.0	27.5	2.0	14.1	1.3	>0.05

*Control – untreated seeds; OinA – seeds treated by ozone generated from humid air; OinO – seeds treated by ozone generated from dry oxygen; *P* – the probability of null-hypothesis (if $P > 0.05$, the difference could not be considered as valid)

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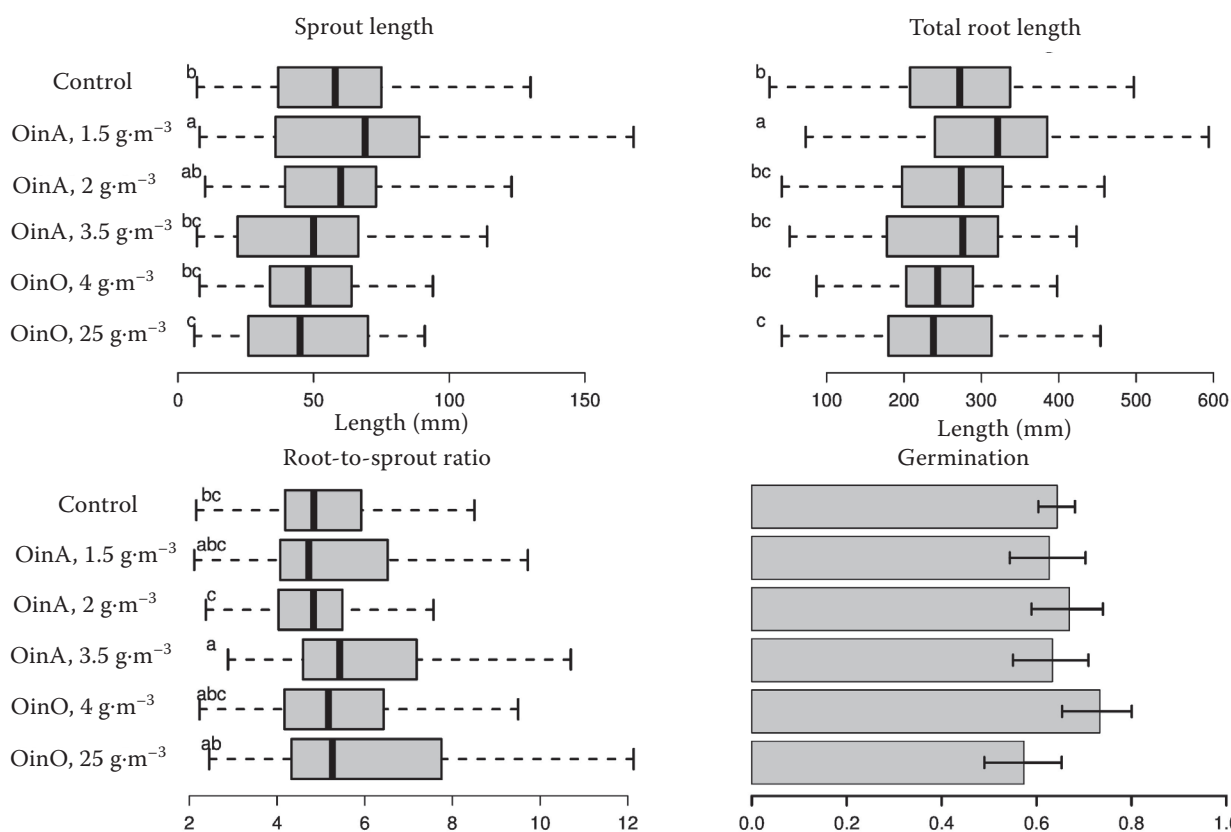


Fig. 4. Morphological characteristics (sprout length, total root length, root-to-sprout ratio) and 7-day germination of the wheat seeds of 2013 yield

*results with the validation of 95%

results of visual examination of the contamination of 7-day-old seedlings are presented in Table 1, containing the data in terms of the proportions of all the seeds in a trial, i.e. 150 grains.

A general scenario of invigoration of the seeds affected by mycelium is in a good agreement with the ideas in (RAILA et al. 2006; MARIQUE et al., 2012; SAVI et al. 2014; ZAHORANOVÁ et al. 2016). Agents of grain fusariosis are well-treated by ozone, and the attributes of their development become less distinguishable as the ozone concentration increases. However, at high ozone concentrations, especially in the case of the treatments with an ozone-oxygen mixture, the damages related to the development of fungi *Aspergillus* appear. This fact is also highlighted in (RAILA et al. 2006). Moreover, close spacing of seeds makes recontamination possible.

The results of measuring the morphological parameters and germination ability of the seeds are given in Fig. 4 (for the seeds of 2013 yield) and in Fig. 5 (for the seeds of 2014 yield). The data are presented in the form of box plot, where the central lines are the medians, the boundaries of the grey

rectangle are quantiles of 25 and 75%, the contour dashed lines are 0.25 and 100%. Significant differences between the experimental groups are shown in the graphs by letters. Identical letters marked groups that do not have statistically reliable differences. The fragment of the figures with germination data has no letter markings, since no significant differences in seed germination were found. The statistical reliability of the differences was determined using the Tukey's Honestly Significant Difference (Tukey's HSD) test.

The wheat treatments by ozone under the conditions considered above do not significantly influence the germination ability of the seeds. Therefore, it can be concluded that the dependence of seed germination on the exposure by surface discharge plasma shown in (ZAHORANOVÁ et al. 2016) does not result from the ozone effect. On the other hand, seed treatments by ozone cause changes in the lengths of roots and sprout. This is very noticeable for the results of the treatment of weakened wheat seeds. The change of the seed morphology appears along with the change in the number of

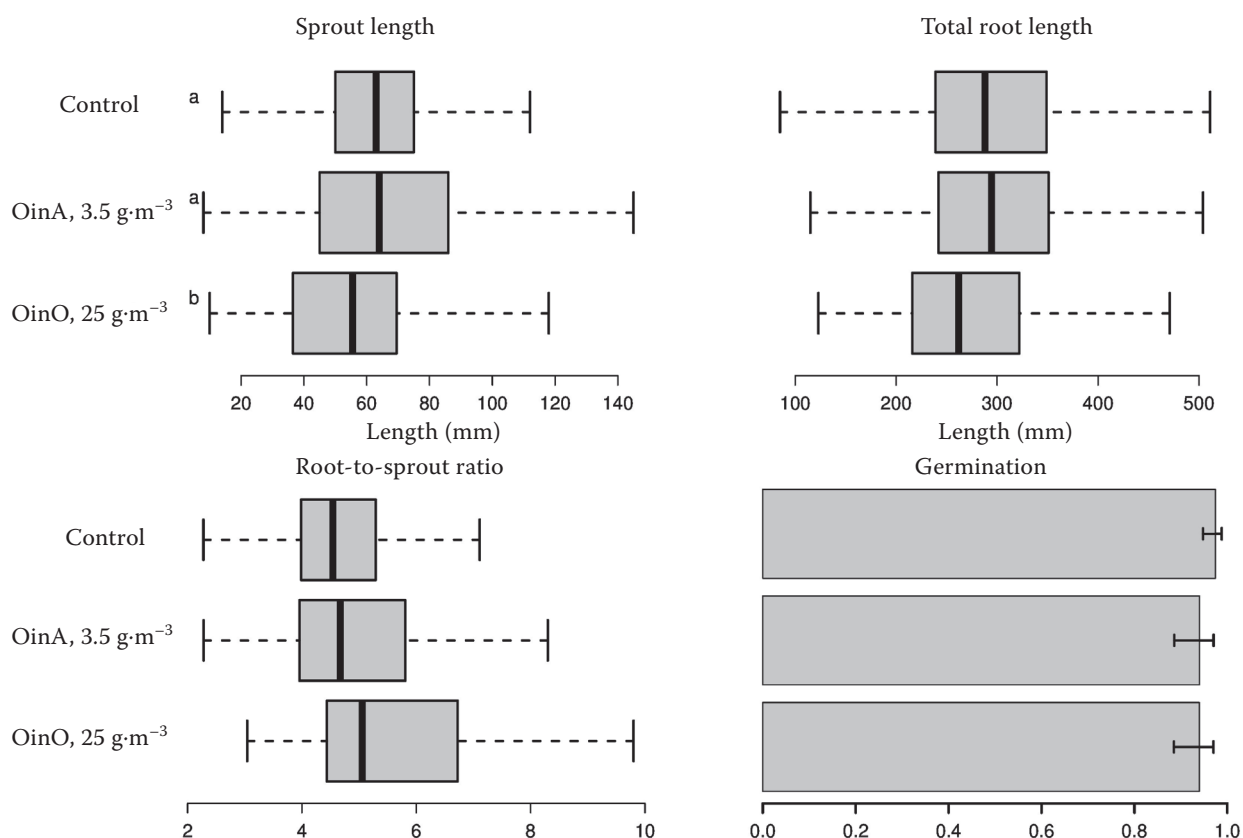


Fig. 5. Morphological characteristics (sprout length, total root length, root-to-shoot ratio) and 7-day germination of the wheat seeds of 2014 yield

*results with the validation of 95%

contaminated seeds. Moreover, not all of the contamination agents are inhibited equally effectively. Healthy seeds quantity is 72%.

The results of seed treatments by ozone generated from humid air and dry oxygen in the similar concentrations do not differ in the germination ability and morphology of the seedlings or in the seed treatment efficiency. This might suggest that reactive nitrogen species do not play a significant role. High concentrations of ozone at the exposure of the seeds do not further improve the efficiency of seed treatment, since when the basic contaminations are eliminated, microorganisms which are more resistant to an oxidative stress and cannot be detected by eye. Seed treatment by ozone in a high concentration generated from dry oxygen causes the reliable inhibition of the sprout contaminations and a relative transfer of biomass into roots, i.e. the increase in the ratio of total length of roots to the length of sprout. This is indicative of the enhanced resistance of a developing plant to different negative factors.

CONCLUSION

The investigation of morphological characteristics and germination ability of the soft spring seeds treated by ozone generated from humid air and dry oxygen has given the following results:

ozone treatment leads to the significant change in morphological characteristics of the seedlings;

ozone treatment does not lead to the change in the germination ability of the seeds;

data on the efficiency of ozone seeds treatment and ozone stimulation of the seeds germination are not correlated.

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