Effect of presowing seed treatment on photosynthetic pigments content and enzyme activity in *Hordeum vulgare* L. plants

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Abstract. The use of nanoparticles in pre-sowing seed treatment increases the resistance of plants to pathogenic microflora, adverse weather conditions and increases the yield of crops. The study aimed to study the effect of molybdenum nanoparticles, a suspension of iron and silicon and preparations based on humic acids on the content of photosynthetic pigments, the activity of antioxidant enzymes in *Hordeum vulgare* L. plants and its productivity. The content of chlorophyll *a* and *b*, carotenoids in different phases of plant development was determined using the standard method with Smashevsky modifications, catalytic activity according to the Ebi method, superoxide dismutase according to Hyannopolitis and Rice with some modifications of Polesskaya. Barley seed treatment with preparations based on iron, silicon, molybnum, bogum and molybdenum nanoparticles M Mo stimulates an increase in the level of photosynthetic pigments and the activity of antioxidant enzymes in plants. Using a composition created based on Fe$_3$O$_4$ (10$^{-3}$ mg/L) and SiO$_2$ (10$^{-4}$ mg/L) nanoparticles in a 1:1 ratio for the presowing treatment of barley seeds increases grain yield.

1. Introduction

Nanotechnology is widely used in all areas of agriculture. In crop production, the use of ultrafine particles or nano preparation for pre-sowing seed treatment and as micronutrient fertilizers provides increased resistance to adverse weather conditions and increased yields of almost all food and industrial crops. Due to its microscopic size (from 1 to 100 nanometers), nanoparticles actively penetrate the plant and act more efficiently than macroparticles of the same elements [1–3]. A promising direction for the use of nanotechnologies is the pre-sowing treatment of seeds with metal nanoparticles, which in this case play the role of protecting them from pathogenic microflora and trigger metabolic processes in plants at the beginning of the growing season [4, 5]. In this regard, studies on the physiological and biochemical indices of vital plant activity under the influence of nanoparticles are of great interest for understanding the adaptive capabilities of plant organisms to technogenic nanomaterials [6–11].

The purpose of these studies is to study the effect of pre-sowing treatment of seed-particle-mizheleza, silicon, molybdenum and preparations based on humic acids, the content of photosynthetic pigments, the activity of antioxidant enzymes in spring barley plants and its productivity.
2. Materials and methods
In the studies, SiO$_2$ NPs with a size of 30.7 ± 0.3 nm and a ζ-potential of 27 ± 0.12 mV, Mo NPs (100–120 nm) manufactured by Plazmotherm (Russia, Moscow, https://plasmotherm.ru), Fe$_3$O$_4$ NPs (80–100 nm, ζ potential 20 ± 0.14 mV) purchased from “Advanced Powder Technologies” (Tomsk, Russia, www.nanosized-powders.com). For preparation, the solutions accurately weighed samples were dispersed in glass flasks with distilled water by ultrasound at a frequency of 35 kHz for 30 minutes. The following concentrations were used for seed treatment: a mixed solution was obtained by mixing a suspension of Fe$_3$O$_4$ ($10^{-3}$ mg/L) and SiO$_2$ ($10^{-4}$ mg/L) in a 1:1 ratio. Prepared LF solutions carried out pre-sowing treatment of seeds by preliminary soaking for 10–15 minutes. Seeds treated with deionized water served as a control. The experiment also involved preparations based on humic acids: Biohumus and Borogum M Mo.

As a test object, we used the seeds of spring barley (Hordeum Vulgare L.) of the Natali variety provided by the Federal State Budget Scientific Institution Scientific Center of the BST RAS (Russia, http://fncbst.ru).

Seeds met all the requirements for the 1st class and guidelines "Procedure for the biological assessment of the effect of nanomaterials on plants using morphological characters." Seeds were not treated with disinfectants, as evidenced by quality documents.

For determination of the pigment content and enzyme activity in plants, the selection of green mass was carried out in the phase of tillering, entering the tube and the beginning of heading. The content of photosynthetic pigments (chlorophyll a and b, carotenoids) was determined by the standard method with Smashevsky modifications [12].

For determination of the activity of catalase, the spectrophotometric method proposed by Ebi was used [13]. The total activity of superoxide dismutase was determined by the ability of the enzyme to inhibit the photochemical reduction of nitrosine negotetrazolium, according to Hyannopolitis and Rice with some modifications, as described by Polesskaya et al. [14, 15].

Statistical analysis was performed using standard ANOVA techniques followed by the Tukey test (SPSS ver. 17.0). Spearman's method was used for determining the correlation coefficient. Differences were considered statistically significant at p <0.05.

Field experiments were conducted on an experimental site in the village of Nezhinka (Orenburg district, Orenburg region, Russia). The total size of the plot is 990 m$^2$, which was further divided into 20 plots with an area of 49.5 m$^2$ (1.65x30). Calcic Pachic Chernozems submitted the soil of the site.

For the destruction of weeds, loosening, levelling the soil and creating a dense bed of seeds before sowing the seeds, double cultivation was carried out on the site. After sowing, the field was rolled in order to create favourable conditions for seed germination. Table 1 shows soil characteristics.

| Indicator   | Value  |
|-------------|--------|
| pH          | 7.2    |
| N-N$_{O}$, mg/kg of soil | 11.9 |
| P$_{2}$O$_{5}$, moving, mg/kg of soil | 44.5 |
| K$_{2}$O, exchange, mg/kg of soil | 246.5 |

3. Discussion of the results
Let us turn to the analysis of the photosynthetic apparatus at different stages of development of barley plants. A variant of seed treatment with a mixture of Fe$_3$O$_4$ + SiO$_2$ showed an increase in the content of chlorophyll a at the beginning of the growing season by 40 %, chlorophyll b in the phase of exit to the tube – by 56 % compared with the control level (Fig 1).

We dare to suggest that our concentration of Fe$_3$O$_4$ ($10^{-3}$ mg/L) contributes to an increase in the level of pigments, which is consistent with the results of Racuciu and Creanga, who noted an increase in the chlorophyll a content under the influence of low concentrations of Fe$_3$O$_4$ NPs [16]. Regarding chlorophyll a, this regularity was preserved only at the beginning of the growing season, since the
content of this pigment significantly decreased in all variants of the experiment for the control during the exit phase into the tube. It should be noted that at this stage of development, the plants felt a lack of moisture – the productive moisture reserves in the soil layer 0-0.5 m were 5.6 mm, and the moisture supply of plants was 30% of the need. In his studies of Levardet al (2012) and Barrios et al (2016) the conditions under which nanoparticles act affect their penetration and movement within the plant, which can cause a different reaction of plants to the same nanoparticle [17, 18]. Oxidative stress caused by drought leads to a decrease in plant photosynthesis [19]. After seed treatment with Biohumus, the level of chlorophyll $a$ and $b$, Borogum M Mo – chlorophyll $b$ significantly increased at the beginning of the growing season ($p > 0.05$). In the later phases of plant development, these preparations did not affect the content of these pigments in plants.

![Figure 1](image_url)

**Figure 1.** The content of photosynthetic pigments in the leaves of *Hordeum Vulgare L.* depending on the preparations: $a$ – chlorophyll $a$, $b$ – chlorophyll $b$, $c$ – carotenoids.
LF molybdenum did not affect the level of chlorophyll $a$ and $b$ at the beginning of the growing season. In the phase of entry into the tube, when the plants experienced drought, the content of chlorophyll $a$ as well as in other variants significantly decreased, and chlorophyll $b$ doubled.

By the time the spike appeared, the content of photosynthetic pigments in all variants of the experiment had remained at the control level, except for the variant with Mo NP, where the content of chlorophyll $a$ increased by 34 % compared with the control variant and chlorophyll $b$ decreased by 33 %.

Analysis of the carotenoid content in barley plants showed that this group of pigments was less sensitive to the NP mixture of Fe$_3$O$_4$ + SiO$_2$ and Mo. The level of carotenoids in these variants remained at the control level during the whole vegetation period of the plants, except the variant with Mo NPs in the spike out phase, where it almost halved. When processing barley seeds with Biohumus, an increase in the level of carotenoids was noted at the beginning of the growing season and in the phase of getting into the tube and Borogum M Mo at the beginning of the growing season by 56 – 58 %.

Under the influence of adverse factors (drought, temperature, low frequencies), the activity of antioxidant enzymes in plants increases. Catalase plays a significant role in reducing the effects of oxidative stress by catalyzing the oxidation of H$_2$O$_2$. In our studies, at the beginning of the plant growing season, the catalase activity was increased in the treatment options for LF seeds of the Fe$_3$O$_4$ + SiO$_2$ and Mo mixture by 67 and 34 % relative to the level of the control variant, which indicates an increased formation of H$_2$O$_2$ in these variants (Fig. 2).

![Figure 2. The activity of enzymes depending on the drugs: a – superoxide dismutase (soda), b – catalase.](image-url)
This assumption is confirmed in studies [20]. In the phase of entry into the tube, when the plants were additionally stressed by drought, the catalase activity in the variants with NP decreased by more than half compared to the control. This process suggests that under oxidative stress caused simultaneously by two factors, drought and NP activity of catalase cannot cope with plant protection.

In the spike exit phase, in all variants, the catalase activity is 2–3 times higher than the control variant. Figure 2 shows when the activity of catalase increases, in the activity of superoxide dismutase, the changes relative to the control variant are insignificant. The existence of a functional relationship and competition between the studied enzymes was indicated by the studies of Huseynova I.M. et al. [21].

Adverse weather conditions (air-soil drought) prevailing during the barley growing season harmed grain formation. The grain yield in the experimental variants was at the control level, except for the variant with the Fe\textsubscript{3}O\textsubscript{4} + SiO\textsubscript{2} mixture, where it was higher by 0.6 centners per 1 ha (Table 2).

| Table 2. The effect of various preparations on barley grain yield of *Nordeumvulgar* L. on grain yield |
|---|---|---|---|
| Options | Grain mass, g from 1 m\textsuperscript{2} | The share of grain in biomass, % | Grain productivity, centners per 1 ha |
| Control | 134 | 31.1 | 8.1 |
| Fe\textsubscript{3}O\textsubscript{4} + SiO\textsubscript{2} | 141 | 36.9 | 8.7 |
| Vermicompost | 127 | 33.2 | 8.1 |
| Borogum M Mo | 168 | 37.5 | 8.0 |
| Mo | 140. | 35.2 | 8.1 |

In the variants, Fe\textsubscript{3}O\textsubscript{4} + SiO\textsubscript{2}, Borogum M Mo, Mo, a tendency toward an increase in the mass of grain from 1 m\textsuperscript{2}, a fraction of grain in the biomass of plants, was noted.

4. Conclusions

Thus, the treatment of barley seeds with preparations based on nanoparticles of iron, silicon, molybdenum, Biohumus and Borogum M Mo stimulates an increase in the level of photosynthetic pigments and the activity of antioxidant enzymes in plants.

Oxidative stress caused by drought leads to a decrease in the intensity of plant photosynthesis. Using a composition created based on Fe\textsubscript{3}O\textsubscript{4} (10\textsuperscript{-3} mg/l) and SiO\textsubscript{2} (10\textsuperscript{-4} mg/l) nanoparticles in a 1: 1 ratio for the presowing treatment of barley seeds increases grain yield.

Further studies to study the effect of pre-sowing seed treatment with nanoparticle-based preparations should be carried out in different climatic conditions with crops that are widespread and significant in agriculture.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical standards: All applicable international, national, and institutional guidelines for animal care and use have been followed.

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