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Spring Wheat Features in Response to Seed Treatment by Metal Nanoparticles

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Abstract—Application of advanced nanotechnologies is one of the main ways to increase crop yields in order to meet the growing global demand for food. This paper investigates the effect of Fe and Zn nanoparticles (NPs) on seed germination and growth of spring wheat seedlings (*T. aestivum* L.) of the Zlata cultivar, as well as leaf anatomical changes, yield, grain quality, and microelement composition of the soil after harvesting. It was found that presowing seed treatment by a composition of Fe NPs at a concentration of $10^{-5}\%$ and Zn NPs at a concentration of $10^{-4}\%$ contributed to a 27% increase in the seed germination energy index and root weight compared to the control. The highest plant height (by 8.2%) and green mass (by 8.5%) were observed in the experimental variant with presowing seed treatment by Zn NPs. The leaf area after presowing seed treatment by iron and zinc NPs increased by 18.2 and 33%, respectively. The highest index of specific leaf area (28% higher than the control), calculated as the ratio of leaf area to green mass, was observed in wheat leaves after seed treatment by Zn NPs. Presowing seed treatment by Fe and Zn NPs separately or in combination led to changes in anatomical parameters (leaf, mesophyll, and epidermis thickness, and conducting bundle area), which were higher than those in the control group of plants whose seeds were treated with water and lower than those in leaves of the plants whose seeds were treated with polymers. Evaluation of the wheat yield structure and grain quality after presowing seed treatment by the composition of metal NPs showed a greater thousand grain weight (1.9 g more than the control) and a higher grains/ear ratio. Presowing treatment of spring wheat seeds by Fe : Zn NPs contributed to higher content of mobile forms of iron, zinc, copper, and phosphorus in the soil after harvest.

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INTRODUCTION

Wheat is a cereal crop that is important for the country's food security. Increasing its production and yield is the main objective of the grain industry in agriculture and at the same time an extremely difficult problem, given such constraints as climatic fluctuations, poor soil conditions, increased risk of epidemic outbreaks of diseases, pests, etc. [12]. Its solution requires introducing innovative technologies that have the potential to increase the sustainability of modern farming systems. In this regard, a special requirement is placed on crop cultivars. Agriculture needs cultivars of new types with the highest possible productivity in various agroeconomic conditions [3]. Grain productivity is greatly affected by the sowing qualities of seeds that give rise to vigorous synchronous seedlings: purity, size, evenness, moisture content, germination ability, and germination energy. Sowing seeds of the highest quality can reduce their consumption and increase productivity by 20% [1, 4]. The development of nanotechnologies and their widespread use in crop production indicate the promising application of products such as nanofibers, nanopesticides, nanoherbicides, and nanosensors for the efficient nutrition of plants and the fight against diseases and pests [4, 5]. Recent studies on the use of nanotechnology in growing crops indicate the active influence of nanoparticles (NPs) on the process of seed germination. The natural process of germination takes a lot of time, but processing seeds by NPs allows achieving high germination rates, which makes the use of nanotechnologies a powerful method to increase seeds' rate of germination. A study on monitoring various NPs—for example, gold, silver, and carbon—on the germination of *Trichilia dregeana* and *Vigna radiata* showed that silver and gold NPs inhibit the rate of germination, while carbon NPs increase it and promote active growth of roots and shoots [5, 6]. Numerous studies showed that various nanomaterials, especially those based on metals and carbon, affect the growth and development of a number of cultivated plants [7, 8]. Positive morphological changes include an increase in not only the percentage and rate of seed germination, but also the length of roots and shoots, their ratio, and vegetative biomass of seedlings of multiple cultivated plants, including corn, wheat, ryegrass, alfalfa, soy, rape, tomato, radish, lettuce, spinach, onion, pumpkin, and cucumber. In addition, application of nanomaterials increases several physiological parameters, primarily photosynthetic activity and nitrogen metabolism in soybean plants [9], spinach [10, 11], and peanut [12]. When using nanotechnology in crop production, an increase in the yield and product quality is observed in terms of the content of nutrients in fruits and grain [13–16]. For example, iron and copper NPs were used as a factor for increasing wheat yields. It was found that copper NPs improve the yield and stress resistance of wheat, due to their effect on the intensity of glycolysis and the Krebs cycle [17]. Researchers' interest in studying the use of nanotechnology in crop production is associated with the unique properties of NPs. In particular, our long-term studies of disperse systems and NPs allowed us to identify the following features of biological effects of NPs. Metal nanoparticles have low toxicity—7–50 times lower than the toxicity of metals in ionic form, demonstrate prolonged and multifunctional effects, stimulate metabolic processes, easily penetrate all organs and tissues, have biological activity associated with structural features of the particles and their physicochemical characteristics, and also exhibit a synergistic effect when interacting with natural polysaccharides [18–22].

The purpose of this study was to investigate the effect of iron and zinc NPs on spring soft wheat seeds, their germination rates, growth of wheat seedlings in laboratory conditions, grain quality, and soil trace elements after harvesting in the field.

MATERIALS AND METHODS

Iron and zinc nanoparticles were obtained by high temperature condensation [23] using the Migen-3 unit [24].

Samples of metal nanoparticles were dispersed in water using the ScientzJY 92-IIN ultrasonic disintegrator (China) in the regime of 0.5 A and 44 kHz for 30 s with a break of 30 s (triple repeated), under cooling the dispersed mixture with ice.

For presowing treatment of wheat seeds, a composition based on Na-carboxymethyl cellulose and polyethylene glycol-400 was developed, into which a suspension of metal NPs was introduced in the desired concentration.

The study was carried out using seeds of spring soft wheat (*T. aestivum* L.) of the Zlata cultivar harvested in 2018. This cultivar has been zoned since 2009 in the first (Northern), second (North-Western), third (Central), fourth (Volga–Vyatka), and seventh (Middle Volga) regions of Russia.

Laboratory tests. For the germination of wheat seeds in laboratory studies, we employed a roll method (GOST 12038-84, RF). Vessels with rolls were placed in a climate chamber with ventilation without lighting, in which a temperature of 20 ± 1°C was maintained.

The germination energy was determined on the third day. The seed germination ability was estimated on the seventh day according to GOST 12038-84, RF.

Leaf anatomy. Microscopy of wheat leaves was carried out using the Axioskop 40 FL microscope (Carl Zeiss, Germany) at a 110-fold magnification.

Leaves were collected from 10 plants in each experiment variant, followed by weighing, scanning, and measuring their area (S) using the AxioVision software. Then the scanned leaves were dried at a temperature of 95°C to a constant weight and weighed. The following indicators were calculated: the ratio of the weight of fresh leaf to its area (m/S) and specific leaf area (SLA, cm^2/g) [25, 26]. These measurements were carried out for the second leaf of the seedling.

To study the anatomical structure of the leaf, fresh sections in the middle of the transverse section of the third leaf were prepared. Measurements were carried out using the AxioVision software. The thickness of the leaf blade was unlike values in different zones, in particular, in the central conducting bundle, side bundles, and between bundles of the leaf blade. We measured the thickness of the leaf and mesophyll in their wide (central conducting bundle) and narrow parts (between the central conducting bundle and a neighboring one), epidermis thickness on both sides of the leaf, and areas of the conducting bundle, phloem, and xylem.

Field trials. Field preparation was carried out on an area of 4 ha, taking into account the fertility passport of the land. The plots were located systematically, the area of the experimental and control plots was 12 m^2, and the experiments triple replicated. The predecessor was winter wheat. The soil was soddy–podzolic medium loamy with the humus horizon thickness of 25 cm. Humus content was 2.2%, mobile phosphorus 155 mg/kg, exchange potassium 94 mg/kg, average soil pH_{kcl} 5.1, exchange calcium 8.7 mEq/kg, and exchange magnesium 3.7 mEq/kg. The average day/night air temperatures in May, June, July, August, and September in the planting region were 18.5/1.9, 20.5/6.1, 25.1/10.6, 23.5/11.1, and 19.5/6.4°C, respectively. The lowest relative daily humidity on average in these months ranged from 30% (May) to 66.3% (September), and the highest average relative humidity ranged from 85.5% (June) to 92.6% (September). Monthly precipitation (mm) was as follows: 84.1 (May), 133.2 (June), 110.6 (July), 68 (August), and 37.9 (September).

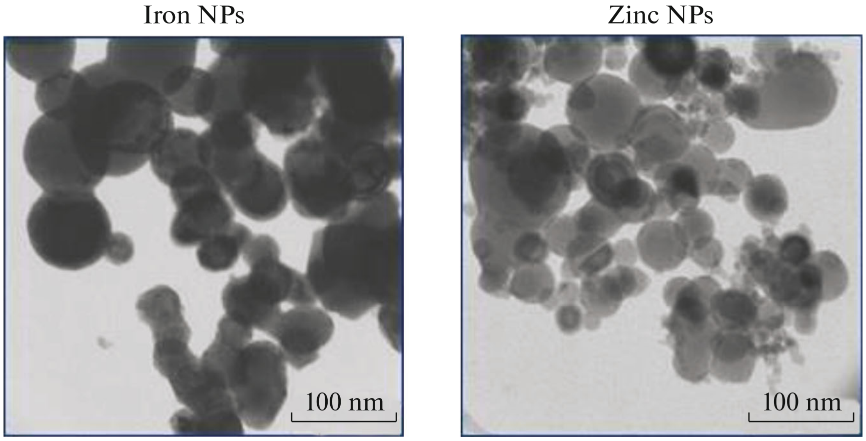

Fig. 1. (Color online) TEM image of iron and zinc nanoparticles (transmission electron microscopy).

The preparation of the field was of fall plowing 23–25 cm deep. Spring cultivation of the field was carried out using the combined Amazone unit. Prepared seeds of the Zlata spring wheat cultivar were sown at the rate of 6 million germinating seeds per 1 ha. Before sowing, mineral fertilizers ("Azofoska") were introduced at the rate of 200 kg/ha. Sowing was carried out to a depth of 5 cm with simultaneous rolling down. During tillering, nitrogen fertilizing with ammonium nitrate was carried out at the rate of 100 kg/ha using the Amazone fertilizer spreader aggregated with the MTZ-82 tractor. After that, chemical treatment with a combination of herbicidal preparations was carried out. Harvesting was done using the Wintersteiger selection combine.

All technological operations during the cultivation of wheat in the experimental and control plots were carried out at the same day and with a single technological aggregate composition. To analyze the crop structure and carry out phenological and biometric observations, three control sites, each 1 m^2 in area, were marked.

Soil elemental composition. To analyze the content of chemical elements after harvesting, soil samples were selected according to GOST 17.4.4.02 [27]. Each averaged sample was composed of 10 individual specimens taken by a drill from each of the experimental variants applying the direct route method to the depth of the arable layer (up to 30 cm). To extract mobile forms (accessible to plants) of macro- and microelements from the soil, various extracts were proposed that differ in the extent of their effect on the soil. To determine mobile phosphorus in the studied soddy–podzolic soil, the Kirsanov method was used (GOST R 54650-2011). To determine mobile forms of micronutrients, we applied an extract system in which an individual extractant was used for each element: acetate–ammonium buffer with pH 4.8 for zinc (Zn), 0.1 M H_2SO_4 for manganese (Mn) and iron (Fe), and 1 M HCI for copper (Cu) (GOST R 50682-94, GOST R 50684-94).

Microelements in the above extracts were determined using the Aanalist-200 atomic absorption spectrometer (PerkinElmer, Sweden).

Statistical data processing. We calculated the mean value *X*, the standard deviation from the mean *SD*, and the standard error of the mean *SE*. The significance of differences between the means of the two groups was calculated according to the Student's *t*-test. The differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical properties of metal nanoparticles. Based on the results of electron microscopy, it was found that iron and zinc NPs are single crystal structures of circular regular shape, covered with a translucent oxide film (Fig. 1).

The size distribution curve for iron NPs was located in the region of 5–150 nm. The average iron particle diameter was 56.0 ± 0.9 nm. The size distribution curve for zinc NPs lied in the region of 0–800 nm. The average diameter of the obtained zinc particles was 104.0 ± 3.7 nm. Metal nanoparticles consisted of a metal core and an oxide film on the surface of the particles, which was formed as a result of passivation of particles by air, aimed to reduce the pyrophoricity of NPs. The data of X-ray phase analysis of the nanosized iron powder showed that the metal crystalline phase of iron Fe-α constituted 27.9%, the rest referred to the metal crystalline phase of iron oxide, γ-Fe_2O_3 (maghemite). The zinc nanosized powder contained a metallic crystalline phase of zinc. X-ray phase analysis detected no oxide phases [22].

Effect of metal NPs on the germination energy, germination ability, and morphometric indicators of wheat seedlings in laboratory conditions. It was found that the use of NPs for presowing seed treatment promotes

Table 1. Effect of presowing seed treatment by Fe and Zn nanoparticles on germination ability, germination energy, and morphometric parameters of wheat seedlings in laboratory conditions*

Group	Germination energy, third day, experiment/ control, %	Germination, seventh day, experiment/ control, %	Root weight per plant, experiment/ control, %	Main root length, experiment/ control, %	Seedling height, experiment/ control, %	Number of germinal roots per plant, experiment/ control, %	Length of germinal roots per plant, experiment/ control, %	Green mass per plant, experiment/ control, %
Fe NPs 10^{-5}%	121.7 ± 17.7	95.2 ± 1.9	115.1 ± 11.4	97.1 ± 4.5	99.2 ± 10.6	102.8 ± 6.0	95. 5 ± 8.5	113.4 ± 9.4
Zn NPs 10^{-4}%	112.1 ± 21.0	91.5 ± 10.5	91.8 ± 6.1	93.5 ± 3.8	108.2 ± 11.0	97.3 ± 5.3	88.0 ± 7.4	108.5 ± 30.4
Fe NPs 10^{-5}% + Zn NPs 10^{-4}%	127.1 ± 5.3	100.0 ± 1.5	127.0 ± 1.5	91.7 ± 4.1	101.1 ± 13.0	98.2 ± 7.2	93.5 ± 9.3	97.0 ± 3.4
Control	100.0 ±1 6.8	100.0 ± 4.5	100.0 ± 18.7	100.0 ± 5.3	100.0 ± 11.7	100.0 ± 6.7	100.0 ± 8.7	100.0 ± 13.0

*Data are given as mean (*X*) ± *SE*.

higher seed germination ability and yield and improves grain quality [14, 17, 27]. Previously, to study the effect of NPs on plants, the technology of seed soaking in suspensions of metal NPs was used [28]. Currently, the most practical and promising direction in agriculture is the coating of seeds with polymer nanocomposites, consisting of polymers and nanosized particles of various nature. This technology makes it possible to retain moisture, accelerate processes of seed swelling, activate metabolic enzymes of seeds, etc., which leads to a higher rate of seed germination, efficient mobilization of seed reserves, and enhances growth of seedlings [6, 29]. In the studies, iron and zinc NPs were introduced into a polymer film consisting of carboxymethyl cellulose (CMC) and polyethylene glycol (PEG), which was formed on the surface of the seeds during the presowing treatment. Table 1 shows the comparative characteristics of the morphometric parameters of wheat seedlings under the action of iron and zinc NPs and their composition as part of the polymer. It can be seen that the presowing treatment of wheat seeds with Fe NPs at the concentration of 10^{-5}% (80 mg/L ton of grain) increases the seed germination energy by 22% and reduces the seed germination ability by 4.8%. On the seventh day of seedling growth, we observed a decrease in the length of the main root and germinal roots by 2.9 and 4.5%, respectively, and an increase in green mass by 13.4% compared with the control.

The use of Zn NPs at the concentration of 10^{-4}% (800 mg/L ton of grain) leads to an increase in the germination energy of wheat seeds by 12.1% and a decrease in their germination ability by 8.5% compared to the control. Seven days later, there was a significant increase in the height of wheat seedlings and their green mass (by 8.2 and 8.5%, respectively) and a decrease in all indicators of the root system development. So, the length of the main root, the number of germinal roots, and their length were lower compared to the control by 6.5, 2.7, and 12%, respectively, which led to a decrease in the root weight in wheat seedlings by 8.2%.

Given that iron and zinc exhibit the properties of physiological synergists, presowing treatment of wheat seeds was carried out using the composition of these elements. In this case, the seed germination energy increased by 27.1% while maintaining 100% germination ability. On the seventh day, the height of wheat seedlings and their green mass remained at the control level. The length of the main root, the number of germinal roots, and their length were reduced in comparison with the control by 8.3, 1.2, and 6.5%, respectively. At the same time, the root weight in wheat seedlings increased by 27.0%. This indicated improved root supply in wheat seedlings, which affected their ability to supply shoots with water and mineral elements during the vegetative phase of plant growth [30].

Thus, presowing treatment of wheat seeds with iron and zinc NPs resulted in the improved morphometric parameters of plants compared with the parameters of the control group, the seeds in which were not treated with metal NPs. Given the results of laboratory experiments for presowing treatment of wheat grain, we carried out field trials using the combination of Fe : Zn nanoparticles in concentrations of $10^{-5} : 10^{-4}$ (%).

Analysis of area and anatomical structure of leaves of wheat seedlings. It is known that leaf area is an important condition for high plant productivity. Leaf area contributes intensive photosynthesis and high gain in biomass; therefore, it significantly affects yield. Under production conditions, leaf area most often increases slowly, therefore, certain measures are necessary for the leaf surface to grow as fast as possible, being in an active state for a prolonged time [31–33]. One of the ways to solve this problem, in addition to agricultural techniques and choosing particular cultivars, is plant growth stimulation in the early stages of the growth season.

Table 2. Parameters of leaves of *Triticum aestivum* L. seedlings of cultivar Zlata after pre-sowing seed treatment by metal NPs*

Variant	S, cm^2	T_2	T_1	m/S	T_2	T_1	SLA (S/m_1)	T_2	T_1
Fe 10^{-5}%	4.29 ± 0.76	0.79	0.96	0.015 ± 0.001	0.78	0.81	592.6 ± 65.93	0.97	0.98
Zn 10^{-4}%	4.84 ± 0.64	1.00	1.00	0.014 ± 0.001	0.97	0.95	623.3 ± 78.27	0.99	1.00
Zn 10^{-4}% + Fe 10^{-5}%	3.69 ± 1.04	0.68	0.12	0.018 ± 0.004	0.59	0.27	558.0 ± 95.64	0.51	0.87
Control 1 (0.5% CMC + 1.25% PEG)	3.63 ± 0.51	0.71		0.017 ± 0.004	0.37		485.7 ± 107.91	0.81	
Control 2 (water)	3.9 ± 0.57		0.71	0.016 ± 0.003		0.37	535.2 ± 30.8		0.81

*Data are given as mean ($X \pm SE$) at $n = 10$; T_2, t-test compared to control 2 (water); T_1, t-test compared to control 1 (0.5% CMC + 1.25% PEG).

Table 2 presents data on changes in the basic parameters of leaves of wheat seedlings at the 14th day of cultivation after presowing treatment of seeds by metal NPs. It can be seen that presowing seed treatment of *Triticum aestivum* L. of the Zlata cultivar by Fe NPs at the concentration of 10^{-5}% led to an increase in leaf area by 18.2% compared to control 1 (seed treatment with polymers –0.5% CMC + 1.25% PEG) and 10% compared to control 2 (seed treatment with water). The use of Zn NPs at the concentration of 10^{-4}% increased leaf area by 33.3% compared to control 2 and 24.1% compared to control 1. The ratio of fresh leaf weight to leaf area (m/S) was higher in the group of plants which presowing treatment was carried out using the composition of nanoparticles at the concentration (Zn 10^{-4}% + Fe 10^{-5}%). The increase in the wheat leaf specific area was most pronounced in the group of plants with pre-sowing seed treatment by Zn NPs at the concentration of 10^{-4}%, increasing this indicator by 28.3% compared to control 1 and 16.5% compared to control 2.

The data obtained indicate that the presowing seed treatment by metal NPs within the composition of the polymer film and the film itself mainly had a positive effect on the indicators of leaf area and their relative values. This, in turn, promoted activation of photosynthesis, efficient and rapid assimilation of nitrogen and carbon, and increased metabolism in leaves, which inevitably affected vegetative and reproductive traits of plants [31, 34].

Let us consider the anatomical features of the structure of wheat leaves after presowing seed treatment by metal NPs. The anatomical structure of the spring wheat leaf in different experimental variants is shown in Fig. 2.

It can be seen that the plate of wheat leaves contains several types of conducting bundles: large ones, which merge with the surface of the plate through distinct sclerenchyma fibers on both sides of the conducting bundle; smaller bundles with well-defined phloem and xylem cells that have a sclerenchyma fiber only to one of the sides, lower or upper, of the leaf blade; small bundles with underdeveloped xylem; and transverse conducting bundles represented by phloem or xylem cells. Some large conducting bundles have a double lining of cells elongated along the longitudinal axis of the leaf plate. Cells of the outer lining contain chloroplasts, while cells of the inner lining of the bundle are represented by typical sclerenchyma fibers.

Table 3 presents the results of quantitative analysis of anatomical indicators. It was found that presowing treatment of wheat seeds by metal NPs changed the quantitative anatomical parameters of the leaf. First, presowing seed treatment by the polymer composition (CMC + PEG, control 1) increased the anatomical parameters of the leaf compared to seed treatment with water (control 2). The introduction of metal NPs into the polymer film in all variants of the experiment increased the studied parameters compared to seed treatment with water (control 2). So, presowing treatment of wheat seeds by Fe NPs at the concentration of 10^{-5}% increased leaf thickness by 1.6 times, mesophyll thickness by 1.7 times, epidermis thickness by 1.8 times on the upper side of the leaf and by 1.6 times on the lower side of the leaf, and the area of the conducting bundle by 3.0 times and its phloem by 2.6 times compared to control 2. These changes can enhance photosynthesis and the formation of plastic substances and intensify metabolism due to increased ascending and descending flows, which will favourably affect the state of plants in the early stages of development. The use of the Zn NPs suspension at the concentration of 10^{-4}% resulted in a 1.2-fold increase in leaf and mesophyll thickness compared to control 2 and a decrease in the same parameters compared to control 1 (the system of polymers). In addition, the use of Zn NPs at the concentration of 10^{-4}% contributed to a decrease in the epidermis thickness and the conducting bundle area compared to control 1. The combination of Fe NPs at the concentration of 10^{-5}% and Zn NPs at the concentration of 10^{-4}% did not affect the anatomical parameters of the leaf compared with control 2 but reduced them compared with control 1.

Field tests of spring wheat. The tests were carried out on the fields of Federal Research Center Nemchinovka (Moscow oblast) in the second selection crop

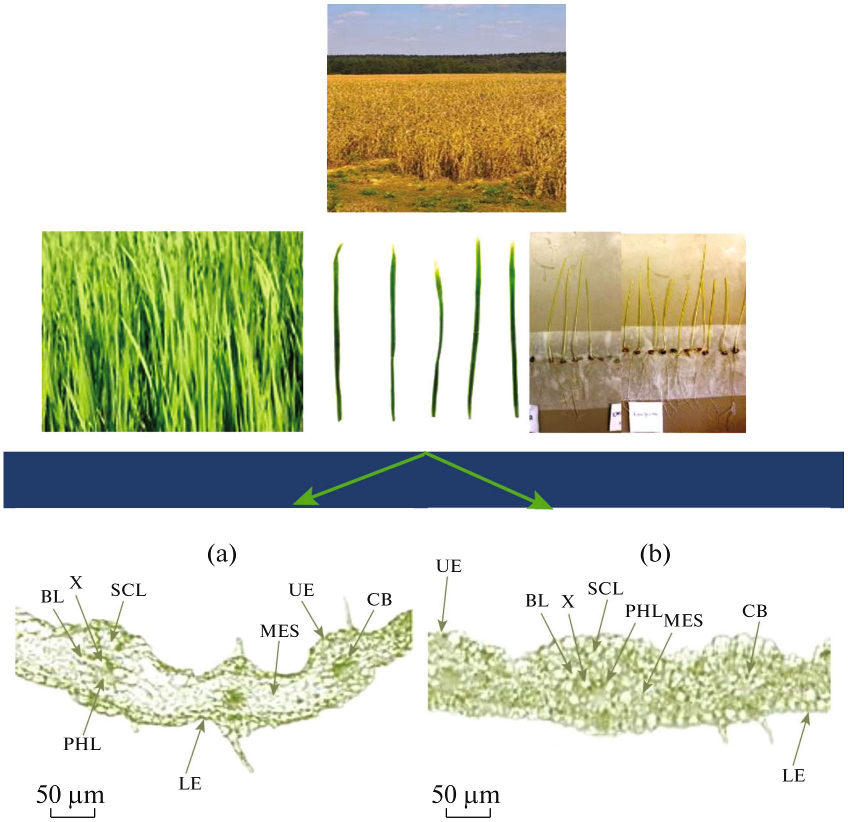

Fig. 2. (Color online) The anatomical structure of the leaf of *Triticum aestivum* L. of cultivar Zlata on the cross section of the plate: (a) control (without treatment by NPs), (b) experiment (presowing seed treatment by composition of Zn NPs $10^{-4}\%$: Fe NPs $10^{-5}\%$): UE, upper epidermis; LE, lower epidermis; MES, mesophyll; CB, conducting bundle; X, xylem; PHL, phloem; SCL, sclerenchyma; BL, bundle lining.

rotation. Field preparation, including the test plot, was described in the Methods section. In the studied variant, a combination of Fe : Zn nanoparticles in concentrations of $10^{-5} : 10^{-4}$ (%) was added to the basic composition of the common preparations.

Table 4 presents characteristics of the Zlata spring soft wheat cultivar in the preharvest period, as well as yield and grain quality of the control and experimental groups. It can be seen that the treatment of seeds by metal NPs against the background of the traditional processing of crops made it possible to retain such an important feature as early maturity. The Zlata cultivar belongs to the group of early ripe cultivars. In the field conditions, the height of plants raised from seeds treated by metal NPs was 3 cm higher than that of the control plants, while maintaining resistance to lodging.

The main indicators that determine spring wheat yields under conditions of the Central Non-Black Earth Territory are the grains/ear ratio and grain size. The thousand grain weight in the studied group was 1.9 g higher than the control with the higher grains/ear ratio, which also positively affected the weight of grains per ear (1.5 and 1.4 g, respectively).

Thus, presowing treatment of wheat seeds by metal NPs contributes to the active growth of plants and the formation of a larger, well-grained ear.

Effect of presowing seed treatment by metal NPs on the content of microelements in the soil after harvesting. The need to study the content of microelements in the soil after harvesting is associated with environmental aspects of using metal NPs in crop production. The high reactivity of NPs can lead to undesirable environmental consequences, altered physicochemical parameters of the soil, including pH, and content of active forms of macro- and microelements, and contribute to changes in the habitat of microorganisms and, as a result, quantitative and qualitative disturbances of soil microbiota [37–39].

Therefore, to minimize the damage to soil biota, we use a polymer coating with metal NPs on seeds during presowing treatment, thereby preventing direct entry of NPs into the soil.

The soil in the studied experimental variants was characterized by an acidic reaction of the medium (pH_{kcl} ranges from 4.7 to 5.5 units). The heavy metal content was below established hygienic standards for soils and did not exceed the limit of permissible con-

Table 3. Anatomical characteristics of leaves of *Triticum aestivum* L. seedlings of cultivar Zlata after presowing seed treatment by metal NPs*

Variant	Leaf thickness in wide part, μm	T_2	T_1	Mesophyll thickness in wide part, μm	T_2	T_1	Leaf thickness in narrow part, μm	T_2	T_1	Mesophyll thickness in narrow part, μm	T_2	T_1	Upper epidermis thickness, μm	T_2	T_1	Lower epidermis thickness, μm	T_2	T_1	Conducting bundle area, μm^2	T_2	T_1
Fe $10^{-5}\%$	201.65 ± 29.16	1.00	0.37	166.05 ± 24.49	1.00	0.17	126.81 ± 18.95	1.00	0.51	95.28 ± 13.97	1.00	0.02	24.95 ± 4.23	0.98	0.06	20.59 ± 3.11	0.99	0.07	12723.06 ± 2446.18	0.98	0.46
Zn $10^{-4}\%$	145.15 ± 4.77	0.98	1.00	117.12 ± 4.94	0.97	0.99	91.60 ± 1.67	1.00	0.98	66.37 ± 2.44	0.99	0.98	15.42 ± 1.20	0.50	0.99	13.28 ± 0.81	0.09	1.00	5230.59 ± 339.26	0.83	0.99
Zn $10^{-4}\%$ + Fe $10^{-5}\%$	133.04 ± 3.30	0.77	1.00	104.24 ± 2.63	0.66	1.00	82.96 ± 4.22	0.89	0.99	58.99 ± 3.65	0.79	0.99	17.84 ± 1.54	0.92	0.99	14.86 ± 0.36	0.86	0.99	5565.36 ± 570.19	0.88	0.99
Control 1 (0.5% CMC + 1.25% PEG)	247.71 ± 19.64	1.00		196.90 ± 16.37	1.00		138.52 ± 12.12	1.00		111.92 ± 12.47	0.99		29.72 ± 2.92	1.00		24.38 ± 2.05	1.00		17509.24 ± 2608.09	1.00	
Control 2 (water)	125.29 ± 5.02		1.00	98.32 ± 5.01		1.00	74.30 ± 2.76		1.00	52.56 ± 3.0		0.99	14.20 ± 0.95		1.00	13.11 ± 0.98		1.00	4000.69 ± 642.78		1.00

*Data are given as mean (X) at $n = 10$; T_2, *t*-test compared to control 2 (water); T_1, *t*-test compared to control (0.5% CMC + 1.25% PEG).

Table 4. Assessment of yield structure, yield, and grain quality of spring soft wheat cultivar Zlata

Indicators	Control (pre-sowing seed treatment without NPs)	Pre-sowing seed treatment by combination of Fe and Zn NPs at concentrations of 10^{-5} : 10^{-4} (%)
Yield, t/ha	5.65	5.63
Heading date	June 12	June 13
Number of plants per 1 m^2, pcs.	334	330
Height, cm	85	88
Ear length, cm	8.1	7.8
Number of spikelets per ear, pcs.	14.2	14.8
Number of grains per ear, pcs.	30.6	31.1
Weight of grain per ear, g	1.40	1.50
1000 grain weight, g	46.3	48.2
Grain moisture content, %	14	14
Septoria (leaf) infection, %	25	25
Protein, %	16.4	16.8
Gluten content, %	36.1	37.7

Table 5. Content of mobile phosphorus forms and microelements* in soil (mg/kg)

Indicators	Control (pre-sowing seed treatment without NPs)	Pre-sowing seed treatment by combination of Fe and Zn NPs at concentrations of 10^{-5} : 10^{-4} (%)
Mobile phosphorus	169 ± 15	251 ± 23
Zinc	1.27 ± 0.11	1.55 ± 0.14
Manganese	72.35 ± 6.70	69.30 ± 6.80
Iron	30.65 ± 2.80	48.90 ± 4.70
Copper	2.60 ± 0.25	2.68 ± 0.25

*Data are given as mean (*X*) at $n = 10$.

centrations. So, the average lead content in the studied soil was 4 mg/kg, cadmium less than 0.5 mg/kg, mercury less than 0.2 mg/kg, nickel 2.2 mg/kg, and chromium 0.5 mg/kg.

The results of determining the content of mobile phosphorus forms and microelements (zinc, manganese, iron, and copper) in the soil from the control variant (presowing seed treatment without metal NPs) and experimental (presowing seed treatment with the combination of Fe : Zn in concentrations of 10^{-5} : 10^{-4} (%)) after harvesting are presented in Table 5.

As can be seen from Table 5, in the variant of using metal NPs during presowing seed treatment, the soil after harvesting was observed to have higher content of mobile phosphorus (P_2O_5), mobile zinc, mobile iron, and mobile copper than the control version. So, under presowing seed treatment by metal NPs, in the soil after harvesting, the content of mobile zinc increased from 1.27 mg/kg (in the control version) to 1.55 mg/kg (in the experimental version) and the content of mobile iron increased from 30.65 mg/kg (in the control version) to 48.9 mg/kg (in the experimental version). At the same time, the content of mobile manganese, on the contrary, was higher in the control variant. Given the fact that the availability of elements for plants is determined by their mobile forms, the increase in mobile phosphorus in the soil after wheat harvesting is not a concern. However, a small increase in the content of mobile forms of iron, zinc, and copper is a signal for careful monitoring of the state of the soil after the use of metal NPs.

CONCLUSIONS

Presowing treatment of seeds by the composition of iron NPs at the concentration of 10^{-5}% and zinc NPs at the concentration of 10^{-4}% contributed to the 27% increase in seed germination energy and root weight compared to the control. The greatest plant height and green mass were observed in the experimental variant of presowing seed treatment by zinc NPs, which were 8.2 and 8.5% greater than the control indicators.

The leaf area under presowing seed treatment by iron and zinc NPs increased by 18.2 and 33%, respectively, and when treated by the combination of NPs by 1.6%. The highest index (28% higher than the control, when seeds were treated with the polymer) of the specific leaf area, calculated as the ratio of area to dry weight, was observed for wheat leaves when treating seeds by zinc NPs.

The anatomical parameters of wheat leaves, the seeds of which were treated by iron NPs at the concentration of $10^{-5}\%$ and by zinc NPs at the concentration of $10^{-4}\%$ separately or as the composition changed in all variants of the experiment, being higher when compared to the group of plants whose seeds were treated with water, but lower when compared to the group of plants whose seeds were treated with the polymer coating.

Presowing treatment of spring wheat seeds by iron and zinc NPs contributed to higher content of mobile forms of iron and zinc in the soil after harvesting this cultivated crop.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. A. A. Kachutova, "Efficiency of grainproduction—basis of food safety of the country," Vestn. NGIEI, No. 3 (22), 76 (2013).
2. Poonam Jasrotia, Prem Lal Kashyap, Ajay Kumar Bhardwaj, et al., "Scope and applications of nanotechnology for wheat production: A review of recent advances," Wheat Barley Res. **10**, 1 (2018). doi.org/ https://doi.org/10.25174/2249- 4065/2018/76672
3. N. V. Davydova, A. M. Rezepkin, and N. K. Volkov, "The breeding value of spring wheat varieties from MOSNIISKH 'Nemchinovka' of various varietal change groups," in *Innovative Developments in Breeding and Crop Cultivation Technology* (2018), p. 72.
4. Pankaj Singh Rawat, Rajeew Kumar, Pradeep Ramet, et al., "Effect of nanoparticles on wheat seed germination and seedling growth," Int. J. Agricult. Biosyst. Eng. **12**, 13 (2018).
5. C. Ma, J. C. White, J. Zhao, et al., "Uptake of engineered nanoparticles by food crops: Characterization, mechanisms, and implications," Ann. Rev. Food Sci. Technol. **9**, 129 (2018). https://doi.org/10.1146/annurev-food-030117-012657
6. B. Sekhon, "Nanotechnology in agri-food production," Nanotechnol. Sci. Appl. **7**, 31 (2014). doi.org/ https://doi.org/10.2147/NSA.S39406
7. R. Nair, S. H. Varghese, B. G. Nair, et al., "Nanoparticulate material delivery to plants," Plant Sci. **179**, 154 (2010).
8. C. M. Rico, S. Majumdar, M. Duarte-Gardea, et al., "Interaction of nanoparticles with edible plants and their possible implications in the food chain," J. Agric. Food. Chem. **59**, 3485 (2011).
9. S. Agrawal and P. Rathore, "Nanotechnology pros and cons to agriculture: A review," Int. J. Curr. Microbiol. App. Sci. **3** (3), 43 (2014).
10. M. Linglan, L. Chao, and Q. Chunxiang, "Rubisco activase MRNA expression in spinach: Modulation by nanoanatase treatment," Biol. Trace. Elem. Res. **122**, 168 (2008).
11. X. M. Liu, F. D. Zhang, Z. B. Feng, et al., "Effects of nano-ferric oxide on the growth and nutrients absorption of peanut," J. Plant Nutr. Fertil. Sci. **11**, 551 (2010).
12. J. P. Giraldo, M. P. Lry, and S. M. Faltermeier, et al., "Plant nanobionics approach to augment photosynthesis and biochemical sensing," Nat. Mater. **13**, 400 (2014).
13. I. P. Olkhovskaya, O. A. Bogoslovskaya, A. G. Yablokov, and N. N. Glushchenko, "Spring barley yield after presowing seed treatment with metal nanoparticles," Nanotechnol. Russ. **14**, 55 (2019). doi.org/ https://doi.org/10.21517/1992-7223-2019-1-2-55-62
14. A. Mehmood, "Brief overview of the application of silver nanoparticles to improve growth of crop plants," IET Nanobiotechnol. **12**, 701 (2018). https://doi.org/10.1049/iet-nbt.2017.0273
15. G. S. Nechitailo, O. A. Bogoslovskaya, I. P. Ol'khovskaya, and N. N. Glushchenko, "Influence of iron, zinc, and copper nanoparticles on some growth indices of pepper plant," Nanotechnol. Russ. **13**, 161 (2018). . https://doi.org/10.1134/S1995078018020052
16. P. Wang, E. Lombi, F. J. Zhao, et al., "Nanotechnology: A new opportunity in plant sciences," Trends Plant Sci. **21**, 699 (2016). https://doi.org/10.1016/j.tplants.2016.04.005
17. F. Yasmeen, N. Raja, A. Razzaq, et al., "Proteomic and physiological analyses of wheat seeds exposed to copper and iron nanoparticles," Biochim. Biophys. Acta Proteins Proteom. **1865**, 28 (2017). https://doi.org/10.1016/j.bbapap.2016.10.001
18. N. N. Glushchenko, O. A. Bogoslovskaya, and I. P. Olkhovskaya, "Physico-chemical regularity of the ultradisperse metal powders biological action," Chem. Phys. **21**, 79 (2002).
19. A. A. Rakhmetova, T. P. Alekseeva, O. A. Bogoslovskaya, I. P. Ol'khovskaya, A. N. Zhigach, and N. N. Glushchenko, "Wound-healing properties of copper nanoparticles as a function of physicochemical parameter," Nanotechnol. Russ. **5**, 271 (2010).
20. O. A. Bogoslovskaja, A. A. Rakhmetova, M. N. Ovsyannikova, I. P. Olkhovskaya, and N. N. Gluschenko, "Antibacterial effect of copper nanoparticles with differing dispersion and phase composition," Nanotechnol. Russ. **9**, 82 (2014).
21. A. A. Rakhmetova, O. A. Bogoslovskaja, I. P. Olkhovskaya, A. N. Zhigach, A. V. Ilyina, V. P. Varlamov, and N. N. Gluschenko, "Concomitant action of organic and inorganic nanoparticles in wound healing and antibacterial resistance: Chitosan and copper nanoparticles in an ointment as an example," Nanotechnol. Russ. **10**, 149 (2015).
22. Chzhao Khuei, Lyu Min', Chen' Yu, et al., "A method of growing plants using metal nanoparticles and a nutrient medium for its implementation," RF Patent No. 2015154325 (2015).
23. M. Ya. Gen and A. V. Miller, Author's certificate of the USSR No. 814432, Bull. Izobret., No. 11 (1981).

24. I. O. Leipunsky, A. N. Zhigach, M. L. Kuskov, et al., "Synthesis of TiH_2 nanopowder via the Guen-Miller Flow-Levitation method and characterization," J. Alloys Compd. **778**, 271 (2019). https://doi.org/10.1016/j.jallcom.2018.11.088

25. L. Sack, P. D. Cowan, N. Jaikumar, et al., "The 'hydrology' of leaves: Coordination of structure and function in temperate woody species," Plant, Cell Environ. **26**, 1343 (2003).

26. F. Vendramini, S. Diaz, D. E. Gurvich, et al., "Leaf traits as indicators of resource-use strategy in floras with succulent species," New Phytolog. **154**, 147 (2002).

27. V. N. Zeyruk, S. V. Vasilieva, M. K. Derevyagina, O. A. Bogoslovskaya, I. P. Olkhovskaya, E. S. Afanasenkova and N. N. Glushchenko, "Effect of the presowing treatment of potato tubers with film coating containing metal nanoparticles on pathologies and yield production," Nanotechnol. Russ. **14**, 248 (2019). doi.org/ https://doi.org/10.21517/1992-7223-2019-5-6-70-78

28. L. V. Kovalenko and G. E. Folmanis, *Biologically Active Nanopowders of Iron* (Inst. Metall. Materialoved. im. A. A. Baikova RAN, Moscow, 2006) [in Russian].

29. L. Gorim and F. Asch, "Seed coating increases seed moisture uptake and restricts embryonic oxygen availability in germinating cereal seeds," Biology (Basel). **6** (2), 31 (2017). doi.org/ https://doi.org/10.3390/biology6020031

30. S. A. Stepanov, N. S. Il'in, E. L. Gagarinskii, et al., "Physiological features of morphogenesis of seedlings of spring soft wheat," Izv. Sarat. Univ., Nov. ser. Ser. Khim. Biol. Ekol., **14** (3), 37 (2014).

31. D. Wang, S. Fahad, S. Saud, et al., "Morphological acclimation to agronomic manipulation in leaf dispersion and orientation to promote 'Ideotype' breeding: Evidence from 3D visual modeling of 'super' rice (Oryza sativa L.)," Plant Physiol. Biochem., **499** (2019). https://doi.org/10.1016/j.plaphy.2018.11.010

32. J. Duan, Y. Wu, Y. Zhou, et al., "Approach to higher wheat yield in the Huang-Huai plain: Improving post-anthesis productivity to increase harvest index," Front. Plant. Sci. **9**, 1457 (2018). https://doi.org/10.3389/fpls.2018.01457

33. X. Ma, X. L. Dai, X. J. Wang, et al., "Stability of a winter wheat population with high yield and high resource use efficiency," Ying Yong Sheng Tai Xue Bao **28**, 3926 (2017). https://doi.org/10.13287/j.1001-9332.201712.025

34. J. L. Osnas, J. W. Lichstein, P. B. Reich, et al., "Global leaf trait relationships: Mass, area, and the leaf economics spectrum," Science (Washington, DC, U. S.) **340**, 741 (2013).

35. O. O. Osunkoya, D. Bayliss, F. D. Panetta, et al., "Leaf trait co-ordination in relation to construction cost, carbon gain and resource-use efficiency in exotic invasive and native woody vine species," Ann. Botany **106**, 371 (2010).

36. Y. Geng, L. Wang, D. Jin, et al., "Alpine climate alters the relationships between leaf and root morphological traits but not chemical traits," Oecologia **175**, 445 (2014).

37. G. A. Achari and M. Kowshik, "Recent developments on nanotechnology in agriculture: Plant mineral nutrition, health, and interactions with soil microflora," J. Agric. Food. Chem. **66**, 8647 (2018). https://doi.org/10.1021/acs.jafc.8b00691

38. M. Simonin and A. Richaume, "Impact of engineered nanoparticles on the activity, abundance, and diversity of soil microbial communities: A review," Environ. Sci. Pollut. Res. Int. **22**, 13710 (2015). . https://doi.org/10.1007/s11356-015-4171-x

39. C. O. Dimkpa, "Can nanotechnology deliver the promised benefits without negatively impacting soil microbial life?," J. Basic. Microbiol. **54**, 889 (2014). https://doi.org/10.1002/jobm.201400298

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