AGRICULTURAL BIOLOGY,
2020, V. 56, Iss. 1, pp. 135-145
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)
ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Plant growth stimulants

UDC 633.11:631.559.2:631.53.027.2
doi: 10.15389/agrobiology.2021.1.135eng
doi: 10.15389/agrobiology.2021.1.135rus

STIMULATING EFFECTS OF PRE-SOWING SEED TREATMENT WITH METAL NANOPARTICLES ON WINTER WHEAT GROWTH AND DEVELOPMENT

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The authors declare no conflict of interests
Acknowledgements:
Authors would like to thank the Ministry of Education and Science of the Russian Federation and the staff of the laboratory of INEPCP FRCCP RAS under the leadership of A.N. Zhigach for the support in the experiments.
Received June 7, 2020

Abstract

The use of nanotechnologies in agriculture is an advanced course enabling to reduce the dependence of crop tonnage and quality on external factors. A special section is represented by studies of pre-sowing treatment of seeds with metal nanoparticles (NPs). In this work, it is shown for the first time that pre-sowing treatment of seeds by metal nanoparticles with specific physicochemical parameters affects morphometric indices of the of winter wheat growth at all stages of its development as well as the plant resistance to pathogens, grain quality, the degree of its damage from fusarium and elemental composition of the soil after harvesting. Effects depended on the type of metal used. Our aims were to study (1) effects of pre-sowing seed treatment with iron, zinc, and copper NPs on the growth parameters and grain quality of winter wheat, and (2) whether this treatment affects the soil after harvesting. Iron, zinc, and copper NPs were obtained by the method of high temperature condensation at the Migen-3 apparatus (Institute for Energy Problems of Chemical Physics RAS, Russia). The shape and size of NPs were evaluated by JSM-7401F scanning electron microscope (JEOL Ltd., Japan). X-ray phase analysis was carried out using an ADP-1 X-ray analyzer (NPO Modern Technologies of Non-Destructive Control, Russia). Field trials were carried out at the validation test site of the Novokuban Branch of the Rosinformagrotech (Krasnodar Territory). The predominant soil type is typical chernozem, with medium humus content, heavy loamy. The sowing of winter wheat (*Triticum aestivum* L.) cv. Stan was performed on October 4, 2016 with a setting seed rate 240 kg/h. The assigned treatments were as follows: control (seeds without treatment), seed treatment with Fe NPs ($5\times10^{-4}\%$), Zn NPs ($1\times10^{-4}\%$), Cu NPs ($5\times10^{-7}\%$); Fe NPs + Zn NPs + Cu NPs ($5\times10^{-4}\% + 1\times10^{-4}\% + 5\times10^{-7}\%$). Soil samples were collected for chemical analysis. For phenological and biometric observations, plants were taken from three locations of 1 m^2 area from each experimental and control plot. Plant height, average root length, thickness of the main stem at the plant bottom, tillering and the depth of the tillering node were measured. Iron, zinc, and copper NPs were round single-crystal structures covered with a semitransparent oxide film. Average diameter of Fe NPs was 27.0±0.51 nm, Zn NPs 54.0±2.8 nm, Cu NPs 79.0±1.24 nm. X-ray phase analysis showed that iron NPs consisted of 53.6% crystalline metal phase, Fe_3O_4 content was 46.4 %, and the oxide film thickness was 3.5 nm. Cu and Zn NPs contained only crystalline metal phases with the similar oxide film thickness, 0.5 to 1.0 nm. Pre-sowing treatment of seeds with Fe NPs affected the height of seedlings, promoted the formation of a developed root system with total root length being 4.5% more ($p \le 0.05$) than in the control group, and increased the seedling stand density by 9.96% ($p \le 0.05$) vs. control. Pre-harvest monitoring of crops revealed an increase in the yield of wheat plant mass after pre-sowing seed treatment with Fe and Cu NPs. Stem length was larger than that of the control (81.3±1.2 cm) by 3.8 and 8 cm, respectively, the average thickness of the main stem at the plant bottom being larger by 6 mm (when processed with Fe NPs) and 5 mm (when treated with Cu NPs) in comparison with the control (44 mm). Plant stands productivity enhancement after Fe and Zn NPs treatments, higher resistance to pathogens (by 3.85 times vs. control) under Fe NPs, a tendency to an increase in the average 1000-grain mass when using NPs of Fe, Zn, and Cu were observed. The crop quality parameters had higher values as compared to the control: in terms of the content of wet gluten by 6.12 % when seeds were treated with Zn and Cu NPs or with NPs composition; the protein mass fraction was larger under treatment with Cu NPs and the NPs composition by 5.1 % vs. control. Pre-sowing treatment with Fe and Zn NPs reduced the prevalence of Fusarium infection in grain by 1.24 and 2.25 times respectively vs. control. Elemental analysis of the soil after harvesting showed a decrease in the content of mobile forms of phosphorus by 27 % and zinc by 48 % after seed treatment with Zn NPs in comparison with the control, and a decrease in the phosphorus mobile forms by 23 % and sulfur by 7 % after pre-sowing treatment with Cu NPs in comparison with the control. The data obtained demonstrate the effective influence of the pre-sowing treatment of seeds by metal NPs on the growth, development and grain quality of wheat.

Keywords: nanoparticles, iron, zinc, copper, yield components, grain quality, soil trace elements

Wheat is an essential food crop for countries with temperate climates; it is consumed by more than half of the world's population. The growing food demand is projected to double the demand for wheat by 2050 [1]. At the same time, wheat farming has ever more exacerbated issues, as new diseases and more aggressive pests appear, water resources are dwindling, arable land is deficient, the climate changes, and weather is unstable [2].

In addition to advanced agricultural technology, the wheat industry uses genetic and breeding breakthroughs, nanomaterials, and nanotechnology to improve yields [2-4]. The yield increase and better crop conditions enabled by nanotechnologically optimized plant nutrition and protection help address not only humanitarian problems such as yields and quality but also the environmental troubles [4, 5]. Agriculture commonly uses Zn, Cu, Fe, Mn nanoparticles (NPs) and oxides thereof. Zinc oxide (ZnO) and copper oxide (CuO) NPs are used in many commercial products including antimicrobials. Recent research proved their effectiveness as fungicides, on account of the ability to inhibit the growth of fungal plant pathogens. So it is not a coincidence that the use of NPs to boost growth and control diseases in plants is becoming a best practice [6-10].

The unique properties of NPs make them an interesting solution in crop production. The authors' long-term studies of dispersed systems and NPs have revealed the following biological effects of NPs. Metal NPs are 7 to 50 times less toxic than ionic metals. They have a prolonged and polyfunctional effect; they stimulate metabolism and easily reach all organs and tissues. Their biological activity stems from their structure and physicochemical properties. Metal NPs increase the effectiveness of natural polysaccharides when used in combination [11-14]. Due to their size, NPs actively penetrate into, and spread across, all issues, thus intensifying physiological, biochemical, and molecular processes of plant germination and growth. Once in a biological fluid, NPs function as a depot: their slow oxidation provides the plant with the micronutrients it needs to grow. These peculiar biological effects of NPs allow using them in crop production; effective NPs concentrations are two orders of magnitude lower than those of metal salts [15]. There is a special niche for research into the pretreatment of seeds with NPs, which increases the yield by 30-40% [16].

This paper is the first to show that the pre-sowing treatment (pretreatment) of seeds with metal NPs of specific physical and chemical properties affects the morphometric growth parameters of winter wheat at all phases of its development and influences the crop resistance to phytopathogens, the quality of grains, the extent of fusariosis-caused damage, and the content of elements in the soil after harvesting. The effects depend on which kind of metal is used.

The goal hereof was to find out how pretreatment of seeds with iron, zinc, and copper NPs, individually and in combination, would affect the growth of winter wheat in all phases, the quality of grains, and the levels of soil elements after harvesting.

Materials and methods. NPs of iron, zinc, and copper were produced by high-temperature condensation [17] on a Migen-3 unit (Institute of Problems of Chemical Physics of RAS, Russia) [18]. Weighed NP samples were dispersed in distilled water using a Scientz JY 92-IIN ultrasonic disintegrator (Ningbo Scientz Biotechnology Co. Ltd, China) at 0.5 A and 44 kHz over 30 s with a pause of 30 s, three repeated treatments; the dispersed mixture being cooled down in an ice bath.

The shape and size of NPs were estimated by transmission electron microscopy (TEM) on a JSM-7401F scanning electron microscope (JEOL Ltd., Japan) at 1 kV. To find out the average NPs diameter, microphotographs were processed in Micran 25 (https://www.micran.ru/), measuring at least 1000 particles across. The data were then used to plot NPs size distribution curves and estimate the average size.

X-ray crystallography (XRC) was run on an ADP-1 unit (Advanced Non-Destructive Testing Technologies, Russia). A cobalt tube was used as the radiation source. Scanning was done at increments of 0.05° with 8 to 10 minutes to accumulate the signal. To find out the phase composition, the obtained interference peaks were processed in Match 3.8.0.137 (http://www.crystalimpact.com/).

Research was carried out with the winter wheat (*Triticum aestivum* L.) variety Stan (Krasnodar Agricultural Research Institute) picked from the varieties allowed for use in the North Caucasus on moderately or highly fertile soils. The Stan is a strong, short-stalk variety resistant to lodging and shedding; it matures early and produces grain that has favorable traits for baking. The plants are about 95 cm tall and show immunity to loose smut when artificially infected. The Stan is resistant to brown, yellow and stem rusts, field resistant to powdery mildew, medium resistant to head smut, moderately susceptible to septoriosis, and susceptible to fusarium ear blight; medium frost resistant, high heat resistant.

Field experiments were carried out at the test fields of the Novokubansk Branch of Rosinformagrotech, a site in the Krasnodar Territory where soil hydration is inconsistent. The farm mostly had typical chernozem with medium humus content and high loam content. The soil was sampled for chemical testing per GOST 17.4.4.02-84. Nature protection. Soils. Methods for sampling and preparation of soil for chemical, bacteriological, helmintological analysis (Moscow, 2018). Each average sample was made up of 10 individual samples taken at the test site by using the technique referred to as “the envelope method” in GOST (five sampling points: one in the center and four placed equidistantly around it forming a square – *translator’s note*); samples were taken from arable layer depth (30 cm). The soils had an enlarged concentration of humus, elevated or high nitrification capacity, low/medium/elevated phosphorus content, medium or elevated potassium content, and neutral or close-to-neutral exchangeable acidity. The sulfur content was low or medium; the manganese, zinc, and copper contents were low. On average, 1 kg of soil contained 37.1 mg of nitrate nitrogen, 20.0 mg of phosphorus, 297 of potassium, 5 mg of sulfur, 3.14 mg of manganese, 0.37 mg of zinc, 0.08 mg of copper, 4.56% of humus; pH 6.16.

Winter wheat variety Stan (RS-1) was sown for production at a time optimal for the central climatic zone: October 4, 2016; the sowing rate was 240 kg/ha. The monthly average temperature was +18.1 °C (September 2016), +10.7 °C (October 2016), +6 °C (November 2016), –2.6 °C (December 2016),

–1.3 °C (January 2017), +0.1 °C (February 2017), +8.3 °C (March 2017), +13.1 °C (April 2017), +17.6 °C (May 2017), +22.6 °C (June 2017), +26.6 °C (July 2017).

Several groups were planted: controls (untreated seeds), seeds treated with NPs of Fe ($5\times10^{-4}\%$), Zn ($1\times10^{-4}\%$), Cu ($5\times10^{-7}\%$), Fe + Zn + Cu ($5\times10^{-4}\% + 1\times10^{-4}\% + 5\times10^{-7}\%$). All the cultivation operations in both the experimental fields and in the control field were carried out on the same day with the same machinery.

Labile phosphorus and potassium compounds were detected per GOST 26204-91. Soils. Determination of mobile compounds of phosphorus and potassium by the Chiricov method modified by CINAO (Moscow, 1992). The extent of fusariosis-induced damage was estimated per GOST 31646-2012. Cereals. Method for determination of the scabby kernels content (Moscow, 2019).

To collect phenological and biometric data, plants were sampled from three sites sized 1 m^2 from each experimental and control field. Density, height, root length, tillering node depth, and tillering were measured in the third/fourth-leaf stage, as well as in the spring once vegetation resumed (6-8 leaves), and once the plants began tillering (parent shoot plus 2-3 tillers). Tillering was measured as the number of tillers per plant.

Two weeks before harvesting, the research team measured plant height, root length, main stem thickness at the bottom, number of grains per ear, number of productive and nonproductive stems. To that end, frames sized 50×50 cm were buried in the fields; all plants would be scooped out within a frame, sorted by yield structure, then counted and measured being triply repeated for each experimental scheme.

Grain quality was assessed in terms of wt.% of wet gluten (GOST 54478-2011. Grain. Methods for determination of quantity and quality of gluten in wheat. Moscow, 2012), wt.% of protein (GOST 10846-91. Grain and products of its processing. Method for determination of protein. Moscow, 2009)), vitreousness (GOST 10987-76. Grain and grain processing products. Methods for determination of vitreousness. Moscow, 2009), hectoliter weight (GOST 10840-2017 Grain. Method for determination of hectolitre weight. Moscow, 2019) and wt.% of moisture (GOST 13586.5-2015. Grain. Method of moisture content determination. Moscow, 2019).

Statistical analysis was made by Microsoft Excel 2010 and Statistica 20 (StatSoft Inc., USA). Means (*M*) and standard errors of the means (±SEM) were calculated. The significance of the differences between the groups was assessed by parametric tests (Student's *t*-test) and non-parametric tests (Wilcoxon's pairwise *W*-test). The significance threshold was $p \leq 0.05$.

Results. Requirements for nanopowders depend on the fields of their application, and that is why NPs characteristics are rather diverse, and so are the methods for their synthesis. The optimal biological activity of NPs depends on their physical and chemical properties such as size, phase and element composition, and oxide film thickness [13, 14]. Besides, the effectiveness of metal NPs depends on their concentration and application mode [15].

According to the TEM images, metal NPs tested herein were monocrystalline structures of regular shape covered with a translucent oxide film (Fig. 1). Thus, Fe particles were sized 5 to 80 nm, 27.0±0.51 nm on average, Zn particles were 54.0±2.8 nm on average, and Cu particles were 79.0±1.24 nm. XRC of Fe particles showed that they were 53.6±4.2% crystalline metal and 46.4% Fe_3O_4; the oxide film was 3.5 nm thick. Cu and Zn particles were crystalline metal only, the oxide film was of an identical thickness (0.5 to 1.0 nm).

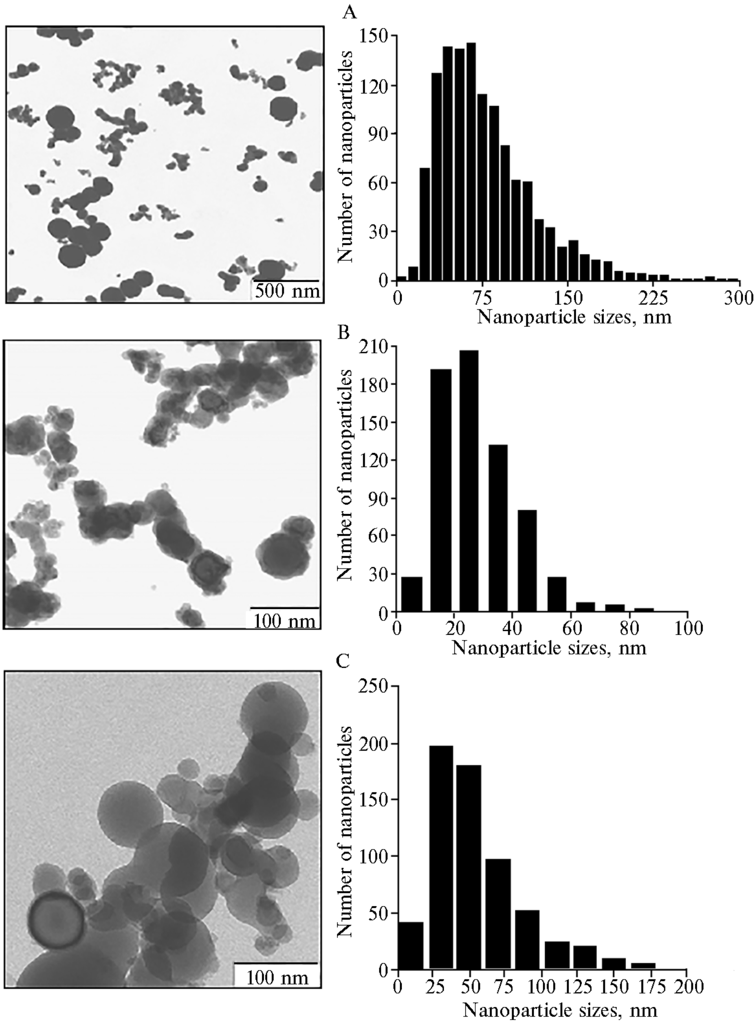

Fig. 1. Nanoparticles of copper (A), **iron** (B), **and zinc** (C) (to the left; images produced by transmission electron microscopy, JSM-7401F, Jeol Ltd., Japan) **and distribution by particle size** (to the right).

Field experiments represented a density of 320±11 plants/m^2 when pretreated, showing a 10% increase against the control (291±9 per m^2) ($p \leq 0.05$). Treatment with Fe NPs significantly increased the height ($p \leq 0.05$) by 2.0±0.5 cm against the controls. Pretreatment with Cu NPs reduced the density by 17.9% ($p \leq 0.05$) whilst insignificantly increasing the height compared to the controls. Pretreatment with Zn NPs and an NP combination of Fe, Zn, and Cu somewhat delayed the growth of plants and the development of their roots; density virtually did not differ from the controls.

When early tillering in the autumn, Zn-pretreated specimens had the largest number of stems (1.5 times that of the controls, $p \leq 0.05$). This trend continued when the plants began tillering in spring (Fig. 2). Pretreatment with Cu NPs and with the combination intensified tillering as well. Tillering node was found at the greatest depth in Fe-pretreated plants in autumn (down to 4.3±0.7 cm), closest to the surface in Zn-pretreated plants (2.35±00.5 cm), compared to 3.13±0.6 cm in the controls. In spring, the look was the same in all the groups (3.6±0.1 cm).

The emergence of roots in the autumn and spring was intensified by pretreatment with Fe NPs, ~~as~~ the roots being respectively 4.5% and 3.8%

longer ($p \le 0.05$) compared to the controls. Many authors have noted the boosting effect that Fe NPs have on the root system and its activity [15, 19, 20].

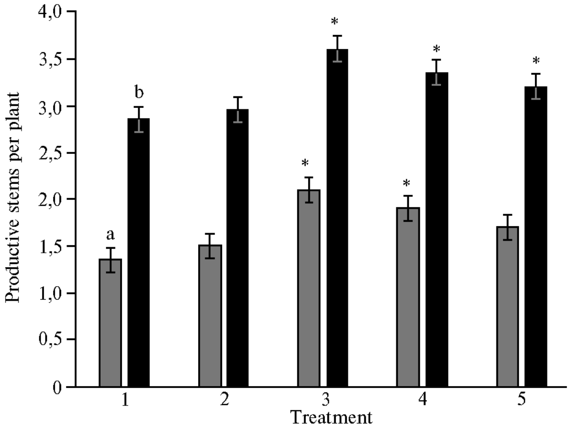

Fig. 2. Tillering of winter wheat (*Triticum aestivum* L.) cv. Stan in the autumn (a) **and spring** (b) **after pre-sowing treatment with NPs:** 1 — control (untreated), 2 — Fe NPs ($5\times10^{-4}\%$), 3 — Zn NPs ($1\times10^{-4}\%$), 4 — Cu NPs ($5\times10^{-7}\%$), 5 — Fe NPs + Zn NPs + Cu NPs ($5\times10^{-4}\% + 1\times10^{-4}\% + 5\times10^{-7}\%$) ($n$ = 290-320, *M*±SEM; Krasnodar Territory, 2017). * Differences between treatment and control groups are statistically significant at $p \le 0.05$.

Thus, before winter, wheat plants pretreated with metal NPs were well-rooted and tillered specimens that looked better than the controls in terms of stem height (Fe and Cu NPs), seedling density (Fe and Zn NPs, as well as the NPs combination), and the tillering node depth (Fe and Cu NPs, as well as the NP combination). Spring growth beginning in this particular case was complicated by the early cold snap and abundant snowing. But no dead or damaged plants were found in the field after the winter.

Pre-harvest monitoring showed NP pretreatment to increase plant height, with the most drastic increase of 9.1% against the controls ($p \le 0.05$) being observed in the Cu group (Table 1). The mean root length varied from 7.8±0.9 cm (NPs combination) to 8.1±1.0 cm (Cu), compared to 8.04±0.5 cm in the controls. Stems were on average 13.6% or 11.4% thicker at the bottom when pretreated with Fe and Cu, respectively, compared to the controls (44.0±1.0 mm), $p \le 0.05$. Zn and combination-pretreated plants had the most stems, 0.8% and 2.0% more than the controls, respectively. Zn pretreatment increased the number of nonproductive stems by 1.3% ($p \le 0.05$), whilst the combination reduced that number by 0.4% ($p \le 0.05$) against the controls. Specimens pretreated with Fe, Zn, and Cu NPs had the least disease-affected stem number compared to the controls. Thus, there were four times less affected stems in Fe-treated plants ($p \le 0.05$). In these groups, the 1000 grains mass exceeded by 1.9%, 1.3%, and 1.1% the control group value.

1. Pre-harvest monitoring of wheat (*Triticum aestivum* L.) cv. Stan pretreated with metal nanoparticles (NPs) (n = 110-120, *M*±SEM; Krasnodar Territory, 2017)

Group	Height, cm	Root length, cm	Stem thickness at the bottom, mm	Number of stems			1000 grain mass, g
				total, plants/m^2	nonproductive, %	disease-affected, %	
Controls (untreated)	87.8±1.2	8.0±0.8	44±1.0	456.0±11.0	1.5	2.4	44.2±0.3
Fe NPs	91.6±0.9	8.0±0.9	50±1.8	446.8±10.1	1.5	0.6	45.0±0.2
Zn NPs	88.3±1.4	7.9±0.6	46±1.4	465.2±9.6	2.8	1.1	44.8±0.2
Cu NPs	95.8±1.3	8.1±1.0	49±1.3	437.2±10.3	1.2	1.6	44.7±0.4
Combination of Fe, Zn, and Cu NPs	92.4±1.0	7.8±0.9	45±0.9	460.0±12.4	1.1	2.4	43.9±0.3

2017 had excessive rainfall; high humidity is known to contribute to the growth of fungal pathogens, including fusariosis. Fungi are known to significantly compromise the yield and quality of grains in cereals; besides, they may end up in food raw materials, foods and feeds, contaminating them with fungal toxins [21]. Given the urgency of the issue, the presence of contaminated grains becomes an important quality metric. Pretreatment with Fe/Zn/Cu and

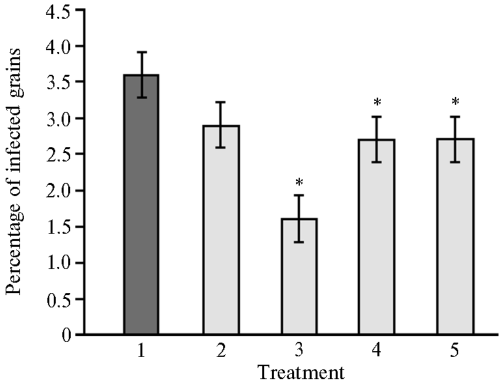

Fig. 3. Number of fusariosis-infected grains of winter wheat (*Triticum aestivum* L.) cv. Stan pretreated with metal nanoparticles (NPs): 1 — control (untreated), 2 — Fe NPs ($5\times10^{-4}\%$), 3 — Zn NPs ($1\times10^{-4}\%$), 4 — Cu NPs ($5\times10^{-7}\%$), 5 — Fe NPs + Zn NPs + Cu NPs ($5\times10^{-4}\% + 1\times10^{-4}\% + 5\times10^{-7}\%$) (*n* = 1000, *M*±SEM; Krasnodar Territory, 2017).
* Differences between treatment and control groups are statistically significant at $p \leq 0.05$

their combination reduced contamination by a factor of 1.24 ($p \leq 0.05$)/2.25 ($p \leq 0.05$), 1.33 ($p \leq 0.05$) against the controls (Fig. 3). Zn NPs have been shown by other authors to effectively inhibit the growth of pathogens, including the fusariosis agent, not only in wheat but also in legumes [22, 23].

Per technical specifications, all the harvested grain from all the groups was Class IV soft wheat (Table 2). Grain quality was consistently higher in pretreated plants compared to the controls. Thus, the mass fraction of wet gluten was 2.6% higher in Fe NPs-pretreated plants, 5.1% higher in the Zn NPs and composition-pretreated plants, and 6.1% higher in the Cu NPs-pretreated plants than in the controls. The mass fraction of protein in grains was 1.6%, 2.5%, 3.4%, and 5.1% higher in Fe, Zn, Cu, and composition-pretreated plants, respectively. Vitreousness and bulk density increased by within 1% against the controls.

2. Grain quality of winter wheat (*Triticum aestivum* L.) cv. Stan pretreated with metal nanoparticles (NPs) (*n* = 1000, *M*±SEM; Krasnodar Territory, 2017)

Metric	Group				
	control (untreated)	Fe NPs	Zn NPs	Cu NPs	combination of Fe, Zn, and Cu NPs
Wt.% of wet gluten	19,6±1,2	20,1±1,4	20,6±1,3	20,8±1,4	20,6±1,2
Wt.% of protein (dry matter)	11,8±0,5	12,0±0,3	12,1±0,2	12,2±0,2	12,4±0,3
Vitreousness, %	45,7±5,4	46,1±4,3	45,9±4,6	46,2±4,3	45,7±3,2
Mass per volume, g/l	767±21	770±30	772±28	767±30	774±31
Moisture content, wt.%	13,4±2,4	13,5±1,8	13,2±2,1	13,2±2,2	12,8±1,8

Therefore, pretreatment of seeds with NPs of Fe ($5\times10^{-4}\%$), Zn ($1\times10^{-4}\%$), and Cu ($5\times10^{-7}\%$), individually and in combination, distinctly affect the germination of seeds, the biological yield of plant mass, the productivity of stems, the resistance to pathogens, and the quality of grains. The causative factor lies in the structural reorganization of leaves, the regulation of the growth of vascular bundles in leaves, stems, and roots, which boosts photosynthesis and improves the suction force of the roots [15]. Active research is going on into how these processes are regulated on the molecular level using transcriptome, proteome, and metabolome analysis [24, 25].

Postharvest soil composition testing revealed neutral soil pH in experimental groups; heavy metal concentrations were within the acceptable limits. The lead content (Pb) was 0.19 to 0.22 mg per kg of soil (the maximum permissible concentration (MPC) is 6.0 mg/kg), cadmium (Cd) was 0.023 to 0.028 mg/kg (MPC 0.10 mg/kg). The availability of nitrate nitrogen ($N\text{-}NO_3$), labile phosphorus (P_2O_5), exchangeable potassium (as K_2O), labile sulfur (S), manganese (Mn), zinc (Zn), and copper (Cu) were largely the same as in the control field soil. However, some changes were observed, too. Pretreatment with Zn NPs reduced P_2O_5 content by 27% and Zn by 48%, bringing the soil into the “scarce” category. Cu NPs pretreatment reduced the availability of P_2O_5 by 23% and S by

7% compared to the controls. Results of the analysis suggest that despite low concentrations, metal NPs may affect the content of elements in the soil. Further studies are expected to find out how exactly metal NPs affect the presence of micronutrients in the soil and its biome. Without a doubt, the fact that zinc and copper NPs may alter the micronutrient composition of the soil must be taken in mind when applying such NPs.

Thus, the finding is that the winter wheat variety Stan has better morphological indicators in all growth phases, better yield quality, and stronger phytophthorosis resistance after seed pretreatment with nanoparticles (NPs) of iron, zinc, copper, or a combination thereof. The best morphometric parameters of early growth and development were observed under the use of Fe NPs (a consistent 12% increase in seedling height, 4.5% in root length, 9.96% in density, and 9.3% in tillering node depth compared to the controls). Right before the harvest, the crops from experimental groups had high stems: 4.3% taller than the controls when treated with Fe, by 9.1% taller when treated with Cu. The main stem was 13.6% and 10.4% thicker at the bottom in these two groups than in the controls. Fe, Zn, and Cu NPs-pretreated plants had the least number of damaged from infections stems. The weight of 1000 grains was increased by 1.9%/1.3%/1.1% against the controls when pretreated with Fe, Zn, and Cu NPs. All the experimental groups had 2.5% to 6.1% more wet gluten, 1.6% to 5.1% more protein in grains (as wt.%). Vitreousness and bulk density improved as well. Fusariosis affected Zn-pretreated plants 2.25 times less than it did in the control; plants pretreated with other NPs or with the combination were 20% to 30% less affected. After harvesting, the soil had 27% less labile phosphorus, 48% less labile zinc in the fields after growth of Zn NPs-pretreated plants; pretreatment with Cu NPs reduced the content of P_2O_5 by 23% and that of S by 7%.

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