Evaluation of Stimulatory, Antifungal and Thermo-Resistant Action of Aqueous Dispersions of Nanoparticles on Seeds of Parental Forms and Reciprocal Hybrids of Winter Wheat

S. N. Maslobrod, G. A. Lupashku, S. I. Gavzer, A. I. Gore, and Yu. A. Mirgorod

Abstract

For the first time in the crop technology, the effect of aqueous dispersions of nanoparticles was investigated on seeds of parental forms and their reciprocal hybrids, making it possible to effectively use this factor in plant breeding and genetic practice. The received data can be used to select optimal pairs for crossing and obtain new promising hybrids. It was shown the stimulatory, antifungal and thermo-resistant action (increase of resistance to pathogenic fungus) of water dispersed solutions of silver and copper nano-particles on seeds of winter triticale. The antifungal effect of the nanofactor is more effective than of the potassium permanganate. In terms of seed germination energy and sprout length of winter wheat (parent varieties and their reciprocal hybrids) we revealed the stimulatory and antifungal action of aqueous dispersions of silver, copper, bismuth, and zinc oxide nanoparticles on the seeds. The maternal effect is revealed—the primary influence of the maternal form on the physiological parameters of the hybrid.

Keywords

Water dispersed solutions • Nanoparticles • Seed germination energy • Antifungal effect • Sprout length • Reciprocal hybrids • Parent varieties

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1 Introduction

Aqueous dispersions containing nanoparticles of various substances have bactericidal and fungicidal effects on a plant object (Ling and Yatts 2005; Zhu et al. 2008; Yatts and Ling 2007; Morgalev et al. 2010; Glushchenko et al. 2006).

They also function as trace elements of the mineral nutrition of plants of prolonged action, increasing the adaptive potential of the plant organism. Nanoparticles are electrically neutral, which allows them to be evenly distributed in the film former and develop a thin layer to envelop the seeds. This provides reliable seed protection from pathogens. Oxidizing gradually in the soil, nanoparticles create unfavorable conditions for pathogenic microorganisms and at the same time are used by plants as trace elements in the process of growth (Zhu et al. 2008; Yatts and Ling 2007; Morgalev et al. 2010; Glushchenko et al. 2006; Panichkin and Raikova 2009; Raikova 2004).

Nanopowders have extremely huge specific surface area (of the order of several hundred square meters per 1 g), therefore, they can be effectively used in micro-doses. Thus, for the pre-sowing treatment of 1 ton of seeds is used only few milligrams of nanopowder (Ling and Yatts 2005), which at the same time ensures ecological safety of both the environment and the bioproduct. Herewith, the toxicity of metal nanopowders is 10–40 times less than the toxicity of salts of the same metals (Glushchenko et al. 2006).

We began intense research on nanotechnology in crop production in 2014 (Maslobrod et al. 2014). The task was to study the effect of aqueous dispersions (AD) of nanoparticles (NP) of various metals and their oxides on seed germination of parental forms (varieties) and reciprocal hybrids of tomato and wheat, as well as on the resistance of these seeds to pathogenic fungi and low temperature (Maslobrod et al. 2017). The data of such studies can be taken into account when selecting parental pairs for crossing in order to obtain productive and environmentally sustainable hybrids. This publication reflects the

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results of ongoing research in this direction using both other types of nanoparticles and genotypes.

2 Materials and Methods

Experiments were conducted in the Laboratory of Applied Genetics of the Kursk South-West State University (Russia) where the nanoparticles were obtained and characterized in detail (Mirgorod et al. 2012, 2013, 2018) (Fig. 1). Seeds of winter triticale (variety *Ingen93*) were used for conducting the methodological experiment. As parental forms, in the main experiments, we took seeds of winter wheat (variety): (1) *Odessa 267* and *Nikonia*, (2) *Accent* and *Select*, (3) *Basarabianka* and *M/M3* and their reciprocal hybrids. Seeds were soaked in AD of NPAg, NPCu, NPBi and NPZnO (concentration and exposure are presented in the results of the study). The seeds were treated with *Helminthosporium avenae* fungus (the main pathogen for cereal seeds) (Methods 1982). Fungus exposure is within 18 h. For comparison, we used 1% potassium permanganate solution ($KMnO_4$) in the methodological experiment, with a 1 h exposure. When evaluating the thermo-resistant effect of the nanofactor, the seeds were subjected to low temperatures (+4 °C) during 12 h. After the treatment, the seeds were germinated in distilled water in *Petri* dishes at the temperature of 20 + 25 °C. We used 200–300 seeds in each embodiment. The following signs were taken into consideration: seed germination energy SGE (number of germinated seeds on the 2nd day), length of the sprout root LR (on the 3rd day), length of the seedling LS (on the 7th day).

3 Results and Discussions

3.1 Methodological Experiment

The aim of this experiment was to compare the antifungal effects induced by potassium permanganate solution

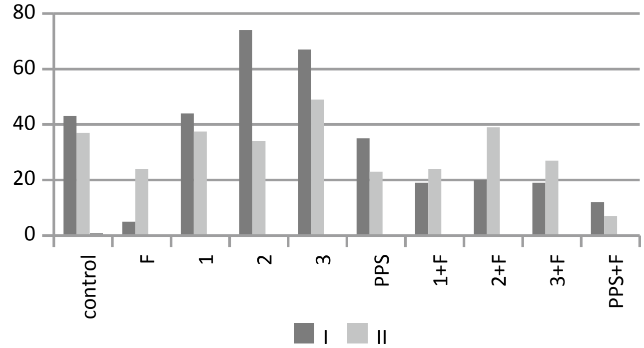

Fig. 2 Germination of triticale seeds (variety *Ingen 93*) under the action of nanoparticles aqueous dispersions of silver (I), of copper (II), *Helminthosporium avenae* fungus (F), potassium permanganate (PPS) (the arithmetic mean error does not exceed 10%) 1,2,3—concentrations of NPAg [I], respectively, 8×10^{-6} mol/l, 16×10^{-7}mol/l, 32×10^{-8}mol/l; of NPCu (II)—8×10^{-6}mg/l, 16×10^{-7}mg/l, 32×10^{-8}mg/l

(PPS) and aqueous dispersions at three different concentrations of NPAg and NPCu. The criterion for assessing the effect was the operational parameter *seed germination energy* (Fig. 2). When using AD of NPAg, stimulation was obtained by 72% and 56% at NP concentration respectively 16×10^{-7} mol/l and 32×10^{-8} mol/l. The antifungal effect was observed from both the nanofactor and PPS. The nanofactor turned out to be more effective than PPS: the *fungus+NPAg* variant was 4 times more effective than the *fungus* variant, and the *fungus+PPS* variant was 2.4 times more efficient. In general, the antifungal effect was 1.7 times higher than that of PPS. When using AD of NPCu, the concentrations were calculated in mg/l. Stimulation was obtained at the concentration of 32×10^{-8} mg/l and it increased by 32%. The antifungal effect was obtained at the NP concentration of 32×10^{-8} mg/l, exceeding the *fungus* variant by 1.6 times. The PPS increased the negative effect of the fungus by 3.4 times. In further experiments, AD of NPBi, NPCu, NPZnO were used at stimulative concentrations: 10^{-7} mg/l, and AD of NPAg at 10^{-7} mol/l concentration.

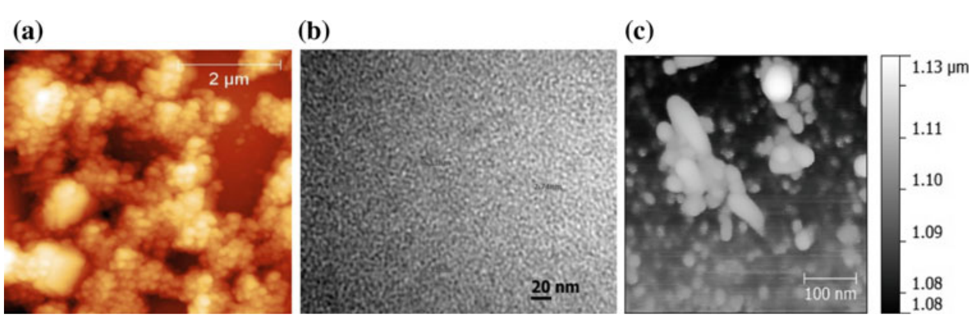

Fig. 1 General view of nanoparticles of copper (**a**), silver (**b**) and bismuth (**c**) used in our research. Images were developed by Mirgorod et al. (2012, 2013, 2018) and reprinted here with permission of the author

3.2 Evaluation of the Stimulatory, Antifungal and Thermo-Resistant Action of Aqueous Dispersions of Nanoparticles on Seeds of Parental Forms and Winter Wheat Hybrids

As can be seen in Table 1, AD of NPBi, by the seed germination energy (SGE) parameter, has a significant stimulatory effect on all the genotypes—the parent forms *Odessa267* and *Nikonia* and their reciprocal hybrids: the excess of SGE is by 1.75, by 1.53, by 1.36 and by 1.69 times. Here there is revealed a predominant influence of the maternal form on the SGE of the hybrid.

Aqueous dispersion of NPZnO also has a stimulating effect on the three genotypes (in the same parental forms and hybrids, the excess of SGE was by 1.20, by 1.57, by 1.44 and by 1.03 times). The maternal effect is again observed (Table 1). With the same genotypes, the stimulating and antifungal action of the nanofactor (AD of NPAg) was tested by a different parameter—the length of the seedling sprout (Table 1). The stimulation effect was obtained only with *Nikonia* seeds. The antifungal effect was observed with *Nikonia, Odessa267-x-Nikonia* and *Nikonia-x-Odessa267*, excess of the *NPAg + Fungus* variant over the *Fungus* variant being respectively by 1.06, by 1.06 and by 1.16 times).

With the seeds of winter wheat of other genotypes: *Accent, Select, Accent-x-Select, Select-x-Accent*, the stimulating and antifungal effect was tested by the length of the germ (Table 2). Here, the stimulation is observed with the AD of NPAg on *Accent-x-Select* seeds (by 5%) and with the AD of NPCu on *Select* seeds (by 9%). The antifungal effect was not found.

3.3 Evaluation of the Thermo-Resistant Effect of Aqueous Dispersions with Nanoparticles on Seeds of Parental Forms and Hybrids of Winter Wheat

We observed a significant stimulation of the seed germination energy in the study aimed to assess the thermo-resistant effect of the nanofactor on the seeds of the parent forms pair *Basarabianka* and *M/M3* and their reciprocal hybrids (Table 3): (1) *Basarabianka*—only in the aqueous dispersion of the mixture of nanoparticles variant (by 12%), (2) *M/M3*—in all variants of the nanofactor: aqueous dispersions of NPBi, NPCu, NPZnO and of the mixture of nanoparticles (maximum stimulation was observed in the latter variant—by 64%), (3) *Basarabyanka-x-hybrid M/M3*—in all variants of the nanofactor (up to 33%), (4) for the hybrid—practically in all variants with the nanofactor (up to 17%).

As can be seen, aqueous dispersions of nanoparticles of three types—bismuth, copper, and zinc oxide, as well as of the mixture of these nanoparticles have increased seeds thermal resistance, i.e. the resistance of *M/M3* seeds and two

Table 1 Seed and seedling parameters of parent wheat forms and reciprocal hybrids after treating the seeds with nanoparticles aqueous dispersions and fungus

Variant	Odessa 267	Nikonia	Odessa267-x-Nikonia	Nikonia-x-Odessa 267
Control	33.5 ± 3.25	30.0 ± 3.83	40.0 ± 3.07	27.5 ± 4.78
ADNPBi	58.5 ± 3.40**	46.0 ± 0.82*	54.5 ± 4.03*	46.5 ± 1.26*
Control	55.0 ± 3.00	38.5 ± 2.50	44.5 ± 4.37	59.5 ± 4.57
ADNPZnO	66.5 ± 2.06*	60.5 ± 4.71*	64.0 ± 3.91*	61.0 ± 6.13
Control	97.6 ± 1.18	91.5 ± 1.09	104.0 ± 1.40	112.3 ± 1.08
ADNPAg	100.0 ± 1.65	99.9 ± 1.18***	99.1 ± 1.18**	110.1 ± 1.17
Fungus	97.6 ± 1.12	90.4 ± 1.20	97.3 ± 1.31	95.9 ± 1.15
ADNPAg + Fungus	90.3 ± 1.11	95.6 ± 1.19**	103.6 ± 1.09**	111.1 ± 1.22***

*, **, *** - differences are significant compared with the control at a confidence level, respectively 0.95, 0.99, 0.999

Table 2 Sprout length of parent wheat varieties and reciprocal hybrids when treating the seeds with nanoparticles aqueous dispersions and fungus, mm

Variant	Accent	Select	Accent-x-Select	Select-x-Accent
Control	81.1 ± 1.27	75.6 ± 1.30	84.4 ± 1.20	84.8 ± 1.08
ADNPAg	81.7 ± 1.06	74.4 ± 1.09	89.0 ± 1.42**	84.0 ± 1.02
ADNPCu	79.6 ± 1.24	82.7 ± 1.11***	85.3 ± 1.45	86.7 ± 1.21
Fungus	76.8 ± 1.28	76.8 ± 1.47	84.2 ± 1.11	84.5 ± 1.25
ADNPAg + Fungus	78.7 ± 1.12	74.2 ± 1.18	80.8 ± 1.10	85.3 ± 1.21
ADNPCu + Fungus	74.2 ± 1.15	74.3 ± 1.19	82.7 ± 1.22	81.6 ± 1.32

*, **, *** - differences are significant compared with the control at a confidence level, respectively 0.95, 0.99, 0.999

Table 3 Seed germination energy of parental forms and reciprocal winter wheat hybrids treated with nanoparticles aqueous dispersions subjected to subsequent action of a temperature of +4 °C, in %

Nanoparticle aqueous dispersions	Basarabianka	M/M3	Basarabianka-x-M/M3	M/M3-x-Basarabianka
Control	48.7 ± 2.40	33.3 ± 3.71	42.7 ± 1.20	34.7 ± 2.68
ADNPBi	54.7 ± 1.76	48.0 ± 1.00**	56.7 ± 4.09*	58.0 ± 4.58**
ADNPCu	50.0 ± 2.31	44.7 ± 3.28*	54.7 ± 3.96**	46.7 ± 0.83**
ADNPZnO	54.0 ± 1.52	47.3 ± 3.17*	56.7 ± 2.33***	41.3 ± 1.77
AD∑NP	61.0 ± 3.27*	54.0 ± 2.54***	48.7 ± 1.67*	46.7 ± 3.84*

*, **, *** - differences are significant compared with the control at a confidence level, respectively 0.95, 0.99, 0.999

Table 4 Length of coleoptiles seedlings of parental forms and reciprocal hybrids of winter wheat, after seeds treating with water dispersions of nanoparticles and subjected to subsequent temperature of + 4 °C, mm

Nanoparticle aqueous dispersions	Basarabianka	M/M3	Basarabianka-x-M/M3	M/M3-x-Басарабянка
Control	23.6 ± 0.88	19.7 ± 0.91	19.7 ± 0.94	20.0 ± 0.98
ADNPBi	30.6 ± 0.96***	29.7 ± 0.91***	25.7 ± 1.48*	23.9 ± 1.07*
ADNPCu	23.4 ± 0.87	21.7 ± 1.02	29.5 ± 1.12***	26.7 ± 0.81***
ADNPZnO	25.6 ± 0.87	20.4 ± 0.80	27.9 ± 0.92***	29.2 ± 0.88***
AD∑NP	25.9 ± 1.00	23.1 ± 1.07*	21.8 ± 0.64	26.3 ± 0.91***

*, **, *** - differences are significant compared with the control at a confidence level, respectively 0.95, 0.99, 0.999

Table 5 Plant mass factors of winter triticale (variety *Ingen-93*) grown in field conditions after pre-sowing seed treatment with nanoparticle aqueous dispersions of Ag

Variant	Number of shoots (%)	Length of the main stem (cm)	Number of stems (pcs.)	Weight of 1000 grain (g)	Yield from a plot (g)
Control	65.9 ± 1.2	95.6 ± 1.03	2.05 ± 0.21	51.5	1551 ± 40
Test	75.4 ± 2.0**	96.3 ± 1.23	2.21 ± 0.20	50.5	2426 ± 45**

*, **, *** - differences are significant compared with the control at a confidence level, respectively 0.95, 0.99, 0.999

reciprocal hybrids to low positive temperature, causing more active germination compared with the control. The predominant influence on one of the parental forms of the hybrid was not found.

For the same objects, the following results were obtained by the *coleoptiles length* parameter (Table 4): (1) aqueous dispersions of NPBi caused significant stimulation in all genotypes (up to 50% in *M/M3*), which generally coincides with the data on the *seed germination energy* parameter, (2) with the hybrids it was observed a stimulation in all variants of AD of NP (with a maximum for NPCu (by 50%) and NPZnO (by 46%). Here, aqueous dispersions of all types of nanoparticles cause a more pronounced increase in the thermo-resistance level in hybrids.

3.4 Effect of Pre-sowing Treatment of Triticale Seeds with Water Dispersion of Silver Nanoparticles on the Productivity of Plants in Field Conditions

A small-plot experiment was carried out (accounting plot being of 4 m^2) in three replications, on the IGPPP field site in order to check the effectiveness of pre-sowing seed treatment (using winter triticale *Ingen93* variety as an example) with water dispersion of silver nanoparticles at a concentration of 10^{-7} mol/l. The same concentration turned out to be simulative in laboratory conditions. The increase in plant productivity (yield from a plot) by 56% (Table 5) was revealed. This growth was caused, in our opinion, by the increase of the following plant mass factors: (1) number of seedlings, (2) length of the main stem and (3) number of productive stems of the plant grown from a single grain.

4 Conclusions

1. For the first time in the crop technology, the effect of aqueous dispersions of nanoparticles was investigated on seeds of parental forms and their reciprocal hybrids, making it possible to effectively use this factor in plant breeding and genetic practice. The received data can be used to select optimal pairs for crossing and obtain new promising hybrids.

2. The research has revealed changes of *seed germination* parameters in winter triticale seeds (*Ingen 93* variety). Aqueous dispersions of silver and copper nanoparticles produce simulative and antifungal effects increasing seed resistance to the pathogenic fungus. The antifungal action of the nanofactor is more pronounced than of the potassium permanganate solution.
3. Simulative and antifungal action on seeds of aqueous dispersions of silver, copper, bismuth and zinc oxide nanoparticles was detected by the parameters: *seed germination energy* and *sprout length.* The study was carried out on winter wheat (parent varieties *Odessa267* and *Nikonia, Accent* and *Select* and their reciprocal hybrids). The effect depends on the genotype of the seed. The maternal effect is revealed—the predominant influence of the maternal form on physiological parameters of the hybrid.
4. After treating the seeds with water dispersions of nanoparticles of bismuth, copper, zinc oxide and a mixture of these nanoparticles, increase of seeds thermal stability is revealed. Winter wheat seeds (parent varieties *Basarabianka* and *M/M3* and their reciprocal hybrids) showed an increase in *seed germination energy* and length of coleoptiles, being exposed to low temperature (+4 °C).
5. In a small-plot experiment in field conditions, an increase by 56% in the productivity of triticale plants (variety *Ingen93*) was obtained as a result of pre-sowing treatment of seeds with water dispersions of silver nanoparticles.

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

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