Influence of pre-sowing priming on the parameters of *Pisum sativum* seedlings

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Abstract. One of the important methods of increasing the yield of cultivated plants is to reduce the yield of crops is pre-sowing treatment of seeds with modern preparations containing various elements and compounds in the nanoform. The aim of the work is to study the influence of pre-sowing treatment of pea seeds by suspension of iron and silicon oxide nanoparticles on germination and morphometric parameters of seedlings. As a result of the experiments with different compositions of seed treatment, it was observed that in the variants of the experiment using a mix of NPsSiO₂ and Fe₃O₄, as well as a variant of Fe₃O₄ in a concentration of 10⁻³ mg/l, the germination of beans reached maximum values. In the course of the experiment in conditions of the laboratory experiment studied the effect of processing on pigments. The restructuring of the pigment apparatus was largely associated with an increase in total chlorophyll and carotenoids against the background of the use of a whole range of solutions for treatment with the maximum effect of increasing their concentration when using Fe₃O₄ (10⁻³ mg/l), SiO₂ (10⁻³ mg/l) and SiO₂ (10⁻⁴ mg/l) by the 35th day of the experiment in the green mass of plants.

1. Introduction

Food production for the world’s growing population necessitates a review of the crop production system. The development and implementation of a seed pre-treatment system ensures high productivity, seed quality and stress resistance [5]. Over the past decade, nanomaterials, or nanoparticles (NPs), which have a high surface area, sorption capacity, and projected ion release kinetics to target sites, have been a common intelligent delivery system in Russia and abroad [12]. With the aim of increasing the efficiency and sustainability of traditional methods of agriculture a number of patents have been registered for products containing low-frequency nanopesticides, nanofillers and nanosensors [5].

The use of metallic NPs in agriculture for the pre-treatment of seeds improves the quality of the sowing material, and increases the resistance of plants to pathogens and the overall performance of crops.
due to the activation of metabolic processes [6, 7]. Nanopreparations have special advantages over traditional means of seed priming: they are not destroyed by heat and light, provide complete moisture to the surface of the plant, can be completely absorbed by plants and remain on the surface during irrigation [6].

In the context of the above, the purpose of our research was to study the effect of colloidal solutions of nanoparticles of nutrients on the germination rate and pigment apparatus of Pisum sativum seedlings.

2. Materials and methods

The SiO$_2$ NPs with a size of 30.7±0.3 nm and a ζ-potential of 27±0.12 mV were acquired from the company "Plasmotherm" (Moscow, Russia, http://plasmotherm.ru). Nanoparticles of Fe$_3$O$_4$ (80–100 nm, z-potential of 20 ± 0.14 mV) were acquired from the company "Advanced Powder Technologies" (Tomsk, Russia, www.nanosized-powders.com).

For the preparation of NP solutions, exact amounts of the preparations were placed in glass flasks with distilled tap water and intensively dispersed by ultrasound at a frequency of 35 kHz for 30 minutes. Fold-dilutions were prepared to reduce the amount of nanomaterials. For seed treatment, the following concentrations were used: for Fe$_3$O$_4$ 10$^{-2}$, 10$^{-3}$ and 10$^{-4}$ mg/l; for SiO$_2$ 10$^{-2}$, 10$^{-3}$ and 10$^{-4}$ mg/l; 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) at a 1:1 ratio. The control version of the experiment included the treatment of seeds in deionized water.

To assess the efficiency of using nanoforms of iron and silicon, a traditional product for pre-sowing seed treatment was used (MivalAgro, acquired from AgroSil company (http://agrosil.ru)). The pea variety "Flagship 12" was used as a test object. Seeds were provided by the FSSI "Samara RIoA" (Russia, http://samniish.ru), complied with all the requirements of the guidelines "The Order of biological assessment of effects of nanomaterials on plants using morphological characters" and were 1st class quality and not treated with disinfectant.

The experiment began with the pre-treatment of seeds of P. sativum in solutions of various agents (NPs SiO$_2$, Fe$_3$O$_4$, MivalAgro) with subsequent drying in air.

The next stage of the experiment involved the germination of plants in opaque plastic containers with soil in a climatic chamber. The containers measured 15 cm long, 12 cm wide and 10 cm high, and were filled with 2 kg of soil.

The soil mass was collected from the 0–20 cm layer, dried, and the inclusions were selected and thoroughly mixed. Each container was sown with 20 pea seeds with germination in a climatic chamber (Pol-eko-1200 KK TOP+; Pol-EKO-Aparatura, Poland) at a relative humidity of 30%, air temperature of 25±2°C, and substrate temperature of 23±2°C. In total, the experiment involved 30 containers. Plant samples were collected and analysed 15, 25 and 35 days after emergence.

There was a fixed germination percentage. The root and shoot lengths of seedlings were recorded using the standard centimetre scale. Measurements were carried out on roots after washing off the soil and drying on filter paper. The tolerance index (TI) of the plants was calculated [16].

The content of photosynthetic pigments (chlorophyll a and b, carotenoids) was determined using the standard method with modifications Smashevsky, 2011 [13].

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

3. Results and discussions

The experiment examining the germination of plants in soil under climatic chamber conditions showed that the maximum germination relative to the control sample was achieved in seeds treated with Fe$_3$O$_4$
NPs at $10^{-3}$ mg/l and Fe$_3$O$_4$+SiO$_2$ at $10^{-3}$ mg/l and $10^{-4}$mg/l (fig. 1). Note that the lowest level of germination was observed in seeds treated with MivalAgro (total ~78% relative to control).

The stimulating effect of the NPs on *P. sativum* was most clearly manifested by the increased growth rate after germination. Thus, the studied compounds had a specific effect on the length of the seedlings, which significantly exceeded the control in the SiO$_2$ treatment at $10^{-3}$ and $10^{-4}$ mg/l at all stages of the study (from 15 to 35 days), and after 25 days in the 10-3 mg/l Fe$_3$O$_4$ and Fe$_3$O$_4$+SiO$_2$ treatment.

MivalAgro significantly stimulated root growth only after 15 days of the experiment, and this stimulation was gradually offset in subsequent periods of growth. At the same time, it had no significant effect on the length of the above-ground organs of peas. Similar results were obtained regarding the length of pea roots, with maximum development in treatments with SiO$_2$ solutions at different concentrations, $10^{-3}$ mg/l Fe$_3$O$_4$ and Fe$_3$O$_4$+SiO$_2$.

The beneficial effects of SiO$_2$ reported by Tahir and his colleagues (2010) were associated with its hydrophilicity Romero-Aranda et al. (2006), and in experiments with wheat led to a significant increase in biomass and yield [10, 14].

Analysis of the photosynthetic apparatus showed a pronounced effect of all the studied concentrations of NPs on the amount of photosynthetic pigments – chlorophylls and carotenoids – after 35 days of germination in vivo, when a sharp increase in the indicator (36–48%) was recorded in the

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**Figure 1.** *P. sativum* germination. Data points with some / no symbols (*) representative no statistical significance at p≤0.05.
leaves of experimental plants (fig. 2). Germination of seedlings within 15 and 25 days did not cause a significant deviation in the content of total chlorophyll and carotenoids from the control values.

Figure 2. Influence of various compounds on the content of photosynthetic pigments in *P. sativum* leaves: 1-control, 2–Fe$_3$O$_4$+SiO$_2$; 3 - Fe$_3$O$_4$ 10$^{-3}$ mg/l; 4 - SiO$_2$ 10$^{-3}$ mg/l; 5 - SiO$_2$ 10$^{-4}$ mg/l; 6 - MivalAgro. Bars are mean ± SEM (standard error of the mean). Data points with some/no symbols (*) represent no statistical significance at p ≤ 0.05.

For individual pigments, it should be noted that the level of chlorophyll a sharply increased when exposed to SiO$_2$ at 10$^{-3}$ and 10$^{-4}$ mg/l and to MivalAgro, which may indicate the activation of the reaction centres and complexes of photosystem I and II. Similar effects of NPs on chlorophyll content were observed by Ghafaryan et al., who examined the impact of low concentrations of iron oxide on soybean (2013) [1]. On the contrary, after treatment of plants with a mixture of Fe$_3$O$_4$ and SiO$_2$, an increase in
the pool of chlorophyll $b$ (by 33.3% relative to the control) was observed (Figure 9). The increase in chlorophyll may not be caused by the effect of the nanometals, but by the iron ions $\text{Fe}^{2+}$, $\text{Fe(OH)}_2$ or $\text{Fe(OH)}_3$[1]. This would be consistent with the results of Ursache-Oprisan et al. (2011), who demonstrated the absence of toxic properties in nano-particles of $\text{Fe}_3\text{O}_4$[3, 15]. Consistent with Racuciu and Creanga (2007), we showed the ability of low concentrations of $\text{Fe}_3\text{O}_4$ NPs to increase the level of chlorophyll $a$ [8]. In addition, we have previously found no stimulating effects of Fe or $\text{Fe}_3\text{O}_4$ on photosynthetic pigments compared to $\text{FeSO}_4$ [4].

Other studies have shown a slight decrease in chlorophyll $a$, $b$ and carotenoids in wheat seedlings treated with $\text{SiO}_2$ nanoparticles [2, 9, 11].

A parallel analysis of the carotenoid content in pea leaves showed that this group of photosynthetic pigments was sensitive to NPs 35 days after treatment. The level of this pigment increased by more than 60%, which indicates an increase in oxidative processes or a primary nonspecific reaction of the plants to stress. A particularly sharp increase was observed after treatment of plants with the $\text{Fe}_3\text{O}_4+\text{SiO}_2$ mix: by 70% compared to control pea samples ($p>0.05$). In general, a deviation in the carotenoid level from the norm (0.1–0.5 mg/g wet weight) can be considered as an adaptive mechanism that provides effective energy absorption and prevents damage to the photosynthetic apparatus.

4. Conclusion
The results obtained during the experiment revealed the stimulating effect of the compositions based on iron oxide and silicon nanoparticles on the sowing of pea beans in the conditions of laboratory experience. So, when using the suspension mix NPs $\text{SiO}_2$ and $\text{Fe}_3\text{O}_4$, as well as a variant of $\text{Fe}_3\text{O}_4$ in a concentration of 10-3 mg/l for pre-sowing treatment, the germination of peas reached maximum values. A change in the pigment apparatus was largely due to an increase in chlorophyll $a+b$ and carotenoids against the background of the use of a whole series of solutions for treatment with the maximum effect of increasing their concentration when using $\text{Fe}_3\text{O}_4$ (10-3 mg/l), $\text{SiO}_2$ (10-3 mg/l) and $\text{SiO}_2$ (10-4 mg/l) by the 35th day of the experiment in the green mass of plants. Thus, the use of compositions for pre-sowing treatment of seeds created on the basis of iron and silicon nanoparticles is a promising direction of development of modern agriculture. And the effects of nanoscale materials should be studied in more detail in the future.

Acknowledgments
The studies were carried out in accordance with the research plan for 2019–2020 of the Federal Research Center for Biological Systems and Agrotechnology’s of the Russian Academy of Sciences (#0761-2019-0004).

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