Influence of iron nanoparticles (Fe_3O_4 and Fe_2O_3) on the growth, photosynthesis and antioxidant balance of wheat plants (*Triticum aestivum*)

Vladimir Kreslavski*, *Anatoly* Ivanov, *Alexander* Shmarev, *Alexandra* Khudyakova, and *Anatoly* Kosobryukhov

Institute of Basic Biological Problems of the Russian Academy of Sciences - a separate subdivision of the FRC PSCBI RAS, Pushchino, Russia

Abstract. More and more attention is paid to the development of technologies using iron nanoparticles in agriculture. In this regard, the effect of treatment of wheat seeds with various concentrations of iron nanoparticles Fe_3O_4 and Fe_2O_3 on the accumulation of biomass, the rate of photosynthesis and respiration, as well as on photochemical activity and antioxidant balance was studied. The seeds were treated for 3 h, germinated for 2 days in Petri dishes, transplanted into sand and grown under light for 18 days without mineral nutrition until the third leaf appeared. At a Fe_3O_4 concentration of 200 mg L^{-1} a significant increase in the dry biomass of the second leaf by 45% and the rate of photosynthesis by 16% was observed. At a concentration of nanoparticles in the form of Fe_2O_3 of 200 and 500 mg L^{-1}, an increase in the rate of photosynthesis in the second leaf was also observed, but not in the biomass of the leaves. The activity of photosystem 2, estimated from the F_v/F_m value, also increased in experiments with nanoiron. However, the activity of antioxidant enzymes, guaiacol-dependent peroxidase and superoxide dismutase, decreased. It is assumed that the acceleration of growth at an early stage of wheat development is associated with an increase in photosynthetic processes.

1 Introduction

In the last decade, nanoparticles (NPs), that is, particles less than 100 nm in size, have been widely used in various areas of the national economy, including medicine, for water purification and in the food industry. However, in agriculture, the use of nanoparticles has not been studied enough so far. Recently, more and more attention has been paid to the study of the ways of action of NPs of various metals, in particular, in the form of iron oxide in order to increase the productivity of plants, as well as reduce the environmental risk of using fertilizers [1–3]. The effect of nanomaterials largely depends on the method of their introduction. Thus, [4] showed that plant growth depends on the method of using nanomaterials. Foliar application of nano-Fe_3O_4 (leaf spraying) significantly improved the

* Corresponding author: vkreslav@rambler.ru

growth and a number of other physiological parameters of *Ocimum basilicum* plants compared to soil application. The use of Fe_3O_4 nanoparticles significantly increased the growth and yield of pea plants by increasing the leaf area. In work [5], an increase in dry biomass, protein content and the number of pods was shown in comparison with the control.

Iron (Fe) is an essential trace element for plants and plays a key role in the regulation of many cellular processes, including chlorophyll biosynthesis, photosynthesis and mitochondrial respiration, as well as redox balance [6]. However, the effect of iron in nanoform on plants is still insufficiently studied. Studies have shown different results on the effect of iron oxide NPs on plants [7–10].

It is assumed that the use of iron NPs increases productivity by enhancing various physiological processes, such as seed germination, photosynthetic activity, plant growth, synthesis of various metabolites, including proteins and nitrogen-containing compounds [2, 11]. It is also assumed that iron NPs can enhance growth by regulating the content of phytohormones, changing the activity of antioxidant enzymes and enzymes of the Calvin cycle [2, 12]. However, at high concentrations, iron NPs can exhibit toxicity [3].

In the presented work, the effect of iron nanoparticles Fe_3O_4 and Fe_2O_3 on growth parameters, rates of photosynthesis and respiration, primary processes of photosynthesis and changes in the activity of antioxidant enzymes in 20-d-old wheat plants under conditions of mineral nutrition deficiency was studied.

2 Materials and methods

2.1 Seeds treatment and plant growth

Wheat seedlings (*Triticum aestivum* L.) were used as the object of the study. The seeds were soaked for 2 h in an aqueous suspension of iron NPs obtained as a result of ultrasonic treatment of Fe_3O_4 (Advanced Powder Technology, Tomsk, Russia, 99%) or Fe_2O_3 (99%, China) for 20 min at 22 kHz using an UZG13 ultrasonic generator (0.1/22 (Russia). The size of the used Fe_3O_4 nanoparticles was 95 ± 10 nm, and the size of Fe_2O_3 nanoparticles – 80 ± 10 nm (China). After treatment, the seeds were germinated in Petri dishes on filter paper. Two days later, germinated seeds of the same size were transferred into vessels (13x13x8 cm), filled with well-washed river sand. Plants were grown without adding nutrients in the soil at a 12 h photoperiod, at a temperature of 24/20 °C (day/night), a relative air humidity of 70–80%, and a light intensity of 300 ± 20 μmol (photons) m^{-2} s^{-1}. During cultivation, the plants were watered with distilled water 3 times a week. 20-day-old plants were used in the experiment. The growth parameters of plants were determined for the 1st and 2nd leaves of seedlings. Plants without nanoiron treatment were used as control.

Fresh and dry leaf weights were determined using an analytical balance (Scout Pro SPU123, Ohaus Corporation, USA). The relative water content (RWC) in the leaves was calculated using the formula: RWC = 100×(FW - DW)/DW, where FW is the wet weight, DW is the dry weight.

2.2 CO_2 gas exchange and photochemical activity

The photosynthesis rate (P_n) was determined using a portable infrared gas analyzer LCPro + (ADC BioScientific Ltd., United Kingdom) in a leaf chamber with an area of 6.25 cm^2 and a saturating light intensity of 1000 μmol (photons) m^{-2} s^{-1}. The rate of dark respiration (R) was determined after turning off the light.

The parameters of photochemical activity were determined using a fluorometer by a JIP test based on the setup described in [13]. Chlorophyll a fluorescence induction curves (OJIP) were recorded using the data of the JIP test for photosystem 2 (PSII) under blue light illumination with an intensity of 6000 μmol (photons) m^{-2} s^{-1}. Fluorescence parameters (F_V/F_m, Y(II), NPQ, Y(NO), and Y(NPQ) were calculated according to [14, 15]. F_V/F_m is the maximum quantum yield of PSII, where F_V is the variable fluorescence equal to the difference between F_m and F_0, F_0 is the minimum fluorescence amplitude (F), and F_m is the maximum fluorescence amplitude. Y(II) is the effective quantum yield of PSII fluorescence was calculated using the formula: $Y(II) = (F'_m - F_t)/F'_m$. NPQ – non-photochemical quenching of fluorescence was calculated as $NPQ = (F_m - F'_m)/F'_m$. Y(NO) – quantum yield of non-photochemical quenching of fluorescence unregulated by light was calculated according to $Y(NO) = 1/(NPQ + 1 + qL \cdot (F_m/F_0 - 1))$. Y(NPQ) – the quantum yield of controlled light-induced non-photochemical fluorescence quenching was calculated as Y(NPQ) = 1-Y(II) -Y(NO).

2.3 The evaluation of activity of antioxidant system

The content of malonic dialdehyde (MDA) was determined according to [16]. The concentration of MDA was measured at 532 and 600 nm using a spectrophotometer (Genesis 10UV, ThermoSpectronic, USA). For comparison, a mixture without plant extract was used. The MDA content was calculated using an extinction coefficient of 155 μmol^{-1} cm^{-1} and is expressed as μmol MDA g^{-1} FM. SOD activity was determined by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium at 560 nm according to [17] with minor modifications. The activity of guaiacol-dependent peroxidase (POD) was determined according to [18]. The estimation of the specific activity of the enzymes was carried out on the basis of the wet weight of the plant material.

2.4 Statistics

Statistical data processing was carried out using the Statistics 10 software (StatSoft Inc., USA). The significance of differences between the mean values of the measured parameters was assessed using the Student's test. Calculations were performed at a given significance level $p \leq 0.05$. The table shows the arithmetic mean values with standard errors.

3. Results and discussion

3.1. Growth parameters

Wheat plants were grown on a sandy substrate without feeding in order to exclude the influence of other nutrients on the growth and other parameters of the seedlings. In experiments with Fe_2O_3, the effect of iron NPs was studied in the concentration range up to 500 mg L^{-1}. The pre-selected concentration of Fe_3O_4 (in the range of 0–200 mg L^{-1}), which gave a noticeable increase in growth and photosynthesis, was 200 mg L^{-1} (Table 1).

Table 1. Physiological parameters of the 2nd leaf of 20-day-old wheat seedlings grown without nutrients (Control, -Fe_3O_4) and from seeds treated with NPs (+Fe_3O_4 200 mg L^{-1}). R is the respiration rate. FW – wet weight, DW – dry weight. Mean values from 5 biological replicates ± SD are shown. * – the values are significantly higher than the control ($p < 0.05$).

Parameters/Variant	-Fe_3O_4	+Fe_3O_4
FW, мг	43.0 ± 3.7	39.9 ± 5.8
DW, мг	8.4 ± 0.7	12.2 ± 1.6*
FW/DW	5.1 ± 0.5	3.3 ± 0.4*
Total leaf DW, mg	20.3 ±1.7	23.9 ±3.4
P_n, μM CO_2 m^{-2} s^{-1}	7.6 ± 0.3	8.8 ± 0.2*
R, μM CO_2 m^{-2} s^{-1}	3.8 ± 0.1	4.0 ± 0.2
SOD, rel. units g^{-1} FW	10.2 ± 0.2	5.1 ± 0.9*
POD, rel. units g^{-1} FW	2.25 ± 0.30	1.38 ± 0.23*
MDA, μM g^{-1} FW	4.4 ± 0.3	4.1 ± 0.2

On the 20th day from the beginning of seed germination, the plants formed the third leaf. The measurement of growth parameters was carried out for the 1st and 2nd leaves. Other physiological and biochemical parameters were determined only for the 2nd developed leaf (see Table 1 and Fig. 1).

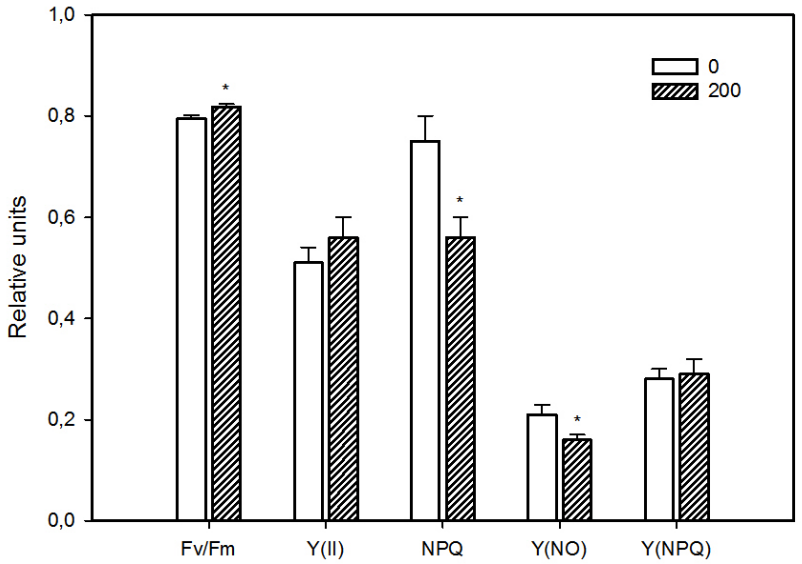

Fig. 1. Fluorescent parameters of the 2nd leaf of 20-day-old wheat seedlings grown without nutrients (0 – without Fe_3O_4) and from seeds treated with NPs (Fe_3O_4 200 mg L^{-1}). Mean values from 5 biological replicates ± SD are shown. * – the values are significantly higher than the control ($p < 0.05$).

Treatment of wheat seeds with Fe_3O_4 nanoparticles improved the growth parameters of plant leaves, which primarily affected an increase in the accumulation of dry biomass (DW) of the 2nd leaf by 45% compared to untreated control plants. DW of the 1st leaf in the experiment with NPs was differed little from the control (weight 9.8 and 9.1 mg L^{-1}, respectively). At the same time, the total dry weight of all leaves was changed little, although a tendency to an increase was observed (20.3 mg in the control and 23.9 mg at 200 mg L^{-1}). There were no significant differences between the variants in terms of the accumulation of wet weight (FW). At the same time, the ratio of wet weight to dry weight

was higher in the control (Table 1). In variants with 8 and 40 mg L^{-1} nanoparticles, no significant differences in the dry weight of the second leaf were found (data not shown). Also, no differences in growth rates were found between plants grown from treated and untreated seeds using a different form of Fe_2O_3 iron NPs (Fig. 2).

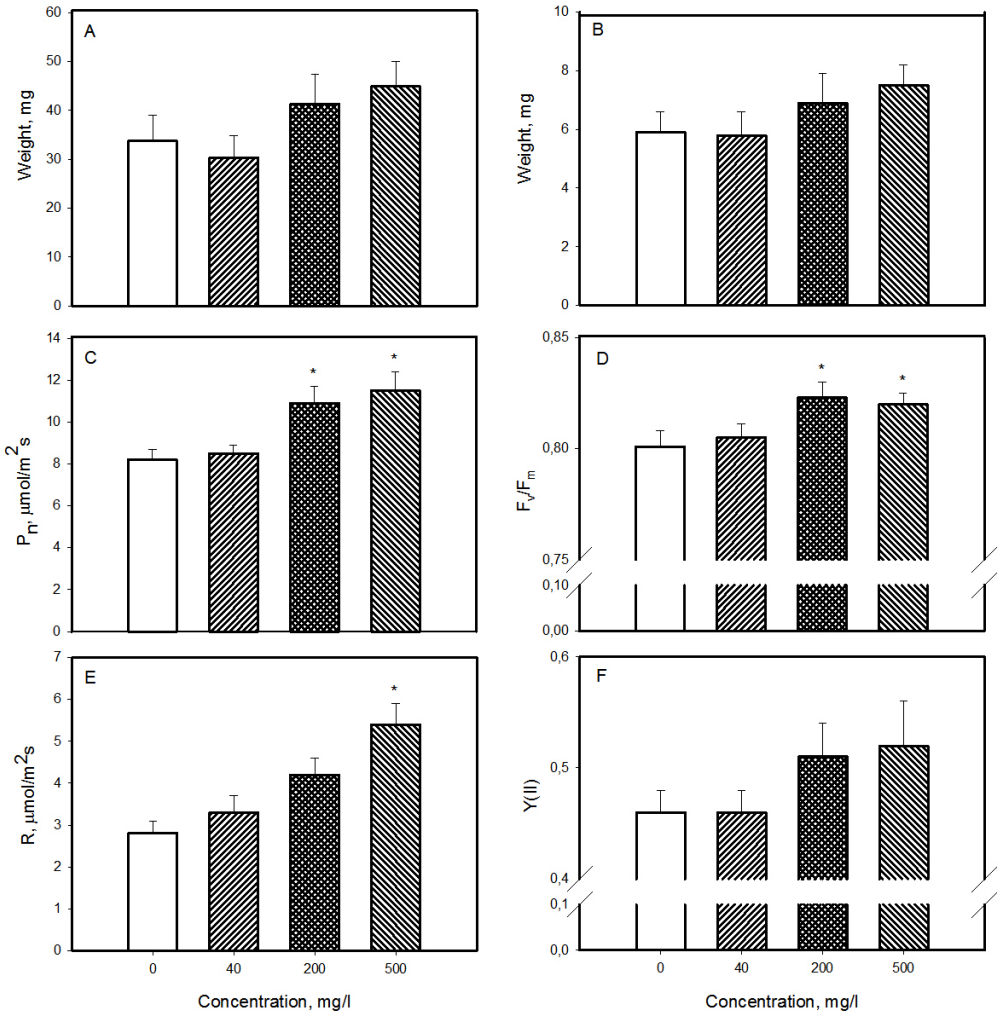

Fig. 2. Physiological parameters of the second leaf of 20-day-old wheat seedlings grown without nutrients (0, without Fe_2O_3) and from seeds treated with iron NPs (+Fe_2O_3 40, 200, 500 mg L^{-1}) A – average fresh weight of the leaf, B – average dry weight of the leaf. C is the rate of photosynthesis. D – maximal quantum yield of PSII (F_v/F_m). E - respiration rate (R). F – Y(II), the effective quantum yield of PSII. Average values from 3 biological replicates ± SD are shown. * – the values are reliably higher than the control ($p < 0.05$).

3.2. Photosynthesis and photochemical activity

Plants grown from seeds treated with Fe_3O_4 showed a 16% increase in photosynthesis (P_n), assessed by the rate of CO_2 fixation (Table 1). At the same time, the rate of dark respiration (R) remained approximately the same in both variants of the experiment. A change in some fluorescent parameters of the leaves of plants grown from seeds treated with Fe_3O_4 in

comparison with the control was also revealed (Fig. 1). Thus, the values of regulated non-photochemical quenching (NPQ) and the quantum yield of non-regulated non-photochemical quenching of fluorescence (Y(NO)) in leaves treated with Fe_3O_4 decreased by 25 and 24%, respectively, as compared to untreated plants. On the other hand, the values of the maximum quantum yield F_v/F_m were higher than in the control. However, in both cases, the value of quantum yields (about 0.80) characterizes the plants as being within the physiological norm. The data obtained are consistent with the fact that under iron deficiency, the efficiency of the functioning of photosystem 2 is reduced [18]. The value of the parameter Y (NPQ), which reflects the efficiency of controlled dissipation of absorbed energy into heat, practically coincided in both variants of the experiment.

The treatment of seeds with Fe_2O_3 (200 and 500 mg L^{-1}) also led to an increase in the rate of photosynthesis of the second leaf as compared to the control (Fig. 2). Moreover, the maximum quantum yield in the control was also lower than in experiments with 200 and 500 mg L^{-1} of Fe_2O_3 NPs. The study of the effect of treatment with iron NPs on the pro/antioxidant balance in the 2nd leaf using the example of Fe_3O_4 showed that in the leaves of treated plants, the activity of such enzymes as superoxide dismutase (SOD) and guaiacol-dependent peroxidase (POD) significantly decreases relative to the control, by 50 and 68%, respectively (Table 1). At the same time, no significant differences were found between the control and experiment with Fe_3O_4 in terms of the content of malondialdehyde.

The effect of NPs on plant growth and development largely depends on the method of application of NPs, their size, and plant species [4, 7]. We hypothesized that under unfavorable conditions, iron NPs could support plant growth and photosynthesis. In our case, long-term cultivation of plants in sand under conditions of a lack of nutrients led to a lower activity of photosystem 2 and a reduced rate of photosynthesis compared to untreated plants. The higher photochemical activity of photosystem 2, estimated as the F_v/F_m ratio, and the decreased activity of antioxidant enzymes, all are consistent with the decreased heat dissipation in plants grown from treated seeds (Figs. 1 and 2).

It was shown in [2] that the use of Fe_2O_3 nanoparticles led to an increase in root length, height and biomass of plants, as well as chlorophyll content in peanut plants. The authors suggested that the use of Fe_2O_3 promotes the growth of peanuts by regulating the content of phytohormones and the activity of antioxidant enzymes (SOD, POD, CAT). In our study, the activity of the antioxidant enzyme SOD in control without Fe_3O_4 was increased, which, along with a lower value of the F_v/F_m value and a higher level of heat dissipation may indicate a weaker oxidative stress in the untreated plants.

Iron NPs, regardless of their shape (Fe_2O_3 or Fe_3O_4) and approximately equal particle size, stimulate wheat photosynthesis. At the same time, in the case of Fe_2O_3, there is a tendency to an increase in the accumulation of wet and dry biomass of the 2nd leaf, while in the case of Fe_3O_4, the wet to dry weight ratio significantly decreased, that is, an increase in the accumulation of dry matter occurred. An increase in biomass during treatment with nanoiron can be explained by an increase in the rate of photosynthesis and, in part, respiration, as well as a change in the pro/antioxidant balance due to the entry of iron ions into plants.

4 Conclusions

The positive effect of iron oxide NPs on growth processes can be associated with a more efficient use of nutrients from seeds in treated plants, as well as with an increase in the rate of photosynthesis and respiration in leaves, possibly due to a change in the redox balance in the presence of a sufficient amount of iron ions in the leaves. It can be assumed that the treatment of seeds with iron oxide NPs will be effective for maintaining the productivity of cereals (wheat, etc.) under conditions of a deficit of mineral nutrients.

Acknowledgements

The reported study was funded by RFBR and NSFC, project number 21-54-53015.

References

1. M.J. Abhilash, Int. J. Pharma Bio Sci., **1,** 1 (2010)
2. M. Rui, C. Ma, Y. Hao, J. Guo, Y. Rui, X. Tang, Q. Zhao, X. Fan, Z. Zhang, T. Hou, S. Zhu, Front. Plant Sci., **7**, 815 (2016)
3. K. Kornarzyński, A. Sujak, G. Czernel, D. Wiącek, Sci. Rep., **10**, 8068 (2020)
4. S.A. Elfeky, M.A Mohammed., M.S. Khater, Y.A.H. Osman, E. Elsherbini, Med. Plants - Int. J. Phytomed. Relat. Ind., **463**, 2051 (2013)
5. A. Al-Sherbini, H.G. Abd El-Gawad, M.A. Kamal and S.A El-feky. American-Eurasian J. Agric. & Environ. Sci., **15(7)**, 1435 (2015)
6. S. Ghasemi, A.H. Khoshgoftarmanesh, M. Afyuni, H. Hadadzadeh, Sci. Hortic., **165**, 91 (2014)
7. H. Wang, X. Kou, Z. Pei, J.Q. Xiao, X. Shan, B. Xing, Nanotoxicology, **5**, 30 (2011)
8. N. Siddiqi, J.K. Harrison, A. Clegg, E.A. Teale, J. Young, J. Taylor, S.A. Simpkins, Cochrane Database Syst. Rev., **3(3)**, CD005563 (2016)
9. M.F. Iannone, M.D. Groppa, M.L. de Sousa, M.B. Fernández van Raap, M.P. Benavides, Environ. Exp. Bot., **131**, 77 (2016)
10. S. Huang, L. Wang, L. Liu, Y. Hou, L. Li, Agron. Sustain. Dev., **35**, 369 (2015)
11. T.L Winder., J.N. Nishio, Plant Physiol., **108**, 1487 (1995)
12. V.D. Kreslavski, A.V. Lankin, S.I. Allakhverdiev, V.Y. Luybimov, G.K. Vasilyeva, G.N. Semenova, F.-J. Schmitt, T. Friedrich, J. Plant Physiol. Biochem., **81**, 135 (2014)
13. V.N. Goltsev, H.M. Kalaji, M. Paunov, W. Bąba, T. Horaczek, J. Mojski, H. Kociel, S.I. Allakhverdiev, Russ. J. Plant Physiol., **63(6)**, 869 (2016)
14. H. Kalaji, A. Jajoo, A. Oukarroum, M. Brestič, M. Zivcak, I. Samborska, M. D. Cetner, Izabela Łukasik, V. Goltsev, R. Ladle, Acta Physiol. Plant., **38**, 102 (2016)
15. M. Uchiyama, M. Mihara, Anal. Biochem., **86**, 287 (1978)
16. S.A. Gupta, R.P. Webb, A.S. Holaday, R.D. Allen, Plant Physiol., **103**, 1067 (1993)
17. B. Chance, A.C. Maehly, Methods Enzymol., **2**, 764 (1955)
18. F. Morales, A. Abadía, J. Abadía, Aust. J. Plant Physiol., **25**, 403 (1998)