Advanced Studies in Biology, Vol. 15, 2023, no. 1, 47 – 54
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https://doi.org/10.12988/asb.2023.91621

Effect of Ferric Iron Oxide Nanoparticles on Ascorbate Peroxidase Activity in Durum and Bread Wheat Seedlings

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Abstract

Under stress conditions, the content of reactive oxygen species (ROS) in plant cells increases, therefore, the intensity of free-radical oxidative processes increases. In response to an increase in ROS, the components of the antioxidant system (AOS) of plant protection are activated. In this regard, the article considers the change in the activity of one of the high-molecular components of plant protection AOS - ascorbate peroxidase (APO) in two-week-old seedlings of durum and bread wheat varieties under the influence of ferric oxide nanoparticles (NPs). In the seedlings of the tested durum wheat varieties, NPs led either to a slight or sharp increase in the activity of the enzyme (Kyrmyzy bughda and Karagylchyg-2), respectively, or to a decrease (Yagut), or practically did not cause any changes (Karabagh) in the APO activity. In the case of bread wheat varieties, under the influence of NPs, an increase in the activity of the enzyme was observed in seedlings of varieties (Daghdash and Kobustan), while in seedlings of varieties (Sheki-1 and Mirbashir-128), a decrease in APO activity was observed. Thus, the obtained results can serve as the basis for the selection of wheat varieties in order to obtain varieties more resistant to abiotic stressors.

Keywords: antioxidants, ascorbate peroxidase, ferric oxide nanoparticles, durum and bread wheat varieties

Introduction

During their life, plants are constantly or periodically exposed to adverse environmental factors, which leads to increased generation of reactive oxygen

species (ROS), the level of which in plant cells is controlled by the antioxidant defense system (AOS) (Kreslavsky et al., 2012). AOS is a multicomponent multilevel self-regulating system, represented in plant cells by high molecular weight enzymes and a number of low molecular weight components. To ensure the most effective protection, all elements of the system are in constant interaction, and maintaining their balance is important for maintaining the viability of plants under stressful conditions (Kolupaev et al., 2011).

One of the plant cell antioxidant enzymes is ascorbate peroxidase (APO; EC 1.11.1.11), a heme-containing enzyme localized mainly in chloroplasts and having a high affinity for hydrogen peroxide, reducing it to water, using ascorbic acid as electron donors, thereby regulating the rate of oxidation of the latter in the cell (Pradedova et al., 2017).

Today, the study of the problem of plant resistance to adverse environmental factors is one of the central tasks of modern biology. On the other hand, with an increase in the rate of consumption of agricultural products and in order to ensure the food security of the population, agricultural production needs to constantly integrate the achievements of science into the agrotechnological process (Kutskir, 2014). In this regard, in recent years, interest has increased in studying the effect of various metal NPs entering the environment, both as a result of natural processes and as a result of the activity of the anthropogenic factor, on biological systems (Siddiqui et al., 2015).

Thus, environmental pollution with high concentrations of NPs of inorganic materials has a negative impact on the physiological and biochemical characteristics of the cells of living organisms. For example, one of the most frequently reported toxic effects of nanoparticles by researchers is the generation of ROS in cells, leading to oxidative stress (Anjum 2015). The high reactivity of ROS and free radicals leads to an acceleration of oxidation reactions that disintegrate the molecular basis of the cell, which, in turn, causes damage to cellular structures. In the case of using nanoparticles in low concentrations, on the contrary, a positive effect of their impact on biological objects is observed. Thus, the effects of NPs on living organisms depend on their concentrations (Chichiricco and Poma, 2015).

Data on changes in the state of the antioxidant system under the influence of various abiotic stress factors were obtained for a number of agricultural crops (Bhagat et al., 2015). In recent years, it has been established that oxidative stress can be caused by nanoparticles based on iron, copper and nickel, which are among the five most used by industrial enterprises worldwide (Buzea et al., 2007). It is assumed that the intensity of development of the biological effects of highly dispersed metals differs from the effects of their oxide forms, and largely depends on the presence of variable valence metals in the composition. The latter are able to release toxic ions from their colloidal matrix and stimulate the production of reactive oxygen species (Mirshra, 2014).

In general, numerous experimental and review articles have been devoted to studying the effect of nanometals on plant organisms (Sharma et al., 2012). However, to date, no studies have been conducted on the effect of various

concentrations of ferric NP oxides on the functioning of AOS components in wheat seedlings. Therefore, the aim of our work was to study the effect of ferric oxide NPs on the activity of ascorbate peroxidase in two-week-old seedlings of durum and soft wheat varieties to assess their tolerance to the action of nanoparticles.

Objects and Methods

The objects of the study were four varieties of durum (Triticum durum. Desf.) - (Karabagh, Yagut, Kyrmyzy bughda and Karagylchyg-2) and soft (Triticum aestivum L.) - (Kobustan, Daghdash, Mirbashir-128 and Sheki-1) wheat purchased from the Research Institute of Agriculture under the Ministry of Agriculture of Azerbaijan. Before sowing, all seeds were disinfected with 0.01% KMnO4 solution for 5 minutes and after washing the seeds three times with distilled water, they were germinated in pots with soil for 14 days, at 12-hour illumination, at a temperature of 24 ± 1°C and humidity 80 ± 5%, preventing the seedlings from drying out in the climatic chamber (Taisite GZX-300E, China). Plants were divided into three groups:

1. Control series (without tillage with iron NPs)
2. 1st series (soil treatment with Fe2O3 at a concentration of 50 mg per 1 kg)
3. 2nd series (soil treatment with Fe2O3 at a concentration of 100 mg per 1 kg)

Soil treatment with iron NPs 20 by 40 nm in size (Skyspring Nanomaterials Inc, USA) was carried out once, taking into account their maximum allowable concentration (MAC): the applied amount exceeded the MAC by 2–4 times, respectively. Each series included 30 seeds of the studied wheat varieties.

The activity of ascorbate peroxidase (APO, EC 1.11.1.11) was determined according to the method (Nakano, Asada,1981) with some modifications. The method is based on determining the rate of decomposition of hydrogen peroxide by ascorbate peroxidase of the test sample with the formation of water and dehydroascorbate. Optical density was recorded on a spectrophotometer (MRC, model UV-200-RS, Israel) at 265 nm. To do this, a sample of plant material (1 g) was homogenized in a chilled mortar with 10 ml of 0.06 M phosphate buffer, pH 7.6, with the addition of 0.3 g of polyvinylpyrrolidone. The ground mass was transferred into a 50 ml volumetric flask, made up to the mark with the same buffer, mixed well, and left for 15 min. The resulting homogenate was centrifuged at 8000g for 10 min at 4°C. The supernatant was used to determine the activity of the enzyme.

The reaction mixture was 50 µl of 0.1 mM EDTA (Biochemica), 50 µl of 0.05 mM ascorbic acid (Sigma-Ultra), 50 µl of 0.1 mM hydrogen peroxide, 2.25 ml of phosphate buffer and 300 µl of the plant extract obtained after homogenate centrifugation. Activity was expressed in nmol per gram wet weight per time unit [nmol g^{-1} min^{-1}]. The calculation of ascorbate peroxidase activity was carried out on the basis of the molar extinction coefficient (E=2.8 mM-1cm-1).

Statistical Analysis

The experiments were carried out in triplicate biological replication and each was reproduced independently 3 times. Statistical processing of the results was carried out using the licensed software package IBM SPSS Statistics. The assessment of the reliability of differences in arithmetic means was carried out on the basis of the Student's coefficient. Differences between groups were considered significant at a two-sided significance level $p \leq 0.05$.

Results and Discussion

According to the results obtained in the course of the studies, the activity of ascorbate peroxidase in control and experimental samples of the studied wheat varieties differed from each other.

Analysis of the data presented in table 1 showed that in two of the tested varieties of durum wheat, Kyrmyzy bugda and Karagylchyg-2, the activity of ascorbate peroxidase in the first and second series of samples increased by (17%; 30%) and (69%; 100%), respectively, compared with the control. In the seedlings of the Karabagh variety, NPs of ferric oxides had practically no effect on the activity of the enzyme, while in the seedlings of the Yagut variety they led to an increase in the activity of APO compared to the control in the first series of samples by 11%, in the second series, on the contrary, the activity of ascorbate peroxidase decreased by compared with control by 19%.

As for bread wheat varieties, the highest activity of the enzyme was observed in the treated seedlings of the Daghdash variety, the lowest in the control samples of the same variety (table 2). In samples of the Kobustan variety, seed treatment with ferric iron NPs, both in the first and second series, led to an increase in the activity of ascorbate peroxidase, which was compared with the control (15%, 37%), respectively. For seedlings of varieties Sheki-1 and Mirbashir-128, in the first series of samples, an increase in enzyme activity was observed, while in the second series of samples, under the action of NPs of ferric oxides, there was a decrease in ascorbate peroxidase activity compared to the control by 10% and 8%, respectively.

Thus, considering the data obtained, we find that less ROS are formed in the first series of samples, therefore, they have a lower intensity of free radical oxidative processes than the second series of samples, with the exception of three varieties Mirbashir-128, Sheki-1 and Yagut. The conducted studies demonstrate the positive or negative effect of ferric NPs oxides on the activity of ascorbate peroxidase in two-week-old seedlings of various durum and soft wheat varieties, which is of great importance for many branches of agriculture.

As is known, NPs are distinguished by unusual physicochemical properties and specific effects on living organisms (Yurin and Molchan, 2015). Recent studies on the use of nanotechnologies in the cultivation of agricultural crops indicate the active influence of NPs on the process of seed germination. The natural germination process takes a long time, but when seeds are treated with NPs, high

germination rates are achieved, making the use of nanotechnology a powerful method to increase seed germination (Ma et al., 2018).

There are data in the literature on the effect of iron NPs and its oxides on the physiological and biochemical processes occurring in plants (Ahmad et al., 2008). It has been shown that, under in vivo conditions, •OH is formed mainly as a result of the iron-catalyzed Haber-Weiss reaction, which is a combination of two elementary processes: the Fenton reaction and the reduction of ferric iron O^{2-}• (Halliwell and Gutteridge, 1986).

$H_2O_2 + Fe^{2+} \rightarrow$•OH + -OH + Fe^{3+}

$Fe^{3+} + O^{2}$•$^{-} \rightarrow Fe^{2+} + O^{2}$

Thus, the increase in the activity of ascorbate peroxidase in our experiments is evidence of the protective function of plants aimed at the reduction of hydroxyl radicals.

In the work of Sokolovskaya-Sergienko, a positive effect of microelement nanopreparations on the content of chlorophyll, the activity of antioxidant enzymes of chloroplasts, and wheat yields was revealed (Sokolovskaya-Sergienko, 2013).

According to some authors, low concentrations of silver NPs increased the energy of germination and the ability to germinate seeds, their growth and development, respiration intensity, and the activity of enzyme systems (Fedorenko et al., 2011). In the studies of Egorov and Shafronov, iron nanopowders increased the yield and quality of grain crops (Egorov et al., 2008).

Thus, the results of studies conducted with NPs are contradictory, and further research in this direction is appropriate. Apparently, the overwhelming effect of ferric iron NP oxides on the activity of ascorbate peroxidase in the second series of accessions of the Yagut, Mirbashir-128, and Sheki-1 varieties is associated with a high concentration of NPs. Differences in the levels of enzyme activity in the studied soft and durum wheat varieties may be associated with their different resistance to high concentrations of oxides of ferric iron NPs.

So, as a result of the experiments, it was revealed that the activity of ascorbate peroxidase in wheat seedlings under the influence of ferric oxide NPs depends on varietal characteristics. In this regard, the study of the mechanisms of the effect of metal oxide NPs on the rate of ascorbic acid oxidation in various wheat varieties deserves further work in this direction.

Conclusions

Based on the data obtained, we conclude that in the seedlings of the first series of tested durum and soft wheat varieties, ferric oxide NPs led to an increase in ascorbate peroxidase activity, while in the second series of seedlings of durum varieties, either a slight increase (Kyrmyzy bughda) or a decrease was observed (Yagut) activity of ascorbate peroxidase, almost no changes were observed in the seedlings of the Karabagh variety, while in the seedlings of the Karagylchyg-2 variety, the activity of the enzyme under the action of iron NPs was 2 times higher compared to the control. In the case of the second series of soft wheat varieties, a

sharp increase in the activity of ascorbate peroxidase was observed in the seedlings of the Daghdash variety, while in the seedlings of the Sheki-1 and Mirbashir-128 varieties, a decrease in the enzyme activity was observed under the action of ferric oxide NPs. In the seedlings of the Kobustan variety, an increase in APO activity was also observed.

Thus, the data obtained make it possible to distinguish the varieties Karagylchyg-2, Kyrmyzy bughda, Daghdash and Kobustan as resistant to the action of ferric oxide NPs, which is of great importance in breeding work to obtain resistant varieties.

Table 1. Ascorbate peroxidase activity in control and experimental samples of durum wheat (nmol g^{-1} min^{-1})

Varieties	Control series	Experimental series	
		I-series (Soil treatment with Fe_2O_3 at a concentration of 50 *mg/kg)*	II-series (Soil treatment with Fe_2O_3 at a concentration of 100 *mg/kg*
Kyrmyzy bughda	23,1710±2,41*	27,0643±2,27*	30,1567±2,59*
Karabagh	26,4827±1,02*	28,1761±1,34*	28,5313±1,53*
Yagut	48,4980±2,49*	53,6482±2,36*	39,1540±2,75*
Karakylchyk-2	34,7020±0,54*	58,5360±0,97*	69,3130±1,04*

*Differences between the control and experimental series are significant at $p \leq 0.05$ significance levels

Table 2. Ascorbate peroxidase activity in control and experimental samples of bread wheat (nmol g^{-1} min^{-1})

Varieties	Control series	Experimental series	
		I-series (Soil treatment with Fe_2O_3 at a concentration of 50 *mg/kg)*	II-series (Soil treatment with Fe_2O_3 at a concentration of 100 *mg/kg)*
Mirbashir-128	27,4900±2,93*	34,2097±2,54*	25,1800±1,59*
Kobustan	26,9040±2,16*	30,8574±1,86*	36,9153±1,62*
Daghdash	22,4867±0,80*	69,4367±1,23*	76,2353±1,47*
Sheki-1	26,0020±0,59*	28,4561±0,93*	23,3130±1,04*

*Differences between the control and experimental series are significant at $p \leq 0.05$ significance levels

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Received: January 21, 2023; Published: February 27, 2023