The activity of ascorbate peroxidase in seedlings of durum and soft wheat varieties under the influence of trivalent ferric oxides nanoparticles

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Abstract – As, it is known, the content of reactive oxygen species (ROS) in plant cells increases under stressful influences, therefore, the intensity of free radical oxidative processes also increases. In response to an increase of ROS, as a rule, activation of the components of the, antioxidant plant protection system (AOS) occurs. In this regard, the article considers the change in the activity of one of the high molecular weight components of the, antioxidant plant protection system – ascorbate peroxidase (APO), in two-week seedlings of durum and soft wheat varieties under the influence of trivalent ferric oxides nanoparticles (NP). In the course of studies found, that the activity of ascorbate peroxidase under the influence of trivalent ferric oxides NPs in wheat seedlings depends on the varietal characteristics. In seedlings of tested durum wheat varieties, ferric oxides NPs led either to an insignificant or a sharp increase in the activity of APO (Gyrmyzy bugda and Garagylchyg-2), respectively, or to a decrease in the enzyme activity (Yagut), or practically did not cause any changes (Garabag) in the activity of ascorbate peroxidase. In the case of soft wheat varieties, under the influence of NPs of ferric oxides, there was a sharp increase in the activity of APO (Dagdash), while in the seedlings of the varieties (Sheki-1 and Mirbashir-128), a decrease in the enzyme activity was observed. An increase in the activity of ascorbate peroxidase, was also observed, in the seedlings of the variety (Gobustan). Thus, the obtained results can serve as the basis for selection of wheat varieties, in order, to obtain varieties more resistant to abiotic stressors.

Keywords – antioxidants, ascorbate peroxidase, nanoparticles of ferric oxides, durum and soft wheat varieties

INTRODUCTION

During life, plants are constantly or periodically exposed to adverse environmental factors, which leads to an increase in the generation of reactive oxygen species (ROS), the level of which in plant cells is controlled by the antioxidant system (AOS) of protection [1, 2]. 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 must be in constant interaction, and maintaining their balance is important to preserve the viability of plants under stress conditions [3, 4].

One of the antioxidant enzymes of a plant cell is ascorbate peroxidase (APO; EC 1.11.1.11), a heme-containing enzyme localized mainly in chloroplasts, which has a high affinity to hydrogen peroxide, reducing it to water, using as donor of electrons ascorbic acid, thereby regulating the rate of oxidation of ascorbic acid in cells [5, 6]. Today, the study of the problem of plants' resistance to adverse environmental factors is one of the central problems of modern biology. On the other hand, with an increase in the rate of consumption of agricultural products, and to ensure food security of the population, agricultural production requires constant integration of science in the agro-technological process [7]. In this regard, in recent years, interest to the study of the influence of nanoparticles (NPs) of various metals entering the environment, both as a result of natural processes and as a result of the activity of an anthropogenic factor on biological systems, has increased [8, 9].

Thus, environmental pollution with high concentrations of NPs of inorganic materials with altered structural and physicochemical properties has a negative effect on the physiological and biochemical characteristics of living organisms' cells. For example, one of the most frequently reported toxic effects of nanoparticles by researchers is the generation of reactive oxygen species (ROS) in cells, leading to oxidative stress [10, 11]. The high reactivity of ROS and free radicals lead to an acceleration of oxidation reactions that disintegrate the molecular basis of cells, which, in turn, causes damage of cellular structures. In the case of using nanoparticles in low concentrations, on the contrary, there is a positive effect of their impact on biological objects. Thus, the effects of NPs on living organisms depend on the concentration and size of NPs [12]. Studying, the accumulated experimental material we come to the conclusion, that there are various theories of the effect of NPs on living systems, at the same time, the mechanisms of their biological activity have not been sufficiently studied, which requires their further more complete study [13].

The data on changes in antioxidant system, in response to various abiotic stress factors, obtained for a number of crops [14, 15]. In recent years, it has been, established, that oxidative stress can be caused by nanoparticles, based on iron, copper and nickel, which are the most used by industrial enterprises worldwide [16]. It is assumed, that the intensity of development of biological effects of highly dispersed metals differs from the effects of their oxide forms, and mainly depends on the presence in its composition of variable valence metals. The last are capable to release toxic ions from their colloidal matrix and stimulate the production of reactive oxygen species [17]. Nano-powders quite easily penetrate the cells of seeds prepared for sowing and actively influence the enzymatic system of physiological and biochemical reactions [18].

In general, numerous experimental and review articles are devoted to the study of the effect of NPs of metals on plant

organisms [19; 20]. However before, no studies, have been carried out, to study the effect of different concentrations of ferric oxides NPs on the functioning of AOS components in wheat seedlings.

Therefore, the aim of our work was to study the effect of ferric oxides NPs on the activity of ascorbate peroxidase in two-week seedlings of durum and soft wheat varieties to assess their tolerance to the influence of nanoparticles.

MATERIALS AND METHODS

The objects of the study were four varieties of durum (Triticum durum. Desf.) - (Garabag, Yagut, Gyrmyzy bugda and Garagylchyg-2) and soft (Triticum aestivum L.) - (Gobustan, Dagdash, Mirbashir-128 and Sheki-1) wheat purchased from the Research Institute of Agriculture under the Ministry of Agriculture of Azerbaijan.

First, all seeds were disinfected with 0.01% $KMnO_4$ solution for 5 minutes and after washing three times with distilled water, control and experimental seeds were germinated in pots with soil within 14 days, under 12-hour illumination, temperature 24 ± 1 °C and humidity 80 ± 5%, avoiding drying in a climatic chamber (Taisite GZX-300E, China) [21]. The plants, were divided into three groups:

1. Control series (without soil treatment with iron NPs)
2. 1-st series (soil treatment with Fe_2O_3 at the concentration of 15 mg per 1 kg)
3. 2-nd series (soil treatment with Fe_2O_3 at the concentration of 30 mg per 1 kg)

The size of ferric oxides NPs is 20*40 nm (Skyspring Nanomaterials Inc, USA). Treatment of soil with NPs, was carried out once, taking into account their maximum permissible concentration (MPC): the applied amount exceeded MPC 2-4 times, respectively. Each series included 30 seeds of the studied wheat varieties.

The method of determination the activity of ascorbate peroxidase (APO, EC 1.11.1.11) was based on determining the rate of decomposition of hydrogen peroxide by ascorbate peroxidase of tested samples with the formation of water and dehydroascorbate [22]. Optical density was recorded on a spectrophotometer (MRC, model UV-200-RS, Israel) at 290 nm.

For this, a sample of the plant material (1 g) was homogenized in a chilled mortar with 10 ml of 0.06 M phosphate buffer, pH 7.6, with addition of 0.3 g polyvinylpyrrolidone. The ground mass, was transferred to a 50 ml volumetric flask, which was filled by the same buffer till the mark, mixed well, and left for 15 min. This homogenate was centrifuged at 8000g for 10 min at 4 °C. The reaction mixture consisted of 50 µl 0.1 mM EDTA (Biochemica), 50 µl 0.05 mM ascorbic acid (Sigma-Ultra), 50 µl 0.1 mM hydrogen peroxide, 2.25 ml phosphate buffer, and 300 µl plant extract obtained after centrifugation of the homogenate. Activity was expressed in nmol per gram of wet weight per unit of time [$nmol \cdot g^{-1} \cdot min^{-1}$]. The calculation of the activity of ascorbate peroxidase was carried out on the basis of molar extinction coefficient ($E = 2.8\ mM^{-1}cm^{-1}$).

The experiments, were carried out in three biological replicates and each was reproduced independently three times. Statistical processing of the results, was carried out, using the licensed IBM SPSS Statistics software package. 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-tailed level of significance $p \leq 0.05$. The diagrams were constructed using the GraphPad Prism 8 software.

RESULT AND DISCUSSION

According to the obtained results of the research, the activity of ascorbate peroxidase in the control and experimental samples of studied wheat varieties differed from each other, the numerical values of which, are presented in the tables below.

Table 1

The activity of ascorbate peroxidase in control and experimental samples of durum wheat varieties, [$nmol \cdot g^{-1} \cdot min^{-1}$]

Varieties	Control series	Experimental series	
		1-st series (soil treatment with Fe_2O_3 at the concentration of 15 mg per 1 kg)	2-nd series (soil treatment with Fe_2O_3 at the concentration of 30 mg per 1 kg)
Gyrmyzy bugda	23,1710 ± 2,41*	27,0643 ± 2,27*	30,1567 ± 2,59*
Garabag	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*
Garagylchyg-2	34,7020 ± 0,54*	58,5360 ± 0,97*	69,3130 ± 1,04*

* Differences between control and experimental series are significant at levels of significance p≤ 0.05

Table2

The activity of ascorbate peroxidase in control and experimental samples of soft wheat varieties, [nmol·g^{-1}·min^{-1}]

Varieties	Control series	Experimental series	
		1-st series (soil treatment with Fe_2O_3 at the concentration of 15 mg per 1 kg)	2-nd series (soil treatment with Fe_2O_3 at the concentration of 30 mg per 1 kg)
Mirbashir-128	27,4900±2,93*	34,2097±2,54*	25,1800±1,59*
Gobustan	26,9040±2,16*	30,8574±1,86*	36,9153±1,62*
Dagdash	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 control and experimental series are significant at levels of significance p≤ 0.05

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

As for soft wheat varieties, the highest enzyme activity, was observed in treated seedlings of Dagdash variety, the lowest in the control seedlings of the same variety. In the samples of Gobustan variety, seed treatment with ferric oxides NPs, led to an increase in the activity of ascorbate peroxidase, in both, first and second series by (15%, 37%), respectively in comparison with the control. In first series of samples Sheki-1 and Mirbashir-128 an increase in the enzyme activity was observed, while in the second series of samples, the activity of ascorbate peroxidase under the influence of NPs of ferric oxides, decreased by 10% and 8% respectively, compared with the control (fig. 2).

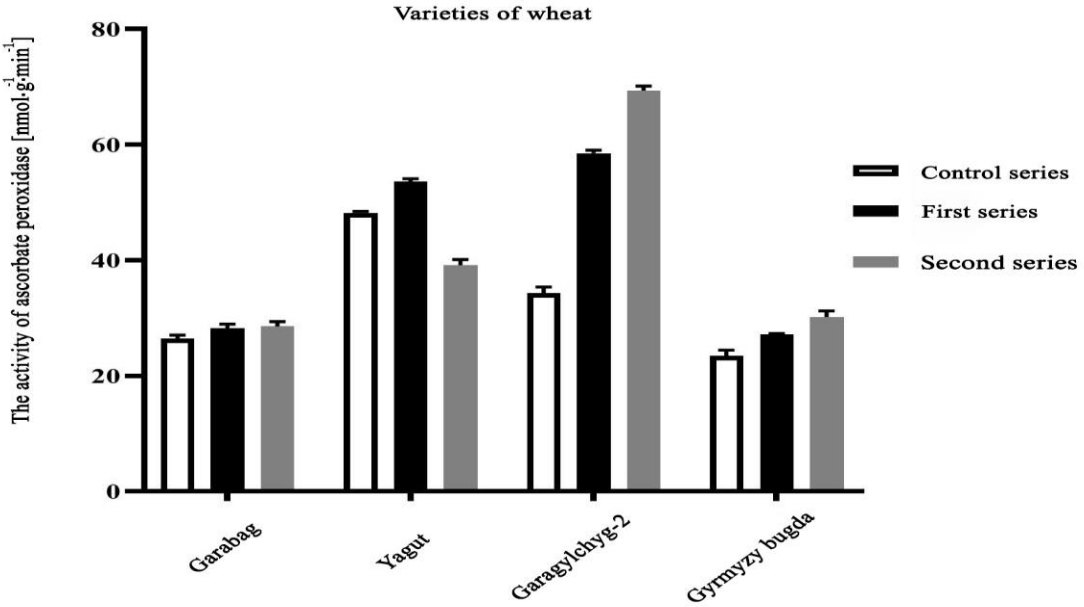

Fig. 1 The activity of ascorbate peroxidase in control and treated seedlings of durum wheat varieties

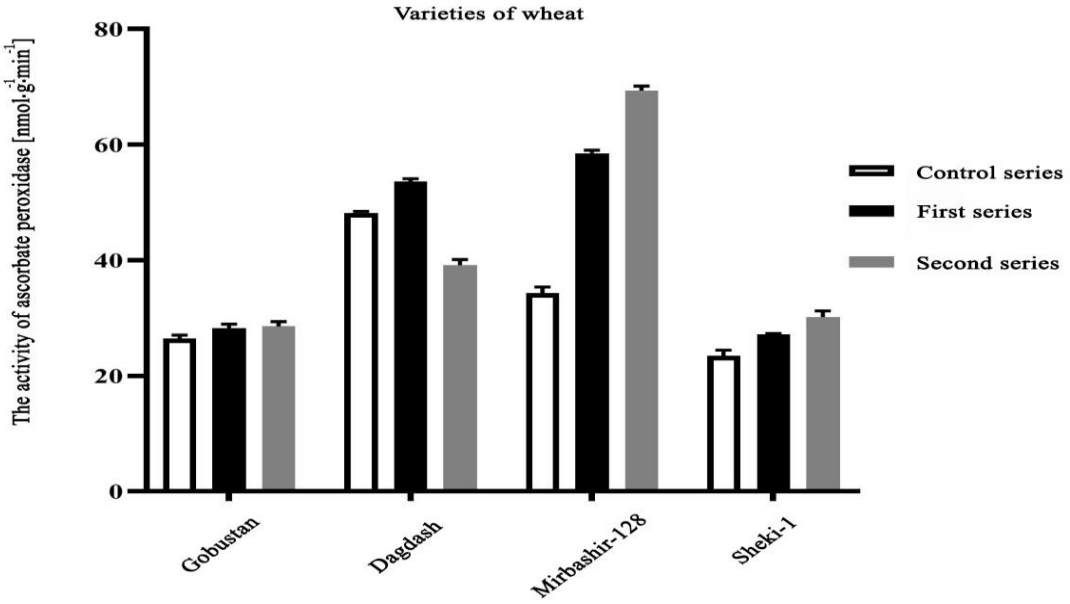

Fig. 2 The activity of ascorbate peroxidase in control and treated seedlings of soft wheat varieties

Thus, considering the obtained data, we find that less ROS is 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.

Our studies demonstrate a positive or negative effect of NPs of ferric oxides on the activity of ascorbate peroxidase in two-week seedlings of various durum and soft wheat varieties, which has important implications for many industries of agriculture. As it is known, NPs are distinguished, by unusual physicochemical properties and specific effects, on living organisms [23]. Recent studies on the use of nanotechnology in growth of crops indicate the active influence of NPs on the process of seed germination. The natural process of germination takes a long time, but in the case of seeds' treatment with NPs, high germination rates are achieved, which makes the use of nanotechnology as a powerful method for increasing seed germination [24].

The literature contains data regarding the effect of iron NPs and its oxides on physiological and biochemical processes in plants [25]. It has been shown that in vivo •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 O_2^-• [26].

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

$$Fe^{3+} + O^{2\bullet-} \rightarrow Fe^{2+} + O_2$$

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

A positive effect of nano-preparations of microelements, on the chlorophyll content, the activity of antioxidant enzymes of chloroplasts and the yield of wheat, was revealed in the works of Sokolovskaya-Sergienko [27]. According to some authors, silver NPs in low concentration increased the germination energy and the ability of seeds to germinate, their growth and development, respiration rate and the activity of enzyme system [28]. In the studies of Egorov and Shafronov, iron nanopowders increased the yield and grain quality of crops [29].

Thus, the results of studies carried out with NPs are contradictory, and further studies in this direction are expedient. Apparently, the suppressive effect of NPs of trivalent ferric oxides on the activity of ascorbate peroxidase, in the second series of samples of Yagut, Mirbashir-128, and Sheki-1 is associated with high concentration of NPs. Differences in the levels of enzyme activity of studied soft and durum wheat varieties can be associated with their different resistance to high concentration of trivalent ferric oxides NPs.

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

CONCLUSION

Based on the obtained data, we conclude that in the first series of all tested wheat varieties NPs of ferric oxides led to an increase in the activity of ascorbate peroxidase, while in the second series of durum wheat varieties the activity of APO had a slight increase (Gyrmyzy bugda) or a decrease (Yagut), or practically no changes were observed in the seedlings of Garabagh, but in the seedlings of Garagylchyg-2, the enzyme activity was two times higher than in the control.

In the case of second series of soft wheat varieties, a sharp increase in the activity of ascorbate peroxidase under the influence of ferric oxides NPs was observed in the seedlings of Dagdash, contradictory a decrease of the enzyme activity was observed in the seedlings of Sheki-1 and Mirbashir-128.

Thus, the obtained data make it possible to distinguish the varieties Garagylchyg-2, Gyrmyzy bugda, Dagdash and Gobustan as resistant to the influence of NPs of ferric oxides, which has importance in breeding work for obtaining resistant varieties.

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