



## EFFECT OF Fe<sub>2</sub>O<sub>3</sub> AND Al<sub>2</sub>O<sub>3</sub> NANOPARTICLES ON THE ANTIOXIDANT ENZYMES IN SEEDLINGS OF *TRITICUM AESTIVUM* L.

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### SUMMARY

During their lifetime, plants are constantly exposed to varied environmental factors, which leads to an enhancement in the generation of reactive oxygen species (ROS), and the antioxidant system (AOS) that controls the level of ROS and protects the plant cells. The latest study considered the changes in the activity of several high molecular weight components of the AOS—ascorbate peroxidase (APO), catalase (CAT), and polyphenol oxidase (POL)—in two-week old seedlings of soft wheat (*Triticum aestivum* L.) cultivars under the influence of different (trivalent ferric oxide and aluminum oxide nanoparticles [NPs]). The study observed the activity of investigated enzymes under the influence of several NPs depends on varietal characteristics in wheat seedlings. In the tested wheat cultivars' seedlings, ferric oxide NPs led to a sharp increase in activity of APO in the cultivars Dagdash and Gobustan. However, in the seedlings of cultivars, Sheki-1 and Mirbashir-128, a decline in the enzyme activity was observed. Concerning the influence of aluminum oxide NPs, the study affirmed that increasing the concentration of NPs leads to increase in the enzyme activity, in addition to the activity of APO. The results concluded that each plant genotype has its mechanisms for removing the harmful effects of aluminum oxide NPs, which include antioxidant enzymes. Thus, the latest findings can help serve as a basis for the wheat cultivars selection with more resistance to abiotic stress conditions.

**Keywords:** *Triticum aestivum* L, nanoparticles, activity, ascorbate peroxidase, polyphenol oxidase, catalase

**Key findings:** The hypothesis on the existence of various resistance mechanisms in *Triticum aestivum* L. genotypes after exposure to aluminum and ferric oxide nanoparticles has been evaluated.

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### INTRODUCTION

The antioxidant system (AOS) of protection is a multi-component and multilevel self-regulating system, represented in plant cells by high-molecular-weight enzymes and several low-molecular-weight components. All the system

elements must be in constant interaction and maintaining their balance to ensure the most effective plant protection and to preserve the plant's life sustainably under stress conditions (Kolupaev *et al.*, 2011; Rico *et al.*, 2015).

Ascorbate peroxidase (APO; EC 1.11.1.11) is one of the antioxidant enzymes

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of a plant cell, localized mainly in chloroplasts with a high affinity to hydrogen peroxide, reducing it to water; it is also used as a donor of electrons to ascorbic acid by regulating the rate of oxidation of ascorbic acid in cells (Polesskaja, 2007; Pradedova *et al.*, 2017). Polyphenol oxidase (POL; EC 1.14.18.1), a copper-containing enzyme, is one of the plant cell terminal oxidases, oxidizing in the presence of a molecular oxygen various phenols and their derivatives with the formation of the corresponding quinones (Olenichenko *et al.*, 2006). Catalase (CAT; EC 1.11.1.6) and peroxidase utilize peroxide surfaces, thus are at the front line of protection from the toxic effects of reactive oxygen species (ROS). They mix coating superoxide anion and peroxide substances in the reaction to a minimum and do not allow these to react to form hydroxyl anion (Anjum *et al.*, 2015). The study of plants' resistance problems to adverse environmental factors is one of the central concerns of modern biology. On the other hand, with an increased consumption rate of agricultural products, and to ensure food security for the expanding population, agricultural production requires constant integration of science in the agro-technological process (Kuckir, 2014). In this regard, interest in the study of nanoparticles' (NPs) influence on various metals, as a result of natural processes and as activity of an anthropogenic factor on biological systems, has increased (Frolova *et al.*, 2011; Kareem *et al.*, 2022).

Accordingly, environmental pollution, with high concentrations of inorganic material NPs that have altered structural and physicochemical properties, harms the physiological and biochemical characteristics of living organisms' cell. For example, one of the most frequently reported toxic effects of NPs by researchers is the generation of ROS in cells, leading to oxidative stress (Manke *et al.*, 2013; Anjuma *et al.*, 2015). The high reactivity of ROS and free radicals leads to an acceleration of oxidation reactions that crumble the molecular basis of cells and causes damage to cellular structures. In the case of using NPs with low concentrations, on the contrary, they effect positive impacts on biological objects. Hence, the effects of NPs on living organisms depend on the NPs concentration (Chichiricco and Poma, 2015). Based on the accumulated experimental materials of different studies, one can conclude that there are various theories on the NPs' effects on living systems, however, the mechanisms of their biological activity have not been sufficiently investigated, which

requires intensive research (Morgalev *et al.*, 2010; Kovaleva *et al.*, 2017).

In recent years, reports validated that oxidative stress can be caused by nanoparticles, based on iron, copper, and nickel used by industrial enterprises worldwide (Buzea *et al.*, 2007). Further, the intensity of biological effects of highly discrete metals is assumed to differ from the effects of their oxide forms and mainly depends on the presence of variable valence metals in their composition. The latter is capable to release toxic ions from their colloidal matrix and accelerate the production of ROS (Mishra *et al.*, 2014). The NPs easily penetrate the cells of seeds prepared for sowing and actively influence the enzymatic system of physiological and biochemical reactions (Kolesnikov *et al.*, 2018). In general, numerous research and review articles devoted to studying the effects of metal NPs on plant organisms (Riahi-Madvar *et al.*, 2012; Sharma *et al.*, 2012). However, no such studies focused about the effect of different concentrations of trivalent ferric oxide and aluminum oxide NPs on the functioning of AOS components in wheat seedlings. Therefore, his research work aimed to study the effects of ferric and aluminum oxide NPs on the ascorbate peroxidase, polyphenol oxidase, and catalase activity in two-week old seedlings of wheat (*Triticum aestivum* L.) cultivars to assess their tolerance to the NPs.

## MATERIALS AND METHODS

The study materials, comprising four wheat (*Triticum aestivum* L.) cultivars, viz., Gobustan, Dagdash, Mirbashir-128, and Sheki-1, were procured from the Agriculture Research Institute, Ministry of Agriculture, in Azerbaijan. The seeds of all the wheat genotypes were first disinfected with 0.01% KMnO<sub>4</sub> solution for 5 min, and after washing three times with distilled water, the control and experimental seeds were germinated in pots with soil within 14 days, under 12-h light. The samples were exposed to a temperature of 24±1°C and humidity at 80±5%, avoiding drying of seedlings in a climatic chamber (Taisite GZX-300E, China), as prescribed in the research work of Lebedev *et al.* (2014). The plants of all the genotypes were divided into three groups (Table 1).

The size of ferric oxide NPs is 20 nm × 40 nm (Skyspring Nanomaterials Inc, USA). Soil treatment with NPs was carried out once by taking into account their maximum

**Table 1.** Soil treatment with various doses of ferric oxide NPs.

No.	Soil treatment
1	Control series (without soil treatment with iron NPs)
2	First series (Soil treatment with Fe <sub>2</sub> O <sub>3</sub> at the concentration of 15 mg kg <sup>-1</sup> )
3	Second series (Soil treatment with Fe <sub>2</sub> O <sub>3</sub> at the concentration of 30 mg kg <sup>-1</sup> )

permissible concentration (MPC), and the applied amount exceeded MPC 2-4 times, respectively. In each series of treatments, 30 seeds were included in the studied wheat cultivars. To study the effect of different concentrations (0.1 mg/l, 0.01 mg/l, and 0.001 mg/l) of aluminum oxide NPs, Karabakh (*Triticum durum* Desf.) and Mirbashir-128 (*Triticum aestivum* L) cultivars were tested. The seeds of the plant samples were treated with powders of Al<sub>2</sub>O<sub>3</sub> NPs, sized at 50 nm (Sigma-Aldrich, Germany).

In determining the activity of ascorbate peroxidase (APO, EC 1.11.1.11), the researchers determined the rate of decomposition of hydrogen peroxide by ascorbate peroxidase of tested samples with the formation of water and dehydroascorbate (Nakano and Asada, 1981). Optical density was recorded on a spectrophotometer (MRC, model UV-200-RS, Israel) at 290 nm.

For this, a plant material sample (1 g) was homogenized in a chilled mortar with 10 ml of 0.06 M phosphate buffer, pH=7.6, and the addition of 0.3 g polyvinylpyrrolidone. The groundmass was transferred to a 50 ml volumetric flask, which was filled with the same buffer till the mark, mixed well, and left for 15 min. This homogenate was centrifuged at 8000 g 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. The activity was expressed in nmol per gram of wet weight per unit of time (nmol·g<sup>-1</sup>·min<sup>-1</sup>). The activity of ascorbate peroxidase was calculated based on the molar extinction coefficient (E = 2.8 mM<sup>-1</sup>cm<sup>-1</sup>).

The polyphenol oxidase (POL, EC 1.14.18.1) activity was determined spectrophotometrically (MRC, model UV-200-RS, Israel) by the increases in optical density at a wavelength of 590 nm. A plant material sample (1 g) was homogenized in 25 ml of potassium-phosphate buffer (0.06M, pH=7.2). The homogenate was centrifuged at 5000g for 10 min. The extraction was performed at a temperature of 4°C. The supernatant was used as sample for analysis. The reaction mixture

contained: 1 ml supernatant, 1 ml of phosphate buffer (pH=7.2), 1 mL of 0.02% diethyl paraphenylenediamine, and 1 ml of 1% pyrocatechin. In the control variant the latter was replaced with 1 ml of distilled water. The activity was expressed in µmol per grams of crude weight per unit time (µmol·g<sup>-1</sup>·min<sup>-1</sup>) (Ermakov *et al.*, 2005).

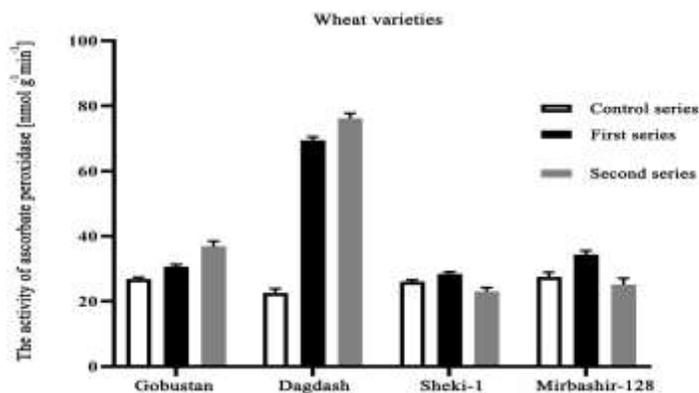
The activity of the catalase (CAT, EC 1.11.1.6) was estimated by the rate of loss of hydrogen peroxide in the incubation medium. The concentration of hydrogen peroxide was determined by the reaction with ammonium molybdate, which gave a persistent colored complex (Koroliuk *et al.*, 1988). Optical density was recorded on a spectrophotometer (MRC, model UV-200-RS, Israel) at 410 nm.

### Statistical analyses

The experiment employed three biological replicates and each replicate was reproduced independently three times. Statistical processing of the results was done using the licensed IBM SPSS Statistics software package. The assessment of the reliability of variations in arithmetic means was carried out based on the Student's coefficient. Differences between groups were considered significant at a two-tailed level of significance p≤0.05. The diagram was constructed using the Graph Pad Prism-8 software.

## RESULTS AND DISCUSSION

The research obtained results that show the activity of ascorbate peroxidase in the control and treated samples of wheat cultivars differed from each other, with the following numerical comparison. Analysis of the data showed the highest enzyme activity was observed in treated seedlings of the cultivar Dagdash, whereas the lowest was in the control seedlings of the same cultivar. In the cultivar Gobustan samples, seed treatment with ferric oxide NPs led to an increase in the ascorbate peroxidase activity in the first (15 mg·kg<sup>-1</sup>) and second (30 mg·kg<sup>-1</sup>) series of treatment by 15% and 37%, respectively compared with the control. In the first series of treatment, the



**Figure 1.** The activity of ascorbate peroxidase in control and two treated series of the seedlings of soft wheat cultivars.

samples of wheat cultivars Sheki-1 and Mirbashir-128 exhibited an increase in the enzyme activity, while in the second series, the activity of ascorbate peroxidase under the influence of NPs of ferric oxides decreased by 10% and 8%, respectively, in both genotypes compared with the control (Figure 1).

Considering the obtained data, the absence of ROS was confirmed (the quantity of ROS was not measured, only the activity of APO supposedly removing the ROS content). Their quantity was not determined, but only what was formed in the first series of wheat samples as having a lower intensity of free radical oxidative processes than in the second series of samples, except for two wheat cultivars Mirbashir-128 and Sheki-1. The study also demonstrated positive and negative effects of ferric oxide NPs on the ascorbate peroxidase activity in two-week-old seedlings of various soft wheat cultivars, revealing significant impacts for the agriculture industry. The NPs are known to be distinguished by unusual physicochemical properties and specific effects on living organisms (Jurin and Molchan, 2015). Past studies also reported the effects of iron NPs and their oxides on physiological and biochemical processes in plants (Ahmad *et al.*, 2008).

Other studies also observed that in vivo  $\bullet\text{OH}$  is formed mainly as a result of the iron-catalyzed Haber-Weiss reaction, which is a combination of two elementary processes, i.e., the Fenton reaction and the reduction of ferric  $\text{O}_2^-$  (Halliwell and Gutteridge, 1986).



Thus, in this study, the increase in the ascorbate peroxidase activity proves its protective function on plants that aim to reduce hydroxyl radicals. The studies of Sokolovskaja-Sergienko (2013) revealed a positive effect of nano-preparations of microelements discovered on the chlorophyll content, the activity of the antioxidant enzymes in chloroplasts, and a positive impact on the wheat yield. According to other past studies, the silver NPs with low concentration increased the energy and ability of the seeds to germinate, improve their growth and development, respiration rate, and the activity of the enzyme system (Fedorenko *et al.*, 2011). In the studies of Egorov *et al.* (2008), they reported that iron nano-powders enhanced the grain yield and improve the quality of crops. Thus, with the past studies' results on the NPs that are contradictory, further rigorous investigations are suggested.

The latest research clarified that the suppressive effect of NPs of trivalent ferric oxides on the activity of ascorbate peroxidase, in the second series for wheat cultivars Mirbashir-128 and Sheki-1, samples contained a high concentration of NPs. The concentration data is available at the beginning of the results section. Differences in the levels of enzyme activity of studied wheat cultivars can be associated with their different resistance to the high concentration of trivalent ferric oxide NPs. As a result, the study further disclosed that the activity of ascorbate peroxidase in wheat seedlings under the influence of NPs of trivalent ferric oxides depends on the varietal characteristics. For this reason, the study of the mechanisms of metal oxide NPs on the rate of oxidation of ascorbic acid in various wheat

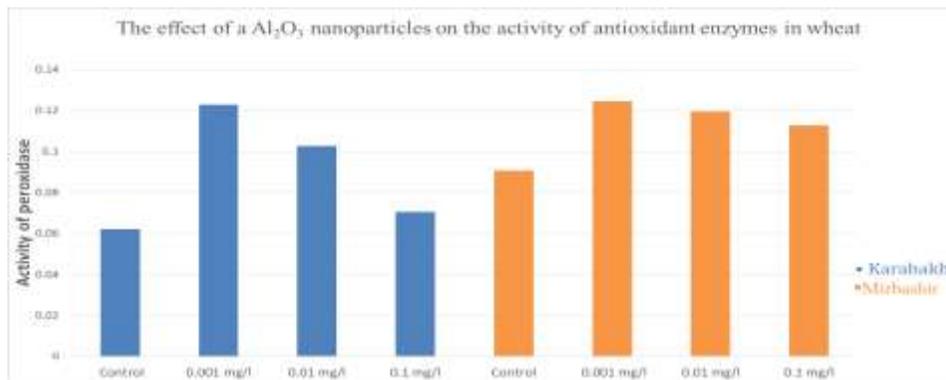
cultivars deserves further examination. Figure 2 shows the effect of different concentrations of aluminum oxide nanoparticles on peroxidase activity.

Different concentrations of NPs affect the activity of the enzyme in different ways. In durum and bread wheat cultivars, Karabakh and Mirbashir-128, respectively, the highest activity of peroxidase is observed at an aluminum oxide concentration of 0.001 mg/l. With an increase in the concentration of NPs in the cultivar Karabakh, the activity of the enzyme decreases, while in the cultivar Mirbashir-128 it remains almost at the same level. When determining the activity of polyphenol oxidase, the study found that at the NP concentration of 0.001 mg/l and 0.01 mg/l, the enzyme activity decreases in both cultivars, whereas at a concentration of 0.1 mg/l it sharply increases (Figure 3).

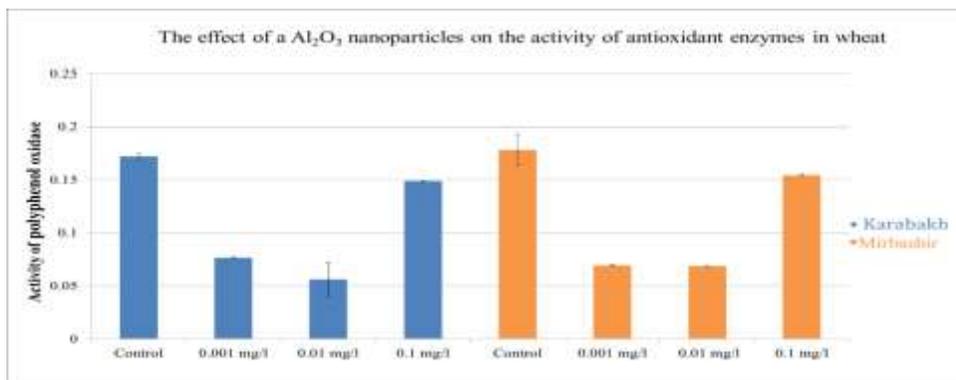
The activity of the catalase enzyme increases as the NP concentration increased (Figure 4). In the past research work of Yanik and Vardar (2018), they studied the effects of Al<sub>2</sub>O<sub>3</sub> nanoparticles using different parameters, such as, H<sub>2</sub>O<sub>2</sub> content, superoxide dismutase

and catalase activity, lipid peroxidation, total proline, photosynthetic pigment, and anthocyanin content in *Triticum aestivum* L. The results indicated that while Al<sub>2</sub>O<sub>3</sub> nanoparticles caused a dose-dependent increase in H<sub>2</sub>O<sub>2</sub> content, superoxide dismutase activity, lipid peroxidation, proline contents, and the catalase activity was decreased compared with the control. Moreover, the total chlorophyll, chlorophyll a, carotenoids, and anthocyanin contents were reduced to the highest concentration of 50 mg/ml. In conclusion, the Al<sub>2</sub>O<sub>3</sub> NP caused oxidative stress in wheat after 96 h.

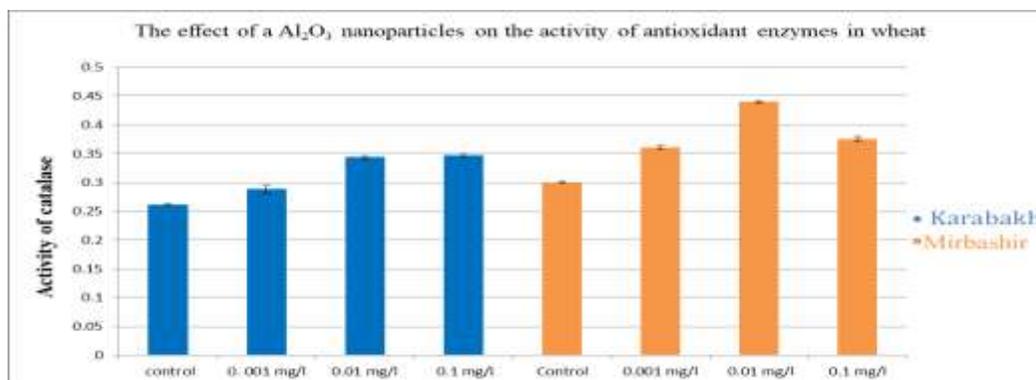
The latest results further authenticated that the activity of peroxidase and catalase enhanced with an increase in the concentration of aluminum oxide NP. The activity of polyphenol oxidase also increased with the high NP concentration, and this is due to peroxidase and catalase complementing each other.



**Figure 2.** Influence of Al<sub>2</sub>O<sub>3</sub> nanoparticles on the activity of peroxidase.



**Figure 3.** Influence of Al<sub>2</sub>O<sub>3</sub> nanoparticles on the activity of polyphenol oxidase.



**Figure 4.** Influence of  $\text{Al}_2\text{O}_3$  nanoparticles on the activity of catalase.

## CONCLUSIONS

In the first series of treatments on the tested wheat (*Triticum aestivum* L.) cultivars, NPs of trivalent ferric oxides led to an increase in the activity of ascorbate peroxidase, while in the second series of soft wheat cultivars, a decrease of the enzyme activity was observed in the seedlings of cultivars Sheki-1 and Mirbashir-128. However, in contradiction, a sharp increase in the activity of APO under the influence of ferric oxide NPs was observed in the seedlings of cultivar Dagdash. Thus, the obtained results make it possible to distinguish the cultivars Dagdash and Gobustan as resistant to the influence of NPs of ferric oxide, which can be used in future breeding programs to develop the resistant cultivars. Based on the obtained data, the study concluded that each plant genotype has its mechanisms for removing the harmful effects of aluminum oxide nanoparticles, which include antioxidant enzymes.

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