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Effect of Iron Oxide Nanoparticles on the Growth of Maize Sprouts Cultivated Under Drought Stress and on the Activity Dynamics of NADP-ISDH Enzyme

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Abstract

The effect of iron oxide nanoparticles (FeNPs-Fe_2O_3) on the growth of maize sprouts (*Zea mays L.*) cultivated under drought conditions and on the activity dynamics of the NADP-ISDH enzyme in the root and leaf cells of the plant has been studied. In the conducted experiments, it has been observed that nanoparticles have a stimulating effect on the samples of the maize plant cultivated under both control and drought conditions. Thus, in 15-day-old sprouts, compared to the control, in the sprouts developed from seeds treated with nanoparticles, the root has advanced by 27.8%, and stem 32.7%. In the samples cultivated under drought conditions, the sprouts developed from seeds treated with nanoparticles, the root has progressed by 21.5% and the stem by 25.9% compared to the other option (only drought). The induction of NADPH-ISDH enzyme has been recorded in root and leaf tissues in all variants related to the growth of sprouts during the first 10 days of the experiment. The highest value of enzyme activity has been recorded in sprouts cultivated under drought stress conditions. On the 15th day of the experiment, a weakening of the enzyme activity has been observed in all variants, and this inhibition has been recorded especially in the variant cultivated under drought stress.

Keywords: abiotic stress, drought stress, maize sprouts, FeNPs, NADP-ISDH

Introduction

Various stress factors slow down the growth of living organisms in most of the cases, including plants, and even cause their destruction when persistent [Heng, et al, 2020]. In general, it is known that stress factors reduce the productivity of plants by 50% to 70% [Kumar, 2020]. The drought factor is also among the main abiotic stress factors seriously affecting the plants [Meeta, et al, 2019]. When drought and salt stress coincide, the soil salinity increases, which often happens in the territory of our republic. Increasing global warming makes drought stress more inevitable. Due to the effect of drought stress, stomata in the leaves close and reduce the transpiration process, photosynthesis weakens, the connection between oxidation and phosphorylation is disturbed, and thus the plant growth is slowed down [Bartels, et al, 2007]. In order to overcome the retarding effect of some abiotic stress factors, the application of nanoparticles is used [Muhammad, et al, 2022]. Nanoparticles protect the cell membrane and the photosynthetic apparatus, increase the photosynthetic efficiency, ensure the accumulation of osmolytes, hormones, and improve the activity of antioxidants. It also increases the resistance of plants to stress [Adnan, et al, 2022].

With its ability to defend itself against stress, the plant tries to show tolerance, but under the conditions of constant stress, such defense cannot ensure the stability of the plant [Mei, et al, 2018]. At the same time, plants give different defense reactions against stress [Mirza et al, 2019]. Therefore, the study of the mechanism of the defense system of plants under stress conditions remains one of the actual problems. NADPH-producing enzymes are one of the systems providing the main reducing potential of the cell and comprising the basis of the plant's defense system. [Ying, 2008]. One of these enzymes is the NADP-dependent Isocitrate dehydrogenase (NADP-ISDH, EC 1.1.1.42) enzyme, which breaks down isocitrate into α-ketoglutarate and CO_2 gas and is accompanied by the formation of NADPH as a reaction product [Leterrier, et al, 2012]. The enzyme is also known to have an antioxidant function in plants.

Taking all these mentioned above into account, our experiments have been conducted in the direction of determining the activity of the enzyme in the leaves and tissues of the cultivated corn plant under the conditions of drought stress and the application of nanoparticles.

Materials and methods

The experiments have been conducted on the Zagatala local improved genotype of maize plant (Zea mays L.) for 15 days. For the variants where nanoparticles were applied, seeds were pollinated with iron oxide (γ- Fe_2O_3 powder, 20-40 nm) nano particles for 10 minutes in a mixer (Vortex mixer Model VM-1000). 1 g of iron oxide nanoparticles were applied for every 300 seeds (0.1 g - 30 seeds). Seed cultivation was carried out in Plant Growth Chamber (GVS 940) at room temperature (23-24°C), with 70% relative humidity, in 300 μmol m^{-2} s^{-1} light intensity, 16/8 hours (light/dark) mode. In order to study the effect of drought stress on corn sprouts, an artificial drought environment has been created. Two of the planted corn samples - the control and the nanoparticle-applied variants - have been watered daily. The other two variants - only drought and nanoparticles applied + drought - have watered every 2 days first and then every 3 days. The biometric indicators of sprouts and the activity dynamics of the NADP-ISDH enzyme have been calculated every 5 days. Enzyme activity has been determined spectrophotometrically, at a wavelength of 340 nm, based on the rate of reduction of NADP in an MRC (Israel) spectrophotometer. μM NADPH/min/g wet weight has been taken as the enzyme unit. The reaction has been carried out at a temperature of 23°C, measurements have been repeated 4 times. Tissue: extraction solution has been taken at the ratio of 1 g : 4 ml.

To prepare the homogenate, the root and stem of the plant have been crushed in a cold mortar with the presence of glass shards and been centrifuged at 9000 g and 4 ºC for 20 minutes to obtain the supernatant enzyme preparation. 100 mM Tris-HCl buffer (pH 8.2) containing 5 mM MgCl2, 2 mM EDTA, 14 mM mercaptoethanol, 5% polyvinylpyrrolidone (PVP), 1% polyethylene glycol (PEG) (pH 8.2) have been used for the preparation of the enzyme, and 50 mM Tris-HCl (pH 8.2) solution containing 2.5 mM $MgCl_2$, 2 mM D,L-isocitrate and 0.5 mM NADP to determine the activity of the enzyme.

To study the organic content of the plant, after drying the 15-day-old sprouts, the organic, wet and dry weight of its roots and leaves have been calculated separately. Determination of heavy metals has been carried out in ICP-MS and atomic absorption spectrometer (Sequential X-ray Fluorescent Spectrometer (S8 TIGER)). The plant samples have been stored in a drying electric oven at a temperature of 65ºC, and the obtained mass was ground in an electric ball mill (Herzog). 2 grams of plant powder have been taken, 2 ml of solid nitric acid has been added to it, after keeping it in a closed oven for a day, it has been dried again at 65°C temperature, placed in a microwave oven (SineoMBES 86) and kept at 150-165°C temperature for 1 hour. Then the solutions have been transferred to graphite cups and dried at a temperature of 65°C until a moist residue has been obtained. After adding liquid 2% HNO_3 to each of the samples, they have been filtered on filter paper and transferred to volumetric flasks. After storing the prepared samples for 2 hours, the measurements have been carried out in the ICP-MS apparatus.

Results and discussion

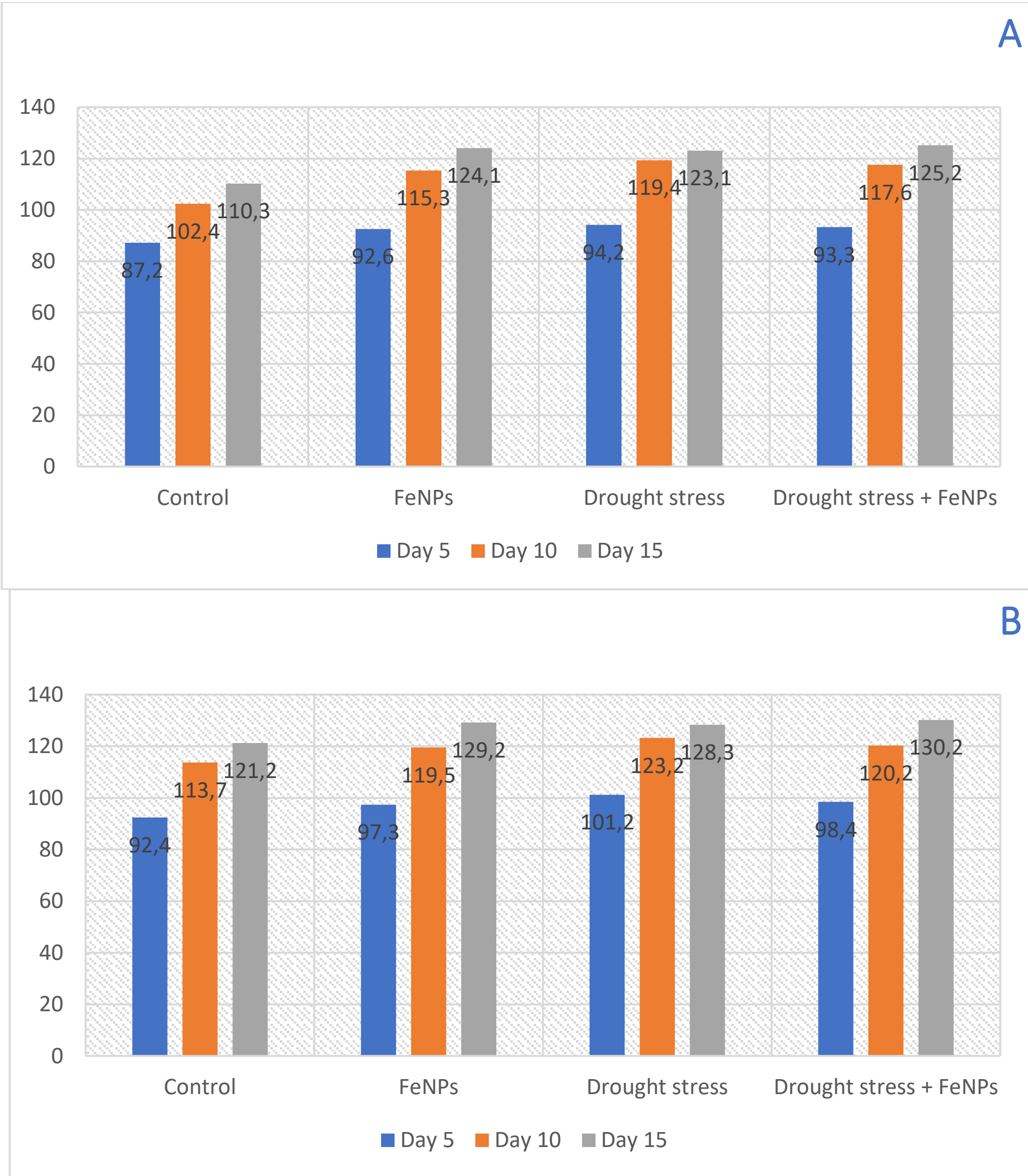
The experiment conducted showed that the water disbalance in the stems and leaves of the plant noticeably delayed the growth of sprouts. The reduction of watering decreased the branching and elongation of hairs of the root system in variants. It was observed that the application of iron oxide nanoparticles improved the growth in both the control variant and the variants cultivated under the influence of drought stress. In the table below, the biometric indicators of the root and stem system of the samples of corn sprouts cultivated under control and stress (drought + FeNPs) conditions are given (Table 1).

Table 1. Biometric indicators of root and stem system (cm) of corn sprouts under control and stress conditions (drought, iron oxide nanoparticles).

	Day 5	**Day 10**	**Day 15**
Control	**root 2.4±01** **stem 4.2±02**	**root 5.1±02** **stem 8.5±02**	**root 10.4±01** **stem 17.4±01**
FeNPs	**root 3.1±02** **stem 5.3±02**	**root 6.4±02** **stem 11.4±02**	**root 13.3±01** **stem 23.1±01**
	root 1.9±02 **stem 3.3±01**	**root 3.5±03** **stem 5.6±02**	**root 5.1±02** **stem 8.1±03**
Drought **Drought + FeNPs**	**root 2.4±01** **stem 3.6±02**	**root 4.1±01** **stem 6.8±01**	**root 6.2±02** **stem 10.2±02**

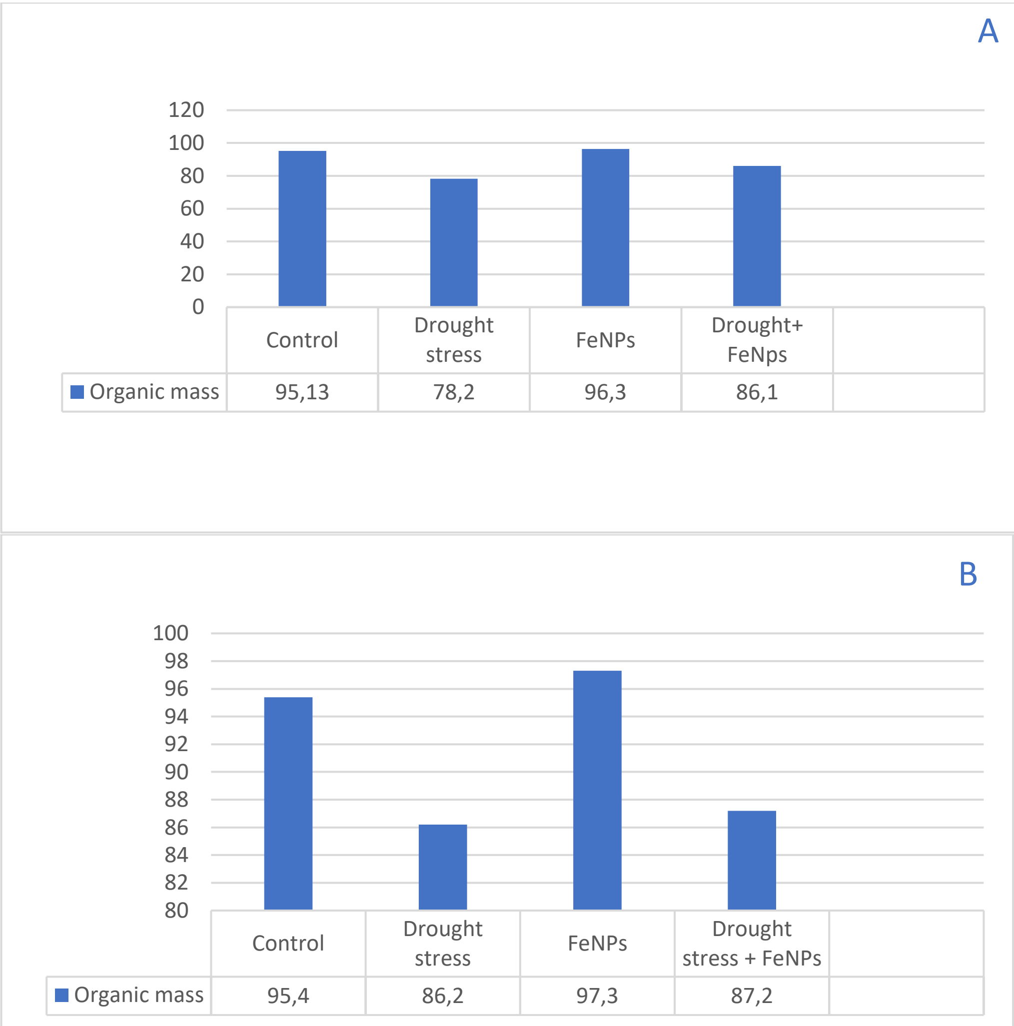
As As can be seen from the table (Table 1.), the growth of the root and stem system of corn sprouts was significantly weakened during the drought stress and was stimulated by the application of nanoparticles. The effect of drought stress was partially reduced in the variants accompanied by the application of nanoparticles. Thus, on the 15th day of the experiment, compared to the control variant, the root system and stem system regressed by 50.9% and 53.4%, respectively, in the samples cultivated under drought stress conditions. Compared to the control variant, in samples where nanoparticles were applied, the root system and stem system of sprouts progressed by 27.8% and 32.7% accordingly. With the application of nanoparticles, the effect of drought stress was reduced by 21.5% in the root system and 25.9% in the stem system.On the 5th, 10th, and 15th days of the experiment, the activity of the NADP-ISDH enzyme was calculated, and the induction of the enzyme during the first 10 days and its gradual inhibition on the 15th day were recorded for all samples (Figure 1).

Figure 1. Activity dynamics of NADPH-ISDH enzyme (µ mol/g wet wgt.) in the root (A) and leaf (B) systems of corn sprouts under control and stress conditions (drought and drought+FeNPs).



As can be seen from the figure (Figure 1, A and B), the value of NADP-ISDH enzyme activity was recorded with an increasing line during the first 10 days in both the root and stem systems with regard to the growth of the corn plant. In samples cultivated under drought stress, the activity of the enzyme reached the highest value in both root and leaf cells on the 10th day of the experiment, but it was sharply inhibited on the 15th day. On the last day of the experiment, the activity of the enzyme reached the highest points in sprouts cultivated under drought stress conditions accompanied by the application of nanoparticles. The drought stress, as well as the application of nanoparticles changed the metabolic character of the corn plant, it also affected the value of organic content in roots and leaves. These values are reflected in the figure below (Figure 2).

Figure 2. The results of analysis (organic content) (mg/kg (in PPm)) of corn plant samples' (Zea mays L., Zagatala locally improved) root (A) and leaf (B) cells under control and stress conditions (drought+FeNPs) dried at 105°C.



As can be seen from the figure (Fig. 2., A and B), the drought stress reduced the value of the plant's organic content, however, the application of nanoparticles had a positive effect on this value.

Conclusion

The negative effect of drought stress on corn sprouts caused a decrease in the organic content of the plant, thereby both the root and leaf systems failed to develop. With the application of iron oxide nanoparticles, the development of sprouts was accelerated compared to the control variant, whereas the drought stress was partially prevented in the variant cultivated under drought conditions. This, in its turn, paves the way for the further use of iron oxide nanoparticles to protect corn plants from drought stress.

A sharp increase in the activity of the NADPH-ISDH enzyme in the initial phase of the growth of sprouts arising out of the occurrence of drought stress allows us to consider that it has a role in the formation of a defense mechanism against stress.

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