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Influence of *Trichoderma asperellum* on nitrate reductase activity and NO₃ accumulation in organs of soybean and barley under chloride saliny conditions

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Abstract

This work investigated the accumulation of NO₃ and nitrate reductase activity (NRA) in the organs of 14 day-old barley and soybean plants grown under salinity conditions (100 mM NaCl) by soaking part of their seeds in a *Trichoderma asperellum* cultural solution. It has been established that seed treatment with *Tr. asperellum* under conditions of chloride salinity significantly and differently restores NPA (in soybeans more in the roots, and in barley in the leaves) and NO₃ accumulation (more in the leaves of barley and in the roots of soybeans) in the organs of both plants, regardless of species. It is assumed that this difference may be associated with the formation of nitrogen-fixing nodules on the roots of the soybean plant.

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Introduction

Land and water resources and their use play an important role in improving food security on Earth. Our planet's population continues to grow every year and is estimated to exceed 9 billion people by 2050. During this period, it will be necessary to annually produce an additional 1 billion tons of grain and 200 million tons of livestock products (Bruinsma, 2009). Improving the use of land unsuitable for cultivation is one of the most important ways to meet the growing population demand for agricultural products in the context of a deteriorating environmental situation (FAO, 2012). Therefore, in our republic, where a significant part of the land is saline and faces drought (Mamedov, 2002), much attention is paid to scientific research devoted to this problem. One of the ways to solve these problems is the use of microorganisms that have a positive effect on the development and productivity of plants as innovative methods used in agriculture to increase the resistance of plants to abiotic and biotic stresses. This approach is one of the new and effective methods of increasing plant resistance to environmental changes (drought, high temperature, salinity, etc.) (Adnan *et al.*, 2019; Alwhibi *et al.*, 2017; Fadji *et al.*, 2022). *Trichoderma* micromycetes are one of these microorganisms and are used as biological control agents for fungal diseases, as well as to reduce the negative effects of drought and salinity on plant nutrition, morphophysiological and biochemical parameters (Bakshaliyeva *et al.*, 2021; Muradov *et al.*, 2019; Yay and Verma, 2018; Woo *et al.*, 2022) They secrete compounds for various purposes, plant hormones (auxins, gibberellins, cytokinins, abscisic acid, ethylene), as well as various organic acids, intracellular amino acids, vitamins and a large number of antibiotics that cause local systemic immune reactions (Benitez *et al.*, 2004; Hexon *et al.*, 2009). Despite a large number of studies on the stimulating and protective effects on various agricultural plants (Dou *et al.*, 2020; Alizadeh *et al.*, 2024; Bandara and Kang, 2024), the role of *Trichoderma* in regulating the activity of the nitrate reductase enzyme and the accumulation of NO₃ in plants in saline environments has not been fully studied.

Therefore, the work investigated the role of *Trichoderma* in eliminating the toxic effect of chloride salts on the activity of the nitrate reductase enzyme and the accumulation of NO₃ in plant organs that differ in the degree of salt tolerance.

Materials and methods

Barley (*Hordeum sativum* L.) and soybean (*Glycine hispida* L.) plants were used in the studies. Before planting, plant seeds were soaked in a culture solution of *Trichoderma asperellum* (*Tr. asperellum*) for 15 hours.

Plants were grown in laboratory conditions on Knop's nutrient medium. The lighting intensity was 4500 lux, the duration of light and dark periods was 12 hours, and the temperature was 23°C. 14-day-old plants were used in the experiments. Experiment scheme: 1. Control (C); 2. C + *Tr. asperellum*; 3. C+100 mM NaCl; 4. C+ 100 mM NaCl + *Tr. asperellum*. The activity of the nitrate reductase enzyme was determined on a spectrophotometer at an optical density of 448 nm (Dorofeev and Peshkova, 2002), and the amount of nitrates was determined at an optical density of 410 nm by the reaction to salicylic acid (Cataldo *et al.*, 1975). Experiments were carried out in 3 analytical and 3 biological replicates and processed statistically.

Results and discussion

One of the important mechanisms of plant adaptation to salt stress is the regulation of the activity of enzymes, including the enzyme nitrate reductase, which play a key role in the absorption and metabolism of nutrients in a saline environment. Considering that, by synthesizing substances for various purposes (phytohormones of plant origin and antioxidants), *Tr. asperellum* stimulate the growth and development of plants, increase their resistance to pathogens, their effect on the activity of the nitrate reductase enzyme and the accumulation of nitrates (NO₃) in the organs of soybean and barley plants was studied.

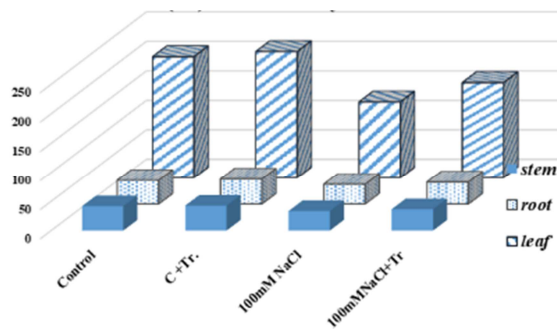


Fig. 1. Nitrate reductase activity in barley organs with the participation of *Tr. asperellum* (Tr) under salinity conditions

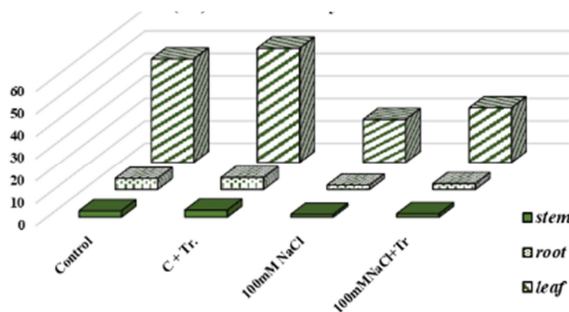


Fig. 2. Nitrate reductase activity in soybean organs with the participation of *Tr. asperellum* (Tr) under salinity conditions

Fig. 1 and 2 show the results of the influence of *Tr. asperellum* on the activity of the nitrate reductase enzyme in the organs of barley and soybean plants in a saline environment (100 mM NaCl). As can be seen, depending on the species, the activity of the enzyme in the plant organs is completely different, and the activity of the nitrate reductase enzyme both at the level of the whole plant and in its organs is higher in the barley plant than in the soybean plant. In both plants, enzyme activity in the leaves is significantly higher than in other organs.

Although salinity with 100 mM NaCl had a negative effect on enzyme activity in both plants, it was more noticeable in soybean plants. Thus, when salinized with 100 mM NaCl, the activity of the nitrate reductase enzyme decreases in the leaves by 37.9%, in the stems by 17.5% and in the roots by 17.3% in barley; in soybean plants these figures are 58.4% in the leaves and 41.7% in the stems and 63.5% in the roots, respectively.

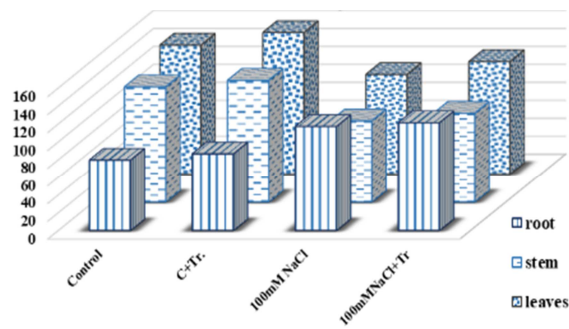


Fig. 3. Accumulation of NO₃ in organs of barley and soybean with the participation of *Tr. asperellum* (Tr) under salinity conditions

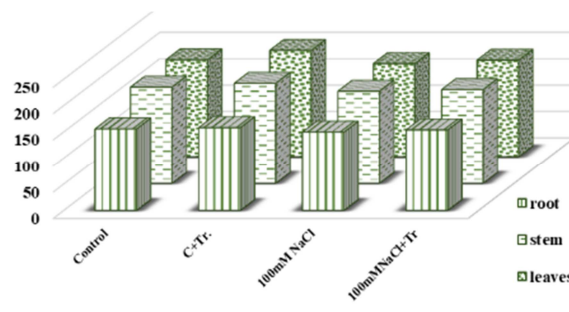


Fig. 4. Accumulation of NO₃ in organs of soybean plant

In both plants, the seeds of which were treated with the *Tr. asperellum* culture solution, enzyme activity increased in all plant organs in both normal and saline environments. In control plants, *Trichoderma* increased enzyme activity in the leaves of the barley plant by 4.2%, in the stems by 2.4% and in the roots by 5.6%. And in the organs of the soybean plant, *Trichoderma* in the control variant increases enzyme activity by 10.4% in leaves, by 10.7% in stems and by 8.2% in roots.

In salinity conditions, the effectiveness of trichoderma is more noticeable. At a salinity of 100 mM NaCl, enzyme activity increases in the barley plant by 25.4% in the leaves, by 9.9% in the stems and by 11.5% in the roots, in soybeans by 27.1% in the leaves, by 25.0% in stems and by 36.8% in roots.

Analysis of the accumulation of nitrates in plant organs shows that they differ depending on the plant species (Fig. 3, 4). As you can see, in the barley plant, nitrates accumulate more in the leaves. However, in

soybeans they accumulate in approximately equal quantities in the leaves and stems of the plant. In both plants, the roots come last in this indicator. At a salinity of 100 mM NaCl, the accumulation of nitrates in all organs is reduced. Despite the decrease in nitrate content in the leaves and stems of the barley plant (22.7 and 29.3%, respectively), at its roots it increased by 47.1%. However, in soybeans, the accumulation of nitrates in the leaves decreases by 3.9%, in the stem by 4.3%, and in the root by 3.4%.

We observed an increase in the accumulation of nitrates in direct proportion to the activity of the nitrate reductase enzyme in the organs of barley and soybeans, the seeds of which were treated with a *Trichoderma* culture solution in normal and saline media. *Trichoderma* more effectively affects the accumulation of nitrates in the barley plant. Depending on the species of plants in normal and saline environments, the influence of *Trichoderma* is also different. More nitrates accumulate in the leaves and stems of a barley plant seeds of which are treated with *Trichoderma* than in a normal medium (3.2% more in the leaves than in a normal medium, and 3.6% more in the stem), and in the roots they accumulate more in a normal environment (5.2% more than in a salty environment). However, in the organs of the soybean plant we observe the opposite. In the leaves and stems of the plant, nitrates accumulate more in a normal environment (in the leaves 6.0% more than in a saline environment, and in the stem 2.2% more), and in the roots, on the contrary, in a saline environment (1.5% more than in normal environment).

Analysis of our results gives reason to say that under chloride salinity, *Trichoderma* micromycetes noticeably increase the activity of the nitrate reductase enzyme in the organs of barley and soybean plants, regardless of species. In the soybean plant this effect is more pronounced. In the barley plant it increases significantly in the leaves compared to other organs, and in the soybean in the roots. However, the opposite trend is observed in the accumulation of nitrates. The participation of *Trichoderma* in a saline

environment leads to a greater accumulation of nitrates in the leaves of barley than in the roots, and in the soybean plant, on the contrary, to a greater accumulation of NO₃ in the roots than in other organs. This difference can also be explained by the formation of nitrogen-fixing nodules on the roots of the soybean plant.

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