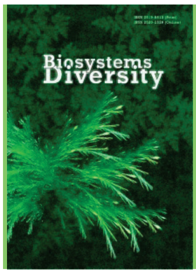

Biosystems Diversity

ISSN 2519-8513 (Print)
ISSN 2520-2529 (Online)
Biosyst. Divers.,
2024, 32(2), 252–259
doi: 10.15421/012427

Chelated forms of trace elements improve antioxidant properties and nodulation potential of soybean-*Bradyrhizobium* symbiosis under insufficient water conditions

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Article info

Received 02.04.2024
Received in revised form 12.05.2024
Accepted 26.05.2024

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Nyzhnyk, T. P., Kots, S. Y., Pukhtaievych, P. P., Kots, T. A., & Vegera, L. V. (2024). Chelated forms of trace elements improve antioxidant properties and nodulation potential of soybean-Bradyrhizobium symbiosis under insufficient water conditions. Biosystems Diversity, 32(2), 252–259. doi:10.15421/012427

The development of new nanotechnological approaches and the appearance of preparations with low concentrations of microelements can serve as a basis for promising solutions aimed at increasing stress-protective properties and tolerance to the adverse factors effects. The aim of the study is to show the effectiveness of seed inoculation with nodule bacteria modified by chelate forms of trace elements germanium, ferrum and molybdenum to stimulate antioxidant properties and improve the functioning of the *Glycine max* (L.) Merr. – *Bradyrhizobium* symbiosis in insufficient water supply conditions. For this, the symbiotic systems of soybean with active virulent *Bradyrhizobium japonicum* B1-20 were used with the addition of chelated forms of ferrum, germanium and molybdenum in a dilution of 1:1000 to the culture medium. The chelator was citric acid. At the phenological stages during active nitrogen fixation by soybeans, two models of plant watering regimes were create optimal at the level of 60% of the full field capacity and insufficient/water stress at the level of 30% of the full field capacity. Microbiological, physiological, and biochemical methods of plant testing were used. It was found that the addition of rhizobia, chelated forms of germanium or ferrum to the culture medium, induces an increase in the antioxidant properties of plants by activating the key enzymatic complexes of superoxide dismutase and ascorbate peroxidase in soybean nodules and leaves under water stress. The use of chelated forms of ferrum or germanium led to the stimulation of the *Bradyrhizobium* nodulation potential, which was accompanied by the optimization of the water status and growth processes of soybean plants in insufficient moisture supply conditions. It was shown that inoculation with rhizobia containing chelated forms of molybdenum induced soybean plants sensitive to water deficit, as evidenced by an unstable reaction of enzyme activity, decrease or increase, in nodules and leaves. It inhibits nodulation processes on soybean roots and at the same time disrupts the water status of plants with insufficient water supply. It was concluded that the addition of chelated forms of germanium or ferrum to the rhizobia culture medium is a promising solution for stimulating the protective antioxidant properties of soybeans, which helps to optimize the physiological state of plants under insufficient water conditions.

Keywords: *Glycine max*; nodule bacteria; ascorbate peroxidase; superoxide dismutase; water potential; water stress.

Introduction

Modern nanotechnological approaches based on the use of biological preparations modified with growth-regulatory properties are promising for use in agriculture to improve the quality and productivity of leguminous crops with minimal damage to the environment (Shang et al., 2019; Liu et al., 2021; An et al., 2022). One of the nanotechnological approaches is the use of biologically active compounds containing trace elements in very low (nano) concentrations by treating seeds or spraying vegetative parts of the plant to stimulate growth and activate metabolic processes, which helps to increase plant productivity (Du et al., 2017). It was shown that metal microelements in very low concentrations, in accordance with the principle of hormesis, induce the activation of stress-protective reactions of the plant, which in turn causes the corresponding rearrangements of their metabolism and the development of tolerance to the action of stress factors (Kole et al., 2013; Du et al., 2015; Taran et al., 2016).

Currently, it has been proven that various forms of chelated forms of metals can have a specific effect on the course of biochemical processes in plant cells due to a similar principle of action with key antioxidant enzymes such as catalase, peroxidase, superoxidase (Wu et al., 2015). Their treatment of seeds or vegetative plants leads to the modulation of the activity of photosystem II by increasing the splitting of water and the release of oxygen, which improves the process of photosynthesis (Pradhan et al., 2013).

It has been proved that metal nanoparticles have increased reactivity and efficiency, and their use as micronutrient fertilizers contributes to the resistance of cultivated plants under adverse growing conditions by increasing the water-absorbing capacity of cells and the accumulation of protective compounds (Khot et al., 2012; Solanki et al., 2015; Prasad et al., 2017; Dimkpa et al., 2017; Abobatta, 2018). It has been shown that the treatment of seeds with metal nanoparticles increased the adaptation of plants to moisture deficiency in critical stages of plant growth and development, which increases their drought resistance (Chhipa & Joshi, 2016; Rahimi et al., 2016; Patra & Baek, 2017; Javaid et al., 2020).

Under modern conditions of soybean cultivation, it is important to address the issue of maximizing the nitrogen-fixing potential of the crop, (Kots et al., 2010; Schogolev & Raievska, 2021; Hlushach & Avksentieva, 2024), which can be achieved through the use of biological products based on effective inoculants modified with chelated forms of trace elements (Morgun et al., 2019; Morgun et al., 2021; Kots et al., 2021). In this direction, there are no unequivocal statements regarding the mechanism of influence of chelated forms of metals in combination with rhizobia on the development of pro-oxidant-antioxidant properties of soybean plants on the one hand and the realization of the nodulation potential of *Bradyrhizo-*

bium in symbiosis with soybean on the other hand. Such studies are relevant for evaluating the possibility of using chelated forms of trace elements to optimize the functioning of leguminous plants in symbiosis with rhizobia under the influence of climate changes induced by insufficient water. They are of theoretical importance because they will reveal the unknown mechanisms of inclusion of protective enzymatic reactions in soybeans in symbiosis with nodule bacteria under the action of water stress and after its action. At the same time, the use of chelated forms of trace elements in a complex with rhizobia will have practical application as a promising element in the cultivation of soybeans in areas with insufficient moisture supply induced by modern climate changes.

The aim of the study is to show the effectiveness of seed inoculation with nodule bacteria modified by chelate forms of trace elements germanium, ferrum and molybdenum to stimulate antioxidant properties and improve the functioning of the soybean-*Bradyrhizobium* symbiosis in insufficient water supply conditions.

The presented studies describe a holistic approach to the effective use of ecological sources of nitrogen in the soil-plant-atmosphere system under climate change; it demonstrates how nature-based environmental solutions can be combined with conventional elements of modern agricultural technologies.

Materials and methods

Conditions for plants growth. Soybean plants (*Glycine max* (L.) Merr.) of the Almaz variety inoculated with nodule bacteria (*B. japonicum*) Tn5 mutant B1-20, modified with chelated forms of trace elements ferrum, germanium and molybdenum, were used in the research. The effective Tn5 mutant of *B. japonicum* B1-20 has been deposited at the Institute of Microbiology and Virology named after Zabolotny National Academy of Sciences of Ukraine under registration number B-7538 (Kots & Vorobei, 2018).

Before sowing, soybean seeds were divided into different equal parts, each of which was inoculated with rhizobia suspensions containing chelated forms of trace elements germanium, ferrum or molybdenum. Separately, there was a part of the seeds that were inoculated with a pure bacterial culture without the content of microelement chelates, which was used as a control for the experiment.

The research was carried out in a pot experiment, where plants were grown on sterile sand with the addition of Hellrigel nutrient medium with 0.25 nitrogen rate. Two modes of plant watering were created – optimal / control with 60% of full field capacity (FC) and insufficient / water stress with 30% FC. The duration of water stress was 14 days in the phenological stages of soybean with active nitrogen fixation, i.e. from the beginning flowering and full bloom. The period of restoration of water supply to 60% FC was within 7 days in the stage of pod formation (Fig. 1). To conduct physiological and biochemical analysis, soybean nodules and leaves were selected in 6 replicates at the stages of beginning flowering, full bloom and pod formation.

Cultivation of nodule bacteria. Cultivation of nodule bacteria was carried out at 26–28 °C for 9 days on a mannitol-yeast medium with the addition of chelated forms of trace elements to ferrum, germanium and molybdenum in a 1:1000 dilution. The chelator was citric acid. Chelated forms of trace elements for our research were kindly provided by "Avatar" Research and Production Company LLC (Kyiv, Ukraine) (Kosinov & Kaplunenko, 2008; Kaplunenko & Kosinov, 2009).

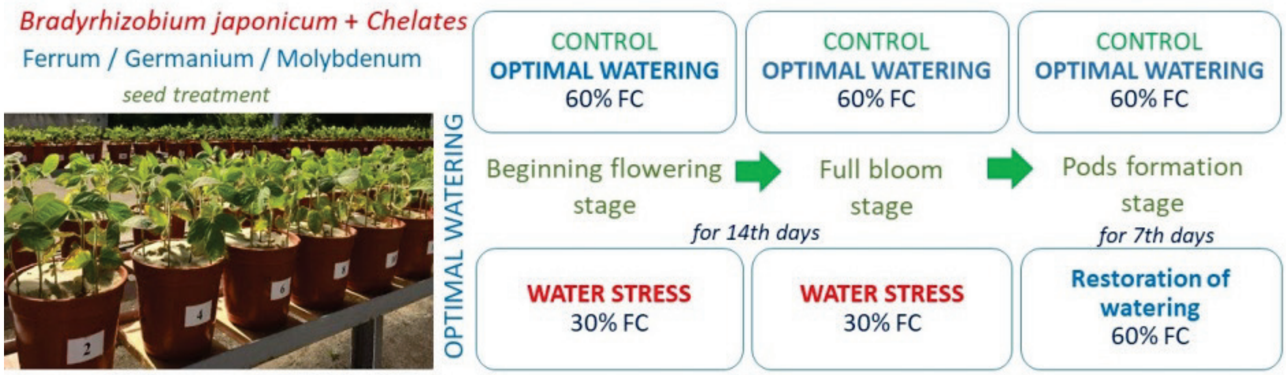

Fig. 1. Schematic representation of the experiment under optimal (60% full field capacity [FC]) and insufficient/water stress (30% FC) conditions of water supply

Erlenmeyer flasks containing 200 mL of culture medium were used for the cultivation of nodule bacteria, where the inoculant was introduced at a concentration of 2% of the total volume of the culture medium. The titer of nodular bacteria when added to the culture medium was 10^8 cells/mL.

Extraction of plant material to determine the activity of enzymes. It included the first step, which was its homogenization in a ratio of 1:2 with 50 mM phosphate buffer (pH 7.5) in a ratio of 1:2 containing 2 mM EDTA, 1 mM PMSF, 5 mM β-mercaptoethanol, and 1% polyvinylpyrrolidone; the second stage – centrifugation at 10,000 rpm for 20 min at 4 °C.

Determination of superoxide dismutase activity. The superoxide dismutase activity (EC 1.15.1.1) was determined according to its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Raychauhuri & Deng, 2000). The reaction mixture had the following composition: 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 μM riboflavin, 63 μM p-nitroblue tetrazolium, 0.1 mM EDTA, and 100 μL enzyme extract. The reaction proceeded for 15 min at a light intensity of 70 μmol quanta/(m^2 • s) when illuminated by fluorescent lamps with a power of 15 W. The optical density was measured using a double-scanning spectrophotometer "UV-1900" (Shimadzu, Japan) at 560 nm. The results are presented in units of enzyme activity (U) per mg of total soluble protein.

Determination of ascorbate peroxidase activity. Ascorbate peroxidase activity (EC 1.11.1.11) was measured by its ability to oxidize ascorbate (Nakano & Asada, 1981). The reaction was initiated by adding 50 μL of the supernatant to the reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.2 mM ascorbate, 0.1 mM H_2O_2. The course of the oxidation reaction was recorded for 2 min by the decrease in optical density at 290 with the help of a scanning two-beam spectrophotometer "UV-1900" (Shimadzu, Japan) (Nakano & Asada, 1981). The results are presented in μmol of oxidized ascorbate (ε = 2.8 $mM^{-1}cm^{-1}$) per mg of total soluble protein per minute.

Total soluble protein was measured based on the standard curve method by using bovine serum albumin (Bradford, 1976).

Nodulation potential of B. japonicum. To determine the nodulation ability of rhizobia, 20 typical plants of each experimental variant were selected and the roots were washed; then the nodules were separated and their average number and mass per plant were calculated, thus determining the nodule formation potential of the studied rhizobia. The results are presented as the number (pieces) and mass (g) of nodules per plant.

Leaf water potential. It was measured using different molar sucrose solutions, where 8–10 leaf disks of the same diameter were immersed. The samples were kept in the solutions for 40–60 minutes and shaken periodically. Two drops of the initial and then the corresponding test solution were applied to the refractometer prism with a glass rod. A solution is found whose concentration has not changed after the plant material samples have been in it (Xue et al., 2006).

Plant growth assessment. This was carried out by weighing the aboveground and underground (root) mass of plants during the stages of soybean ontogeny: beginning flowering, full bloom and pod formation. The results are presented per plant based on 8 plants per pot.

Statistical data analysis. All statistical analyses were performed using Statistica ver. 13.3 software package (StatSoft, 2011). The significance of differences in the physiological parameters was tested, and the results of the tests are presented in the Figures and Tables as x ± SD (mean ± standard deviation). The physiological and biochemical parameters were compared using the Kruskal-Wallis test (ANOVA) for the studied variants (*Rhizobium* vs. chelated forms of trace elements).

Results

Enzymatic reaction in soybean nodules and leaves. Under optimal conditions of soybean plants cultivation, an increase was recorded in superoxide dismutase activity in nodules formed by rhizobia with the addition of chelated forms of germanium by 54.0–77.6% in the full bloom and pod formation stages (Fig. 2a). In the variants with soybean inoculation with rhizobia modified with ferrum or molybdenum chelates, no significant differences in the levels of enzyme activity in nodules were found compared to the control (without chelates).

Water stress, the general dynamics of superoxide dismutase activity in soybean nodules was observed for all experimental variants – an increase in the beginning flowering stage and a decrease in the full bloom stage with the intensity of the stress factor (Fig. 2b). In soybeans inoculated with rhizobia containing ferrum chelates, a decrease in enzyme activity in nodules in the full bloom stage and under the influence of molybdenum chelate in the beginning flowering stage was detected compared to the control (rhizobia without chelates). In the variant with molybdenum chelate, under the influence of water stress, a decrease (beginning flowering stage) and an increase (full bloom stage) in the levels of superoxide dismutase activity in nodules were recorded compared to the control (Fig. 2b).

After resumption of watering in the stage of pod formation, the highest activity of the enzyme was recorded in the nodules of soybean variant without chelates, which was 3.20 times higher than its level in soybeans that were not exposed to stress (Fig. 2). After stress, in all variants of the experiment with soybean inoculation with rhizobia with chelated forms of trace elements, almost the same levels of superoxide dismutase activity were found in nodules, approaching the same level as in plants with optimal watering (Fig. 2).

Under optimal conditions of soybean cultivation from beginning of flowering to pod formation stage, a decrease of ascorbate peroxidase activity was observed in nodules inoculated with rhizobia containing chelated forms of ferrum and germanium compared to other experimental variants (Fig. 3a).

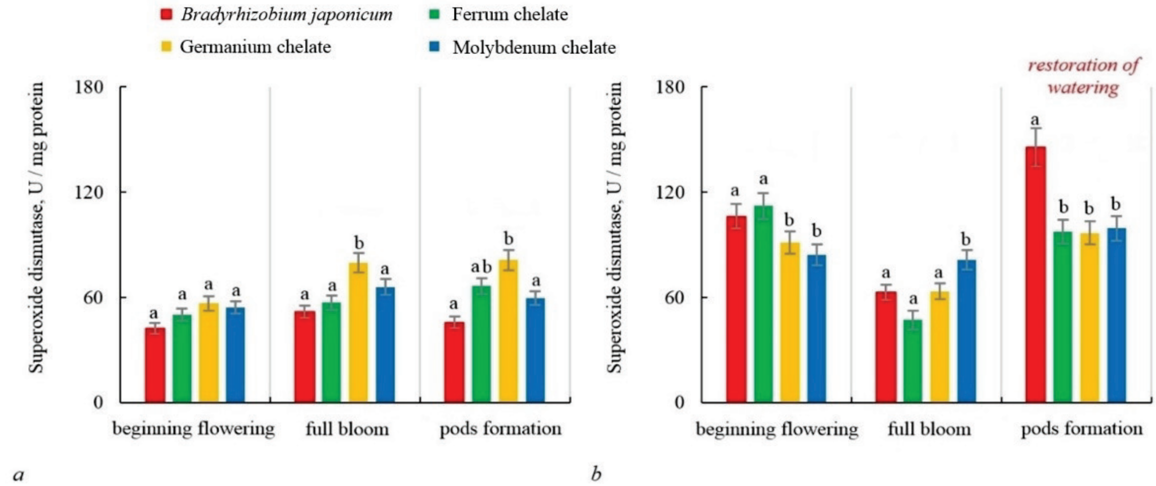

Fig. 2. Influence of soybean seeds inoculation with *B. japonicum* containing chelated trace elements on superoxide dismutase activity in soybean nodules under optimal (*a*) and insufficient (*b*) water supply (x ± SD, n = 6): letters indicate statistically significant differences between experimental variants for each phylogenetic stage according to Kruskal-Wallis test; data compared to the control (*Bradyrhizobium*) are significant *b* – P < 0.05, *bb* – P < 0.01; *a* – differences are not significant at P > 0.05

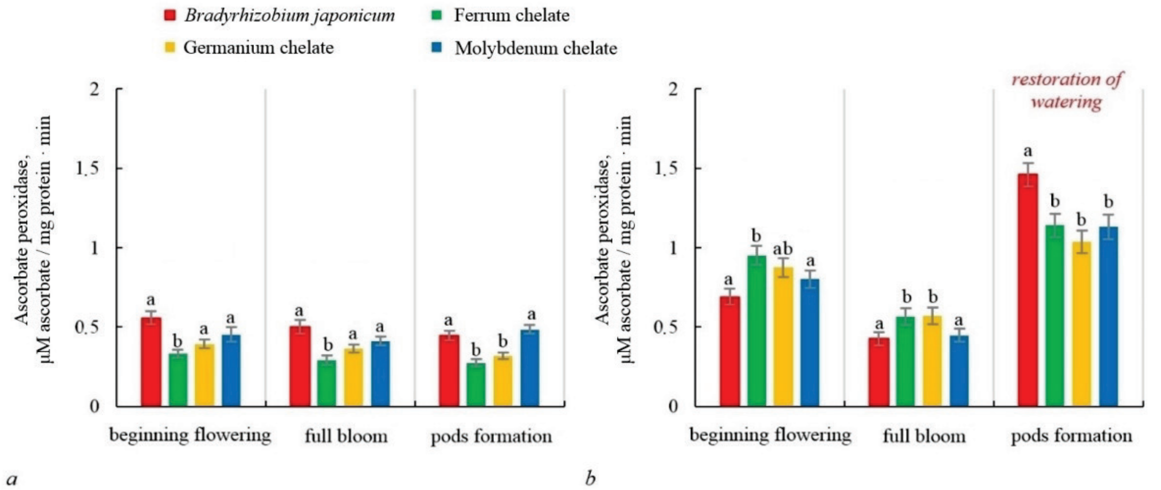

Fig. 3. Influence of soybean seeds inoculation with *B. japonicum* containing chelated trace elements on ascorbate peroxidase activity in soybean nodules under optimal (*a*) and insufficient (*b*) water supply (x ± SD, n = 6): letters indicate statistically significant differences between experimental variants for each phylogenetic stage according to Kruskal-Wallis test; data compared to the control (*Bradyrhizobium*) are significant *b* – P < 0.05, *bb* – P < 0.01; *a* – differences are not significant at P > 0.05

Water stress in the beginning of the flowering stage induced an increase in ascorbate peroxidase activity in soybean nodules of variants inoculated with rhizobia containing chelated forms of trace elements compared to the control (rhizobia) (Fig. 3b). The total level of ascorbate peroxidase activity in soybean nodules decreased under prolonged exposure to water stress in the full bloom stage compared to the beginning flowering stage. In the variants with ferrum and molybdenum chelates, higher (by 30%) levels of enzyme activity were found in nodules in the full bloom stage compared to other experimental variants (Fig. 3b).

After the restoration of watering, in the stage of pod formation, an intensification of ascorbate peroxidase activity levels in soybean nodules was recorded in all experimental variants. The highest activity of the enzyme was found in soybean variant without the use of chelated forms of metals (Fig. 3b). In the variants with chelated forms of trace elements, its activity in nodules was approximately the same after stress.

Under optimal conditions of water supply, a decrease in the levels of superoxide dismutase activity in the leaves of soybean variants with the addition of chelated forms of ferrum and germanium to the rhizobia inoculation suspension was recorded compared to the control (Fig. 4a). In soybeans inoculated with rhizobia containing molybdenum chelate, no significant differences in the levels of superoxide dismutase activity in leaves were found compared to the control.

Water stress during the beginning flowering of soybean plants, did not induce changes significant changes in the levels of superoxide dismutase activity in the leaves between the studied variants were recorded (Fig. 4b). Under a prolonged moisture deficit in the full bloom stage, an increase in the level of enzyme activity was observed in the leaves of soybean inoculated with rhizobia containing molybdenum chelate.

After the restoration of soybean watering in the stage of pod formation, approximately the same level of superoxide dismutase activity was observed in the leaves in all experimental variants, which approached the levels of enzyme activity in the leaves of plants that were not exposed to stress (Fig. 4b).

Under optimal water conditions, there were no significant differences between the experimental variants in the levels of ascorbate peroxidase activity in soybean leaves during the beginning of flowering and full bloom stages (Fig. 5a). In the stage of pod formation, compared to the previous stages of ontogeny, a general increase in the levels of enzyme activity in soybean leaves was shown, especially in variants with ferrum and germanium chelates.

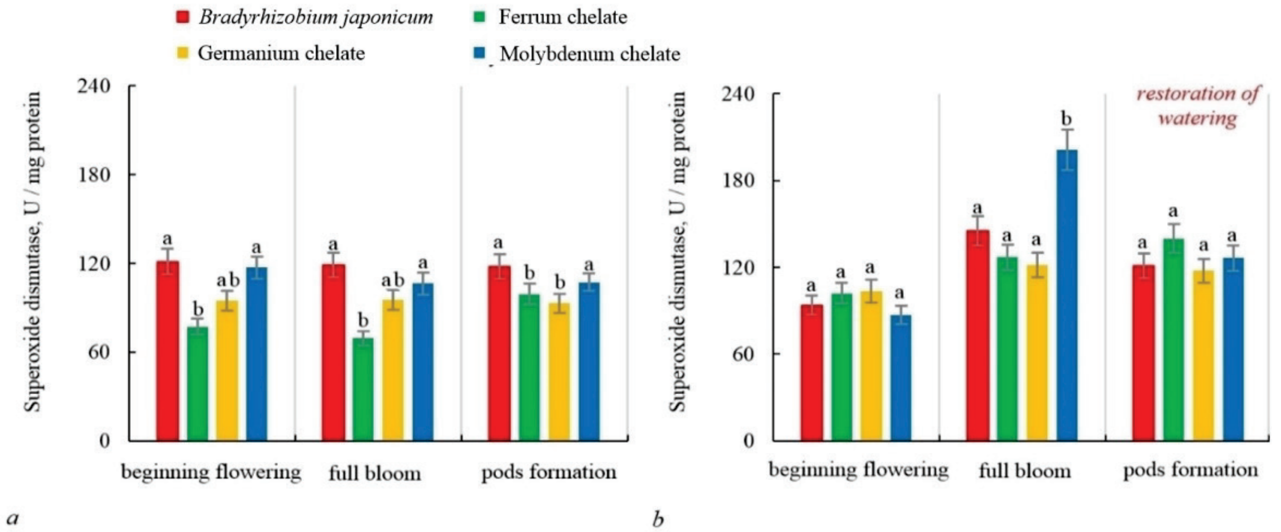

Fig. 4. Influence of soybean seeds inoculation with *B. japonicum* containing chelated trace elements on superoxide dismutase activity in soybean leaves under optimal (*a*) and insufficient (*b*) water supply (x ± SD, n = 6): letters indicate statistically significant differences between experimental variants for each phylogenetic stage according to Kruskal-Wallis test; data compared to the control (*Bradyrhizobium*) are significant *b* – P < 0.05, *bb* – P < 0.01; *a* – differences are not significant at P > 0.05

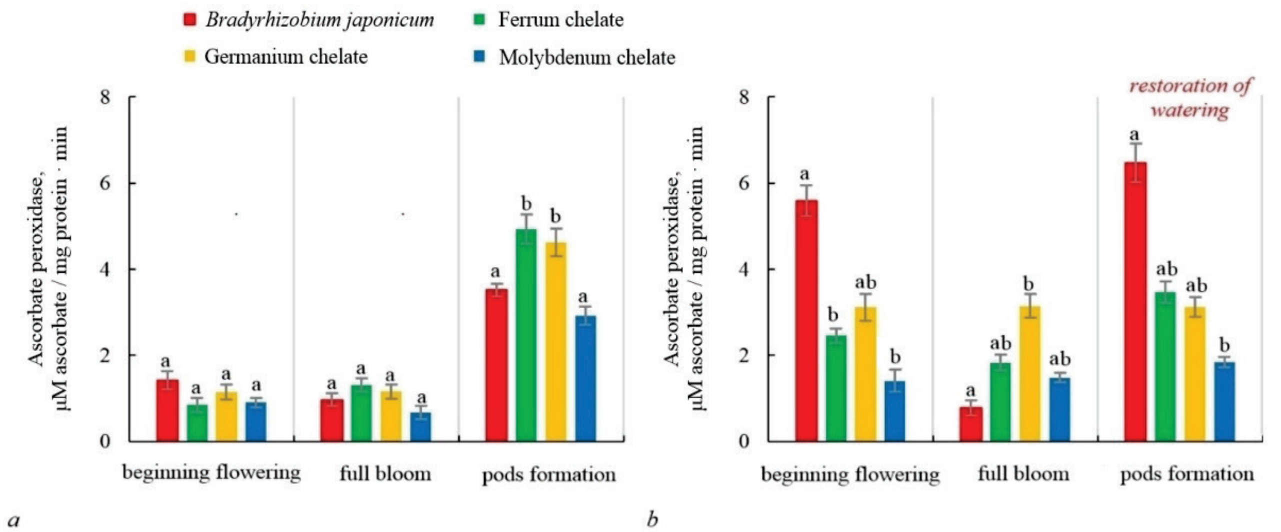

Fig. 5. Influence of soybean seeds inoculation with *B. japonicum* containing chelated trace elements on ascorbate peroxidase activity in soybean leaves under optimal (*a*) and insufficient (*b*) water supply (x ± SD, n = 6): letters indicate statistically significant differences between experimental variants for each phylogenetic stage according to Kruskal-Wallis test; data compared to the control (*Bradyrhizobium*) are significant *b* – P < 0.05, *bb* – P < 0.01; *a* – differences are not significant at P > 0.05

During water stress in soybeans inoculated with rhizobia without the use of chelates, unstable dynamics of ascorbate peroxidase levels in leaves was recorded, which was manifested by an increase (beginning of flowering stage) and decrease (full bloom stage) of the enzyme activity (Fig. 5b). In the variants with chelated forms of trace elements, a stable dynamic of ascorbate peroxidase activity in leaves was found during stress, the levels of which were lower in the beginning of flowering stage and higher in the full bloom stage only due to changes in the enzyme activity in the control.

After the restoration of watering in the pod formation stage, a significant increase in the activity of ascorbate peroxidase in soybean leaves of the variant without chelates was shown compared to other variants of the experiment (Fig. 5b). In soybeans inoculated with rhizobia containing chelates, no significant changes in the levels of enzyme activity in the post-stress period were recorded compared to their previous level of activity under water stress.

Physiological reaction of soybean plants. Under optimal conditions of water supply, there were no statistically significant changes in the value of leaf water potential between the experimental variants (Fig. 6). The effect of water stress led to a violation of the water status of plants in the variant without the use of chelates, as evidenced by a significant decrease in the value of leaf water potential, compared to soybean plants under optimal watering (Fig. 6). During the recovery period, higher indicators of water potential were found in soybean leaves of variants using ferrum chelates (32.1%) and germanium (33.6%) compared to the control (rhizobia). In the variant with molybdenum chelate, its value was at the level of the control (Fig. 6).

The positive effect of using a rhizobial suspension containing chelates of trace elements for seed inoculation on the vegetative mass of plants was established. In the experimental variants with the addition of chelated forms of metals to the inoculation suspension, an increase in the aboveground mass of plants in the beginning of flowering and full bloom stages, as well as in the underground mass of plants (root) in the stage of pod formation, was observed compared to soybean plants of the variant without chelates (Table 1). Among the studied variants, the most pronounced positive effect on the vegetative mass of plants under optimal growing conditions was recorded in the variant with soybean inoculation with rhizobia in combination with ferrum and germanium chelates (Table 1).

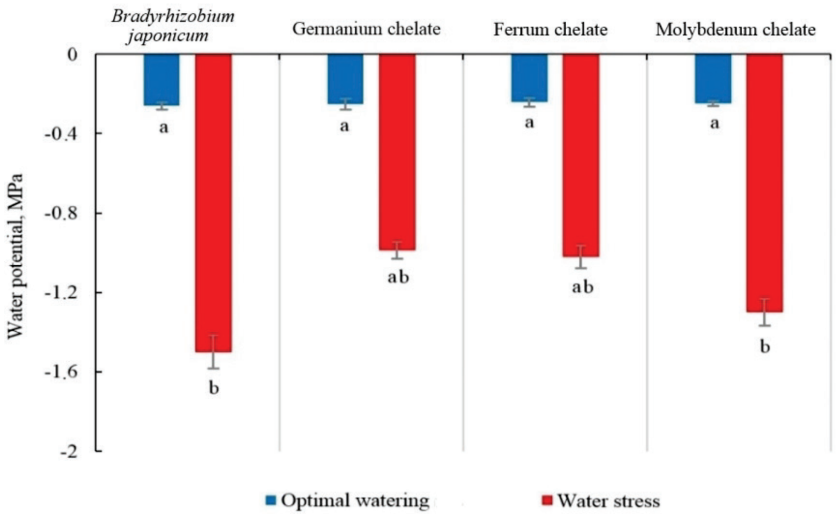

Fig. 6. Influence of soybean seeds inoculation with *B. japonicum* containing chelated trace elements on water potential in soybean leaves under different water supply (x ± SD, n = 8): summarized values of the indicator during the beginning of flowering, full bloom and pod formation stages are shown; letters indicate statistically significant differences between experimental variants according to Kruskal-Wallis test (ANOVA); data compared to the control (Rhizobium) are significant bb – P < 0.01, bbb – P < 0.001; a – differences are not significant at P > 0.05

Table 1
Effect of seeds inoculation with *B. japonicum* containing chelated trace elements on plants biomass under optimal water supply (x ± SD, n = 8)

Variant	Phenological stages					
	beginning of flowering		full bloom		pod formation	
	above-ground, g	root, g	above-ground, g	root, g	above-ground, g	root, g
B. japonicum	3.72 ± 0.22^{a}	3.07 ± 0.18^{a}	4.72 ± 0.36^{a}	3.97 ± 0.32^{a}	6.35 ± 0.51^{a}	2.92 ± 0.18^{a}
Ferrum chelate	4.25 ± 0.31^{a}	2.33 ± 0.12^{b}	6.15 ± 0.46^{b}	3.18 ± 0.21^{a}	6.73 ± 0.64^{a}	3.66 ± 0.24^{b}
Germanium chelate	4.44 ± 0.33^{b}	2.27 ± 0.13^{ab}	4.91 ± 0.34^{a}	2.77 ± 0.11^{b}	6.79 ± 0.61^{a}	3.81 ± 0.16^{b}
Molybdenum chelate	4.29 ± 0.38^{a}	1.89 ± 0.11^{b}	4.57 ± 0.31^{a}	2.09 ± 0.12^{b}	5.63 ± 0.26^{a}	4.37 ± 0.41^{b}

Note: letters indicate statistically significant differences between experimental variants within a column of the table according to Kruskal-Wallis test (ANOVA).

Insufficient water supply led to inhibition of the growth of vegetative mass of plants in the variant without the use of chelated forms of trace elements, compared to soybean plants under optimal watering (Table 2). In soybeans inoculated with rhizobia containing chelated forms of trace elements, an increase in these indicators was found compared to the control under dehydration conditions. The most significant effect on the vegetative mass of plants under water stress was found in the variant using germanium chelate, compared to other variants of the experiment (Table 2).

In the post-stress period, a more positive dynamic in the growth of the root system of plants was observed in the variants using seed inoculation with rhizobia containing chelated forms of trace elements, compared to plants of the variant without chelates (Table 2).

The use of rhizobia containing chelated forms of germanium or ferrum for seed inoculation under optimal water supply conditions led to stimulation of nodulation processes, which was manifested in the increased number and mass of nodules on soybean roots from the beginning of flowering stage to the pod formation (Table 3). When applying the inoculation suspension containing molybdenum chelate, no significant differences in the process of nodule formation on soybean roots were found compared to soybeans of the variant without the use of chelates (Table 3).

Water stress during the beginning of flowering stage induced inhibition of the nodulation process in soybean variant without chelates, as the number and mass of nodules was by 43.1% and 66.4% lower compared to the same indicators in soybean under optimal watering (Table 4). Under prolonged dehydration during the full bloom stage, an increase in the number of small nodules was recorded on the roots of soybean, the mass of which was 65.2% less compared to soybean nodules under optimal watering. A similar tendency of nodule formation on soybean roots under water stress was recorded in the variant with the complex use of rhizobia and molybdenum chelates for inoculation of soybean seeds (Table 4).

In the variants with seed inoculation with rhizobia containing chelated forms of ferrum or germanium trace elements, a significant increase in the number and mass of nodules on the roots under dehydration conditions was found compared to similar indicators in soybeans without the use of chelates (Table 4). In the post-stress period during pod formation stage in soybeans, activation of nodulation processes was observed, especially in the variant using chelated forms of germanium in the inoculation suspension (Table 4, Fig. 7).

Table 2
Effect of seeds inoculation with *B. japonicum* containing chelated trace elements on plants biomass under water stress (x ± SD, n = 8)

Variant	Phenological stages					
	beginning of flowering		full bloom		pod formation restoration of watering	
	above-ground	root	above-ground	root	above-ground	Root
B. japonicum	2.84 ± 0.18^{a}	1.61 ± 0.11a	2.52 ± 0.13^{a}	1.03 ± 0.07^{a}	3.19 ± 0.22^{a}	2.18 ± 0.12^{a}
Ferrum chelate	3.73 ± 0.41^{b}	2.34 ± 0.16^{b}	3.91 ± 0.31^{b}	1.86 ± 0.12^{b}	3.82 ± 0.38^{a}	3.51 ± 0.22^{b}
Germanium chelate	3.52 ± 0.16^{b}	2.14 ± 0.12^{b}	4.12 ± 0.34^{b}	2.17 ± 0.18^{b}	4.79 ± 0.39^{b}	3.23 ± 0.21^{b}
Molybdenum chelate	3.66 ± 0.26^{b}	2.04 ± 0.12^{b}	2.34 ± 0.16^{a}	1.49 ± 0.12^{a}	3.29 ± 0.28^{a}	4.12 ± 0.34^{b}

Note: see Table 1.

Table 3
Effect of seeds inoculation with *B. japonicum* containing chelated trace elements on nodule formation in soybean under optimal water supply (x ± SD, n = 8)

Variant	Phenological stages					
	beginning of flowering		full bloom		pod formation	
	number, pcs.	mass, g	number, pcs.	mass, g	number, pcs.	mass, g
B. japonicum	12.31 ± 0.83^{a}	0.238 ± 0.016^{a}	16.31 ± 1.14^{a}	0.328 ± 0.021^{a}	20.61 ± 1.14^{a}	0.399 ± 0.028^{a}
Ferrum chelate	24.02 ± 1.21^{b}	0.327 ± 0.023^{b}	25.02 ± 1.75^{b}	0.341 ± 0.024^{a}	33.62 ± 2.31^{b}	0.675 ± 0.047^{bb}
Germanium chelate	28.01 ± 1.26^{b}	0.371 ± 0.026^{b}	28.31 ± 2.19^{b}	0.403 ± 0.028^{b}	30.61 ± 1.41^{b}	0.481 ± 0.031^{b}
Molybdenum chelate	15.62 ± 0.91^{a}	0.198 ± 0.014^{a}	22.02 ± 1.43^{b}	0.263 ± 0.017^{a}	27.33 ± 1.81^{a}	0.429 ± 0.028^{b}

Note: see Table 1.

Table 4
Effect of seeds inoculation with *B. japonicum* containing chelated trace elements on nodule formation in soybean under water stress (x ± SD, n = 8)

Variant	Phenological stages					
	beginning flowering		full bloom		pod formation restoration of watering	
	number, pcs.	mass, g	number, pcs.	mass, g	number, pcs.	mass, g
B. japonicum	7.04 ± 0.41^{a}	0.081 ± 0.005^{a}	14.04 ± 0.87^{a}	0.114 ± 0.006^{a}	16.61 ± 0.72^{a}	0.171 ± 0.012^{a}
Ferrum chelate	13.02 ± 0.86^{b}	0.238 ± 0.017^{b}	18.02 ± 1.12^{a}	0.334 ± 0.021^{b}	29.32 ± 2.01^{b}	0.331 ± 0.024^{b}
Germanium chelate	31.31 ± 2.32^{bbb}	0.204 ± 0.013^{b}	36.03 ± 2.31^{b}	0.337 ± 0.024^{b}	34.33 ± 2.32^{b}	0.506 ± 0.032^{bb}
Molybdenum chelate	11.31 ± 0.72^{a}	0.137 ± 0.008^{a}	16.31 ± 1.14^{a}	0.115 ± 0.008^{a}	30.04 ± 2.14^{b}	0.291 ± 0.021^{b}

Note: see Table 1.

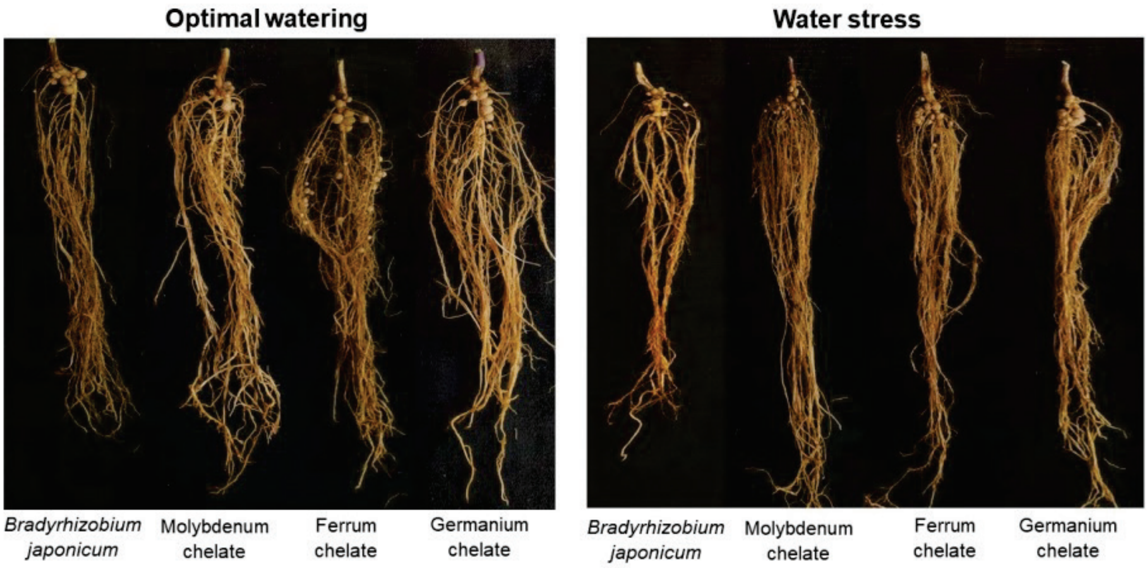

Fig. 7. Formation of nodules on soybean roots during the recovery period after water stress

Discussion

Modern nanotechnological approaches are promising in the study of legume-rhizobial symbiosis and involve the use of effective strains of nodule bacteria in a complex with chelated forms of trace elements to improve the tolerance of soybean plants in conditions of water shortage induced by climate change (Morgun et al., 2019; Morgun et al., 2020; Kots et al., 2022). Their use in agricultural production will help to provide plants with nitrogen and essential microelements in an accessible form. However, such studies are few and far between and require more detailed research for widespread implementation in production.

It was shown that inoculation of seeds with rhizobia modified chelated forms of trace elements stimulates the activity of superoxide dismutase in nodules and at the same time reduces its level in soybean leaves under optimal plant watering. This tendency of the enzyme activity was most pronounced in soybean nodules when using inoculation with rhizobia in combination with germanium chelate, and in leaves – with ferrum chelate. This proves that as a result of the complex action of inoculation with rhizobia containing chelated forms of trace elements, changes in the level of the enzyme occur even under optimal plant growing conditions.

The general dynamics of the superoxide dismutase level in soybean nodules during water stress action for all variants were recorded – activation of levels at the initial stages of dehydration in the beginning of the flowering stage and its decrease during prolonged stress in the full bloom stage. In the leaves, on the contrary, a significant intensification of superoxide dismutase activity levels was found under prolonged exposure to the stress factor induced by water shortage.

The recorded changes in the activity levels of superoxide dismutase in soybean nodules, as well as in plant leaves under water stress, may indicate that the enzyme is involved in the neutralisation of excessive amounts of superoxide anion radicals produced in cells. The decrease in enzyme activity levels in soybean nodules under prolonged exposure to water stress may indicate the depletion of the enzyme pool due to its enhanced functioning under significant development of oxidative stress.

The greatest amplitude of the superoxide dismutase reaction in nodules (increase and decrease) during water stress was recorded in the variants with inoculation of rhizobia together with ferrum chelate, as well as in the variant without the use of chelates. The use of molybdenum chelate with rhizobia showed the highest level of superoxide dismutase in soybean leaves under long-term dehydration conditions. When germanium chelate was added to the inoculation suspension, the smallest changes in enzyme levels were observed in soybean nodules and leaves under water stress. During the recovery period during the pod formation stages in soybeans, the restoration of superoxide dismutase levels in nodules and leaves to the optimum was recorded when rhizobia modified with trace elements were used.

The observed dynamics of superoxide dismutase activity in nodules and leaves is due to the degree and duration of the stress factor (water deficit), as well as the sensitivity of soybean-rhizobial symbiosis to water stress. It is known that the activity of superoxide dismutase can change depending on the course of metabolic processes in plant cells under the influence of stress factors (Laxa, 2019). A decrease in the ascorbate peroxidase level in nodules formed by rhizobia containing chelated forms of trace elements was recorded under optimal soybean irrigation. At the same time, activation of enzyme levels in soybean leaves during full bloom and pod formation stages were observed in variants with rhizobia inoculation and chelated forms of ferrum or germanium.

The general tendency of changes in the levels of ascorbate peroxidase activity in soybean nodules and leaves in all experimental variants under water stress was revealed – an increase in the beginning of flowering stage and a decrease in the full bloom stage of soybean, as well as intensification of the enzyme activity levels in nodules after the restoration of plant watering. The most pronounced changes in enzyme activity in soybean nodules and leaves in response to water supply conditions were observed in variants with inoculation of rhizobia containing molybdenum chelate and without the use of chelates. In the variant without chelates, very unstable changes in ascorbate peroxidase activity in leaves were found, which were manifested in the intensification and decrease of the enzyme activity during stress, as well as in the rapid increase of the enzyme activity after stress. This indicates significant disruption of the functioning of the ascorbate peroxidase enzyme complex induced by excessive development of oxidative processes in plant due to dehydration.

We found that the activity of ascorbate peroxidase in leaves was most sensitive to water supply conditions at the period of pod formation in soybeans. At the same time, activation of the general level of enzyme activity in soybean leaves was observed in all variants of the experiment. From one side, this could be due to ontogenetic changes in plant metabolism, due to the gradual transition to the formation of a generative organ – the seed. From the other side, it could be due to the needs of plants for moisture during this reproductive period, growth and development of soybeans, which increase to a critical level and account for about 60–70% of the total amount of water for the entire growing season (Gaonkar & Rosentrater, 2019). Deficiency in water supply of plants induces significant changes in physiological and biochemical processes, which are accompanied by activation of enzymatic systems of antioxidant protection.

The highest levels of activity of the studied enzymatic complexes, especially in plant leaves, were found under the conditions of water supply restoration in the stage of pod formation. In our opinion, this is primarily due to the sensitivity of the photosynthetic apparatus of plants to moisture conditions. It was established that the violation of its effective functioning in case of lack of water occurs as a result of an imbalance in the transport of electrons and excessive production of reactive oxygen species (Hasanuzzaman et al., 2019, 2020). Increasing the level of reactive oxygen species in cells is important in inducing signaling systems that stimulate antioxidant defence systems (Xie et al., 2019), the major enzymes of which are superoxide dismutase and ascorbate peroxidase. Therefore, the effective inclusion of enzymatic antioxidant systems under conditions of water stress is necessary to maintain the pro-oxidant-antioxidant balance for the optimal functioning of the soybean-*Bradyrhizobium* symbiosis.

Water stress caused a violation of the water status and inhibition of biomass growth of soybean plants in the case when trace element chelates were not used in the inoculation suspension. However, the use of seed inoculation with rhizobia in combination with chelated forms of trace elements contributed to the preservation of plant water potential and had a positive effect on the growth of the aboveground mass and root system of plants under prolonged water stress. Among the studied variants in soybeans inoculated with rhizobia containing germanium chelate, the preservation of the water status of plants by the indicator of leaf water potential under water stress was noted. In turn, this could have a positive effect on the complex of processes that ensure the maintenance of plant metabolism, their growth and development under conditions of water imbalance.

In the variants using seed inoculation with rhizobia containing chelated forms of ferrum or molybdenum, there is also a positive trend in the growth of vegetative mass of plants after the restoration of soybean watering in the stage of pod formation. However, in these experimental variants (chelated forms of ferrum and molybdenum), soybean plants were more sensitive to water supply conditions compared to variant with germanium chelate.

According to the intensity of vegetative mass growth of plants under the influence of stress factors, including insufficient water supply, it can be concluded that the self-regulation processes of the plant organism develop, including its ability to restructure metabolic processes and adapt to growing conditions (Mamenko, 2021; Naeem et al., 2024). It is known that the degree of development of the plant's vegetative mass depends on the supply of a whole range of plastic substances that are important for the formation of reproductive organs and the next harvest (Kots et al., 2010; Mamenko, 2019).

The addition of chelated forms of germanium or ferrum to the inoculation suspension contributed to the increase of nodulation processes under different moisture conditions, which was manifested in the increase in the number and mass of nodules on soybean roots from the beginning of flowering stage to pod formation. There was no positive effect of inoculation of seeds with rhizobia containing chelated forms of molybdenum on the formation of soybean symbiotic apparatus under optimal and insufficient water supply conditions. This was manifested in the absence of significant changes in the growth of the number and mass of nodules on the roots of soybean inoculated with rhizobia containing chelated forms of molybdenum.

Conclusion

The use of rhizobia with chelated forms of germanium or ferrum for seed inoculation induces the activation of key enzymatic complexes of superoxide dismutase and ascorbate peroxidase in leaves and nodules, which helps to optimise the water status and growth processes of plants, and also has a positive effect on the nodulation ability of rhizobia under water stress.

Soybean plants inoculated with rhizobia modified with a chelated form of molybdenum were sensitive to moisture deficiency, manifested by an unstable response, activation or reduction, of enzymes in nodules and leaves, and inhibition of plant growth and nodulation processes.

The addition of chelated forms of germanium or ferrum metals in a dilution of 1:1000 to the rhizobia culture medium is a promising solution for stimulating the protective antioxidant properties of soybeans, which helps to optimize the physiological state of plants under insufficient water conditions.

The research was carried out within the framework of the state budget topic "To study the features of the formation and functioning of soybean and alfalfa symbiotic systems under the influence of external factors" No. 0121U107432, which was funded by the National Academy of Sciences of Ukraine.

The authors declare no conflict of interest.

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