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EFFECT OF SALINITY ON THE SOYBEAN PLANTS SYMBIOTIC AND PHOTOSYNTHETIC APPARATUSES ACTIVITY UNDER RHIZOBIA INOCULATION WITH THE ADDITION OF Ge AND Mo NANOCARBOXYLATES

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Background. Soybean is one of the main oil and protein crops in the world, which occupies the largest cultivated area among legumes. However, adverse growing conditions, particularly salinity, can cause significant yield losses. It is known that salt stress affects morphological indices and physiological processes in soybean plants. Given the fact that the main factors contributing to the soybean-rhizobial symbiosis productivity are nitrogen fixation and CO_2 assimilation, it is important to find ways to optimize these processes, in particular under salinity conditions.

Materials and Methods. The research was conducted on symbiotic systems created with the participation of soybean plants (*Glycine max* (L.) Merr.) of the Samorodok variety and nodule bacteria *Bradyrhizobium japonicum* RS08 strain, cultivated with the addition of Ge and Mo nanocarboxylates.

Results. It was revealed that salinity inhibited the activity of the symbiotic and photosynthetic apparatuses in soybean. With that, the degree of the symbiotic apparatus activity suppression under salinity conditions decreased over time. The addition of Ge nanocarboxylate to the inoculation suspension had a positive effect on the net CO_2 assimilation rate in plant leaves under salinity at the bean-filling stage, and Mo – at all studied development stages. The transpiration rate closely correlated with the net assimilation rate, although the degree of its suppression by salinity was much less than that of photosynthesis. Positive correlations were found between nitrogen-fixing activity and the calculated net CO_2 assimilation rate by the whole plant. A close positive correlation was found between the net assimilation rate at the bean filling stage and the grain productivity of soybean plants. At the same time, under inoculation with a suspension of rhizobia with the addition of Mo nanocarboxylate, the weight of the grain from the plant was the largest both among the control plants (without salinity) and among the salinity treatments.

Conclusions. The obtained results indicate that, according to all investigated physiological parameters, inoculation of soybean seeds with *Bradyrhizobium japonicum* RS08 strain with the addition of Mo nanocarboxylate was the most effective in maintaining the activity of the symbiotic and photosynthetic apparatuses and, ultimately, grain productivity of plants both under normal conditions and under salinity.

Keywords: *Glycine max* (L.) Merr., *Bradyrhizobium japonicum*, salinity, nitrogen-fixing activity, photosynthesis, metal nanocarboxylates, productivity

INTRODUCTION

In terms of its importance, soybean occupies a special place among legumes, as it is one of the main oil and protein crops in the world. For Ukraine, this is a profitable export-oriented culture, which every year maintains its position in crops areas.

Under optimal conditions, soybean productivity is about 4 t/ha (Langemeier, 2024), but unfavorable growing conditions, in particular soil salinity, can cause yield reduction up to 50% or its complete loss (Ilangumaran *et al.*, 2021; Rasheed *et al.*, 2022). Under salinization, the quality and quantity of free amino acids, protein, oil, sucrose, and starch in soybean grain decrease (Do *et al.*, 2018; Nadeem *et al.*, 2019).

Salt stress significantly affects morphological indices and physiological processes in leguminous plants, including soybean (Mykhalkiv *et al.*, 2023). Its negative effect on seed germination, seedling emergence, growth, leaf length, plant height and weight, number of nodules in soybean was noted (Shu *et al.*, 2017; Kataria *et al.*, 2019). Elevated salt concentrations in the root zone lead to hyperosmotic and hyperionic conditions that reduce the uptake of water and nutrients, causing osmotic stress (van Zelm *et al.*, 2020). High concentrations of Na^+ and Cl^- ions entering the above-ground part of plants and getting into the leaves cause the disruption of cytoplasm ion homeostasis and metabolic processes, which ultimately inhibits the process of photosynthesis and reduces soybean productivity (Yang & Guo, 2018; Rasheed *et al.*, 2022; Xu *et al.*, 2023). Ionic and osmotic stresses, in turn, lead to secondary stresses: an imbalance of nutrients in the cell and the accumulation of toxic compounds that can be destructive to enzymes, DNA, and lipids (Ullah *et al.*, 2019; Dawood *et al.*, 2022).

Pre-sowing inoculation with highly effective nodule bacteria strains to optimize the formation and functioning of soybean-rhizobial symbiosis is a mandatory component of modern agrotechnologies of soybean cultivation. Given the fact that nitrogen fixation and CO_2 assimilation are the main components of leguminous plant productivity, it is important to find ways to optimize these processes not only under normal, but also under stress conditions, in particular salinity. It has been shown that the process of symbiotic nitrogen fixation is more sensitive to salinity than the process of photosynthesis (Otie *et al.*, 2021). Therefore, it is the use of effective microbial preparations that is one of the main ways of increasing the resistance of legumes and their productivity under the impact of this factor.

The creation of new combined forms of microbial preparations, which, in addition to the bacterial component, contain other factors that increase the effectiveness of leguminous-rhizobial interaction, such as microelements, is relevant today. It was shown that the use of nanocarboxylates of Mo, Fe, and Ge as components of the rhizobia cultivation medium to obtain an inoculant stimulates the nitrogen-fixing activity of symbiotic system and the photosynthetic rate in soybean leaves (Morgun *et al.*, 2019). Pre-sowing treatment of soybean seeds with rhizobia cultured with Ge and Fe nanocarboxylates mitigated the negative effects of drought on nitrogen fixation and CO_2 assimilation, and also promoted their recovery after stressor removal (Kiriziy *et al.*, 2022). An increase in the chlorophylls and carotenoids contents under the drought with the use of germanium nanocarboxylate as part of the inoculation suspension was revealed, and a conclusion was made about the ability of this compound as a suspension component to ensure a high level of the soybean pigment system protective mechanisms activity against water deficit (Kots *et al.*, 2022).

Therefore, the aim of this work was to study the effect of germanium and molybdenum nanocarboxylates as components of *Bradyrhizobium japonicum* inoculation suspension on the effectiveness of soybean-rhizobial symbiosis functioning – nitrogen-fixing activity, CO_2 assimilation and transpiration processes, and grain productivity of plants under salinity conditions.

MATERIALS AND METHODS

The research was carried out on symbiotic systems created with soybean plants (*Glycine max* (L.) Merr.) of the Samorodok variety (selection of the Institute of Feed Research and Agriculture of Podillya, National Academy of Agrarian Sciences of Ukraine) and nodule bacteria *Bradyrhizobium japonicum* of the highly active RS08 industrial strain obtained from the nitrogen-fixing microorganisms museum collection of the Department of Symbiotic Nitrogen Fixation of the Institute of Plant Physiology and Genetics NAS of Ukraine.

The rhizobia cultures were grown at 26–28 °C on the Yeast Extract Mannitol (YEM) media (g/L): KH_2PO_4 – 0.5, $MgSO_4$ – 0.2, NaCl – 0.1, yeast extract – 1.0, mannitol – 10.0) for 7 days, and inoculants were prepared the bacterial titer of which was 10^8 cells/mL. Cultivation of bacteria was carried out by the method of periodic incubation on circular rockers in Erlenmeyer flasks containing 200 mL of YEM media, with a rotation speed of the rocker of 220 rpm. In experimental treatments, solutions of Ge or Mo nanocarboxylates were introduced into the medium for growing rhizobia in dilutions of 0.001 and 0.005% respectively. These microelements were chosen for experiments because it is known that Ge exhibits antioxidant properties in biological systems (Liu *et al.*, 2016). Mo is an essential micronutrient that serves a crucial role in nitrogen metabolism as a cofactor for nitrate reductase and nitrogenase (Cakmak *et al.*, 2023). In legumes, Mo is particularly important for atmospheric nitrogen fixation, as it directly affects nitrogenase functioning in root nodules (Yang *et al.*, 2020).

The microelement preparations used were kindly provided by the Scientific and Production Company ”AVATAR” LLC (Ukraine, Kyiv). They were obtained in two stages: 1 – obtaining an aqueous colloidal solution of nanoparticles by dispersing granules of the corresponding highly purified metal with pulses of electric current in deionized water; 2 – production of metal carboxylate by the reaction of direct interaction of the obtained nanoparticles with citric acid.

In our previous studies on the effect of Ge and Mo nanocarboxylates on the growth of rhizobia in pure culture, the effect of concentrations of these drugs in dilutions of 0.001 and 0.005% was studied (Kots *et al.*, 2018). It was shown that for Ge, the optimal dilution for culture growth was 0.001%, and for Mo, the difference between these two concentrations was insignificant. Therefore, taking into account that Mo is necessary for the functioning of the key nitrogen fixation enzyme – nitrogenase, we chose the concentration of 0.005% for seed inoculation by this drug.

Soybean seeds were sterilized for 15 min with 70% ethanol, and washed under running water for 1 h. Before sowing, the seeds were inoculated with suspensions of nodule bacteria *B. japonicum* RS08 without or with the addition of metal nanocarboxylates for 1 hour, and sown in the substrate.

Soybean plants were grown in pots pre-sterilized with a 20% H_2O_2 solution, on washed river sand (10 kg per pot, 7 plants per pot), under natural lighting and optimal moisture (70% of full moisture content, FC). The source of mineral nutrition was Hellriegel's mixture with 0.25 of nitrogen rate (the full nitrogen rate is 0.708 g of $Ca(NO_3)_2$ per 1 kg of substrate), to which microelements were added. The treatments were replicated ten times.

In order to create salinity in the pots of the corresponding treatments, NaCl was added at the rate of 0.25 g/kg of sand at the preparation of the substrate, which corresponded to a salinity level of 30 mM at a substrate humidity of 70% FC. Based on the literature, this level of salinity for soybean can be considered quite moderate. Thus, L. Chen *et al*. (2024) studied the effect of 100 mM salinity on seedlings of 33 soybean genotypes. It was shown that all genotypes could grow at this level of salinity, although the degree of growth inhibition varied greatly between genotypes. However, these studies were conducted on uninoculated plants without taking into account the negative effect of salinity on rhizobia and the formation of a symbiotic apparatus. Therefore, we chose a salinity level of 30 mM to allow the formation of a functionally active symbiotic apparatus.

The scheme of experiment included the following treatments: 1) *B. japonicum* RS08 (intact rhizobia); 2) *B. japonicum* RS08 + Ge; 3) *B. japonicum* RS08 + Mo; 4) *B. japonicum* RS08, NaCl; 5) *B. japonicum* RS08 + Ge, NaCl; 6) *B. japonicum* RS08 + Mo, NaCl. When analyzing the results of each inoculation treatment, the treatment without salinization was considered as the control.

Indices of symbiotic effectiveness and growth parameters were determined at the stages of budding, bean formation, and bean filling. Grain productivity was determined at full maturity. Biometric indices (the total and specific leaf weight, and grain weight) were determined in 5 replications. Nitrogen-fixing activity (NFA) of root nodules was determined in 4 replications. The net CO_2 assimilation (A_N) and transpiration (T) rates were determined in 3 replications.

NFA was determined at the stages of budding and bean formation by the level of nodules acetylene-reducing activity by the acetylene method (Hardy *et al.*, 1968), and was expressed as the amount of ethylene (μmol) produced by the nodules of an individual plant within a one-hour incubation period. The gas mixture was analyzed on an Agilent Technologies 6850 Network GC System gas chromatograph (USA) with a flame ionization detector, on a column (Supelco Porapak N) at a thermostat temperature of +55 °C and a detector temperature of +150 °C. The carrier gas was helium (20 mL/min). The sample volume of the gas mixture was 1 cm^3. Pure ethylene (Sigma-Aldrich, USA) was used as a standard.

The A_N and T were recorded under controlled conditions with the EGM-5 gas analyzer (PP Systems, USA) at the stages of budding, bean formation, and bean filling. The middle part of the third from the top intact leaf was placed in a temperature-controlled chamber (25 °C) and illuminated by a TA-11 LED spotlight (50 W, color temperature 5200 K).

The illumination at the chamber level was 1500 µmol/($m^2 \cdot s$) of photosynthetically active radiation. Conditioned atmospheric air with humidity about 10 mbar was blown through the chamber at a rate of 1 L/min. Gas exchange parameters were calculated according to conventional methods (Busch *et al.*, 2024).

At the stages of budding and bean formation, the total weight of leaf plates from the whole plant, and the weight per unit area of the leaf surface (specific leaf weight, SLW) by weighing cuttings of a known area were determined.

All statistical analyses were performed using STATISTICA ver. 7.0 software package. Significance of differences between samples was evaluated by one-way analysis of variance (ANOVA), where differences were considered significant at $P < 0.05$ using Tukey's test. In the table and graphs arithmetic means and their standard errors (x±SE) are presented.

RESULTS AND DISCUSSION

Determination of NFA of soybean plants revealed that it was almost an order of magnitude higher at the stage of bean formation than at the budding stage (**Fig. 1**). This is explained by the rapid development of the symbiotic apparatus within the period between these stages, and the increase in its power. Treatment of rhizobia with Ge nanocarboxylate significantly increased this index in the control plants (without salinization) compared to plants inoculated with intact rhizobia and with Mo nanocarboxylate at both studied stages. Salinization led to a significant decrease in NFA at the budding stage compared to control indices in all inoculation treatments. At the stage of bean formation, a significant difference with control plants was found only in the treatment with rhizobia with Ge nanocarboxylate, while in the inoculation treatments with intact rhizobia and ones with Mo nanocarboxylate, the difference with control plants was insignificant.

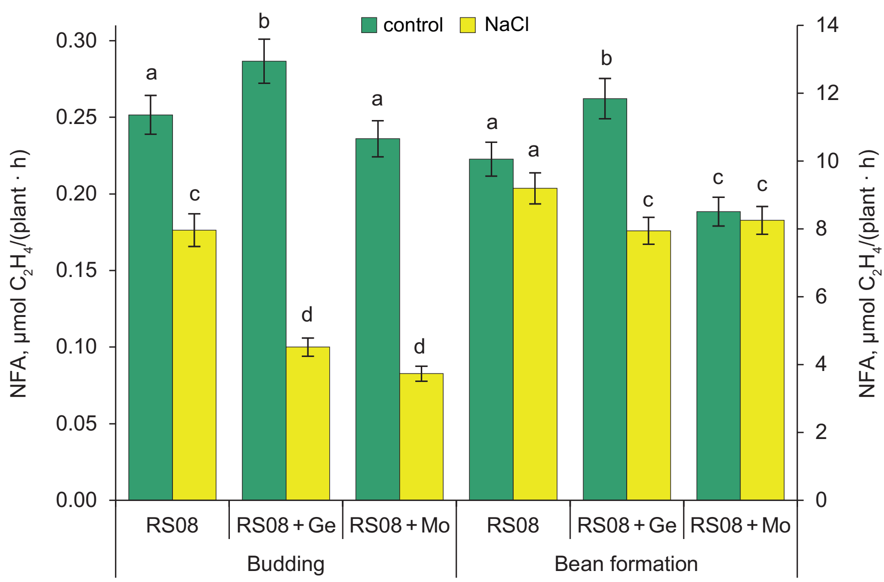

Fig. 1. Nitrogen-fixing activity (NFA) of soybean plants inoculated with intact rhizobia and with the addition of Ge and Mo nanocarboxylates under normal conditions (control) and salinity (30 mM NaCl) (different letters indicate significant differences among treatments at the same developmental stage, as determined by Tukey's post-hoc test following a one-way ANOVA ($P < 0.05$)) (x±SE; n = 4)

At the same time, one cannot fail to note a rather unexpected result: under salinity, the NFA of plants inoculated with rhizobia with Ge and Mo nanocarboxylates was lower than under inoculation with intact rhizobia at both investigated stages of soybean plants development. It can be assumed that treatment of rhizobia with metal nanocarboxylates has a stimulating effect and activates their vital activity. Thus, in our previous study of the direct influence of Ge and Mo nanocarboxylates, a stimulating effect on the growth of rhizobia in pure culture was revealed (Kots *et al.*, 2018). Under normal conditions, this influence further contributes to the formation of an active symbiotic apparatus, which can be seen in the example of control plants inoculated with rhizobia with Ge nanocarboxylate. However, under salinity conditions, this may be undesirable, since the activation of metabolic processes increases their vulnerability to stressors and leads to their inhibition compared to intact rhizobia.

This assumption is indirectly confirmed by the decrease over time in the suppressive effect of salinity on the symbiotic apparatus of plants inoculated with rhizobia with metal nanocarboxylates (see **Fig. 1**). Thus, if at the budding stage under salinization, in the latter cases NFA was 47–57% compared to inoculation with intact rhizobia, at the stage of bean formation it was 86–90%. At the same time, at this stage, in the treatment with inoculation with rhizobia and Mo nanocarboxylate, the difference with control plants was insignificant, as well as under inoculation with intact rhizobia, which indicates the adaptation of the symbiotic apparatus of plants to salinity conditions.

According to the literature data, soybean plants show quite wide genotypic variability in salinity tolerance. The level of this stressor used in our experiments (30 mM) refers rather to the lower part of the range of their salt tolerance (Zhou *et al.*, 2023). Therefore, when studying the formation and functioning of the symbiotic apparatus under salinity, the possibility of different degrees of its influence both directly on the micro- and actually on the macrosymbiont should be taken into account. This especially applies to studies of the photosynthetic apparatus activity, since, on the one hand, its functioning directly depends on the supply of plants with nitrogen, and on the other hand, on the conditions of the root system functioning, including soil salinity.

In our opinion, this can explain some differences in the results of measurements of the soybean leaves A_N from that of plants NFA (**Fig. 2**). It should be noted that **Fig. 2** shows the data on the specific CO_2 assimilation activity of the photosynthetic apparatus per unit area of the leaf surface, and not on the whole plant (we will return to this issue later).

Incorporation of Mo nanocarboxylate into the inoculation suspension was found to have a positive effect on the plants photosynthetic apparatus adaptation to salinity – in this case, the difference with the control plants in the A_N was insignificant at all measurements. At the same time, under salinity, in the treatments with inoculation with intact rhizobia, and with the addition of Ge nanocarboxylate, a significant decrease of this index was observed compared to the control plants at the stages of budding and bean formation. Also, at these stages, the A_N in leaves of plants inoculated with rhizobia with Ge nanocarboxylate was the lowest among the studied treatments. However, at the stage of bean filling, under salinity the A_N in plants of this treatment was equal to the control and the treatment with rhizobia with Mo nanocarboxylate, whereas the lowest A_N was observed in the treatment with inoculation with intact rhizobia.

Differences in the response of symbiotic and photosynthetic apparatuses to the inoculation of soybean plants with a suspension of rhizobia with the addition of metal nanocarboxylates can be explained by the direct effect of the latter on the seeds. It is known that such preparations differ from ordinary inorganic salts of microelements by high penetration into cells and biological activity (Kolbert *et al.*, 2022).

There is evidence in the literature that Mo has a positive effect on physiological processes in soybean, in particular on nitrogen metabolism and photosynthesis. Mo affects the photosynthetic apparatus mainly by improving the nitrogen supply of plants. This trace element plays a key role in nitrogen metabolism as a cofactor of nitrate reductase (the predominant activity of which is concentrated in plastids, including chloroplasts), and also as a cofactor of nitrogenase in root nodules, which is especially important for legumes. That is, in the latter case, it determines the activity of atmospheric nitrogen fixation (Cakmak *et al.*, 2023; Yang *et al.*, 2020). In turn, the chlorophyll content in leaves and the activity of CO_2 assimilation strongly depend on the nitrogen status of the plant, since the majority of the total pool of nitrogen-containing compounds is concentrated in the photosynthetic apparatus (Evans & Clarke, 2019).

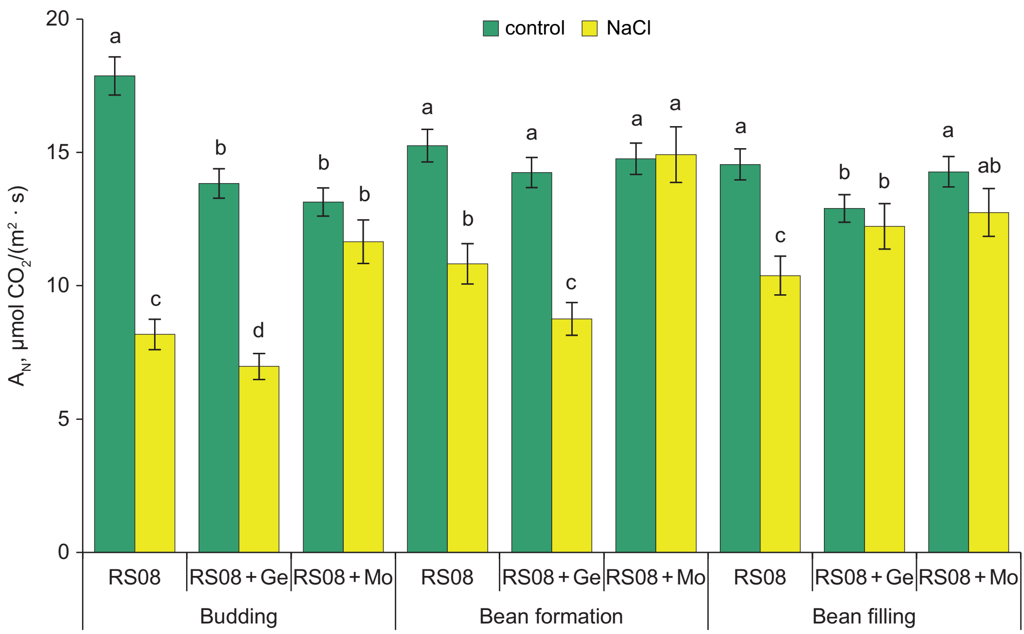

Fig. 2. Net CO_2 assimilation (A_N) rate in leaves of soybean plants inoculated with intact rhizobia and with the addition of Ge and Mo nanocarboxylates, under normal conditions (control) and salinity (30 mM NaCl) (different letters indicate significant differences among treatments at the same developmental stage, as determined by Tukey's post-hoc test following a one-way ANOVA ($P < 0.05$)) ($x \pm SE$; $n = 3$)

These provisions are confirmed by numerous experimental data. Thus, foliar Mo fertilization of soybean and maize plants has been shown to increase leaf nitrate reductase activity, nitrogen and protein content, RuBisCO activity, net photosynthesis, and grain yield. These results indicate that Mo fertilization can effectively enhance nitrogen metabolism and carbon fixation, leading to increased yield (Oliveira *et al.*, 2022). In experiments of other authors, Mo application significantly improved soybean growth and yield indices, including leaf area index, total plant biomass, transpiration rate, stomatal conductance, and yield (Jamali *et al.*, 2023). Application of Mo to wheat plants grown on different nitrogen sources (NH_4NO_3, NO_3^-, NH_4^+) significantly improved chlorophyll content and chloroplast configuration in all variants, and also increased the net photosynthesis. The authors concluded that Mo supply increased the photosynthetic rate not only through chlorophyll synthesis and chloroplast configuration, but also through mineral nitrogen uptake and assimilation, which may be useful for developing technology for using Mo to strengthen the photosynthetic apparatus of plants (Imran *et al.*, 2019).

In our work the stimulating effect of Ge on the A_N was also manifested, but only under salinity at the stage of bean filling.

The peculiarities of the salinity effect on the leaves transpiration rate under different inoculation treatments were generally similar to those indicated in the discussion of the results regarding the A_N, with the difference that the degree of the first index reduction relative to control plants under the stressor impact was, as a rule, smaller than for the second one (**Fig. 3**). This shows that salinity level used in our experiments had a stronger effect on the chloroplasts photosynthetic apparatus than on the stomata functioning. They only adjusted to the rate of CO_2 absorption from the leaf intercellular spaces, reducing or increasing their aperture in accordance with changes in the CO_2 assimilation rate by the chloroplasts of the mesophyll cells. This is confirmed by high coefficients of positive correlation between the A_N and T for plants of all treatments, which were: at the budding stage – r = 0.97 (P <0.01), at beans formation stage – r = 0.99 (P <0.01), at bean filling stage – r = 0.84 (P <0.05).

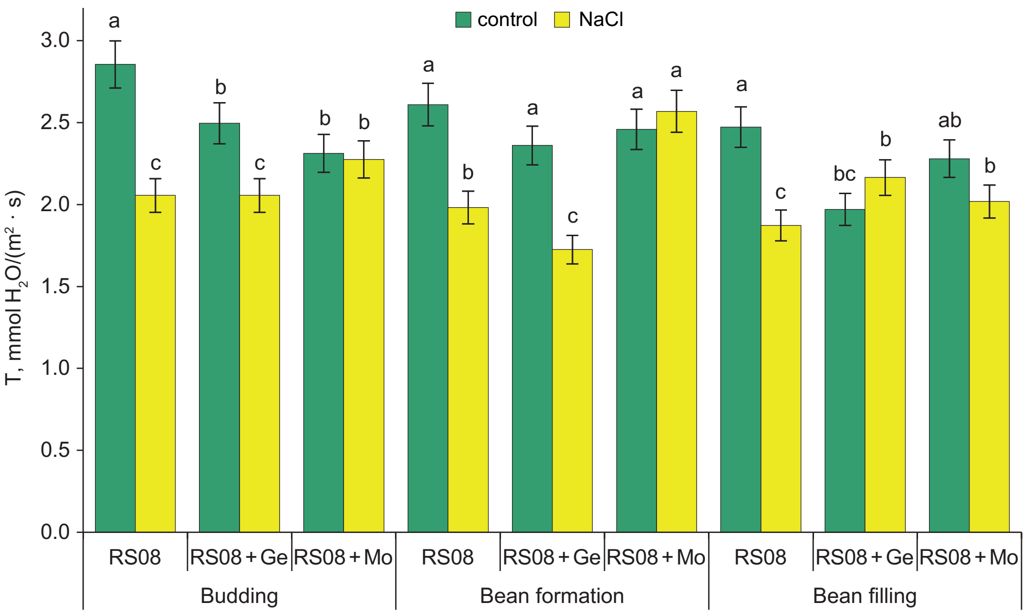

Fig. 3. Transpiration (T) rate in leaves of soybean plants inoculated with intact rhizobia and with the addition of Ge and Mo nanocarboxylates, under normal conditions (control) and salinity (30 mM NaCl) (different letters indicate significant differences among treatments at the same developmental stage, as determined by Tukey's post-hoc test following a one-way ANOVA (P <0.05)) (x±SE; n = 3)

Usually, in legumes, there is a fairly close correlation between NFA and A_N, due to the fact that at a limited level of mineral nitrogen nutrition, the main source of its bound forms is the symbiotic apparatus (Kiriziy *et al.*, 2022). In turn, during the period of intensive growth of vegetative organs, the main sink of nitrogen-containing compounds in the plant is the photosynthetic apparatus, which contains the majority of this element in a plant organism (Luo *et al.*, 2021), and the leaves themselves, accumulate it 2–3 times as much as other vegetative organs (Evans & Clarke, 2019).

However, in our experiment, no correlation was found between the A_N, determined for a unit of leaf surface area, and the NFA of soybean plants. In part, this can be explained by the complex effect of the applied factors on physiological processes. On the one hand, it is salinity as an osmotic stressor that also has a toxic effect on plants and rhizobia (Ferdous *et al.*, 2018; Chen *et al.*, 2024). On the other hand, metal nanocarboxylates also affect the micro- and macrosymbiont. All this affects the symbiotic and photosynthetic apparatus functioning, and is not always unidirectional. In addition, it should be taken into account that the activity of the former was calculated for the whole plant, while the activity of the latter was determined per unit area of the assimilation surface.

To reduce these indices to a single denominator, the amount of CO_2 that can be absorbed by a whole plant depending on the total area of its leaf surface was evaluated. Knowing the leaf plates weight from the whole plant and their SLW, the whole plant assimilation surface area was calculated and, based on the directly determined specific A_N in the leaf, the amount of total CO_2 assimilation by the whole plant was evaluated (**Table**).

Parameters of the assimilation surface and an estimated CO_2 assimilation rate of soybean plants inoculated with intact *Bradyrhizobium japonicum* RS08 strain and with the addition of Ge and Mo nanocarboxylates, under normal conditions and salinity (30 mM NaCl) (x±SE; n = 5)

Treatment	Leaf weight, g/plant	Leaf specific weight, g/m^2	Leaf area, m^2/plant	Net assimilation rate, μmol CO_2/(plant · h)
Budding				
RS08	1.67±0.05^a	152.2±4.6^a	0.0110±0.0003^a	707±21^a
RS08+Ge	1.66±0.05^a	141.7±4.3^a	0.0117±0.0004^a	582±17^b
RS08+Mo	1.75±0.05^a	125.0±3.8^b	0.0140±0.0004^b	663±20^a
RS08, 30 mM NaCl	1.79±0.05^a	126.0±3.8^b	0.0142±0.0004^b	418±13^c
RS08+Ge, 30 mM NaCl	2.16±0.07^b	139.1±4.2^c	0.0156±0.0005^c	390±12^c
RS08+Mo, 30 mM NaCl	1.26±0.04^c	157.5±4.7^d	0.0080±0.0002^d	337±10^d
Bean formation				
RS08	3.28±0.10^a	140.1±4.2^a	0.0234±0.0007^a	1286±39^a
RS08+Ge	3.54±0.11^a	131.2±3.9^b	0.0269±0.0008^b	1382±41^b
RS08+Mo	2.60±0.08^b	147.0±4.4^a	0.0177±0.0005^c	939±28^c
RS08, 30 mM NaCl	4.04±0.12^c	158.5±4.7^c	0.0255±0.0008^b	995±30^c
RS08+Ge, 30 mM NaCl	3.64±0.11^a	144.4±4.3^a	0.0252±0.0008^b	795±24^d
RS08+Mo, 30 mM NaCl	2.51±0.08^b	123.4±3.7^d	0.0203±0.0006^d	1091±33^e

Note: different superscript letters indicate significant differences among treatments at the same developmental stage, as determined by Tukey's post-hoc test following a one-way ANOVA ($P < 0.05$)

At the budding stage in all treatments under salinity, the total CO_2 assimilation by the whole plant was lower than in control plants. At the same time, in the treatment with Mo, where at this stage the A_N under salinity was the highest (see **Fig. 2**), CO_2 assimilation by the whole plant was the lowest as a result of the lower leaf area. At the bean formation stage under salinity, in the treatments with intact rhizobia and with Ge nanocarboxylate the total CO_2 assimilation by the plant was significantly lower than the control values, and in the treatment with Mo nanocarboxylate it was higher. In the plants of the first two treatments, this difference was caused by a low specific A_N, and in the third – on the contrary, by its higher value (see **Fig. 2**).

After calculating the indices of the CO_2 assimilation rate by the whole plant, we determined their relationship with NFA (**Fig. 4**). Quite high correlation coefficients were found between these parameters, which confirms general physiological ideas about the close relationship between symbiotic and photosynthetic apparatuses functioning in leguminous plants. At the same time, our results indicate that for a correct study of these relationships at the level of the whole plant, the influence of the long-term effect of a stress factor (in our case, salinity) on its morphology should be taken into account.

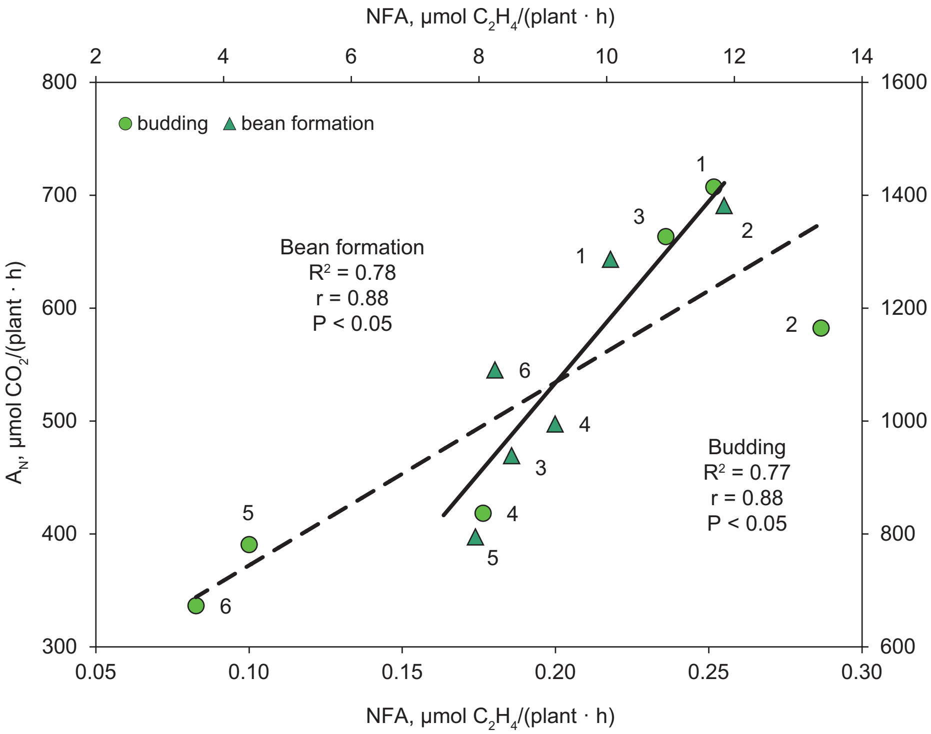

Fig. 4. Correlations between nitrogen-fixing activity (NFA) and the total CO_2 assimilation by the soybean plants inoculated with intact rhizobia and with the addition of Ge and Mo nanocarboxylates at the budding stage (lower X axis and left Y axis, dash and dash line) and bean formation stage (upper X axis and right Y axis, solid line). The figures near the data markers correspond to the treatment numbers: 1 – *B. japonicum* RS08 (intact rhizobia); 2 – *B. japonicum* RS08 + Ge; 3 – *B. japonicum* RS08 + Mo; 4 – *B. japonicum* RS08, NaCl; 5 – *B. japonicum* RS08 + Ge, NaCl; 6 – *B. japonicum* RS08 + Mo, NaCl

During the reproductive period, the vegetative growth of annual plants usually slows down, and the majority of assimilates is directed to the growth of reproductive organs.

Therefore, it is the activity of the photosynthetic apparatus during maturation that determines the productivity of economically valuable parts of plants (Faralli & Lawson, 2020). This axiom was once again confirmed by a rather close correlation found in our experiments between the A_N during the period of bean filling and the grain productivity of soybean plants (**Fig. 5**).

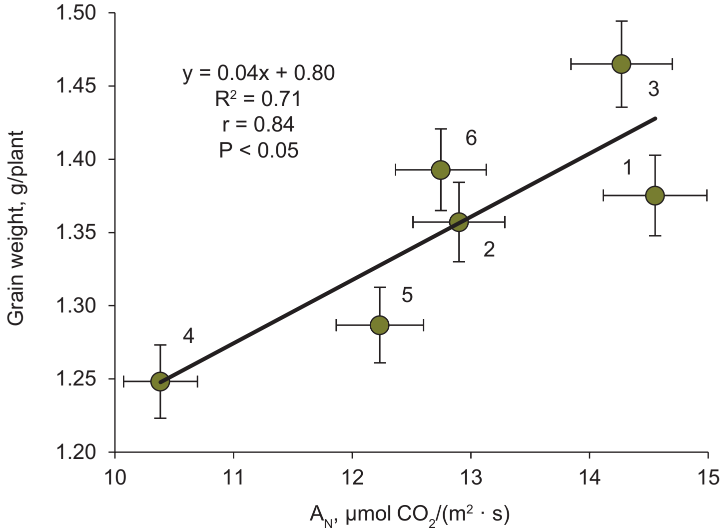

Fig. 5. Correlation between net CO_2 assimilation (A_N) rate in leaves of soybean plants at the bean filling stage and the grain productivity of soybean plants inoculated with intact rhizobia and with the addition of Ge and Mo nanocarboxylates. The figures near the data markers correspond to the treatment numbers: 1 – *B. japonicum* RS08 (intact rhizobia); 2 – *B. japonicum* RS08 + Ge; 3 – *B. japonicum* RS08 + Mo; 4 – *B. japonicum* RS08, NaCl; 5 – *B. japonicum* RS08 + Ge, NaCl; 6 – *B. japonicum* RS08 + Mo, NaCl

At the same time, it should be noted that for the data on the A_N at the stages of budding and beginning bean formation, such a correlation was absent, since during this period the majority of assimilates was obviously still directed to vegetative growth.

If we analyze the location of the treatments markers along the trend line, which approximates the relationship between the A_N and the grain productivity of plants, it can be seen that in its upper part are the control treatments (without salinity) and the treatment under salinity with inoculation with rhizobia suspension with Mo nanocarboxylate (6) (see **Fig. 5**). Next in descending order is treatment with salinization and inoculation with rhizobia with Ge nanocarboxylate (5) and, finally, at the very bottom is the treatment with salinization and inoculation with intact rhizobia (4). The highest grain productivity was obtained in the treatment without salinity and inoculation with rhizobia with Mo nanocarboxylate (3). The same inoculation treatment under salinity (6) reached the grain productivity level of treatments without salinity with inoculation with intact rhizobia (1) and rhizobia with Ge nanocarboxylate (2).

CONCLUSIONS

Experiments on the inoculation of soybean plants with rhizobia cultivated with the addition of Ge and Mo nanocarboxylates revealed that symbiotic and photosynthetic apparatuses react differently to both inoculation treatments and soil salinity. Obviously, this is due to different effects of metal nanocarboxylates in the inoculation suspension on rhizobia and soybean seeds. It was shown that the degree of the symbiotic apparatus activity suppression under salinity decreases over time, while at bean formation stage, in the treatment with the addition of Mo nanocarboxylate to the inoculation suspension, NFA of plants grown on salinity practically equaled the control ones.

Salinity also had a negative effect under inoculation with intact rhizobia on the functioning of the photosynthetic apparatus at the budding, bean formation, and bean filling stages. The addition of Ge nanocarboxylate to the inoculation suspension had a positive effect on the net CO_2 assimilation in plant leaves under salinity at the bean filling stage. The addition of Mo showed a similar effect at all studied stages of development. The transpiration rate was closely correlated with the CO_2 assimilation, although the degree of its suppression by salinity was much less than that of photosynthesis. This indicates that the effect of the osmotic component of the applied salinity level (30 mM NaCl) on the leaves stomatal apparatus was weaker compared to its toxic effect on the chloroplasts of the mesophyll cells. Positive correlations between NFA and the calculated CO_2 assimilation by the whole plant were revealed at the budding and bean formation stages.

A close positive correlation was found between the net CO_2 assimilation at the bean filling stage and the grain productivity of soybean plants. At the same time, in the treatments with seed inoculation with a suspension of rhizobia with the addition of Mo nanocarboxylate, the weight of the grain from the plant was the largest both among the control plants (without salinity) and among the plants grown under salinity. In the latter case, this index was equal to control plants inoculated with intact rhizobia, and with the addition of Ge nanocarboxylate.

Therefore, according to all the investigated physiological indices, inoculation of soybean seeds with the *Bradyrhizobium japonicum* RS08 strain with the addition of Mo nanocarboxylate proved to be the most effective in maintaining the activity of symbiotic and photosynthetic apparatuses and, ultimately, grain productivity of plants both under normal conditions and under salinity. The results of these studies may serve as a basis for improving the technology of producing bacterial fertilizers for pre-sowing inoculation of soybean seeds in order to increase its yield.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

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AUTHOR CONTRIBUTIONS

Conceptualization, [S.K; L.M; I.O]; methodology, [S.K.; L.M; I.O; D.K]; validation, [S.K.; L.M; D.K.]; formal analysis, [L.M; I.O; D.K.]; investigation, [S.K.; L.M; I.O; D.K.]; resources, [L.M; I.O; S.K.; D.K.]; data curation, [L.M; I.O; S.K.; D.K.]; writing – original draft preparation, [L.M; I.O; D.K.]; writing – review and editing, [L.M; S.K.; D.K.]; visualization, [L.M; S.K.; D.K.]; supervision, [L.M; S.K.; D.K.]. All authors have read and agreed to the published version of the manuscript.

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ВПЛИВ ЗАСОЛЕННЯ НА АКТИВНІСТЬ СИМБІОТИЧНОГО І ФОТОСИНТЕТИЧНОГО АПАРАТІВ РОСЛИН СОЇ, ІНОКУЛЬОВАНОЇ РИЗОБІЯМИ З ДОДАВАННЯМ НАНОКАРБОКСИЛАТІВ Ge І Mo

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Вступ. Соя є однією з головних олійних і білкових культур у світі, вона займає найбільші посівні площі серед бобових рослин. Однак несприятливі умови росту, зокрема, засолення, можуть спричинити значні втрати врожаю. Відомо, що сольовий стрес істотно впливає на морфологічні показники та фізіологічні процеси у рослин сої. Зважаючи на те, що основними складовими формування продуктивності соєво-ризобіального симбіозу є азотфіксація й асиміляція CO_2, важливо здійснювати пошук можливостей оптимізації цих процесів, зокрема, в умовах засолення.

Матеріали та методи. Об’єкт дослідження – симбіотичні системи, створені за участю рослин сої (*Glycine max* (L.) Merr.) сорту Самородок і бульбочкових бактерій

Bradyrhizobium japonicum штаму PC08 із музейної колекції азотфіксувальних мікроорганізмів ІФРГ НАНУ, культивованих із додаванням нанокарбоксилатів Ge і Mo. Методи дослідження – мікробіологічні, біохімічні та фізіологічні, статистичний аналіз.

Результати. Виявлено, що засолення пригнічувало активність симбіотичного та фотосинтетичного апаратів у сої. Разом із тим, ступінь пригнічення активності симбіотичного апарату за умов засолення з часом зменшувався. Додавання до інокуляційної суспензії нанокарбоксилату Ge позитивно вплинуло на інтенсивність фотосинтезу листків рослин на засоленні у фазу наливання бобів, а Mo – в усі досліджені фази розвитку. Інтенсивність транспірації тісно корелювала з інтенсивністю фотосинтезу, хоча ступінь її пригнічення на засоленні був значно менший, ніж фотосинтезу. Виявлено позитивні кореляційні залежності між азотфіксувальною активністю і розрахунковою інтенсивністю асиміляції CO_2 цілою рослиною. Знайдено тісний позитивний кореляційний зв'язок між інтенсивністю фотосинтезу у фазу наливання бобів і зерновою продуктивністю рослин сої. Водночас у варіантах інокуляції суспензією ризобій з додаванням нанокарбоксилату Mo маса зерна з рослини була найбільшою як серед контрольних рослин (без засолення), так і серед варіантів на засоленні.

Висновки. Отримані результати свідчать, що за всіма дослідженими фізіологічними показниками інокуляція насіння сої *Bradyrhizobium japonicum* штаму PC08 з додаванням нанокарбоксилату Mo виявилася найбільш ефективною для підтримання активності симбіотичного і фотосинтетичного апаратів та, в кінцевому підсумку, зернової продуктивності рослин як за нормальних умов, так і в умовах засолення.

Ключові слова: *Glycine max* (L.) Merr., *Bradyrhizobium japonicum*, засолення, азотфіксувальна активність, фотосинтез, нанокарбоксилати металів, продуктивність

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