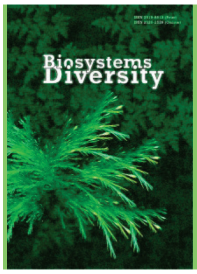

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Composition of pigment complex in leaves of soybean plants, inoculated by *Bradyrhizobium japonicum*, subject to metal nanocarboxylates and various-levels of water supply

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A distinctive feature of legumes is the ability to combine two most important processes: photosynthesis and nitrogen fixation. However, the course of those processes, and therefore seed potential of those crops depend on a number of biotic and abiotic factors, the commonest being drought. Therefore, interest in physical-biochemical resistance of the plant organism to abiotic stress factors is increasing, as well as search for optimum ways to increase its adaptability. Success of adaptation of a plant's organism to unfavourable environmental factors is known to largely depend on optimal functioning of assimilative apparatus. Some indicators of the condition of the apparatus are the content and ratio of photosynthesis pigments. Therefore, we aimed at determining the reaction of the pigment complex of *Glycine max* (L.) Merr. plants, grown against the background of optimal and insufficient watering, to inoculation of seeds with rhizobia bacteria *Bradyrhizobium japonicum*, cultivated using nanocarboxylates of chromium, cobalt, iron, copper and germanium. Research has shown that utilization of germanium nanocarboxylate as a component of inoculative suspension led to the highest content of chlorophylls in leaves of soybean of the studied variants in the blossoming phase during optimal watering, as well as significant increase in the content of carotenoids compared with the control plants regardless of the level of watering. At the same time, this element caused no significant effect on the chlorophyll content in plants grown in drought. It was confirmed that among soybean plants that were in stress conditions (blossoming phase) for two weeks, the highest content of chlorophylls was in leaves of plants grown from seeds inoculated with rhizobial suspension with addition of chromium and copper nanocarboxylates, which caused 25.3% and 22.8% increase in chlorophyll *a*, 29.4% and 32.3% in chlorophyll *b* and 26.4%% and 23.8% in them respectively, compared with the control. Furthermore, chromium and copper nanocarboxylates stimulated the content of carotenoids in the same plants, though it was less expressed than after adding germanium nanocarboxylate. The highest content of photosynthetic pigments in plants after the watering was resumed (phase of bean formation) was in cases of applying chromium and germanium nanocarboxylates. It was confirmed that the most efficient way to protect the pigment complex of soybean plants during drought was using chromium and germanium nanocarboxylates as components of inoculation suspension. The results we obtained indicate the possibility of applying chromium nanocarboxylate in the technology of cultivating soybean in the conditions of water deficiency as an effective way to improve biosynthesis of chlorophylls, as well as using germanium nanocarboxyllate as a component that provides a high level of activity of protective mechanisms of the pigment system of soybean, associated with resisting stress caused by water deficiency.

Keywords: rhizobia; *Glycine max*; chromium nanocarboxylate; germanium nanocarboxylate; drought; photosynthetic pigments.

Introduction

An important physiological feature that reflects the condition of a plant organism, specifics of the course of productive process and the degree of adaptation of plants to the action of stress factors is the content and ratio of photosynthetic pigments (chlorophylls *a* and *b*, carotenoids, anthocyanins and others) (Croft & Chen, 2018). Furthermore, changes in the pigment complex, their dynamics and rates of accumulation are among the indirect parameters of efficiency of legume-rhizobium symbiosis (Adams et al., 2016). There is a presumption that the nitrogen-fixating activity of symbiotic apparatus is determined not only by the area of photosynthesizing surface but also by the content of chlorophyll in it. On the example of the model of non-chlorophyll mutants of pea, it was determined that plants with low chlorophyll level (3.0% of the norm) developed normal symbiotic apparatus, though no fixation of molecular nitrogen occurred. Further increase in the content of pigment in the leaf increased the activity of nitrogenase, though at the level of chlorophyll equaling 40–60% of the norm, the differences in the activity of nitrogenase were practically leveled out.

The significance of photosynthetic pigments is also manifested in the constantly growing interest in studying them: only over the recent 25 years, the number of studies focusing on the influence of environmental factors on changes in chlorophyll has exceeded 2 thou (Esteban et al., 2015; Priadkina & Morgun, 2016). Pigments are photoacceptors which consume quanta of a noticeable share of the solar spectrum and are involved in conversion of light energy into energy of chemical bonds (Ashraf & Harris, 2013). Chlorophylls are the most important in this process. They take part directly in formation of the structure of photosynthetic apparatus, performing the key role in photosynthetic and photochemical reactions by converting solar radiation in storage of chemical energy by consuming quanta of light and transmitting their energy to reaction centers of photosystems and dividing the charges within reaction centers (Kaliaha & Kozel, 2020). However, there is no agreement about what is the optimum amount of chlorophyll in leaves. Some researchers think that its level should be low. They explain that destruction of photosynthesis apparatus would be prevented by excess of consumed energy at the cost of decrease in the amount of consumed light. Thus, low content of chlorophyll in the leaf may ensure its more effective work. Despite this fact, most researchers still tend to think that plants with higher level of chlorophyll consume more energy, thus intensifying photosynthesis (Lawlor, 2009). In particular, a study focused on the relationship between the content of chlorophyll and parameters of photosynthetic activity in the leaf of a yellow-green mutant of solid wheat compared with initial variety with usual green leaves, revealing that quantum yield of transport of electrons of photosystem II and photochemical inhibition of fluorescence of chlorophyll in it were lower (Li et al., 2013). The authors concluded that in the conditions of chlorophyll deficiency, photosynthesis activity is inhibited (Priadkina & Morgun, 2016).

Carotenoids are also involved in collecting light energy. Moreover, they perform light-protective functions, play the role of antioxidants that remove excessive free radicals, formed in the process of photosynthesis (Ristic et al., 2007). In spite of the important role of pigments in functioning of leaf lamina, variation in their content may inform us about the physiological condition of leaf apparatus (Croce & van Amerongen, 2014), and decrease in their content may be a noticeable indicator of stress.

As is known, synthesis of chlorophylls and carotenoids in leaves significantly changes as a response to the influence of environmental factors. Stress factors: extreme temperatures, salinization, heavy metals, drought, and acidity of soil cause significant decrease in the content of photosynthetic pigments through inhibition of biosynthesis, or ruination (Sokolovska-Sergiienko & Stasik, 2008). Such changes in pigment complex may lead to malfunctioning of electron transport, and therefore decrease in photosynthetic ability in most green plants.

Over the recent years, it is drought that among numerous stress factors has been the commonest and most significant abiotic factor that noticeably decreases the yield of agricultural crops. Water deficiency causes serious changes in physiological processes of plant organism: inhibition of growth and development, loss of turgor pressure, decrease in rates of photosynthesis and absorption of carbon, impediment of mineral nutrition and gas exchange in leaves, which influences its productivity to a great degree (Farooq, 2009; Chen, 2014). Inhibition of photosynthetic processes is one of the main causes of decrease in productivity of plants in the conditions of water deficiency, because they are the fastest to react to water deficiency. It has been confirmed that when soil moisture is decreased, absorption of carbon dioxide in photosynthesis is inhibited. First of all, inhibition of photosynthesis during drought is caused by partial closing of stoma. Deeper dehydration of photosynthesizing tissues leads to significant changes in photosynthesis metabolism. Decrease of activity of photosystems I and II and photophosphorylation, decrease in activity and content of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo) in those conditions were experimentally proven (Vu et al., 1987).

A promising way of increasing tolerance of plants to the action of unfavourable environmental factors may be applying nanopreparations, specifically micro-fertilizers, in technology of cultivation of crops (Muszyńska & Labudda, 2019). Microelements are highly specific elements, most of which are catalizers that are able to accelerate a number of biochemical reactions of photosynthesis, respiration, and metabolism. However, minimizing the role of microelements down to only catalyst activity would be wrong. Those elements affect the direction of biochemical reactions as a result of the action toward biocolloids. They are involved in the processes of development of cellular and tissue structures of plants, functions of electron-transporting chains, transduction of hormone signals and functions of genetic apparatus. By their catalyst action, they allow plants to use the essential nutrition elements most efficiently, which in turn positively influences the productivity of plants and quality of yield (Morrissey & Lou, 2006). Presence and possibility of absorption of nutrition elements, especially in critical phases of development of plant organism, influence of specific elements on its adaptation to stress conditions gives an opportunity to influence the structures of elements of yield of plants subject to unfavourable environmental factors. It has to be noted that an important factor is the form in which microelements enter the plants, because they are only absorbed in mobile (water-soluble) form. Despite advantages of micro-fertilizers in the forms of ions of metal salts such as cheapness and availability, they also have a number of disadvantages, specifically: poor availability for plants, efficiency only on low-acidic and acidic soils, salinization and contamination of soils by heavy metals.

Using nanotechnologies, the efficiency of microelements may be improved by converting them into biologically active form. Nanoparticles of microelements belong to the inorganic class, have a nucleus, and their external membrane is formed by atoms of metals. They are characterized by high level of biological activity, change in physical-chemical parameters and toxic properties, compared with microparticles of the same elements (Wang et al., 2016; Du et al., 2017). Drugs based on such microelements have a number of advantages compared with drugs based on inorganic salts. Specifically, they are hardly toxic, they completely dissolve in water and are well absorbed by plants, do not delaminate under the influence of heat and light, do not lose their activity during long storage, are stable in pH range of soil and do not bind into poorly-solved compounds, are not ruined by microorganisms. Having small sizes of particles, those preparations are characterized by high specific surface, and therefore extremely high reaction ability and high penetrability into plant tissues, which intensifies physiological and biochemical processes. They provide increase in tolerance to unfavourable weather conditions (high, or low air temperature, drought, insufficient amount of oxygen in soil, accumulation of pesticides) and increase in yield (by 1.50–2.00 on average) in almost all industrial and technical crops (Anderson do Espirito et al., 2021). By acting through the enzymic system or indirectly binding with biopolymers of plants, microelements may stimulate or inhibit growth processes, development and reproductive function of plants. Microelements with catalyst action allow plants to more effectively use essential elements of nutrition, which in turn improves the productivity of plants and quality of yield.

In spite of the aforesaid, we aimed at determining the reaction of pigment complex of plants *Glycine max* (L.) Merr. grown against the background of optimal and insufficient watering to inoculation of seeds with rhizobia bacteria *B. japonicum*, cultivated using chromium, cobalt, iron, copper and germanium nanocarboxylates.

Materials and methods

The objects of the study were soybean plants of Almaz variety, inoculated with the active strain of rhizobia bacteria *B. japonicum* B1-20, cultivated using nanocarboxylates of cobalt (Co), iron (Fe), germanium (Ge), chromium (Cr) and copper (Cu), added to their growth media. We used microelements provided by AVATAR Ltc Scientific Industrial Company (Ukraine, Kyiv). They were obtained in two stages: 1 – obtaining aqueous colloid solution of nano-particles of microelements by dispersing highly purified granules of corresponding metals using electric stream impulse in de-ionized water; 2 – obtaining carboxyllates of metals through reaction of direct interaction between obtained nanoparticles and edible carbonic acid.

Bacterial culture was cultivated in test tubes in yeast mannitol agar (YMA) at the temperature of +28 ºC for 6–7 days. Rhizobia bacteria were washed off the surface of agarized medium with sterile water, and suspension of Erlenmeyer flask (200 mL) was inoculated with liquid medium, which in corresponding variants contained chelate metals in the ratio of 1:1,000. Inoculated material was introduced to flasks in the concentration of 2% of the volume of growth medium. Freshly prepared suspension of rhizobia which contained nano-metals was cultivated for 6 days at the temperature of +26…+28 ºC on rocket with velocity of 220 rpm, which provided constant aeration of the growth medium. Bacterial titer of the suspension equaled 10^8 cells/mL. Purity of the bacterial culture was checked by its inoculation onto MPA medium, on which rhizobia do not grow. Prior to the inoculation, the seeds were sterilized using 70% ethanol solution for 15 min and rinsed in tap water. Then, for 1 h, we performed bacterization with rhizobia (10^8 cells/mL).

Vegetative experiments were carried out on a plot of the Institute of Physiology of Plants and Genetics of the National Academy of Sciences of Ukraine. The plants were grown in 4 kg vessels in the natural light and temperature, optimal (60% capillary fringe (CF) or insufficient (30% CF) watering, and we used rinsed river sand as substrate. The source of mineral nutrition was nutritive Hellriegel's mixture, nitrogen-impoverished – 0.25 of the norm (1 norm of nitrogen corresponds to 708 mg $Ca(NO_3)_2 \cdot 4H_2O$ per 1 kg of substrate). Moisture of the substrate was supported by the controlled watering. We caused a two-week long drought (the period of three true leaves – blossoming), and then watering was resumed up to 60% CF.

The scheme of the experiment included the following variants: 1. *B. japonicum* B1-20, 60% CF; 2. *B. japonicum* B1-20 + Cr nanocarboxylate, 60% CF; 3. *B. japonicum* B1-20 + Co nanocarboxylate, 60%

CF; 4. *B. japonicum* B1-20 + Fe nanocarboxylate, 60% CF; 5. *B. japonicum* B1-20 + Cu, nanocarboxylate 60% CF; 6. *B. japonicum* B1-20 + Ge nanocarboxylate, 60% CF; 7. *B. japonicum* B1-20, 30% CF; 8. *B. japonicum* B1-20 + Cr nanocarboxylate, 30% CF; 9. *B. japonicum* B1-20 + Co nanocarboxylate, 30% CF; 10. *B. japonicum* B1-20 + Fe nanocarboxylate, 30% CF; 11. *B. japonicum* B1-20 + Cu nanocarboxylate, 30% CF; 12. *B. japonicum* B1-20 + Ge nanocarboxylate, 30% CF. The control was the variant with seed inoculation with rhizobia, without addition of nanocarboxylates, both in optimal conditions (control 1) and insufficient (control 2) watering.

The samples of the plant material were taken for the analysis in the phase of blossoming and formation of beans. We determined the content of photosynthetic pigments (carotenoids, chlorophylls *a* and *b*) in leaves of soybean of third layer spectrophotometrically using ShimadzuUV-1900 (Japan) at the wavelengths of 480, 649 and 665 nm according to the method (Wellburn, 1994) and expressed in mg/g of raw weight of leaves. The obtained extracts were dissolved in dimethyl sulfoxide (1:9), which was taken into account in final re-calculation of the content of pigments.

The tables present mean arithmetic values and their standard errors (x ± SE). Significance of differences between the selections was evaluated using the method of single-factor disperse analysis (ANOVA), where the differences were considered significant when P values were less than 0.05 (taking into account Bonferroni correction).

Results

In the blossoming phase, the highest content of chlorophylls *a*, *b* and their total was seen in plants in the variant using germanium nanocarboxylate, which exceeded the control according to the studied parameters by 22.1%, 20.9% and 22.9% respectively. Using copper and cobalt nanocarboxylates as a component of inoculative suspension caused the tendency toward increase in the content of chlorophyll *a* and overall chlorophyll.

The lowest effect on the content of chlorophylls in leaves was taken by iron nanocarboxylate. According to all parameters, the plants of this variant were at the level of control (Fig. 1–3). Content of chlorophylls increased in leaves of soybean plants inoculated by suspension of rhizobia containing chromium and copper nanocarboxylates and cultivated in the conditions of insufficient water supply, compared with the plants of the control variant 2. Specifically, we observed increase in the concentration of chlorophyll *a* (by 25.3% and 22.8%), chlorophyll *b* (by 29.4% and 32.3%) and their total (by 26.4% and 23.8%) respectively. We observed a tendency toward increase in the concentrations of chlorophylls *a* and *b* and their total in plants influenced by cobalt nanocarboxylares compared with leaves of plants of control 2. After using germanium nanocarboxylate, the content of chlorophylls in leaves was at the same level as the control. A similar situation was seen after exposure to iron nanocarboxylate, though content of chlorophyll *a* in plants of this variant tended to decrease.

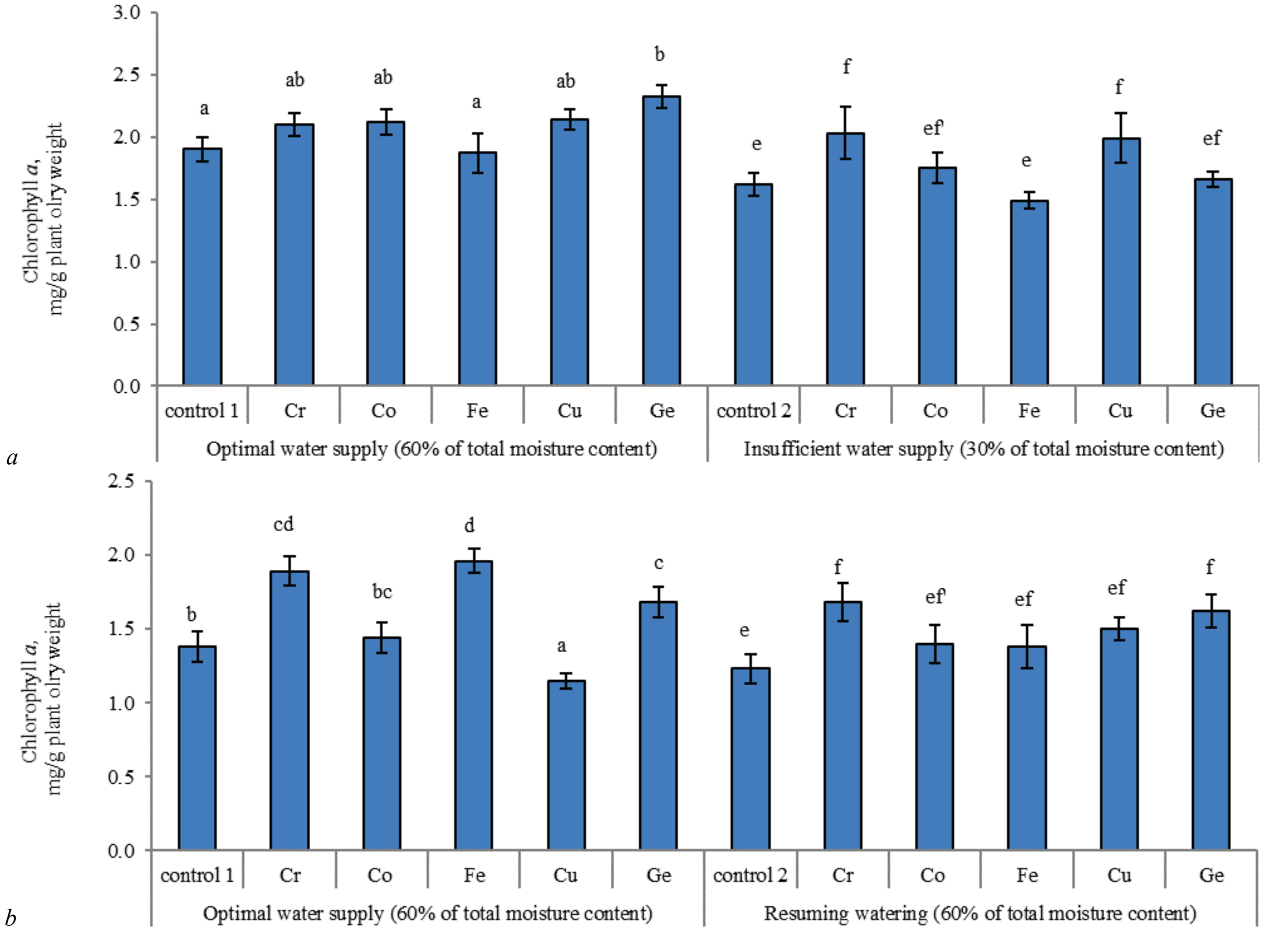

Fig. 1. Content of chlorophyll *a* in leaves of soybean plants subject to metal nanocarboxylates and different water supply: *a* – blossoming phase, *b* – phase of formation of beans (x ± SE, n = 8); different letters in columns indicate values that significantly differ one from another among the variants as according to comparison using Tukey test (P < 0.05) taking into account Bonferroni correction (letters *a*, *b*, *c*, *d* – significantly between the variants with optimal water supply; letters *e*, *f* – significantly between the variants with insufficient water supply and resumption of watering to optimal level

The results of our studies indicate the ability of some nanocarboxylates to positively influence the content of carboxylates in soybean plants. In particular, in the phase of blossoming, while being optimally watered, the plants the seeds of which had been inoculated with rhizobia and addition of cobalt and copper nanocarboxylates exceeded control 1 by 17.1%, and those inoculated with addition of germanium nanocarboxylate – by 24.4% respectively (Fig. 4). The studies revealed that in the conditions of two-week insufficient watering, the plants that had been inoculated with rhizobia and addition of chromium, cobalt, copper and germanium nanocarboxylates to their growth media, exceeded control 2 by 51.6%, 32.2%, 41.9% and 87.1% respectively. In the phase of formation of beans, in optimal conditions of watering, the most positive effect on the content of pigment complex in soybean plants was exerted by chromium, iron and germanium nanocarboxylates (Fig. 1–3). Chromium nanocarboxylate increased the amount of chlorophyll *a*, chlorophyll *b* and their total by 36.9%, 51.7% and 40.1% respectively compared with control 1 plants. Iron nanocarboxylates caused increase in the concentration of all studied pigments compared with the same control by 42.0% – chlorophyll *a*, 48.3% – chlorophyll *b*, 43.1% – overall chlorophyll *a* and *b* and 20.0% – carotenoids (Fig. 1–4). Using germanium nanocarboxylate as a component of inoculation suspension led to increase in chlorophyll *a*, chlorophyll *b* and their total compared to control 1 plants by 21.7%, 31.0% and 23.3%. We saw decreases in chlorophyll *a* and overall chlorophyll by 15.5% and 16.8% in the variant using copper nanocarboxylates.

In soybean plants the watering of which had been resumed following a two-week drought, we observed significant increase in all the studied parameters in variants treated with chromium and germanium nanocarboxylates. Specifically, according to the contents of chlorophyll *a*, chlorophyll *b* and their total, and also carotenoids, an increase was recorded by 36.6%, 33.3%, 36.0% and 24.2% compared with control 2 after action of chromium nanocarboxylate and by 21.1%, 25.9%, 22.0% and 19.1% after effect of germanium nanocarboxylate, respectively. Chlorophyll *a* and total of chlorophylls *a* and *b* in leaves of soybean inoculated with rhizobia, modified by copper nanocarboxylates, tended to increase by 21.9% and 20.0% compared with control 2, and also increase occurred in concentration of carotenoids (Fig. 1, 3, 4). After using inoculation of cobalt nanocarboxylate in suspension, the content of chlorophyll b was higher by 30% compared with plants of control 2 and chlorophyll *a* and overall chlorophyll tended to increase. Under the influence of iron nanocarboxylates, the studied parameters were at the level of control variant 2 (Fig. 2).

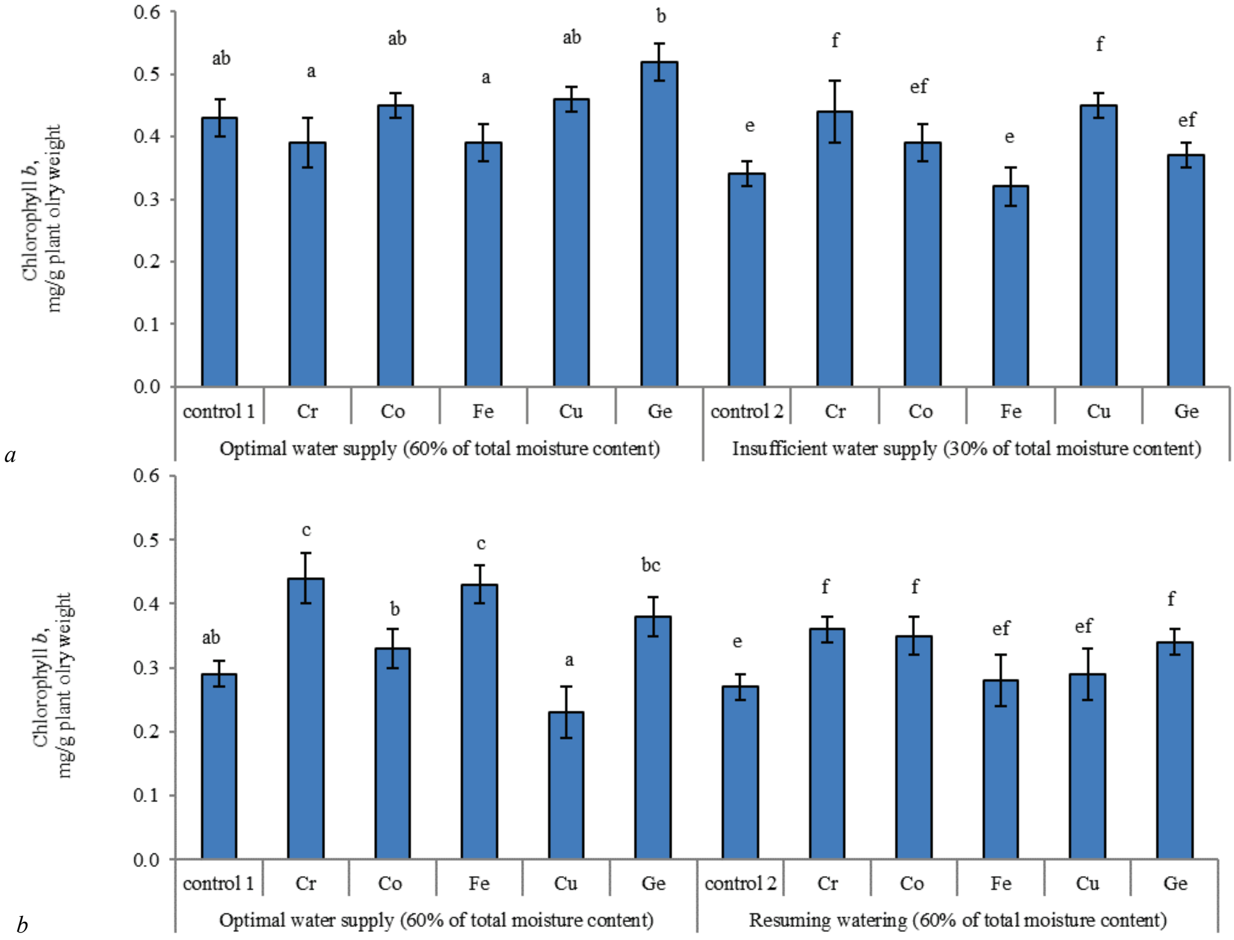

Fig. 2. Content of chlorophyll *b* in leaves of soybean plants subject to metal nanocarboxylates and different levels of water supply: *a* – blossoming phase, *b* – phase of formation of beans (x ± SE, n = 8); different letters in columns indicate values that significantly differ one from another according to comparison using Tukey test ($P < 0.05$) taking into account Bonferroni correction (letters *a*, *b*, *c* – significantly between the variants with optimal water supply; letters *e*, *f* – significantly between the variants with insufficient water supply and resumption of watering to optimal level)

Ratio of concentrations of a/b chlorophyll in leaves of soybean plants ranged 4.0 to 5.4 (Table 1) and significantly depended on type of microelement, development phase of plants and level of their water supply. At the same time, the highest vales of the studied parameter were recorded in the phase of blossoming in plants exposed to influence of copper nanocarboxylates, accounting for 5.2 (optimal water supply), which was 13.0% higher than the ratio in leaves of control 1 and 5.0 (insufficient water supply). Furthermore, in the phase of bean formation, in the conditions of optimal water supply, in leaves of soybean plants grown from seeds inoculated with *B. japonicum* B1-20 with additions of chromium nanocarboxylates, we observed maximal value of ratio of a/b chlorophylls which exceeded control 1 by17.4%.

In the phase of blossoming, in the conditions of optimal water supply, and subject to cobalt nanocarboxylate and conditions of insufficient water supply and subject to chromium nanocarboxylate, we recorded maximal decrease in parameters of chlorophyll ratio by 13.5% and 10.4% in the leaves of plants compared with corresponding control variants respectively.

In the phase of blossoming, in the conditions of optimal watering, subject to cobalt nanocarboxylates and in the conditions of insufficient watering, subject to chromium nanocarboxyllates, we observed maximal decrease in ratio of chlorophylls by 13.5% and 10.4% respectively compared with leaves of plants of corresponding control variants.

In the conditions of insufficient water supply, maximal ratio of chlorophylls to carotenoids was seen in leaves of plants of control variant 2 (Table 2). Treating seeds with chromium, cobalt and iron nanocarboxylates caused decrease in the studied parameter by 17.2%, 18.7% and 21.9% respectively compared with control 2. The lowest ratio of chlorophylls to carotenoids was observed in plants grown from seeds inoculated with rhizobia, modified using germanium nanocarboxylate – it decreased by 45.3% compared with control 2.

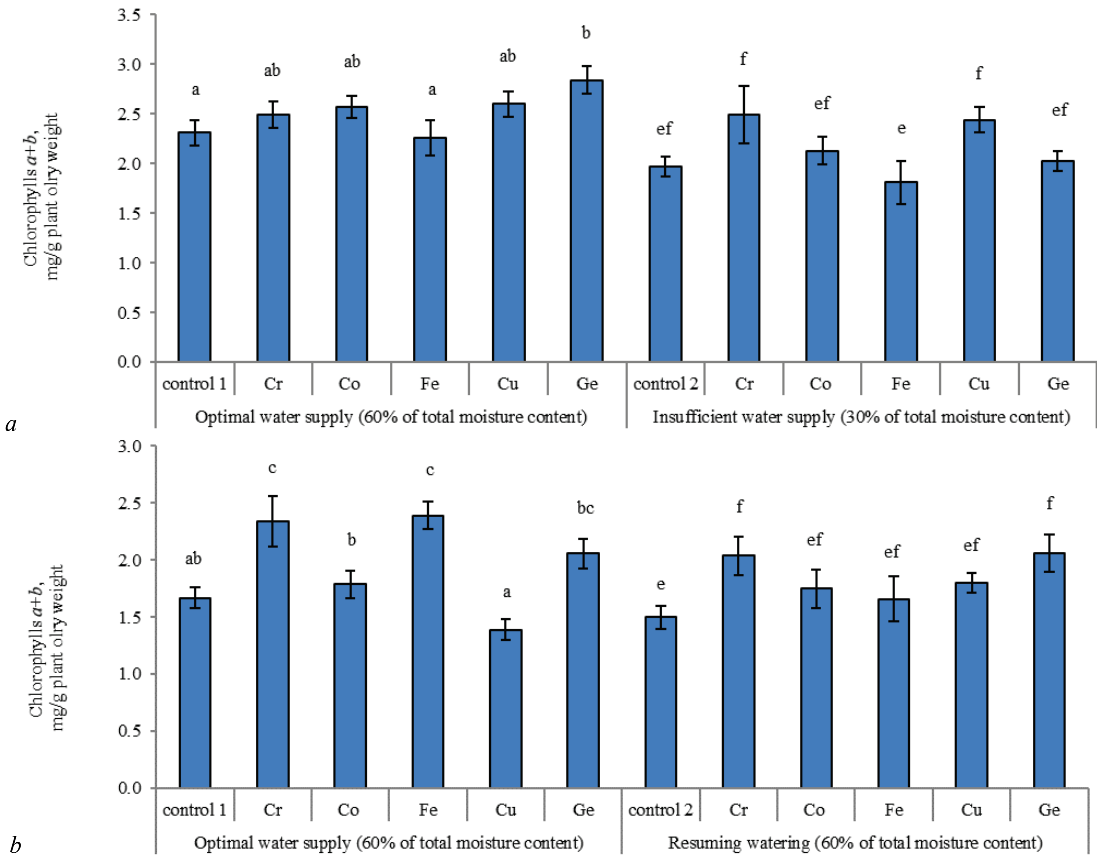

Fig. 3. Total chlorophyll *a* and *b* in leaves of soybean plants subject to influence of metal nanocarboxylates and different water supply: *a* – blossoming phase, *b* – phase of bean formation (x ± SE, n = 8); different letters in columns indicate values that significantly differed one from another between the variants as a result of comparison using the Tukey test ($P < 0.05$) taking into account Bonferroni correction (letters *a*, *b*, *c* – significantly between the variants with optimum water supply; letters *e*, *f* – significantly between variants with insufficient water supply and resumption of watering to optimal level

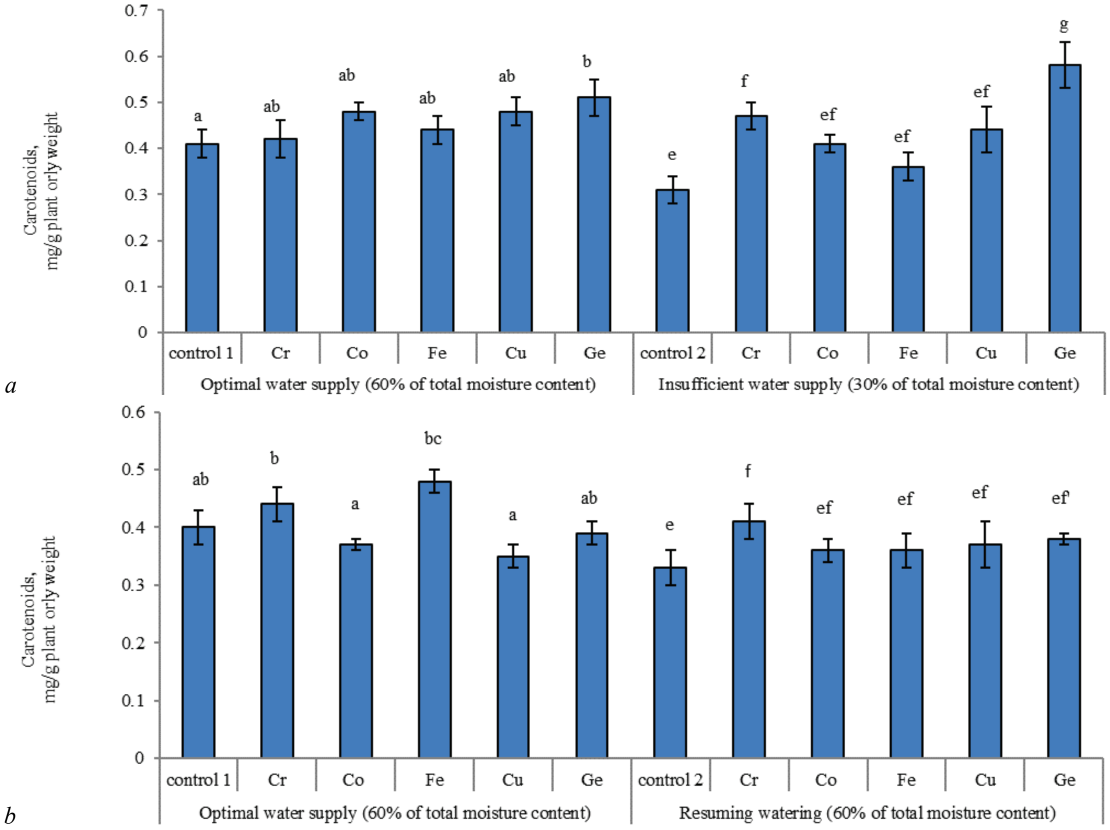

Fig. 4. Concentration of carotenoids in leaves of soybean plants subject to influence of metal nanocarboxylates and different water supply: *a* – blossoming phase, *b* – phase of bean formation (x ± SE, n = 8); different letters in columns indicate values that significantly differed one from another between the variants as a result of comparison using the Tukey test ($P < 0.05$) taking into account Bonferroni correction (letters *a*, *b*, *c* – significantly between the variants with optimum water supply; letters *e*, *f*, *g* – significantly between variants with insufficient water supply and resumption of watering to optimal level

Table 1
Ratio of contents of a/b chlorophyll in leaves of soybean plants grown in the conditions of different watering and influence of metal nanocarboxylates

Variant	Phase of plant development	
	blossoming	bean formation
Watering level	optimal (60% CF, capillary fringe)	
B. japonicum B1-20 (control 1)	4.56 ± 0.11^{b}	4.58 ± 0.15^{f}
B. japonicum B1-20 + Cr nanocarboxylate	4.67 ± 0.29^{bc}	5.38 ± 0.11^{g}
B. japonicum B1-20 + Co nanocarboxylate	4.00 ± 0.23^{a}	4.71 ± 0.16^{fg}
B. japonicum B1-20 + Fe nanocarboxylate	4.93 ± 0.15^{bc}	4.79 ± 0.21^{fg}
B. japonicum B1-20 + Cu nanocarboxylate	5.17 ± 0.12^{c}	4.65 ± 0.13^{fg}
B. japonicum B1-20 + Ge nanocarboxylate	4.38 ± 0.19^{ab}	4.46 ± 0.24^{fg}
Watering level	insufficient (30% CF)	resumption to optimal (60% CF)
B. japonicum B1-20 (контроль 2)	4.76 ± 0.11^{de}	4.79 ± 0.16^{j}
B. japonicum B1-20 + Cr nanocarboxylate	4.29 ± 0.15^{d}	4.61 ± 0.13^{hj}
B. japonicum B1-20 + Co nanocarboxylate	4.36 ± 0.23^{de}	4.49 ± 0.28^{hj}
B. japonicum B1-20 + Fe nanocarboxylate	4.56 ± 0.22^{de}	4.65 ± 0.15^{hj}
B. japonicum B1-20 + Cu nanocarboxylate	5.04 ± 0.12^{e}	4.42 ± 0.32^{hj}
B. japonicum B1-20 + Ge nanocarboxylate	4.42 ± 0.15^{de}	4.49 ± 0.17^{hj}

Note: different letters of upper indices in columns of tables indicate values that significantly differ one from another between the variants as a result of comparison using Tukey test ($P < 0.05$) taking into account Bonferroni correction (letters *a*, *b*, *c* – significantly between variants with optimal water supply (blossoming phase); letters *d*, *e* – significantly between variants with insufficient water supply (blossoming phase); letters *f*, *g* – significantly between variants with optimal water supply (phase of bean formation); letters *h*, *j* – significantly between the variants after resumption of watering to optimal (phase of bean formation).

Table 2
Ratio of concentrations of chlorophylls/carotenoids in leaves of soybean plants grown
in the conditions of different water supply and influence of metal nanocarboxylates

Variant	Phase of plant development	
	blossoming	bean formation
Watering level	optimal (60% CF, capillary fringe)	
B. japonicum B1-20 (control 1)	5.63 ± 0.16^{ab}	4.17 ± 0.13^{fg}
B. japonicum B1-20 + Cr nanocarboxylate	5.93 ± 0.11^{b}	5.32 ± 0.26^{gh}
B. japonicum B1-20 + Co nanocarboxylate	5.35 ± 0.25^{ab}	4.84 ± 0.21^{g}
B. japonicum B1-20 + Fe nanocarboxylate	5.13 ± 0.15^{a}	4.98 ± 0.15^{gh}
B. japonicum B1-20 + Cu nanocarboxylate	5.41 ± 0.12^{ab}	3.97 ± 0.11^{f}
B. japonicum B1-20 + Ge nanocarboxylate	5.56 ± 0.40^{ab}	5.28 ± 0.18^{gh}
Watering level	insufficient (30% CF)	resumption to optimal (60% CF)
B. japonicum B1-20 (control 2)	6.35 ± 0.13^{e}	4.54 ± 0.17^{jk}
B. japonicum B1-20 + Cr nanocarboxylate	5.29 ± 0.11^{de}	4.97 ± 0.41^{jk}
B. japonicum B1-20 + Co nanocarboxylate	5.19 ± 0.26^{de}	4.86 ± 0.12^{jk}
B. japonicum B1-20 + Fe nanocarboxylate	5.03 ± 0.19^{d}	4.61 ± 0.14^{j}
B. japonicum B1-20 + Cu nanocarboxylate	5.54 ± 0.15^{de}	4.86 ± 0.25^{jk}
B. japonicum B1-20 + Ge nanocarboxylate	3.48 ± 0.36^{c}	4.81 ± 0.15^{jk}

Note: different letters of upper indices in the columns of the table indicate values that are significantly different among one another between the variants according to comparison using the Tukey test ($P < 0.05$) taking into account Bonferroni correction (letters *a*, *b* – significantly between the variants with optimal water supply (blossoming phase); letters *c*, *d*, *e* – significantly between the variants with insufficient water supply (blossoming phase); letters *f*, *g*, *h* – significantly between the variants with optimal water supply (phase of bean formation); letters *j*, *k* – significantly between the variants after resumption of watering to optimal (phase of bean formation).

Discussion

Success of adaptation of plants to influence of stressors to a great degree depends on optimal functioning of assimilative apparatus. Some of the parameters of condition of the apparatus are the content and ratio of photosynthetic pigments (Amunova & Lisitsin, 2019). Quantitative and qualitative changes in the pigment system is a sensitive parameter of not only the condition of photosynthesis apparatus, but also physiological condition of plants and orientation of adaptive reactions during exposure to negative environmental factors. Different stress factors, including drought, usually lead to significant decrease in chlorophylls and carotenoids. Decrease in their concentrations may be a consequence of impeding their biosynthesis, or ruination. In turn, decrease in the amount of photosynthetic pigments obstructs the transport of electrons, and therefore decreases photosynthesis activity in most green plants (Grzeszczuk et al., 2018).

As is known, the dominating form of chlorophyll in plants is chlorophyll *a*, represented in both the reaction centers of photosystems and light-collecting complexes of chloroplasts. Level of chlorophyll *a* in flag leaf of wheat is used as a marker of screening to drought tolerance (Hassanzadeh et al., 2009). Chlorophyll *b* exists only in light-collecting complexes (Kume et al., 2018). The decrease in the content of chlorophylls in soybean leaves which we observed during insufficient watering was expected, because prolonged soil drought can cause significant decrease in chlorophylls *a*, *b* and their total (Manivannan et al., 2007; Kanbar et al., 2011; Gholamin & Karimpour, 2019), which may be a result of damage to chloroplasts, specifically the structure of thylakoid membranes and photosystem II by active oxygen species (Khayatnezhad & Gholamin, 2012). We determined that metal nanocarboxylates are able to influence the content of chlorophylls in leaves of soybean plants (Fig. 1–3). In particular, using those compounds as components of inoculation suspension for pre-sowing treatment of seeds caused increase in the content of chlorophylls in leaves of soybean depending on the level of its watering and had a protective effect on the pigment complex of plants grown in the conditions of insufficient water supply (Fig. 1, 4). At the same time, degree of their influence largely depended on the microelement applied. In the conditions of optimal watering, the highest contents of chlorophyll *a*, chlorophyll *b* and their total were observed in the variant with germanium nanocarboxylate (Fig. 1). The stimulating action exerted by nanocarboxylate of this element was obviously conditioned by its ability to influence the activity of antioxidant enzymes. A collective of scientists from Korea in a number of studies with plants *Oplopanax elatus* (Nakai) confirmed that exogenous germanium is able to increase antioxidative activity and increase the activity of radicals in DPPH and ABTS plants. Moreover, total content of phenol and flavonoids in plants during treatment with 50 mg of GeO_2 was higher than in the control (Kim et al., 2016; Liu et al., 2016).

The literature data indicate that copper has a significant effect on the synthesis of chlorophylls, specifically it plays a role directly in the processes of their formation and makes chlorophylls resistant to destruction, stabilizing them and therefore providing prolongation of functions of photosynthetic activity of the green organs, impeding the processes of physiological aging of plastids and increasing the productivity of plants. Scientists attribute such an ability of copper to stabilization of chlorophyll-protein-lipoid complexes by this element. Furthermore, the ability of insignificant concentrations of copper to stimulate the synthesis of chlorophylls in barley plants was confirmed (Bernal et al., 2006). The results of our studies indicate that copper nanocarboxylates as a component of inoculating suspension increased the level of chlorophylls in leaves of soybean plants grown in drought conditions, compared with leaves of soybean of control 2 (Fig. 1).

There is not much data about the positive influence of cobalt on synthesis and content of chlorophyll, and the stimulating effect of this element on those pigments in soybean leaves which we observed may have been indirect, i.e. they activate the process of symbiotic fixation of molecular nitrogen that supplies a significant amount of nitrogen to plants, which they require for chlorophyll synthesis. Cobalt is one of the essential elements of symbiotic nitrogenation and a component of three cobalt-dependent enzymes that take part in formation of rhizome bulbs. It stimulates the development of bacteroid tissues, promotes increase in the amount of ribosomes in plant and bacteroid cells, and at the same time, increase occurs in the activity of bacteroids in rhizome bulbs of legumes. Moreover, cobalt may indirectly influence on the plant organism, increasing intensity of respiration, photosynthesis, cellular reproduction and increasing the overall content of water in the tissues, which is especially relevant in the conditions of insufficient water supply. There are data, according to which cobalt stimulates the synthesis of chlorophyll and decreases its breakdown (Hu et al., 2021).

The effect of using chromium nanocarboxylate as a component of inoculation suspension greatly depended on the conditions of the plants' cultivation. In particular, the examined element – even though having a stimulating effect – caused lower increase in soybean plants that received optimum watering compared with experiments with germanium, copper, and cobalt nanocarboxylates. At the same time, in the conditions of insufficient watering of soybean plants grown from seeds inoculated with rhizobia with additions of chromium nanocarboxylate, there was observed a maximal content of chlorophyls among all the studied variants (Fig. 1–3). Chromium is not an essential element for plants. Furthermore, large concentrations of this element may be highly toxic to the plant organism, therefore leads to formation of a number of carcinogenic substances that cause chromosome deviations, mutations, damage to DNA, inhibition of enzymes of nitroreductase, glutamate synthase and glutamine synthase, chlorophyllase. This results in decrease in the content of nitrate nitrogen, intensity of growth processes, destruction of thin structure of chloroplasts and their membranes, etc. Despite this fact, a number of scientists have found a positive effect of chromium on plants. As is known, small concentrations of this element are able to stimulate the activity of a number of enzymes, particularly catalase, proteinase and others. The treatment of seeds of maize with solution of potassium chromate was confirmed to increase the productivity of photosynthesis and concentration of chlorophylls (Sharma et al., 2020).

Iron nanocarboxylate showed the least expressed positive effect on the concentration of chlorophylls. Its action significantly depended on the level of water supply and phase of plant development. However, there are data in the literature which suggest that treatment of rapeseed with aqueous suspension of iron nanopowder stimulated growth of the root system, increased the area of photosynthetic surface and water-holding ability of leaves, increased the tolerance of plants to a number of diseases and thus increased qualitative and quantitative parameters of productivity of the studied plants. Similar results were obtained for mustard and flax. Efficiency of influence of iron nanopowders on the development of plants was confirmed by large-scale studies that resulted in determining significant increase in productivity of green mass of grain crops and their influence. As known, iron in nanoform is easily absorbed by enzymic systems of plants and improves the tolerance of plants to negative environmental factors (Li et al., 2021). As confirmed, this element plays an important role in the development of the structure and functions of chloroplasts, and also regulates chlorophyll synthesis. Particularly, it was shown that about 80.0% iron is present in photosynthesizing cells of plants, where it is needed for biosynthesis of cytochromes and other gem-containing molecules, including chlorophylls, proteins of electron-transport system, and also needed for constructing clusters with sulfur. To form chlorophyll, the same metabolic pathway is used as for the synthesis of structure of heme, which is a non-protein component of a number of cell enzymes. In the conditions of iron deficiency, rates of formation of precursors of porphyrin (delta-aminolevulinic acid) decrease, resulting in decrease in photosynthetic units in leaves (without damage to photosynthesis apparatus). Furthermore, it was found that concentrations of iron and chlorophyll in green plants correlate well with each other (Rout, 2015).

Other than chlorophylls, a constant component of photosynthesis systems is carotenoids – polyfunctional pigments that perform the role of supporting light-collecting pigments in the processes of photosynthesis, protect chlorophyll from destruction during oxidative stress caused by unfavourable environmental factors. They absorb light in blue parts of the spectrum and to a lower degree in green parts of it, transmitting energy to chlorophyll. Our research found that in the conditions of two-week influence of insufficient water supply, there was an increase in the content of carotenoids in almost all variants we examined, compared with plants of control 2. Moreover, we determined that using chromium and germanium nanocarboxylates as components of inoculation suspension increased content of carotenoids in leaves not only compared with control 2, but also compared with plants of similar variants grown in optimal conditions of water supply (Fig. 4). Such an accumulation of yellow pigments in leaves may be considered adaptive reaction of plants, oriented at increase in resistance of photosynthesis apparatus and prevention of photodynamic destruction, and therefore decrease in general stress (Young, 2006). In addition, growth of concentrations of such pigments plays a protective role, protecting chlorophyll from photoacidification, and – similarly to catalase – they block accumulation of peroxide that deleteriously affects the cells. Positive effect of metal nanocarboxylates on the concentration of photosynthetic pigments in leaves of soybean plants grown with insufficient watering well correlates with the literature data suggesting that nanodrugs of biogenic metals increase tolerance of biological systems to unfavourable weather conditions (Rastogi, 2017).

Over the period of resumption of watering (bean formation phase), the content of pigments in leaves of soybean decreased compared with the previous phase of development in most examined variants. Such an effect has likely been caused by re-distribution of metabolites that form grains in this phase of development of plants. At the same time, the highest concentration of photosynthetic pigments was seen in variants with use of chromium and germanium nanocarboxylates depending on the level of watering. It has to be noted that we found a significant stimulating effect of iron nanocarboxylate on the monitored parameters of plants grown in the conditions of watering in the same development phase of plants (Fig. 1–4).

If the study of the content of photosynthetic pigments allows us to evaluate the influence of unfavourable environmental factors on plants, then changes in ratios of chlorophyll *a* to *b* serve as parameters of tolerance and physiological state of plants, and in the conditions of drought they may be markers of screening of stress tolerance (Takai et al., 2010). The complex of photosystem II is known to contain 200 chlorophyll *a* molecules, 100 molecules of chlorophyll *b*, and also carotenoids and other important constituents. Changes in this ratio may result in changes in functioning of photosynthesis apparatus. The highest parameters in chlorophyll *a/b* ratio which we found in leaves of soybean of all examined variants were caused by high concentration of chlorophyll *a* (Table 2). Such a result may be due to the fact that the analysis was conducted on young leaves of the upper layer of soybean plants, where chlorophyll *a* content is higher than in leaves of other layers. In the blossoming phase, decrease in ratio of chlorophylls *a/b* compared with respective control, lowest values in variants treated with cobalt and both levels of water supply, as well as application of chromium and germanium to plants grown in the conditions of insufficient water supply is obviously a consequence of higher content of chlorophyll *b* in those variants, i.e. those plants had greater antenna pigment. Such results are coherent with the data of other authors, according to whom, heat shock notably decreases chlorophyll *a/b* ratio in leaves of plants (Fomishyna et al., 2009).

A no less important parameter of physiological condition of plants and their ability to tolerate stress factors is ratio of overall content of carotenoids to chlorophylls. In normal conditions, this parameter is stable, though very sensitive when subject to extreme environmental factors. High values of ratio of chlorophylls to carotenoids, which we found in leaves of soybean plants of control 2, indicate low content of carotenoids in leaves of soybean plants of this variant, and therefore low protective abilities of those plants. And by contrast, the decrease in ratio of chlorophylls to carotenoids in the conditions of insufficient watering in variants with using metal nanocarboxylates, compared with control 2, indicate increase in relative level of carotenoids that carry out protective function of chlorophylls by neutralizing oxidative stress.

Conclusions

We found changes in the amount of photosynthetic pigments in leaves of soybean plants subject to metal nanocarboxylates – they depended on the level of watering, phases of development of plants and metal that had been added to the rhizobia growth medium. At the same time, the most efficient method of protecting the pigment complex in the conditions of drought was using chromium and germanium nanocarboxylates. The study revealed that using chromium nanocarboxylates as a component of inoculation suspension led to the highest level of chlorophylls in leaves of soybean plants grown with insufficient watering among all the studied variants. Such a result indicates an opportunity of introducing nanoforms of this element to the technology of soybean cultivation in the conditions of insufficient water supply as an effective way of influencing biosynthesis of chlorophylls, and therefore crop yield.

Treatment of seeds of soybean with rhizobia bacteria *B. japonicum* B1-20 and germanium nanocarboxylates was confirmed to significantly increase the content of carotenoids in leaves of soybean plants subject to water deficiency, indicating high-level activity of protective mechanisms of the pigment of system of plants of this variant, related to stress tolerance caused by water deficiency.

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