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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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) (Crost & 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 systems and dividing the charges within reaction centers (Kaliaha & Khorshidi, 2017) and transferring their energy to reaction centers (Adams et al., 2016). Pigments are photoacceptors which consume quanta of noticeable share of the solar spectrum and are involved in conversion of light energy into energy of chemical bonds (Asrifa & 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 (Kalista & 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 interfering 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 yel-

Keywords: rhizobia, Glycine max; chromium nanocarboxylate; geranium nanocarboxylate; drought; photosynthetic pigments.

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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 22.6% 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 nanocarboxylate 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; geranium nanocarboxylate; drought; photosynthetic pigments.

The number of studies focusing on the influence of environmental factors on changes in chlorophyll has exceeded 2 thou (Esteban et al., 2015; Pradikara & Morgan, 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 (Asrifa & 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 (Kalista & 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 photosynthetic 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 interfering 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 yel-

Biosyst. Divers., 2022, 30(1)
Caroteneoids 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 caroteneoids in leaves significantly changes as a response to the influence of environmental factors. Stress factors: extreme temperatures, salinity, heavy metals, drought, and acidity of soil cause significant decrease in the content of photosynthetic pigments through inhibition of biosynthesis, or ruination (Sokolowska-Sergienko & Stasiak, 2008). Such changes in pigment complex may lead to malfunctioning of electron transport, and therefore decrease in photosynthetic ability in most green plants.

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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 Ltd. Scientific Industrial Company (Ukraine, Kyiv). They were obtained in two stages: 1 – obtaining aqueous colloidal solution of nano-particles of microelements by dispersing highly purified granules of corresponding metals using electric stream impulse in de-ionized water; 2 – obtaining carboxylates 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 °C for 6–7 days. Rhizobia bacteria were washed off the surface of agarized medium with sterile water, and suspension of Erkenmeyer 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 °C on rocket with vel.

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% of capillary fringe (CF) or insufficient (30% CF) 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%
Biosyst. Divers., 2022, 30(1)

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 dispersive analysis (ANOVA), where the differences were considered significant when *P* values were less than 0.05 (taking into account Bonferroni correction).

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 chlorophyll *a* in plants of this variant tended to decrease.

### 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 nanocarboxylates 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.

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**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
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).

![Graph](image)

**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 (\(x \pm SE, n = 8\)); 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 values of the studied parameter were recorded in the phase of blossoming in plants exposed to influence of cobalt 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* В1-20 with additions of chromium nanocarboxylates, we observed maximal value of ratio of \(a/b\) chlorophylls which exceeded control 1 by 17.4%.

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 nanocarboxylates, 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 (\( x \pm SE, n = 8 \)); 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 (\( x \pm SE, n = 8 \)); 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.
to chloroplasts, specifically the structure of thylakoid membranes and chlorophylls (Gholamin & Karimpour, 2019), which may be a result of damage to the 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 nutrition. 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., 2016).

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 (Hassanazadeh 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-seeding 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 micronerol element 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 has revealed 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 GeO₂ 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. Scientif-

### Table 1

| Variant                  | Watering level | Phase of plant development | Mean ± SE | \*abde |
|--------------------------|----------------|-----------------------------|----------|-------|
| B. japonicum B1-20 (control 1) | 4.56 ± 0.11*   | blossoming                  | 4.38 ± 0.15* |
| B. japonicum B1-20 + Cr nanocarboxylate | 4.67 ± 0.29*   | optimal (60% CF, capillary fringe) | 5.38 ± 0.11* |
| B. japonicum B1-20 + Co nanocarboxylate | 4.00 ± 0.23*   | 4.71 ± 0.16* |
| B. japonicum B1-20 + Fe nanocarboxylate | 4.93 ± 0.15*   | 4.79 ± 0.21* |
| B. japonicum B1-20 + Cu nanocarboxylate | 5.17 ± 0.12*   | 4.65 ± 0.13* |
| B. japonicum B1-20 + Ge nanocarboxylate | 4.38 ± 0.19*   | 4.46 ± 0.24* |
| B. japonicum B1-20 + Fe + Ge nanocarboxylate | 5.04 ± 0.12*   | 4.42 ± 0.32* |
| B. japonicum B1-20 + Cu + Ge nanocarboxylate | 4.42 ± 0.15*   | 4.49 ± 0.17* |

Note: different letters of upper indices in columns indicate values that significantly differ among one another between the variants according to comparison using the 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

| Variant                  | Watering level | Phase of plant development | Mean ± SE | \*abde |
|--------------------------|----------------|-----------------------------|----------|-------|
| B. japonicum B1-20 (control 1) | 5.63 ± 0.16*   | blossoming                  | 4.17 ± 0.13* |
| B. japonicum B1-20 + Cr nanocarboxylate | 5.93 ± 0.11*   | optimal (60% CF, capillary fringe) | 5.32 ± 0.26* |
| B. japonicum B1-20 + Co nanocarboxylate | 5.35 ± 0.24*   | 4.84 ± 0.21* |
| B. japonicum B1-20 + Fe nanocarboxylate | 5.13 ± 0.15*   | 4.98 ± 0.15* |
| B. japonicum B1-20 + Cu nanocarboxylate | 5.41 ± 0.12*   | 3.97 ± 0.11* |
| B. japonicum B1-20 + Ge nanocarboxylate | 5.56 ± 0.46*   | 5.28 ± 0.18* |
| B. japonicum B1-20 + Fe + Ge nanocarboxylate | 6.35 ± 0.13*   | 4.54 ± 0.17* |
| B. japonicum B1-20 + Cu + Ge nanocarboxylate | 5.29 ± 0.11*   | 4.97 ± 0.41* |
| B. japonicum B1-20 + Cu + Fe + Ge nanocarboxylate | 5.19 ± 0.26*   | 4.86 ± 0.12* |
| B. japonicum B1-20 + Cu + Co nanocarboxylate | 5.03 ± 0.19*   | 4.61 ± 0.14* |
| B. japonicum B1-20 + Fe + Cu + Ge nanocarboxylate | 5.54 ± 0.15*   | 4.86 ± 0.25* |
| B. japonicum B1-20 + Fe + Co + Ge nanocarboxylate | 3.48 ± 0.36*   | 4.81 ± 0.15* |

Note: different letters of upper indices in columns indicate values that significantly differ among one another between the variants according to comparison using the 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, h – significantly between 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 (Aramonova & Listian, 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 nutrition. 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., 2016).

Biosyst. Divers., 2022, 30(1)
nists attribute such an ability of copper to stabilization of chlorophyll-protein-lipid complexes by this element. Furthermore, the ability of insignificant concentrations of copper to stimulate the synthesis of chlorophylls in barley plants was confirmed (Bermal et al., 2006). The results of our studies indicate that copper nanocarboxylates as a component of inoculation 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 chlorophylls 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 inoculation suspension increased the level of chlorophylls 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 (Fig. 1–4). 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 photodestruction, 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 environmental factors. (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 anterona 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 (Fornishyna 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 chlorophyll. 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 system of plants of this variant, related to stress tolerance caused by water deficiency.

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