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Symbiotic nitrogen fixation of soybean-rhizobium complexes and productivity of soybean culture as affected by the retardant chlormequat chloride

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The effect of pre-sowing inoculation of the soybean (*Glycine max* (L.) Merr.) cultivar ‘Podilska 1’ seeds, with the effectual strain of *Bradyrhizobium japonicum* 71 t, and the subsequent spraying the plants (within the stage of budding) with the antigibberellic preparation of chlormequat chloride in regard to the soy-rhizobium complex formation, as well as the symbiotic nitrogen fixation activity have been studied within the experiment. The anatomic- morphological and physiological parameters of the source and sink functioning as a system, the peculiarities of the crop formation and its quality affected by inoculation with strain 71 t and the subsequent retardant preparation were tested. As it goes out of the data, the use of the retardant in addition to inoculation proved to be effectual, for its use caused limitation of the linear growth of the plant, stimulating branching of the stem and resulted in growth of leaves in number, including the overall area of laminae per plant. Chlormequat chloride was a proven cause of more powerful mesostructure formation, previously due to the enhanced development of chlorenchyme towards growth of pure photosynthesis productivity. Moreover, the retardant caused the formation of a more powerful source-sink sphere of the plant, which provided with assimilates both the processes of symbiotic assimilation and the processes of formation and development of beans and seeds. The results obtained in the research testify to the strengthening of mutual interdependence of bean- rhizobium complexes: the number and weight of the nodules on the roots of inoculated plants increased due to the preparation. In consequence of the enhanced provision of bacteria with assimilates the nitrogen efficacy of the bean-rhesobium complexes essentially increased. It is also important to mention that the formation of plant productivity depended on enhanced supply of the processes of symbiotic nitrogen fixation with assimilates which led to a shift in the peak of activity of nitrogenase to a later stage of ontogenesis - the phase of green bean. Under conditions of the seed inoculation with strain 71t, the activity of nitrate reduction was higher than that of control, and increased from the phase of flowering to the phase of bean formation both in the roots and in the leaves of soybean plants. The use of chlormequat chloride against the background inoculation with the strain did not influence the activity of the ferments in the roots, though reduced it in the leaves, especially in the phase of bean formation. Hence, the effect of retardant chlormequat chloride on the nitrogen redistribution is directly carried out through stimulation of the forming and functioning of the bean-rhisobium complex, and not through the activation of nitrate reduction. Because of increased branching of the stems and placing of a greater number of beans, the formation of a larger leaf surface and the growth of photosynthetic activity per unit of the blade surface area, improvement of the nitrogen nutrition increased the yield capacity of the crop and improved the quality of the products – the contents of nitrogen in the seeds increased and the contents of unsaturated fatty acids in the soybean oil increased either. The suggested formula of the preparation did not cause the accumulation of chlormequat chloride in soybean seeds above the allowed norms.
Keywords: *Glycine max* (L) Merr.; morphogenesis; production process; retardants; symbiotic nitrogen fixation

Introduction

Formation and functioning of the bean - rhizobium complexes determine to a considerable extent the provision of plants with bound nitrogen, which significantly affects the yield of legumes. An efficient influence on the interaction between a legume plant and rhizobia depends also on the physiological state of a plant on the one hand, and the virulence and activity of the bacteria – on the other. In connection with the search of ways to mobilize the internal reserves of nitrogen fixators is carried out to achieve maximum intensification of the process of biological fixation of atmospheric nitrogen (Lee et al., 2012; Alam, F. et al., 2015). Distinctive features of high nitrogen fixation availability also manifested themselves in being competitive as far as aboriginal micro flora of the soil and resistance to stress factors of bacterial preparations is concerned. An effective symbiosis of nodule bacteria with a host plant is determined under the condition of sufficient photosynthesis products, which, being the source of energy for the processes of nitrogen fixation and ammonia assimilation, are distributed from the macrosymbiont to the microsymbiont (Kots et al., 2011). It was also established that the retardant growth regulators (retardants) caused changes not only in the plant but in the growth rate of their different organs. The formation of spare assimilators during the inhibition of linear growth of the plant enhances redistribution of the photosynthesis products towards economically valuable organs, which contributes to the increase of crop yields (Kuryata & Golunova, 2018; Kuryata & Kravets, 2018; Kuryata & Polyvanyi, 2018). Therefore, the processes of storage and redistribution of photoassymilates (among the plant organs influenced by growth regulators) should be considered from the viewpoint of donor-acceptor relations of the plant ("source-sink" system). According to this concept the photosynthesis processes are considered to be a donor, whereas the acceptor – the growth processes, accumulation of reserve substances and the active metabolism zone with autotrophic nutrition (Yu et al., 2015; Bonelli et al., 2016), or the interaction between the storage organs and growth processes at the heterotrophic stage of seedling development (Poprotska & Kuryata, 2017). For legume plants the analysis of source-sink relationship can not be limited only to the specifics of the redistribution of assimilates between the vegetative and generative organs of plants, growth processes and photosynthesis, since the legume - rhizobia complexes serve as additional centres for the traction – redistribution of assimilates. The properties of symbiotic nitrogen fixation within the source - sink system have not been studied well enough yet.

Source and sink spheres of the plant are connected by the system of hormonal and trophic ties (direct, inverse), which provide the correlation between the processes of growth and photosynthesis. The use of synthetic growth regulators enables artificially to alter morphogenesis (Yang et al., 2016), the effectiveness of the vegetative phase and photosynthetic processes therein dealt with to regulate plant loading with fruit and seeds (Zhang et al., 2013; Kasem & Abd El-Baset, 2015; Carvalho et al., 2016; Koutroubas & Damalas, 2016). In fact, the use of such preparations makes it available to prognosticate different degrees of strain in the source - sink relationship and find out a model according to which, anatomic- morphological, physiological changes, the redistribution of assimilates occurs between the organs of experimental plants (Yan et al., 2013, 2015; Wang et al., 2016).

Development and functioning of the root nodules occurs with the participation of phytohormones. In particular, the nodule bacteria can autonomously synthesize gibberellins; however the treatment of soybean seeds with the gibberellin slowed the formation of nodules and had a negative effect on the processes of nitrogen fixation (Zhang et al., 2013). Proceeding from the above mentioned, it is advisable to analyze the effect of preparations with the antigibberellic mechanism (the retardants) on the establishment and functioning of leguminous - rhizobium complex. The retardants are a group of synthetic growth regulators, different by their chemical nature, which are used to inhibit growth processes (Espindula, 2010; Kasem, Abd El-Baset, 2015; Carvalho et al., 2016), increase plant resistance to adverse environmental factors (Li et al., 2010; Peng et al., 2014; Fahad et al., 2016), as well as product quality regulation (Souza et al., 2010; Panyapruek et al., 2016). These preparations have different chemical properties as their structure is concerned, though almost all of them cause the same effect – slowing the division and stretching of cells, being followed by inhibition of growth in general, without causing any abnormal deviations. In this regard, they can enhance branch shooting (Rogach et al., 2016), forming more powerful source area in the plant (Matsoukis et al., 2015; Sousa Lima et al., 2016). With sufficient activity of the assimilation apparatus, the artificial limitation of the vegetative organs growth influenced by retardants causes the redistribution of assimilates towards fruit formation, root crops, tubers, often resulting in increased yields and improved quality of crop production (Pavlista, 2013; Macedo et al., 2017). The physiological effect of the retardants being known is carried out either by blocking the gibberellin synthesis or by blocking the hormone-sink complex formation, in consequence of which the effect of the already synthesized gibberellin is not brought into action (Sang-Kuk & Hak-Yoon 2014; Rademacher, 2016; Hedden & Thomas, 2016, Taniguchi et al., 2018,). The recent researches have proved that the use of different groups of retardants cause not only slower linear growth but they also stimulate branching of the stem, placing more flowers and beans, which significantly increases yield-capacity (Pobudkiewicz, 2014; Kasem & Abd El-Baset, 2015; Koutroubas, 2016; Rogach et al, 2018). There are a lot of publications devoted to effectual retardant and inoculation usage apart, whereas there is no data in concern with the efficacy of joint application of the soybean seeds inoculatied with highly effective *B. japonicum* strains and further retardant treatment to optimize the production process. There is practically no data concerning the retardant effect on the activity of nitrogen fixation and nitrate reduction, which are the key enzymes in dinitrogen fixation and incorporation of soil nitrates into metabolism.

Among various groups of the retardants the increasingly used one is chlormequat chloride, which does not detect any mutagenic and blastogenic properties, it can be neither accumulated nor decomposed in the organism, and within 48 hours it gets removed from it, which, to a large extent, determines its wide use in crop production.

In view of the above mentioned, the purpose of this research was to study the way chlormequat chloride preparation influences the formation and efficiency of the symbiotic soybean - *B. japonicum* system, morphogenesis, yield-capacity and quality of soybean crops. Symbiotic Nitrogen Fixation of Soybean - Rhizobium Complexes and Productivity of Soybean culture as affected by the retardant chlormequat chloride.

Materials and methods

The vegetation research was done in 2016 and deals with soybean (*Glycine max* (L.) Merrill) cultivar 'Podilska 1' and the strain of slow-growing nodule bacteria *B. japonicum* 71t from the collection of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. 'Podilska 1' – medium - late - ripe cultivar, grain use direction. The applicant – Institute of Agroecology and Biotechnology of NAAS, Kyiv. The cultivar passed a state variety trial and in 1997 it was introduced to the register of cultivars of Ukraine. The bush is compact of 80-100 cm high; the stem is green with reddish pubescence and a straight end. The flower is medium, purple (8-12 in the bundle), the pod is slightly bent, and there are 2-4 seeds in a bean. The seed is oval, yellow without pigmentation; the scar is brown, oval. The yield capacity is 14.9 centner per hectare, the fat content is 24.2%, the contents of oil is 241 kg ha^{-1}, the crude protein content is 35.3%, the protein yield is 332 kg ha^{-1}, the lying flat resistance is 5.0 points, crop shedding - 4.8, drought - 4.2. Recommended cultivation zone – forest - steppe.

The plants were raised in 15-kilogramme containers in soil culture with the nutrient medium of VNIS in the vegetarian house of the biological department at Vinnytsia State Pedagogical University. The soybean seeds were inoculated with strain 71 t on the day of their sowing. Before being sown the seeds were sterilized with 70% ethyl alcohol for 20 minutes, washed in running water and inoculated. The seeds for the control variant were washed without any inoculation to follow. Each vessel contained 5 plants. During the experiment 60% soil moisture was maintained, and the lighting was natural.

The plants were treated at the budding stage with 0.5% water solution of chlormequat chloride (CCC, α-chloroethyltrimethylammonium chloride) -[Cl-CH_2-CH_2N $(CH_3)^3$]+C_1 -by spraying the leaves (in morning hours) until they got fully wet. The preparation is obtained by means of dichloroethane interaction with trimethylamine within in a single stage under pressure at the temperature of 80-90 °C. It is white crystalline substance decomposed at the temperature of 245 °C, insoluble in the hydrocarbons, but soluble in water: solubility is 74 % at 20 °C. LD50 for white rats is 640 mg kg^{-1}, the maximum daily dose for a human is 0.07-0.09 mg. The maximum level of the preparation in food is 0.1-0.3 mg kg^{-1}. The substance is low toxic, the antigibberellic effect of it deals with inhibition of the ents-kauren-synthase activity at the synthesis of copalyl pyrophosphate from geranilgrananoyldifosfate in the process of gibberellin formation.

The morphometric indices - height of the plants, number of leaves, total area of the blade surface, number and weight of the nodules on the roots were determined in each phase of the plant development. The net photosynthetic productivity (NPP) was determined as the dry substance increasing in mass per unit of the leaf surface area of the fixed material by means of «Micmed-1» microscope and MOV-1-15x microdrive micrometer. The vegetative material was fixed with the mixture consisting of equal parts of ethyl alcohol, glycerine, 1% aqueous formalin solution. The cell sizes were measured by means of the preparations macerated with 5% acetic acid in 2 N hydrochloric acid. During the vegetation, in accordance with the stages of development, (flowering, the mass formation of beans, the phase of green bean) a fixed number of plants were regularly selected for further research. The plants for biochemical analysis were fixed by liquid nitrogen and dried up in a drying cabinet at the temperature of 60 °C. All the experiments had fivefold recurrence.

Nitrogen-fixing (nitrogenase) effectuality was determined by the level of acetyleneresistant activity of the root nodules by means of the acetylene method. The roots with nodules were placed in glass bottles of 100 cm^3 and closed hermetically with rubber membranes through which acetylene was injected in. Its final concentration in the gas mixture of the bottles equaled 10%. The duration of incubation of the analyzed plant samples - 1 hour. After that, the gas mixture (containing the ethylene produced in the result of acetylene reduction by nitrogenase activity) was separated with a flame-ionizing detector on Chromatograf-504 (Mera Elwro, Poland). The gases were separated on an oven (0.40 130 cm) with Parapac N at 80 °C. The gas - carrier was nitrogen (50 ml/min). The volume of the gas mixture under consideration was 0.5-1.0 cm^3. Neat ethelen was used for the standard. The nitrate reduction activity in the laminae and roots was determined by vacume infiltration 0.1n. KNO_3 (30 min. at 27 °C) followed by determination of the optical density of the solution in wave-length 540 nm after the addition of Griss reagent. In the seeds, towards the end of vegetation, the total contents of nitrogen was measured by Keldall method and the oil was extracted with a Soxhlet appliance. The solvent was petroleum ether with the boiling point of 40-65°C (AOAC, 2010).

Determination of fatty acids contents in the soy oil (quantitative components) was indicated by gas chromatography method on chromatograph Crystal-2000 (AOAC, 2010). The conditions of chromatography: glass cylinders 1500 × 2 mm filled in with sorbent (fraction 0.16 - 0.20 mm) intertop-super+5% neoplex 400 were used. The gas- bearer was nitrogen. Its passing rate equaled 70 ml/min.

The temperature of the heating oven – 2000 °C, the evaporator – 2300 °C, the flame ionizing detector – 2400°C. The analytical recurrence of studies was fivefold.

The CCC residuary amounts in the soy seeds were indexed by thin-layer chromatography in a thin layer of cationite on the plates Silufol UV-254 (Czech). 23% sulfuric acid was used as a mobile solvent. For development the chromatograms were submerged in 11% water solution of phosphoric-molybdenum acid followed by thirty-minute time flushing in water, and finally, the plate was dipped in 1% PbCl2 solution of 10% HCl. The amount of CCC was established by determining the optical density magnitude of the analysed chromatogram samples and the standard solutions, which were measured with a spectrophotometer SF-46 (Russia) in wavelength - 730 nm and compared.

The statistical analysis of the data was performed imploying program Statistica, version 6 (StatSoft Inc., USA). The reliability of the difference between the control and experimental variants was tested by the t-criterion of the Student. The tables and figures represent the arithmetic mean values and their standard errors.

Results and discussion

It has been established, that the outcome of the seed inoculation with strain 71t and the use of chlormequat chloride significantly influenced the growth processes and the formation of the soy source sphere. In particular, the seed inoculation with strain 71t had a stimulating effect and conditioned a better development of the experimental plants in comparison with those of control. The complex application of inoculation and the retardant resulted in the plant height decrease, whereas it caused an increase in the stem branching (Figure 1).

Figure 1. Influence of strain *B. japonicum* 71t and chlormequat chloride on growth of cultivar ‘Podilska 1’: 1 - control, 2 - inoculation with strain 71t, 3 - inoculation with strain 71t+0.5% chlormequat chloride treatment.

The effect of retardants on the photosynthetic effectuality of the plant is realized, to a certain extent, through the anatomical and morphological changes of the photosynthetic apparatus, primarily owing to the plant leaf surface area shaping (Kiriziy et al., 2014; Kuryata, Polyvanyi, 2018). With this, the amount of carbon dioxide absorbed by the whole plant and, accordingly, the mass of the newly formed plastic substances can be characterized both by the intensity of photosynthesis per unit of a leaf surface and the total leaf area of the very same plant correspondingly. The data obtained show that under the conditions of strain *B. japonicum* 71t and the combined use of inoculation with antigibberellic preparation of chlormequat chloride, the quantity of leaves and the total laminae area of the soybean plants essentially increased, especially when inoculation and the retardant preparation were in combination (Table 1).

Table 1. Effect of inoculation and chlormequat chloride on morpho-physiological characteristics of soybean leaves.

Period of vegetation	Indicator	Control	Strain *B. japonicum* 71t	Strain *B. japonicum* 71t+0.5% CCC
Flowering	Total number of leaves, pcs.	6.33 ± 2.12	9.88 ± 3.11*	9.56 ± 1.65*
	Leaf surface area, cm^2	191.77 ± 3.66	268.27 ± 3.24*	258.21 ± 2.32*
	NPP g/m^2·day	1.30 ± 0.23	2.37 ± 0.33*	2.11 ± 0.21*
Mass formation of beans	Total number of leaves, pcs.	8.20 ± 3.67	11.58 ± 2.47*	13.63 ± 2.07*
	Leaf surface area, cm^2	230.30 ± 2.04	298.66 ± 2.24*	296.94 ± 3.68*
	NPP g/m^2 day	1.44 ± 0.23	2.41 ± 018*	3.54 ± 0.23*
The phase of green bean	Total number of leaves, pcs.	10.83 ± 2.04	13.17 ± 3.22	23.08 ± 3.04*
	Leaf surface area, cm^2	280.84 ± 4.22	321.12 ± 5.84*	344.56 ± 5.32*
	NPP g/m^2 day	0.98 ± 0.41	1.27 ± 0.17	2.44 ± 0.21*

* – significant differences P ≤ 0.05

While comparing the net productivity of photosynthesis (NPP) according to the experimental variants (Table 1) the increase of the photosynthetic activity of soy leaves is quite apparent in cases under the influence of inoculation and especially in the complex use of rhizobia with chlormequat chloride. The enhancement of photosynthetic processes caused by the retardants was also noted by other authors (Kumar et al., 2012). Since this parameter characterizes the photosynthetic activity of a leaf area fraction, it is expedient to analyze the peculiarities of anatomical structure of the laminae that deals with the photosynthetic processes and is called "mesostructure" in the scientific literature.

A number of the data in the literature devoted to the effect of retardants on the mesostructural characteristics of the leaves indicate changes in their structure after being influenced by the preparations with antigibberellic mechanism. The effect of retardants of different classes on the plants of oil poppy (Kuryata & Polyvanyi, 2018) and tomatoes (Kuryata & Kravets, 2018) manifested itself in thickening of the leaf blade on the account of the mesophyllic cells increasing in size. The data obtained proves similar changes to occur in the leaves of soybean plants affected by CCC and inoculation with highly effective strains. As it is seen, it is chlormequat chloride being used after the preliminary seed inoculation with strain 71t, that caused the largest thickening of leaves. In addition, the volume of thin walled cells of leaf parenchyma (according to the experimental variants) also reached the maximum value in case with the consistent inoculation with the strain and application of chlormequat chloride. For all that, the use of the preparations did not fundamentally affect the cell size of the spongy parenchyma. The use of strain 71t in combination with chlormequat chloride caused changes in the peritoneal apparatus of the leaf, increasing the stomata in number per unit of the abaxial leaf surface, which serves an important indicator of the intensity of gas exchange and transpiration processes (Table 2).

Table 2. The effect of inoculation with strain *B. japonicum* 71 t and treatment with chlormequat chloride on the mesostructure indices of Soybean leaves of cultivar 'Podilska 1' (phase of bean formation).

Variant	Thickness of a leaf blade, μm	Volume of parenchyma cells, $μm^3$	Cell length of spongy parenchyma, μm	Cell width of spongy parenchyma, μm	Number of stomata per mm^2 of the abaxial leaf surface
Control	162.4 ± 3.62	2146 ± 91	31.4 ± 2.46	25.8 ± 2.54	168.3 ± 2.20
Strain 71 t	270.4 ± 5.89*	2836 ± 38*	35.0 ± 1.82	28.2 ± 1.82	176.1 ± 1.38*
Strain 71 t+ 0.5% CCC	305.3 ± 5.27*	4562 ± 41*	32.9 ± 2.14	27.3 ± 0.63	183.3 ± 2.76*

* – significant differences P ≤ 0.05

Thus, the increase of the NPP index is mostly apparent in the variant with strain 71t and the retardant due to the leaf blade thickening and better development of the assimilative parenchyma. The retardant application against the background of inoculation with strain *B. japonicum* 71t influenced positively the anatomical and morphological parameters of the soybean photosynthetic apparatus, which is an important prerequisite for optimizing the formation of bean-rhizoidal complexes and increasing the crop capacity of the yield.

An important indicator of complementary interaction of macro- and micro-symbionts is the virulence with the nodule bacteria, which is defined by the nodule number. We have established an increase in the nodule activity when using the highly effective strain *B. japonicum* 71t in comparison with that of control (spontaneous inoculation, Table 3). The maximum number and weight of the nodules were observed in case with the retardant treatment against the background of strain 71t, the highest indices of them were marked in the phase of green bean.

Table 3. The effect of inoculation and retardant application on the formation of a nitrogen fixing mechanism of cultivar 'Podilska 1' soybean.

Variant	Development phase					
	Flowering		Bean formation		Green bean	
	number of nodules, pcs	dry substance nodules, g	number of nodules, pcs	dry substance nodules, g	number of nodules, pcs	dry substance nodules, g
Control	5.7 ± 0.11	0.03 ± 0.02	10.4 ± 0.22	0.15 ± 0.03	18.6 ± 0.27	0.16 ± 0.21
Strain 71 t	32.4 ± 0.14*	0.24 ± 0.04*	62.0 ± 0.31*	0.43 ± 0.07*	71.2 ± 0,24*	0.53 ± 0.06*
Strain 71t+ 0.5% CCC	32.0 ± 0.32*	0.25 ± 0.11*	63.2 ± 0.20*	0.51 ± 0.10*	73.0 ± 0.12*	0.61 ± 0.19*

* – Significant differences P ≤ 0.05.

The virulence of nodule bacteria is important in the shaping of effective symbiosis; however, the main distinctive value in this process belongs to the nitrogen-fixing efficacy of the formed nodules. The correlation between the photosynthetic activity of plants and the development of nodules is apparent (Kots et al., 2011). The period of the bean formation in soybean plants is accompanied by rearrangement of assimilates towards the generative organs, and, in consequence of that, the active growth of the vegetative mass of the plants ceases, the acetylene reductive activity of the root nodules decreases. Under the conditions of effective symbiosis of nodules by the end of the reproductive phase, they become to grow old. In the ineffective nodules, the bacteria in the infected cells do not turn into formed bacteroids and age rapidly. Consequently, the increase in the productivity of symbiotic soybeans can be achieved by maintaining high nitrogen-fixing activity and the intensity of photosynthetic processes in the generative phase of development, and the essential condition of such a combination is the high potential activity of the photosynthetic apparatus of plants just after their flowering. We have established that in consequence of inoculation with strain 71t *B. japonicum* nitrogen activity of soybean plants increased in all investigated phases of development against spontaneous inoculation (Figure 2).

Maximum acetylene-reducing activity fell on the period of bean formation. The CCC preparation essentially increased the nitrogen efficiency of the symbiotic complex *B. japonicum* - soybean. The complex application of the treatments was also followed by a shift in the nitrogenase peak activity towards a later stage of ontogenesis - the phase of green bean.

Thus, the formation of a bigger leaf surface of soybean plants with the simultaneous optimization of leaf mesostructure under the effect of CCC caused better providing the bacteroids with the products of photosynthesis thereby increasing their nitrogen activity. In addition to the dinitrogen fixation, a part of the nitrogen, the plant consumes, is carried out by certain prokariotes from the soil in the nitrate form, which dominates the bound forms of nitrogen. A key role in this process is performed by assimilatory nitrate reduction activity. The rate of nitrate reduction in the leaves depends on the nitrates supply from the roots, their distribution between the metabolic and vacuolar pools. Aging of leaves makes for a decrease in the activity of assimilating enzymes, including nitrate reduction in the roots and rootstock, which, in its turn, causes the synthesis of nitrogen gas in the nodules (Kots et al., 2011).

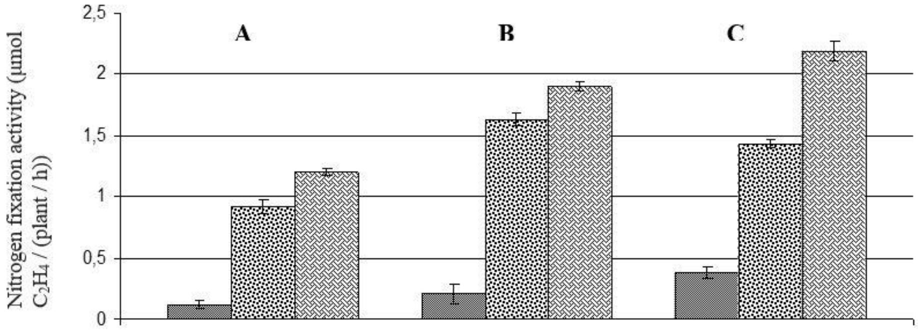

Figure 2. Dynamics of general nitrogenase activity of soy under the influence of strain 71 t *Bradyrhizobium japonicum* and chlormequat chloride: - control, - inoculation with strain 71t, - strain 71t + 0.05% chlormequat chloride.

We have also established that under the conditions of seed inoculation with strain 71t, the activity of nitrate reduction was higher than that of control and increased from the phase of flowering to the phase of bean formation both in the roots and in the leaves of soybean plants (Figure 3). The application of CCC against the background of inoculation with this strain did not affect the ferment activity in the roots, and even reduced it in the leaves, especially in the phase of bean formation.

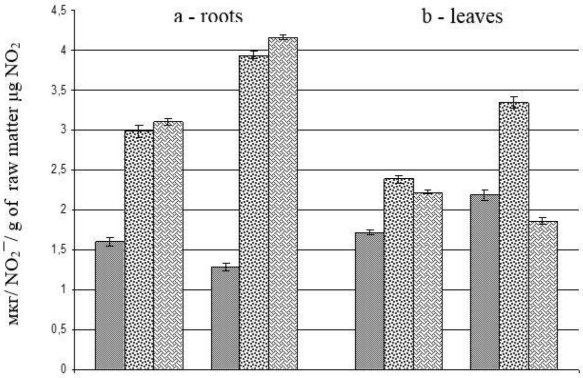

Figure 3. Activity of nitrate reduction in the leaves and roots of soybean plants against the pre-sowing inoculation with strain *Bradyrhizobium japonicum* 71 t and treatment with chlormequat chloride: - control, - inoculation with strain 71t, - strain 71t + 0.05% chlormequat chloride: a – the phase of flowering, b - the phase of bean formation.

In recent researches the antigibberellic preparations are said to cause a rearrangement in the flow of assimilates in plants (Rogach et al, 2018). Such a character of changes in source - sink relations is determined by the inhibition of the meristem activity in the vegetative organs of plants and the emergence of new acceptor centres – fruit or vegetative storage facilities, which increase yields (Kuryata, Kravets, 2018). We found that the soy plants, being influenced by retardant chlormequat chloride, reacted similarly. Its effect against the bacteriation of seeds with strain *Bradyrhizobium japonicum* 71t led to the formation of greater number of beans and growth of the crop capacity (Table 4).

Table 4. The structure of soybean crop cultivar 'Podilska 1' when inoculated with strain *Bradyrhizobium japonicum* 71 t and treatment with chlormequat chloride.

Variant	Number of beans per plant, pieces	Weight of the seeds on a plant, g	Mass of 1000 seeds, g	Oil contents, %	Nitrogen, %
Control	12.4 ± 0.98	4.2 ± 0.33	155.3 ± 1.14	21.1 ± 1.76	4.11 ± 0.12
Strain 71 t	17.6 ± 1.33*	6.82 ± 0.72*	182.5 ± 2.38*	22.0 ± 1.32	5.44 ± 0.26*
Strain 71 t+0.5% CCC	23.2 ± 2.02*	9.96 ± 0.53*	184.2 ± 2.61*	22.2 ± 1.46	5.50 ± 0.21*

* – significant differences P ≤ 0.05.

Nevertheless, the data obtained indicate that treatment with chlormequat chloride practically did not affect the oil contents, though; it made for the nitrogen accumulation in the seeds.
The oil chromatographic analysis according to the experimental variants enabled to determine the following oils: palmitic (C 16:0) stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), α - linolenic (C 18:3) and arachidic (C 20:0) higher fatty acids (Figure 4).

The inoculation with *Bradyrhizobium japonicum* 71 t and further treatment with chlormequat chloride induced an increase in the correlation of unsaturated / saturated higher fatty acids indicating an improvement in the oil quality. So, in the control higher fatty acids equalled 5.91, under the effect of the retardant- 6.08, in the complex treatment - inoculation with strain 71t+CCC preparation - 6.21. However, bringing the most of hidden reserves of synthetic growth regulators into use ought to be taken under a reliable control, especially as far as the residual quantities of preparations in the products is concerned. We have also established that the residual amount of CCC in the experimental variant was 0.006 mg/kg, whereas the retardant permissible norm equals 0.1 mg kg^{-1} (State Sanitary Rules and Regulations (8.8.1.2.3.4.-000-2001) in Ukraine). Thus, the suggested regulation of CCC application was not followed by its excessive accumulation in the soy seeds.

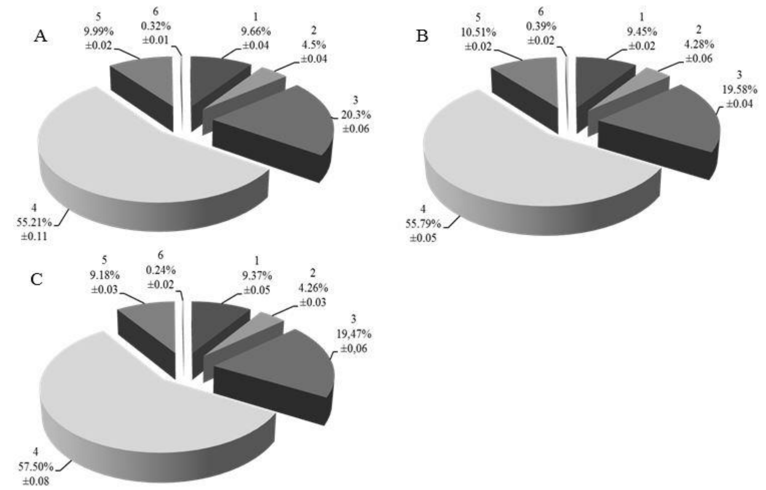

Figure 4. The contents of higher fatty acids in the soybean oil from the plants of Podilska 1 inoculated with strain *Bradyrhizobium japonicum* 71t and 0.5% chlormequat chloride treatment (%): A - control, B - inoculation with strain 71t, C - strain 71t+0.05% CCC: 1 – 16:0, 2 – 18:0, 3 – 18:1, 4 – 18:2, 5 – 18:3, 6 – 20:0.

Results and discussion

Source and sink spheres of a plant are bound directly and inversely by a system of connections (hormonal, trophic), which provide mutually interdependent correction of photosynthetic and growth processes. The use of synthetic growth regulators enables artificially to alter morphogenesis, regulate plant loading within fruit and seeds. In fact, the use of such preparations makes it available to stimulate a plant to different degrees of stress in the source-sink relationship, and, parallel to that, make out anatomic- morphological, physiological and biochemical changes, that occur during redistribution of the assimilates between the plant organs (Yan et al., 2015; Wang et al, 2016; Kuryata & Kravets, 2018).

The plant treatment with CCC preparation during the budding period, the soy seeds being prelimenarily inoculated with an effectual strain - *Bradyrhizobium japonicum* 71t, enhanced significant changes in the donor-acceptor system, optimizing the formation and functioning of the bean - rhizobia complexes. Since the effectuality of symbiotic nitrogen fixation is largely dependent on the provision of the process with photoassimilates, the retardant application proved to be efficacious because it resulted in increase of the photosynthetic apparatus of the plant in consequence of increased stem branching, leaves growth in number, as well as their total area within each plant. Effectiveness of chlormequat chloride was especially apparent during more powerful leaf mesostructure formation being accompanied with blade thickening as well as chlorenchyma tissue, walled and spongy parenchyma cells growing in volume and linearly, number of the abaxial leaf stomata increasing in quantity. Such rearrangements in mesostructure conditioned essential increase of the photosynthesis net productivity index. Thus, application of the retardant caused formation of more developed source sphere of the plant, which provided both the processes of symbiotic assimilation and carpogenesis with the assimilates. Under the retardant condition the enhanced formation of the bean - rhizobium complex manifested itself in growth of nodules on the roots of inoculated plants both in number and in mass. Under the effect of CCC the nitrogenase activity increased, that can be explained by more intensive flow of assimilates towards them in consequence of the photosynthetic productivity getting stronger. Thus, the bean - rhizobia complexes, along with the generative organs, function as additional traction centers for the redistribution of photoassimilates. Not less important for the formation of plant productivity is the fact that the enhanced assimilation processes of the symbiotic nitrogen fixation also caused a shift in the peak activity of nitrogenase towards a later stage of ontogenesis - the green bean phase.

In addition to free nitrogen fixation owing to the nitrogenase, a certain amount of nitrogen is consumed by the plants from the soil in the nitrate form, which is dominant among the bound forms of nitrogen. Under the condition of seed inoculation with strain 71t, the activity of nitrate reduction was higher than that of control and was increasing permanently from the phase of flowering to the phase of bean formation both in the roots and in the leaves of the soybean plants. The use of chlormequat chloride against the background inoculation with the strain did not affect the activity of the ferments in the roots, and even reduced them in the leaves, especially in the phase of bean formation. So the effect of the retardant on the

nitrogen redistribution is realized through stimulation of the soybean-rhezobium complex, – its formation and function, but not through the activation of nitrate reduction. The complex use of strain 71t *B. japonicum* and chlormequat chloride resulted in increased crop yields with the up to date toxicological and hygienic requirements.
Thus, the complex application of retardant chlormequat chloride and inoculation of soybean seeds with strain 71t *B. japonicum* resulted in the enhanced branching of the stem, increased productivity of the plant due to the formation of a more effectual photosynthetic apparatus, placing a greater number of beans as well as optimization of the processes of symbiotic nitrogen fixation.

Conclusion

The use of CCC in the budding phase against the background of pre-sowing soy seed inoculation with *Bradyrhizobium japonicum* 71t strain causes the correction of source-sink relations in the plant, contributes to a more efficient source sphere formation, and enhances the formation of soybean-rhizobia complexes increasing their nitrogenase activity. Due to the increased branching of the stem and placing a greater number of beans, forming a larger leaf surface and increasing the photosynthetic activity per unit of the laminae area; the improved nitrogen nutrition provides good crop capacity and improves the product quality on account of the increased nitrogen contents in the seeds as well as increase in the contents of unsaturated fatty acids in the soybean oil.

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