

Physiological-biochemical responses in seeds and plants of pease (*Pisum sativum* L.) at initial stages of ontogenesis at action of biological products and growth regulators of plants.

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The purpose. To probe physiological-biochemical responses in seeds, roots and shoots of pease at initial stages of ontogenesis at use of biological products and growth regulators of plants. **Methods.** Seeds of pease (*Pisum sativum* L.) of grade Glians were treated with working solutions of growth regulator of plants AKM and microbial specimen Rizobofit. Seeds were germinated in containers with sand in a thermostat at temperature of $20\pm 2^{\circ}\text{C}$. Intensity of peroxide oxidation of lipids in tissues of germinating seeds, young plantlets and roots was evaluated according to the content of malonic dial (MD) which was determined with the help of spectrophotometric method and recalculation for dry matter (DM). That index, and also mass of dry matters was determined at different stages of growth of pease BBCH (00, 03, 05, 08, 12, 13, 14, 15) according to practical standards. **Results.** It is determined that presowing treatment of seeds with growth regulator of plants AKM and its mixture with microbial specimen Rizobofit during heterotrophic nutrition makes active metabolic processes in mericarp, stimulates germination, increases dry mass of roots by 23 and 37% and decreases intensity of POL processes by 37,5 and 24% in comparison to the control. With transfer to autotrophic type of feed the dry mass of seed-lobe intensely drops at treating with AKM and its mixture with Rizobofit. That process is accompanied by activation of growth processes in roots and plantlets, and increase in their mass. POL intensity in roots drops, that testifies to formation of adaptive response to physiological and chemical stresses at germination and formation of nodules. **Conclusions.** It is proved that these specimens manifest phyto-stimulating and adaptogenic effect on the processes of germination of seeds and initial propagation of roots and shoots of pease (*Pisum sativum* L.).

Key words: dry mass, peroxide oxidation of lipids, seed-lobe, plantlets, roots of pease, microbial specimen, growth regulator of plants.

<https://doi.org/10.31073/agrovisnyk201807-02>

Formulation of the problem. The process of seed germination and the appearance of stairs is an important stage in the formation of a grain crop, especially seedlings. It is accompanied by a number of biochemical and morphophysiological processes that begin at the stage of bubbling and cause the transition of the seed from the state of forced rest to the state of active growth. With intensive water absorption under favorable environmental conditions, which is a prerequisite for the beginning of growth, capable of germination of seeds, in the families of the mountain metabolic processes are activated and the intensity of respiration increases. This causes the stimulation of triggers for the formation of active forms of oxygen (AFC) involved in the process of seed germination and activation of the antioxidant system [1].

However, the excessive accumulation of active forms of oxygen in cells, which is the general response of plants to the action of abiotic and biotic factors of the environment, stimulates the process of lipid peroxidation, which leads to the development of oxidative stress, which causes damage to the structural and functional integrity of cell membranes and violation in the course of germination processes and the growth of young plants [2].

Thus, the study of physiological and biochemical reactions in seeds, roots and sprouts in the initial stages of germination is important for optimizing the methods of pre-seed treatment of seeds and increasing the resistance to adverse stress factors.

Analysis of recent research and publications. The metabolic activity in the seeds of seedlings begins at become bloated and increases with further germination, which is regulated by the whole set of environmental factors. Due to the violation of the prooxidant-antioxidant balance, which occurs when excessive AFO formation caused by the intensification of the respiration process, the peas seeds during this period undergoes stress, which leads to delay in germination or even its complete inhibition. The degree of development of oxidative stress and the nature of its effect on seeds at germination can be estimated by the intensity of peroxide oxidation of lipids biomembranes (POL). Due to the fact that MDA is one of the most stable products of POL, its content in tissues can be used to evaluate the activity of this process in the seeds at the beginning of germination and the study of the growth potential of plant pea seedlings [2].

The innovative direction of modern science is the development of exogenous methods regulation and stabilization of adaptive plants possibilities through the use of environmentally safe microbial preparations and physiologically active substances of synthetic and natural origin [3,4,5].

The use of microbial preparations allows for complementary binding surface glycopolymers rhizobia in the initial contacts with the host plant and act as signaling molecules in the formation and functioning of symbiotic systems of the nitrogen fixing of nitrogenase complex, and as a result enhance the synthesis of compounds phytohormonal origin, increase seed germination and stimulate growth and development of plants [6].

Exogenous growth regulators perform a complex action. Penetrating through the membrane of cells, they are able to accelerate the transfer of genetic information, membrane processes, cell division, enzymatic reactions, photosynthesis, respiration and nutrition, which leads to the intensification of growth processes in the plant organism [7, 8]. By stimulating the natural protective mechanisms of a plant organism, they substantially increase the resistance of plants to adverse environmental factors [9, 10, 11]. Biologically active substances can change the course of microbiological processes in the plant's rhizosphere and increase nitrogen activity not only those strains of rhizobia that were used for inoculation but also aboriginal microorganisms in zone of sown seeds, which is important for the formation of bean-rhizobial symbiosis in the cultivation of leguminous cultures [12, 13, 14].

However, the processes of regulating the adaptive and phyto-stimulating possibilities of seedlings pea plants at the initial stages of the body by physiologically active substances in combination with microbic preparations are not sufficiently studied.

Goal. Investigate physiological and biochemical reactions in seeds, roots and sprouts of seedlings in the initial stages of ontogenesis for the use of biological agents and plant growth regulators.

Materials and methods. The research was carried out at the Laboratory of Soil Quality and Plant Quality Monitoring at the Research Institute of Agricultural Technologies and Ecology of the Tavria State Agrotechnological University. In a laboratory double-factor study (AKM - factor A, [12] Risobophyte - factor B), seeds of peas (*Pisum sativum* L.) of Glance type are used. Seeds are sprouted in a sandbox in a thermostat at a temperature of 20 ± 2 °C before the development stage BBCH 08 without light, then - in the illumination. Before seed germination, the working solutions of preparations were treated at a rate of 20 liters of a working solution per 1 ton of seed according to the scheme (Table 1). The repetition of the options in the study - six times.

The intensity of the peroxide oxidation of lipids in the tissues of the ache, sprout, and root was evaluated by the content of malondialdehyde (MDA), which was determined by spectrophotometric method and counted on a dry weight (DW).

This indicator, as well as the mass of dry weight, was determined at BBCH (00, 03, 05, 08, 12, 13, 14, 15) stages of peas in accordance with generally accepted methods [15,16,17]. The variance and correlation analysis and statistical estimation of the average indicators were carried out according to the method of Yeşchenko V.O. and the program "Statistica-6" [17].

1. Scheme of experiment

Option, №	Preparation	Rate
1 (control)	-	-
2	Risobophyte	(0,5 l/t)
3	AKM	(0,3 l/t)
4	AKM+ Risobophyte	(0,3 l/t) + (0,5 l/t)

Research results. The activation of AFO due to the accumulation of malonic dialdehyde (MDA) is one of the mechanisms of metabolic recovery processes in the release of seeds from rest. As the results of the study, pre-planting seed incrustation with Plant Growth Regulator (PGR) AKM contributed to a 9.5% decrease in MDA content in dry seeds (VVSN-00) compared to control. Influence of bioproduct Risobophyte on the intensity of POL is unreliable (Fig. 1).

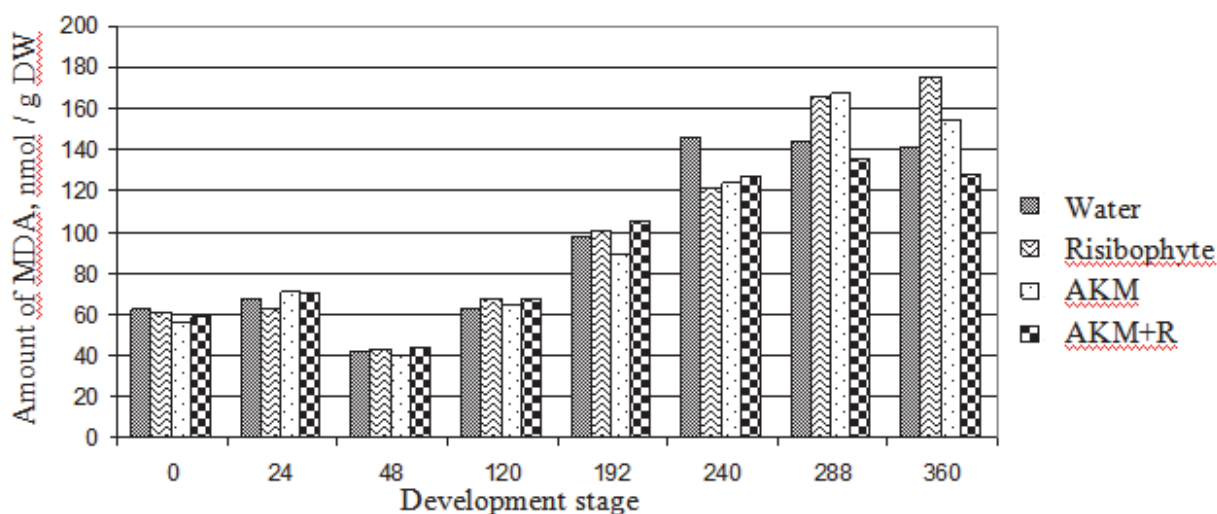


Fig. 1 Amount of MDA in cotyledon of peas, nmol / g of dry weight

In the process of active water absorption (VVSN-03), the intensity of metabolism increases and the content of MDA increases to a greater extent by the use of AKM and its mixture with Risobophytes (1.3 - 1.2 times), to a lesser extent (1 time) in unprocessed seeds and inoculated microbial preparation. The share of the influence of the growth regulator (factor A) was 43.1%. The effect of microbial preparation was insignificant (factor B) - 0.4%, and the interaction of these factors was the highest and were 56.5%.

Investigation of the effect of pre-seed treatment of peas on physiological and biochemical germination processes indicates the relationship between the distribution of dry matter in the cotyledon and the content of MDA. Thus, in the stage of curling of the primary root (VVSN 05), the dry weight of cotyledon decreases due to the active consumption of nutrients (Table 2).

2. Dry weight of one cotyledon of peas, mg. $M \pm m$, $n = 10$

Development stage	Without AKM		With AKM	
	1 (c) (without risobophyte)	2 (with risobophyte)	3 (without risobophyte)	4 (with risobophyte)
00	241,10±1,70	239,75±3,17	234,33±2,17	243,33±3,11
03	209,08±0,42	210,58±0,46	218,63±0,22*	211,62±0,65*♦
05	202,33±2,50	201,83±3,95	205,17±2,02	202,00±0,19
08	167,50±7,29	168,38±4,26	165,88±1,23	176,13±0,51
12	105,25±2,45	102,25±0,87	104,75±1,15	104,63±1,37
13	77,37±0,79	52,63±2,24	64,00±2,31	74,38±0,65

14	45,37±1,80	44,13±44,13	49,00±2,74	40,63±1,95
15	16,87±0,22	17,67±0,79	16,50±0,14	18,88±0,51

In tabl. 2-4:

* the reliability of the difference between 3 and 1, 4 and 2, $P \leq 0.05$

● between 2 and 1, 3 and 4, $P \leq 0.05$

◆ Between 4 and 1, $P \leq 0.05$

The most intensely indicated processes occur in the seed treated with AKM and its mixture with Risobophyte, which is confirmed by an increase in the dry weight of the root in these variants by 23-37% compared with control and a level decrease of oxidative stress by reducing the content of MDA from 376.35 nmol / g DM (control) to 235.29 nmol / g DM (option 3) and up to 287.39 nmol / g DM (option 4) (Table 3, Fig. 2).

The process of consumption of nutrients of the ache of the acne is continued in all variants and in the growth stage of the hypopoietic (VVSN 08), which is associated with the intense growth of the roots and sprouts. The largest increase in dry weight of the roots was observed in the variant for the use of microbial preparation Risobophyte, which was 1.5 times greater than control (Table 3).

It should be noted that the intensity of the POL in the roots for processing by the active strain of the rhizobia decreased by 1.4 times, and in the opposite side, the seed increased by 1 time compared with the control, which may be due to damage to the seed membrane by bacteria and an increase in the absorption of water (fig.2).

3. Dry weight of peas roots in recount of biological unit, mg. $M \pm m$, $n = 10d$

Development stage	Without AKM		With AKM	
	1 (c) (without risobophyte)	2 (with risobophyte)	3 (without risobophyte)	4 (with risobophyte)
05	4,29±0,36	4,21±0,12	5,29±0,14*	5,86±0,08*●◆
08	13,44±0,40	19,89±0,11●	16,83±0,10*	15,17±0,10*●◆
12	33,75±0,58	34,46±0,27	39,63±1,08*	34,38±0,07●
13	41,13±0,79	37,50±1,94	41,67±0,58	40,92±0,51*
14	45,83±1,06	43,42±1,31	46,75±0,58	45,33±1,9
15	67,38±0,36	56,67±1,04●	94,88±1,58*	80,5±0,29*◆

There was no significant effect on the growth of dry weight of the sprout, and the highest intensity of POL was observed for the combined use of AKM with Risobophyte and was 1.3 times more than in the control (Fig. 2).

Thus, during the period of heterotrophic feeding, the accumulation of dry weight in young roots and sprouts depends on the activity of the transformation of the grains, which is most intensely observed in the pre-treatment of AKM and its mixture with Risobophyte. In the transition to an autotrophic type of feeding (BBCH - 12-13) in the cotyledon metabolic activity increases and its dry weight is significantly reduced in all variants, and the cost of dry matter amounted to 48.5%. However, the most significant difference in comparison with control (17-32%) was in the variant for premeditated inkruiting of seeds of AKM and inoculation with Risobophyte (Table 2). This dependence is confirmed by a decrease in the content of MDA in the indicated variants by 15-16% compared to untreated seeds (Fig. 1). Between the content of MDA and dry weight in the germinating seedlings, a strong inverse correlation relationship was established ($r = -0.921 -0.949$). The influence of the growth regulator AKM (factor A) on the decrease of DW grains was highest (39.7%), the interaction of factors (36.8%) was less influenced.

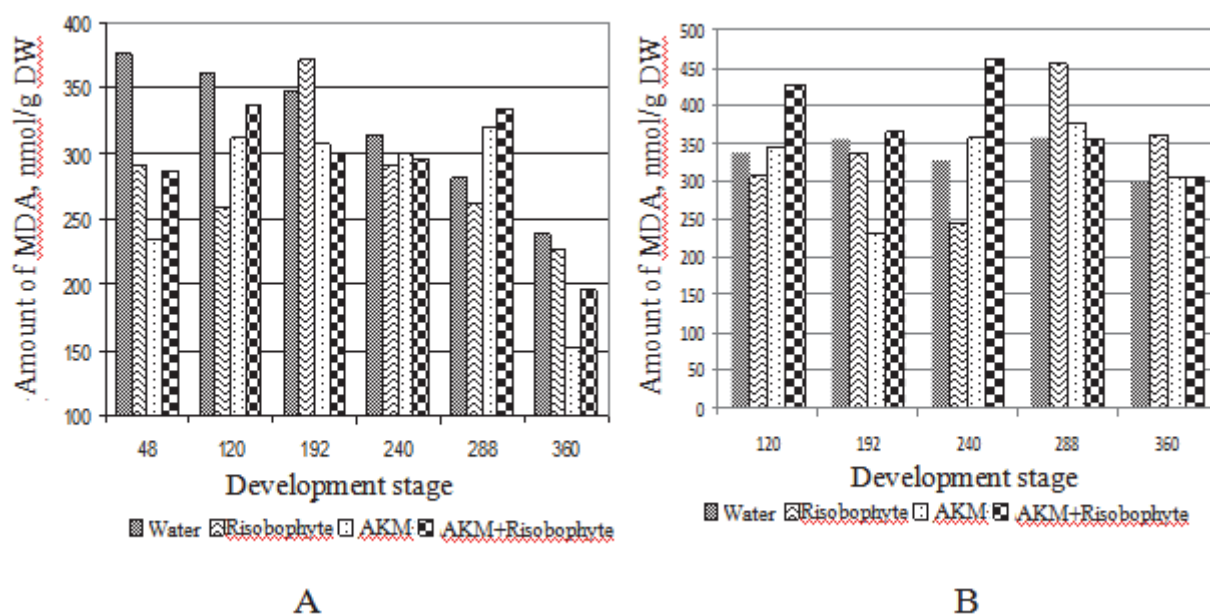


Fig. 2 Amount of MDA in roots (A) and sprouts (B) of pea, nmol / g of dry weight

The activation of the metabolism is also confirmed by stimulation of growth processes in the horns and sprouts, which is manifested in the accumulation of dry matter to a greater extent using the growth regulator (by 17.5 - 18% more than in the control), and less - by the use of the microbial preparation (2 - 9%) respectively (Table 3, Table 4).

4. The dry weight of pea germs in recount of biological unit, mg. $M \pm m$, $n = 10$

Development stage	Without AKM		With AKM	
	1 (c) (without Risobophyte)	2 (with Risobophyte)	3 (without Risobophyte)	4 (with Risobophyte)
08	13,11±0,68	12,5±0,29	11,89±0,22*	12,33±0,19
12	36,25±0,14	32±0,14●	33,42±0,30*	35,75±0,43*●
13	44,67±1,23	48,75±0,87●	52,83±0,30*	40,00±0,14*●◆
14	53,38±0,94	57±0,72●	63,00±0,14*	59,00±0,02*●◆
15	67,00±1,63	74,75±0,72●	72,88±0,07*	69,38±0,22*●◆

In the whole, during the investigated stages of peas development, between the content of MDA and dry weight in pea germ, a strong inverse correlation relationship was established with the combined action of AKM and Risobophyte ($r = -0.726$), which was weakened to medium and weak with other treatment options ($r = 0.187 - 0.445$).

We found that the accumulation of dry weight in the roots at the stage of four true leaves with stipules slightly slowed down, and MDA drastically cut, especially in options for handling growth regulators AKM and AKM with Risobophyte (1.2 times more than in the controls) (Fig. 2). Clearly this indicates the formation of the roots of adaptive physiological response to stress caused by triggering the formation of nodules and the consequent intensification of oxidative metabolism in tissues.

However, it should be noted that excessive formation of reactive oxygen species and the short-term stage (BBCH 15) MDA content in roots in these treatment options significantly reduced in 1,2 and 1,6 compared to untreated seeds (Fig. 2) and the content dry weight increased by 19.5 - 41% according to the control (Table 3), which can be explained by the antioxidant effect of the application of RGP AKM and its mixture with microbial drug Risobophyte and adaptation to the conditions of the root system growth.

Between the content of MDA and dry weight in the roots of peas, a strong inverse correlation relationship was established ($r = -0,574-0,957$). The factor A of plant growth regulator had the greatest influence on growth of roots (78.8%).

Thus, in the period of autotrophic feeding during all phases of peas sowing development, the presence of phytoimmunoassay and adaptogenic effect on germination, seed growth and development of young roots and sprouts was carried out by AKM and its combination with Risobophyte.

Conclusions

According to the results of the study, it was found that during the period of heterotrophic survival the greatest effect on the processes of prophecy is that we take part in actions and games, including in other places, including in other places, by 23% and 37 %, as well as those who review their rights, proxies, the MDA content is reduced by 37.5% and 24% less compare with control.

In the primary autotrophic feeding, the dry weight of cotyledons is intensively reduced for the processing of AKM and its mixture with Risobophyte, which is accompanied by the activation of growth processes in roots and sprouts and an increase in their weight. The intensity of POL in the roots decreases, which indicates the formation of adaptive respond to the physiological and chemical stress in the prophylaxis and the formation of tubers. The intensity of the POL in the germ throughout the entire phase of the growing changes ambiguously, which requires further research.

During the investigated stages of plant development, peas have an inverse correlation between the content of MDA and the dry weight of the ache ($r = -0.921$ to -0.949), between the MDA and the dry weight of the roots ($r = -0.574$ to -0.826) and between the MDA and dry weight of germs ($r = -0.445$ to -0.726).

Consequently, AKM and its mixture with Risobophyte exhibit phyto-stimulating and adaptogenic properties and can be used for active to learn more about the settlement.

References

1. Honchar L.M., Shcherbakova O.M. (2015). Vplyv peredposivnoho obroblennia nasinnia na fiziolohe-biokhimichni protsesy pid chas prorostannia nasinnia nutu. [Influence of pre-planting seed treatment on physiological and biochemical processes during germination of seeds of Nut]. *Naukovyi visnyk NUBiP Ukrainy. Serii «Ahronomiia»*. V. 210. 1:54 – 58. [\[in Ukrainian\]](#).
2. Batsmanova L.M., Taran N.Iu. (2010). Skrynih adaptivnoho potentsialu roslyn za pokaznykamy oksydnoho stresu. [Screening adaptive capacity of plants by indicators of oxidative stress]. Kyiv: Aveha. 79. [\[in Ukrainian\]](#).
3. Kao C.M., Li S.H., Chen Y.L., Chen S.S. (2005). Utilization of the metal-cyano complex tetracya-no-nickelate by *Azotobacter vinelandii*. *Lett. Appl. Microbiol.* 41(2):216–220.
4. Mikhieiev V.H. (2012). Vplyv rehulatoriv rostu y inokuliatsii nasinnia na produktyvnist fotosyntezy posyv soi. [Influence of growth regulators and inoculation of seeds on the productivity of photosynthesis of soybean crops]. *Visnyk TsNZ APV Kharkivskoi oblasti* [Bulletin of the Central Scientific Research Center of the Kharkiv region]. 13:172–178. [\[in Ukrainian\]](#).
5. Murach O.M., Volkohon V.V. (2014). Formuvannia symbiotychnoho aparatu horokhu za vplyvu bakterialnykh preparativ, mikroelementiv i stymulatora rostu. [Formation of symbiotic apparatus of peas for the influence of bacterial preparations, trace elements and growth stimulator]. *Ahroekolohichni zhurn.* 4:55–59. [\[in Ukrainian\]](#).
6. Sherstobaieva O.V., Chabaniuk Ya.V., Kalynych O.M. et al. (2011). Biolohichna aktyvnist u ryzosferi soi za kompleksnoi inokuliatsii. [Biological activity in soybean rhizosphere for complex inoculation]. *Ahroekolohichni zhurn.* 2:77–80. [\[in Ukrainian\]](#).
7. Hrytsaienko Z.M., Ponomarenko S.P., Karpenko V.P., Leontiuk I.B. (2008). Biolohichno aktyvni rehovyny v roslynytsvi. [Biologically active substances in crop production]. Kyiv: ZAT «NICH LAVA». 57–98. [\[in Ukrainian\]](#).

8. *Alekseyevych M., Vanyk M., Kononchuk A., Kononchuk O.* (2013). Optyimizatsiia fiziolooho-biokhimichnykh protsesiv u soi zastosuvanniam rehulatoriv rostu roslyn ta molibdenu. [Optimization of physiological and biochemical processes in soy using plant growth regulators and molybdenum]. *Problemy ta perspektyvy nauk v umovakh hlobalizatsii: mater. IKh Vseukr. nauk. konf. Ternopil: TNPU im. V. Hnatiuka.* 229–233. [[in Ukrainian](#)].

9. *Cygankova V.A., Andrushevich Ya.V., Babayanc O.V. et al.* (2013). Povyshenie regulyatorami rosta immuniteta rastenij k patogennym gribam, vreditelyam i nematodam. [Increase in plant immunity growth regulators to pathogenic fungi, pests and nematodes]. *Fiziologiya i biokhimiya kulturnykh rastenij.* 45(2):138–147. [[in Russian](#)].

10. *Artiushenko T.A.* (2011). Vplyv ahrostymulinu na riven fiziologichnoi adaptatsii horokhu do sumisnoi dii spoluk nikeliu i kadmiiu. [Effect of agrostimulin on the level of physiological adaptation of peas to the joint action of nickel and cadmium compounds]. *Rehuliatytsiia rostu i rozvytku roslyn: fiziolooho-biokhimichni i henetychni aspekty* [Regulation of growth and development of plants: physiological and biochemical and genetic aspects] : mater. II mizhnar. nauk. konf. (Kharkiv, 11 – 13 october 2011). *Kharkiv.* 161–162. [[in Ukrainian](#)].

11. *Kandan A., Ramiah M., Vasanthi V.* (2005). Use of *Pseudomonas fluorescens*-based formulations for management of tomato spotted wilt virus (TSWV) and enhanced yield in tomato. *Biocontrol science and technology.* 15(6):553–569.

12. *Komok M.S., Volkohon V.V., Dimova S.B.* (2012). Fiziologichno aktyvni rehovyny yak zasib pidvyshchennia efektyvnosti mikrobynykh preparativ dlia soi. [Physiologically active substances as a means of increasing the effectiveness of microbial drugs for soy]. *Mikrobiologhiia v suchasnomu silskohospodarskomu vyrobnytstvi.* [Microbiology in modern agricultural production]: mater. VIII nauk. konf. molodykh uchenykh (Chernihiv 25 – 27 September 2012). Chernihiv: TsNP. 37–41. [[in Ukrainian](#)].

13. *Koc S.Ya., Morgun V.V., Patyka V.F. i dr.* (2011). Biologicheskaya fiksatsiya azota: monogr.: v 4-h t. T. 2: Bobovo-rizobialnyj simbioz. [Biological fixation of nitrogen: monograph]. Kiev: Logos. 523. [[in Russian](#)].

14. *Ponomarenko S.P., Terek O.I., Gricaenko Z.M. et al (Iutinskaya G.A., Ponomarenko S.P. Eds)* (2010). Bioregulyatsiia mikrobnorastitelnykh sistem. [Bioregulation of microbial-plant systems]. Kiev: Nichlava. 472. [[in Russian](#)].

15. *Musiienko M.M., Parykova T.V., Slavnyi P.S.* (2001). Spektrofotometrychni metody v praktytsi fiziologhii ta ekolohii roslyn. [Spectrophotometric methods in the practice of physiology and ecology of plants]. Kyiv: Fitosotsiotsentr. 200. [[in Ukrainian](#)].

16. *Palamarchuk V.D., Polishchuk I.S., Kalenska S.M. et al.* (2013). Biologhiia ta ekolohiia silskohospodarskykh roslyn. [Biology and ecology of agricultural plants]. Vinnytsia. 724. [[in Ukrainian](#)].

17. *Yeshchenko V.O., Kopytko P.H., Kostohryz P.V. et al.* (2014). Osnovy naukovykh doslidzhen v ahronomii. [Fundamentals of research in agronomy]. Vinnytsia: PP «TD Edelweis i K», 332. [[in Ukrainian](#)].