BIOMICS/БИОМИКА

ISSN 2221-6197 http://biomicsj.ru

EFFECTS OF *AZOSPIRILLUM* LECTIN ON WHEAT SEEDLING GROWTH UNDER SALT STRESS

Kupryashina M.A.*, Alen'kina Sv.A.

Institute of Biochemistry and Physiology of Plants and Microorganisms, Saratov Scientific Centre of the Russian Academy of Sciences (IBPPM RAS), 13 Pr. Entuziastov 410049, Saratov, Russian Federation,
*E-mail: kupryashina_m@mail.ru

Resume

Azospirillum brasilense, which has the potential to stimulate plant growth, belongs to plant-growth-promoting bacteria. The positive effect of *Azospirillum* on plants may be due to different mechanisms. Azospirilla can assist in mitigation of many kinds of abiotic stress. Lectins are glycoproteins with different molecular masses and carbohydrate specificities. *Azospirillum* lectins are polyfunctional molecules. They promote plant growth and enzyme activity, they also can alter the plant cell content of stress metabolites, which attests that they can induce adaptation processes in wheat seedling roots. We investigated the dose-dependent effects of the lectin from *A. brasilense* strain Sp7 on the roots of 4-day-old wheat seedlings (*Triticum aestivum* L. cv. Saratovskaya 29) grown under salt stress. A low lectin concentration (0.3 mM L^{-1}) improved seedling growth and alleviated the stress-induced growth inhibition. However, higher concentrations of lectin (1.2 mM L^{-1}) did not affect the growth of the stressed seedling roots. In the roots of 4-day-old wheat seedlings under salt stress, the Sp7 lectin decreased total protein content and lipid peroxidation, which causes membrane damage, but increased the content of secondary metabolites such as total phenolics and total flavonoids. We conclude that the *Azospirillum* lectins are involved in adaptational changes in wheat seedling roots, due to which the relationship between bacteria and their hosts can be regulated under changing soil and climatic factors.

Keywords: *Azospirillum* lectin, Wheat, Salt tolerance, Protein content, MDA, Phenolics, Flavonoids.

Citation: Kupryashina M.A., Alen'kina Sv.A. Effects of *Azospirillum* lectin on wheat seedling growth under salt stress. *Biomics*. 2022. V.14(4). P. 315-321. DOI: 10.31301/2221-6197.bmcs.2022-31

Introduction

Soil salinity is a major abiotic stress that limits plant growth and productivity. Wheat (*Triticum aestivum* L. cv. Saratovskaya 29) is moderately salt tolerant; however, its growth decreases strongly at high salinity levels, especially during germination. Actually, wheat is more sensitive to salt stress at germination than at later stages. Research on plant adaptation to salinity shows that this process unevenly during ontogenesis. The greatest sensitivity to high salt concentrations is shown by plants in the first stages of development, i.e., seed germination and emergence of seedlings [Ashraf, 2009].

The major salinity-induced damage to plants is caused by sodium ion accumulation, which is toxic to most organisms [Dong et al., 2010]. NaCl is the predominant salt in most saline environments. When plant roots are subjected to a high-NaCl environment, the external Na^+ and Cl^- establish a large electrochemical gradient that drives the influx of salt ions, disrupting ion homeostasis and ultimately injuring the seedlings.

The root is the first part of the plant to respond to salt stress, by sending chemical signals through the stem to the leaf [Meloni et al., 2003]. Salinity causes oxidative stress in plants.

The causes of oxidative stress are the formation of reactive oxygen species, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^•$), and singlet oxygen ($^1O_2$). Accumulation of reactive oxygen

species causes serious functional disorders of cells. Reactive oxygen species have extremely high reactivity and lead to the oxidation of lipids, proteins, and nucleic acids [Velarde-Buendıa et al., 2012].

According to Ashraf [2009], the generation of the reactive oxygen intermediates under salt stress action causes damage to proteins, nucleic acids, and lipids, and eventually leads to apoptosis and cell death. The oxidative injury of the proteins consists in amino acid modifications, breakage of the peptide chain, aggregation of cross-linked reaction products etc., while the oxidation of some amino acid residues generates the oxo groups which amplify the susceptibility of proteins to proteolysis [Georgiadou et al., 2016].

Among the primary mechanisms of cell damage under oxidative stress, peroxidation of fatty acid residues in membrane phospholipids is leading. This reduces their hydrophobicity and disrupts the stability of membranes, changes the functioning of membrane-dependent enzymes, increases the permeability of membranes for ions, and the ability to selectively accumulate substances disappears. In this case, the salts enter the cell passively, and this further increases the damage to the cell [Ashraf, 2009]. As a rule, the processes of lipid peroxidation are evaluated by the rate and amount of formation of one of the end products of oxidation - malondialdehyde (MDA).

All flavonoids, to one degree or another, are involved in the antioxidant defense of cells. In accordance with the generally accepted point of view, the antioxidant properties of flavonoids are explained by their ability to serve as traps for free radicals, as well as to chelate metal ions involved in radical processes [Es-Safi et al., 2007].

Exploring the potential of plant-growth-regulating chemicals for minimizing the generation of free radicals and mitigating membrane damage is a stepping stone to the management of salt stress in plants [Verma, Mishra, 2005]. Taking into account the need for the greening of agriculture, the search for substances produced by higher plants, fungi and microorganisms is relevant.

Most of the soil microorganisms can colonize plants, associating with them and increasing growth and yields. These microorganisms are commonly called plant-growth-promoting rhizobacteria (PGPR). Among the PGPR, members of the genus *Azospirillum* are well known and commonly used in agriculture [Puente et al., 2018]. Nitrogen-fixing azospirilla have received attention on account of their growth in close association with the roots of various grasses and cereals, their widespread geographic occurrence, and their promotion of plant growth and development. These bacteria benefit plants in several direct and indirect ways. The beneficial effects of PGPR have been attributed to biological nitrogen fixation; production of plant hormones such as IAA, which promote root development and enhance water uptake and nutrient absorption; production of compounds that increase membrane activity and proliferation of root tissues; mitigation of stressor effects; and control of numerous plant pathogens [Puente et al., 2018]. The mechanisms of plant-mediated biocontrol include the ability of the bacteria to induce resistance-enhancing defense responses in plants. Although research in this area is active, an open question remains: which of the above factors has priority over the others? Recently much more attention has been paid to the analysis of the role of PGPR in improving plant growth under stress [Díaz-Zorita et al., 2015].

It is an indisputable fact that lectins, molecules of a protein nature, are involved in the establishment of intercellular biological interactions in N_2-fixing systems. New data have emerged on the role of bacterial lectins in such interactions. Alen′kina et al. [2014] showed that bacterial surface lectins, along with other factors, are involved in this process.

A lectin was isolated from the surface of *A. brasilense* Sp7 that was found to be a polyfunctional glycoprotein. All effects of this lectin, such as the promotion of seed germination, mitogenic and enzyme-modifying activities, and the ability to change the plant-cell content of stress metabolites, are dose dependent [Alen'kina et al., 2014; 2018; Alen'kina, Nikitina, 2020; 2021]. Many lectin-induced effects were obtained with low lectin concentrations.

We hypothesize that like other growth regulators, lectins may have a dose-dependent effect on wheat growth and defense against salt stress. To test this hypothesis, we investigated the effects of different lectin concentrations on the growth of wheat seedling roots. Specifically, we examined total protein content, lipid peroxidation, and the content of secondary metabolites such as total phenolics and flavonoids.

Materials and Methods

Lectin was isolated from the surface of *A. brasilense* Sp7 (IBPPM RAS Collection of Rhizosphere Microorganisms, http://collection.ibppm.ru). The lectin nature of the purified material was confirmed by hemagglutination assay as described by Alen'kina et al. [2018]. Fifty-microliter portions of successive twofold dilutions of lectin solutions were added to the wells of a microtitration plate, with PBS as a control. Washed trypsin-treated rabbit erythrocytes were added at a concentration of 2% in PBS and were incubated at room temperature for 2 h. The minimum lectin concentration that gave hemagglutination was recorded as the hemagglutination titer. Protein concentration was assayed by the Bradford method [1976].

Animals were cared for and handled in compliance with the Guide for the Care and Use of Laboratory Animals, the European Convention for the Protection of

Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the legislation of the Russian Federation. The use of the animals was also approved by the institution where the experiments were done.

Seeds of wheat (*Triticum aestivum* L. cv. Saratovskaya 29; Federal Center of Agriculture Research of the South-East Region, Saratov, Russia) were surface sterilized in 70% (v/v) ethanol for 1 min and were washed five times with sterile water. For laboratory modeling of conditions of salinity, wheat seeds were germinated in Petri dishes on filter paper with the addition of lectins (concentration of 0.1–1.2 mM L^{-1}; used concentrations were selected in preliminary experiments) and 1% NaCl. The roots of seedlings grown in distilled water at 25°C were used as a control. The sample size was 20 seeds in three replicates for each variant. In the experiments, we used 4-day-old seedlings. The germination capacity of caryopses was determined. To measure the growth parameters of seedling roots, morphometric parameters were used: the number and length of roots and their wet weight.

For experiments on total protein content, lipid peroxidation, total phenolics and total flavonoids roots were simultaneously exposed for 2 h to lectin (concentration, 0.1–1.2 mM L^{-1}) and 1% NaCl. Seedlings grown at 25°C were the control group.

Fresh roots (1 g) were homogenized separately in 4 ml of 50 mmol/L sodium phosphate buffer (pH 7.0), 0.1 mmol L^{-1} EDTA–Na_2, 1% (w/v) polyvinylpyrrolidone, and 0.05% (w/v) Triton X-100 in an ice bath. The homogenate was centrifuged at 12000g for 15 min at 4ºC.

The supernatant liquid was used to estimate the root content of protein by the method of Bradford [1976], by using bovine serum albumin as the standard.

From fresh roots, the studied substances were extracted for 20 min with 50% methanol at 0.1 g 10 mL^{-1} in a sonication bath (Julabo, Germany) containing ice-cold water. The extracts were vacuum filtered by using Whatman No. 1 filter paper, and the supernatant liquids were used for the total phenolic and flavonoid content quantification. The total phenolic content in the plant extracts was determined by the Folin–Ciocalteu assay [Makkar et al., 2007]. The aluminum chloride ($AlCl_3$) colorimetric assay was used [Marinova et al., 2005] to estimate the total flavonoid content. For a comparative analysis of variants, activity was expressed in relative units.

Lipid peroxidation in leaf tissue was determined by measuring the amount of malondialdehyde (MDA) by using thiobarbituric acid. Fresh seedling roots (1 g) were homogenized in 4.0 mL of 10% trichloroacetic acid and were centrifuged at 5000g for 10 min at 4ºC. The supernatant liquid was assayed for MDA by the method of Wu et al. [2012]. The results are presented as relative units.

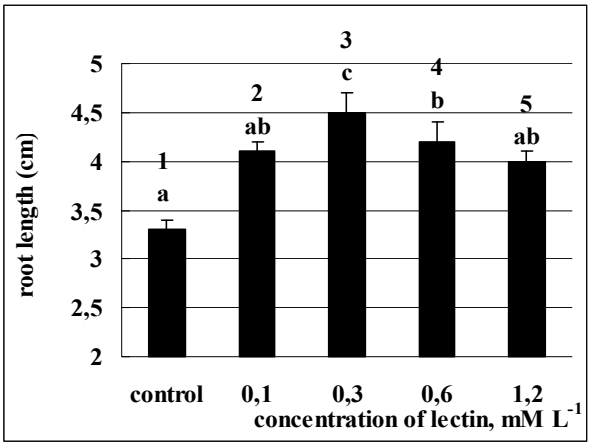
The tables and graphs show the mean values with standard deviations (n = 3). Data were subjected to analysis of variance (ANOVA), and means were compared by Tukey's test at $p \leq 0.05$ by using a computer-based statistical software package (AGROS, version 2.09; Department of Statistical Analysis, Russian Academy of Agricultural Sciences).

Results and Discussions

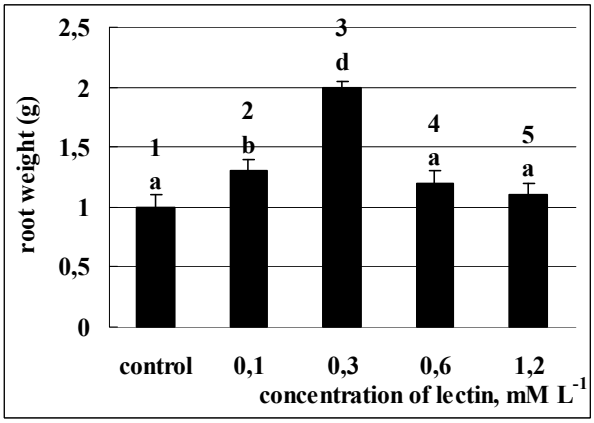
Seed-soaking treatment generally enhances seed germination, which results in better seedling establishment and an enhanced ability to cope with abiotic and biotic stress [Velarde-Buendıa et al., 2012; Rengasamy et al., 2015]. Our results clearly show that seedling root growth (root weight, number of seminal roots, and root length) in 4-day-old wheat plants was improved by soaking seeds in a lectin solution.

Under salt stress, the lectin affected wheat seedling root growth in a dose-dependent manner. The length of the lectin-soaked roots increased significantly after 4 days of growth, as compared to the controls (water-soaked seeds; the root length in the control averaged 3.3 ± 0.2 cm). Root length was maximal (4.5 ± 0.1 cm) at 0.3 mM L^{-1} of lectin (Fig. 1). The fresh weight of the lectin-treated roots was significantly higher than that of the controls (Fig 1) (control value, 1.0 ± 0.2 g). The lectin caused almost no changes in the number of seminal roots. The number of seminal roots in the control was 3.3 ± 0.2. No significant differences in the number of seminal roots were observed between the experimental treatments used or between the treatments and the controls (Fig. 1).

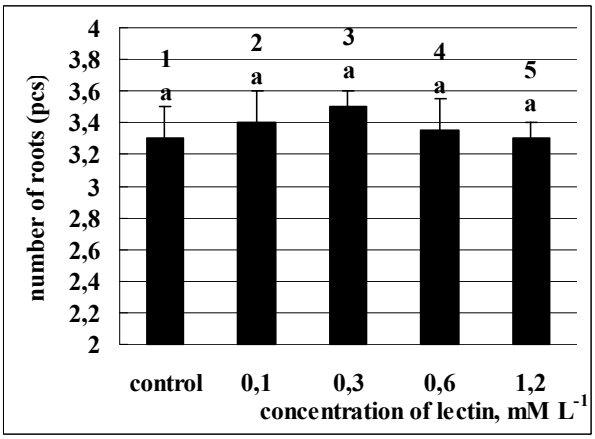
The earliest changes in response to adverse external factors occur at the level of the outer membrane of the plant cell, the plasmalemma. One rapid and nonspecific cell-membrane response caused by any stress is an enhancement of membrane lipid peroxidation, which is a major cause of plant damage and death. The lipid peroxidation product MDA is an indicator of the oxidative injury to the cell membranes [Darko et al., 2011]. Pretreatment with 0.3 mM L^{-1} of lectin significantly reduced the MDA level in the stressed roots. The lipid peroxidation product MDA is an indicator of the oxidative injury of cell membranes [Darko et al., 2011]. Under simulated saline conditions, exposure to *A. brasilense* Sp7 lectin resulted in maximum reduction in MDA after 60 min of incubation with seedling roots. Furthermore, the root MDA content changed parabolically with increasing lectin concentration and was the lowest at 0.3 mM L^{-1} of lectin under salt stress. NaCl treatment markedly increased the root content of MDA, as compared to the control (Fig. 2). In the control samples, the content of MDA was 3.2 μmol g^{-1} root wet weight.



A



B



C

Fig. 1. Effect of *A. brasilense* Sp7 lectin on root length (A), root weight (B), and seminal root number (C) after salt treatment. Results are expressed as mean ± SE. Different letters indicate significantly different values ($P \leq 0.05$). (*1*) Control. (*2–5*) Lectin concentrations of 0.1, 0.3, 0.6 and 1.2 mM L^{-1}, respectively.

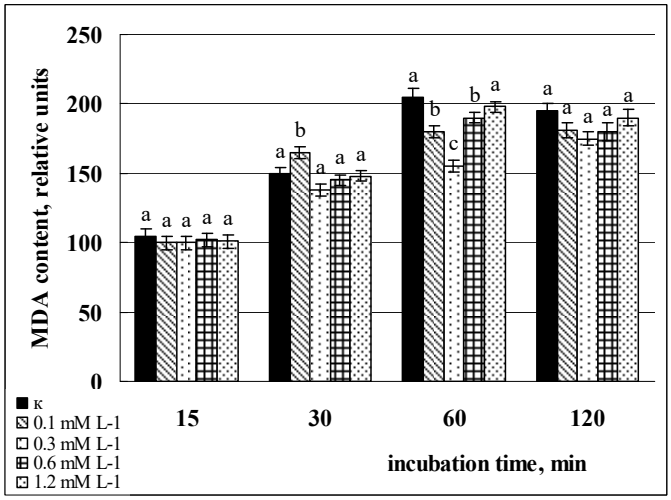


Fig. 2. Effect of the *A. brasilense* Sp7 lectin on MDA content in wheat seedling roots after of salt treatment. Results are expressed as mean ± SE. Mean separation among treatments was done by Duncan test at $p \leq 0.05$. Mean values followed by different letters are significantly different

Another important factor in plant defense against stress is variation in total protein content. Changes in total protein content may be associated with the inhibition of protein synthesis and with protein degradation [Georgiadou et al., 2016].

Salt stress led to a decrease of protein in the roots by 47% after the root treatment within an hour. The levels lower than the control might be due to the inhibition or the suppression of the genes responsible for protein synthesis, as result of salt stress [Salama and El Fouli, 2008]. Also, the decline in the soluble protein amount results from the action of proteolytic enzymes.

The use of 0.3 mM L^{-1} of lectin significantly alleviated the stress-caused decrease and increased the protein content by 30%, as compared to non-lectin-treated plants. Root protein content was also increased by lower and higher lectin concentrations (0.1 and 0.6 mM L^{-1}, respectively) but to a lesser extent than in the previous case. However, the use of 1.2 mM L^{-1} of lectin did not lead to a significant change in root protein content, as compared to that in non-lectin-treated plants under salt stress. Under unstressed conditions, no lectin concentration caused any changes in the root content of protein (Table 1).

The results showed that lectin was able to offset the decrease in soluble protein content caused by salt stress. This may be related to the inhibition of proteolytic enzymes by lectin, which has been shown previously [Alen′kina et al., 2014].

Table 1.

Effect of different lectin concentrations on total soluble protein content in wheat seedling roots after salt treatment

Treatment	Exposure time (min)			
	15	30	60	120
Control (μg g^{-1} wet weight)	30	25	15	27
0.1 mM L^{-1}	101 ± 2a	104 ± 3a	110 ± 3c	106 ± 3a
0.3 mM L^{-1}	101 ± 3a	110 ± 4a	130 ± 4d	105 ± 4a
0.6 mM L^{-1}	100 ± 3a	105 ± 2a	120 ± 2c	99 ± 3a
1.2 mM L^{-1}	100 ± 2a	100 ± 3a	98 ± 3b	97 ± 2a

Results are expressed as mean ± SE. Different letters indicate significantly different values ($p \leq 0.05$), according to Duncan's multiple range test.

Phenolic compounds are obligatory components of higher plant cells and perform various functions in them. They participate in redox processes (components of the electron transport chains of respiration and photosynthesis) and in immune responses; they are used as a reserve energy material; they regulate plant growth and development; and they protect cells from stress. Being highly reactive, these secondary metabolic compounds can inactivate free radicals, thus protecting cells from ROS. There are sporadic data indicating that the content of phenolics increases in stressed plant tissues. Phenolic acids and flavonoids are the most common phenolics in wheat and occur in both free and bound forms at different concentrations. We evaluated the content of phenolics in lectin-treated wheat seedling roots.

Under simulated saline conditions, total phenolic content increased the most after 60-min incubation of 0.3 mM L^{-1} of lectin with seedling roots. The increase was 50%, as compared with the control (Fig. 3). After 120 min of incubation under salinity stress, the total phenolic content decreased to the control level (Fig. 2)

Combined exposure of roots to 0.3 mM L^{-1} of lectin and to salt stress resulted in an increase in flavonoid content, which peaked after 1 h of exposure (1.5 mg catechin equivalents g^{-1} root wet weight). The increase was 60%, as compared with the control (Fig. 4).

Thus, low lectin concentrations increase the phenolic content in wheat roots. The increase in the content of phenolic compounds under the influence of lectins can be explained by the synthesis of these antioxidants to bind free radicals and block the process of peroxidation.

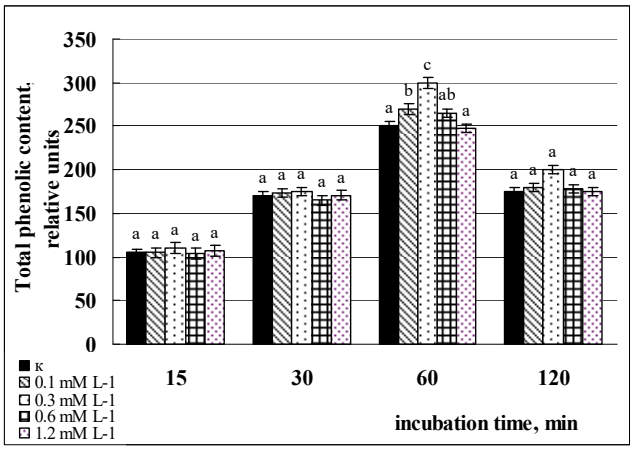


Fig. 3. Effect of the *A. brasilense* Sp7 lectin on total phenolic contentin wheat seedling roots after of salt treatment (1% NaCl). Mean separation among treatments was done by Duncan test at $p \leq 0.05$. Mean values followed by different letters are significantly different.

The regulation of plant growth by the lectins is complex in nature, as evidenced by the concentration differences. These differences can be explained by the influence of the examined stress factors on the binding of the lectins to the receptors located on the roots. The detected concentration dependences can be very useful for understanding plant adaptation as affected by the lectins as plant growth regulators.

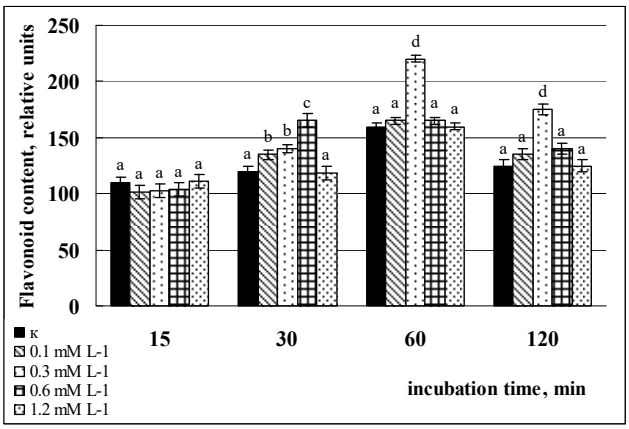


Fig. 4. Effect of the *A. brasilense* Sp7 lectin on flavonoid content in wheat seedling roots after of salt treatment (1% NaCl)**.** Mean separation among treatments was done by Duncan test at $p \leq 0.05$. Mean values followed by different letters are significantly different.

Conclusions

The lectin of *A. brasilense* Sp7 acts dose-dependently on wheat seedling roots under salt stress. Treatment with a low lectin concentration increases the fresh weight and root length of the stressed seedlings. Lectin treatment increases antioxidant enzyme activity, decreases lipid peroxidation, and increases the total soluble protein content. Low lectin concentrations increase the phenolic content in wheat roots. We conclude that the azospirilla lectins are influence on adaptational process in wheat seedlings. The plant-beneficial properties of the lectins, in combination with the growth-promoting activity of *Azospirillum* bacteria, conduce to increased plant productivity. Our data permit correction of the current views about the mechanisms that govern associative plant–bacterium interactions.

Acknowledgements

This work was supported by the Ministry of Education and Science of the Russian Federation (project No. 121031100266-3).

We thank Dmitry Vorobyov for his help in conducting the experiments and processing the results.

Competing interests

The authors have declared that no competing interests exist.

References

1. Alen'kina S.A., Bogatyrev V.A., Matora L.Yu., Sokolova M.K., Chernysheva M.P., K.A. Trutneva, Nikitina V.E. Signal effects of the lectin from the associative nitrogen-fixing bacterium *Azospirillum brasilense* Sp7 in bacterial–plant root interactions // *Plant and Soil*. 2014. V. 381. P. 337-349. doi: 10.1134/S0026261715050021
2. Alen'kina S.A., Romanov N.I., Nikitina V.E. Regulation by *Azospirillum* lectins of the activity of antioxidant enzymes in wheat seedling roots under short-term stresses // *Brazilian Journal of Botany*. 2018. V. 41. P. 579-587. doi: 10.1007/s40415-018-0489-1
3. Alen'kina S.A., Nikitina V.E. Effect of *Azospirillum* lectins on the ascorbate peroxidase activity and ascorbic acid content in wheat seedling roots exposed to abiotic stresses // *Applied Biochemistry and Microbiology.* 2020. V. 56. P. 211-218. doi: 10.1071/SR21092
4. Alen'kina S.A., Nikitina V.E. Stimulating effect from lectins of associative bacteria of the genus *Azospirillum* on the germination and morphometric characteristics of spring wheat sprouts in simulated abiotic stress // *Russian Journal of Plant Physiology*. 2021. V. 68. P. 315-321. doi: 10.1134/S1021443721010027
5. Ashraf M. Biotechnological approach of improving plant salt tolerance using antioxidants as markers // *Biotechnology Advances*. 2009. V. 27. P.84-93. doi: 10.1016/j.biotechadv.2008.09.003
6. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // *Analytical Biochemistry*. 1976. V. 72. P. 248-254. doi: 10.1016/0003-2697(76)90527-3
7. Darko E., Fodor J., Dulai S., Ambrus H., Szenzenstein A., Kiraly Z., Barnabas B. Improved cold and drought tolerance of doubled haploid maize plants selected for resistance to prooxidant tert-Butyl hydroperoxide // *Journal of Agronomy and Crop Science.* 2011. V. 197. P. 454-465. doi: 10.1111/j.1439-037X.2011.00479.x
8. Díaz-Zorita M., Fernández-Canigia M.V., Bravo O.A., Berger A., Satorre E.H. Field evaluation of extensive crops inoculated with *Azospirillum* sp. In: Cassan F.D., Okon Y., Creus C.M. (eds) Handbook for *Azospirillum* technical issues and protocols. Springer International Publishing, Cham, 2015. P. 435-445. doi: 10.1007/978-3-319-06542-7_24
9. Dong H.Z., Kong X.Q., Luo Z., Li W.J., Xin C.S. Unequal salt distribution in the root zone increases growth and yield of cotton // *European Society for Agronomy.* 2010. V. 33. P. 285-292. doi: 10.1016/j.eja.2010.08.002
10. Georgiadou E.C., Ntourou T., Goulas V., Manganaris G.A., Kalaitzis P., Fotopoulos V. Temporal analysis reveals a key role for VTE5 in vitamin E biosynthesis in olive fruit during on-tree development // *Front. Plant Sci*. 2015. V. 6. P. 871.

doi: 10.3389/fpls.2015.00871

11. Es-Safi N.E., Kollmann I., Khlifi S., Ducrot P.H. Antioxidants effect of compounds isolated from *Globularia alypum* L. Structure-activity relationship // *LWT-Food Science and Technology.* 2007. V. 40, P. 1246-1252. doi: 10.1016/j.lwt.2006.08.019
12. Makkar H.P.S., Sidhuraju P., Becker K. Plant secondary metabolites. Totowa: Humana Press Inc. 2007. 122 s. doi: 10.1007/978-1-59745-425-4_1
13. Marinova D., Ribarova F., Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables // *Journal of Chemical Technology and Metallurgy.* 2005. V. 40. P. 255-260.
14. Meloni D.A., Oliva M.A., Martinez C.A., Cambraia J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress // *Environmental and Experimental Botany.* 2003. V. 49. P. 69-76. doi: 10.1081/PLN-100104983
15. Puente M.L., Gualpa G.L., Lopez G.A., Molina R.M., Carletti S.M., Cassán F.D. The benefits of foliar inoculation with *Azospirillum brasilense* in soybean are explained by an auxin signaling model // *Symbiosis*. 2018. V. 6. P. 41-49. doi: 10.1007/s13199-017-0536-x
16. Rengasamy K.R.R., Kulkarni M.G., Stirk W.A., Van Staden J. Eckola new plant growth stimulant from the brown seaweed *Ecklonia maxima* // *Journal of Applied Physiology*. 2015. V. 27. P. 581-587. doi: 10.1007/s10811-014-0337-z
17. Salama Z. A., El Fouly M. Evaluation of the efficiency of some Egyptian wheat *Triticum aestivum* L. cultivars to Zn deficiency through peroxidase activity and protein profile techniques // *Notulae Botanicae Horti Agrobotanici Cluj-Napoca.* 2008. V. 36. P. 42-46. doi: 10.15835/nbha36266
18. Velarde-Buendıa A.M., Shabala S., Cvikrova M., Oxana D., Pottosin I. Salt-sensitive and salt-tolerant barley varieties differ in the extent of potentiation of the ROS-induced K^+ efflux by polyamines // *Plant Physiology and Biochemistry*. 2012. V. 61. P. 18-23. doi: 10.1016/j.plaphy.2012.09.002
19. Verma S., Mishra S.N. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system // *Journal of Plant Physiology*. 2005. V. 162. P. 669-677. doi: 10.1016/j.jplph.2004.08.008
20. Wu H.L., Wu X.L., Li Z.H., Duan L.S., Zhang M.C. Physiological evaluation of drought stress tolerance and recovery in cauliflower (*Brassica oleraces* L.) seedlings treated with methyl jasmonate and coronatine // *Journal of Plant Growth Regulation*. 2012. V. 31. P.113-123. doi: 10.1007/s00344-011-9224-x