CROP PRODUCTION

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HALOTOLERANT PHOSPHATE SOLUBILIZING IAA–SYNTHESIZING REPRESENTATIVES OF *PANTOEA* GENUS PROMOTE PROLINE SYNTHESIS IN WHEAT SEEDLINGS UNDER SALT STRESS

Research article

Abstract

Two halotolerant bacterial strains A1 and A15 of *Pantoea* genus were isolated from the plants growing in saline soils near the waste banks of Solikamsk technogenic biotope (Perm krai, Russia). Microorganisms were able to produce indole-3-acetic acid (IAA) from L-tryptophan and solubilize insoluble phosphates. Maximum accumulation of IAA occurred at the stationary growth phase. In the experiment with wheat seedlings inoculated with isolated strains under salt stress condition, proline concentrations in fresh weight on day 3 were 3 and 2.4 times higher than the control ones reaching 616 and 485 µg/g for A1 and A15 with 1% NaCl respectively. However the higher values of protein content in the variants with salt were achieved only within 7 days, accounting 113% and 138% of the respective total protein amount in the control with 1% of salt for A1 and A15 respectively.

Keywords: spring wheat, salt stress, phosphate solubilization, IAA production, proline accumulation, biofertilizers.

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ГАЛОТОЛЕРАНТНЫЕ ФОСФАТСОЛЮБИЛИЗИРУЮЩИЕ ИУК-СИНТЕЗИРУЮЩИЕ ПРЕДСТАВИТЕЛИ РОДА *PANTOEA* СТИМУЛИРУЮТ СИНТЕЗ ПРОЛИНА В ПРОРОСТКАХ ПШЕНИЦЫ В УСЛОВИЯХ СОЛЕВОГО СТРЕССА

Научная статья

Аннотация

Два галотолерантных бактериальных штамма рода *Pantoea* были изолированы с растений, произрастающих на территории солеотвалов Соликамского месторождения калийных солей (Пермский край, Россия). Микроорганизмы были способны к фосфатсолюбилизации и синтезу индолил-3-уксусной кислоты (ИУК). Максимум накопления ИУК происходил, когда культура выходила на стационарную фазу роста. В эксперименте по влиянию штаммов на накопление пролина в сырой массе проростков пшеницы при солевом стрессе на третий день, были достигнуты наибольшие значения, превышавшие контрольные в 3 и 2.4 раза. При этом существенное увеличение содержания общего белка в вариантах с солью наблюдалось на 7 сутки эксперимента, составив 113% и 138% для А1 и А15 по сравнению с контролем.

Ключевые слова: яровая пшеница, солевой стресс, фосфатсолюбилизирующая активность, синтез ИУК, накопление пролина, биоудобрения.

1. Introduction

Plants are subjected to a wide range of environmental stresses that negatively affect their growth, metabolism and yield. The major environmental constraints are drought and salinity [1], [2]. These factors affect plant production in arid regions and at global scale. Soil salinization is thought to be one of the world's most significant environmental soil hazards, which results in severe land degradation [3].

In order to protect cells and tissues from water deficit and osmotic stress plants accumulate organic and inorganic solutes in the cytosol [1]. One of the most widely studied solutes is proline that accumulates in response to abiotic stress conditions, i.e. salt stress and acts as an osmolyte and antioxidant, helping plants to maintain cell turgor [2], [4], [5]. Also proline serves as a rapidly available source of nitrogen, carbon and reduction equivalents [2].

Among strategies to facilitate plant growth in saline soils the use of plant growth-promoting bacteria (PGPB) especially extremophilic as biofertilizers might be promising offer alternatives to chemical fertilizers [6, 7]. PGPB are able to facilitate nitrogen fixation, phosphate solubilization and exopolysaccharide production, which add up to better seedling germination and biomass production [8]. Furthermore, diverse groups of microorganisms, including soil, epiphytic and endophytic bacteria, were found to synthesize indole-3-acetic acid (IAA) that plays a key role in cell division and elongation [9]. Also many soil bacteria are able to produce various organic acids and convert insoluble phosphorus into soluble forms used by plants. Thus, PGPB usage might be a promising strategy to facilitate plant growth in saline soils [6], [7].

Wheat is a major cereal crop in many parts of the world. Its productivity is influenced by a variety of abiotic stresses such as drought, salinity and heat [10]. In wheat seedlings positive correlation was observed between proline levels and osmotic potential [11].

This study aimed to determine the influence of halotolerant phosphate solubilizing IAA-synthesizing associative microorganisms on proline accumulation in wheat under salt stress conditions.

2. Materials and methods

We used two bacterial strains – A1 and A15 – isolated from rhizosphere and phylloplane of field sowthistle (*Sonchus arvensis* L.) and red clover (*Trifolium pratense* L.) growing in saline soils of the Solikamsk technogenic biotope (Perm krai, Russia). Plant samples were collected at (N59°34'45.29" E56°45'16.01"; N59°37'56.09" E56°45'53.79"). For isolation of nitrogen-fixing bacteria, strains were routinely maintained in liquid and agarised media without nitrogen sources (g/L): A – $MgSO_4 \cdot 7H_2O$ (0.2), $CaCl_2$ (0.02), NaCl (2.0), glucose (1.0); B – $Na_2HPO_4 \cdot 5H_2O$ (1.5), KH_2PO_4 (0.7). Using the 16S nucleotide sequence analysis the isolates were identified as *Pantoea vagans* A1 and *Pantoea vagans* A15. Sequence data were deposited in GenBank under accession numbers KP115326 and KM235113. For inoculation experiments bacterial cells were harvested, washed and resuspended in 1% NaCl solution to get optical densities of 1.0 at 590 nm.

Spring wheat seeds (*Triticum aestivum* L. cv. Ekado-70) were surface sterilized by immersion in 1:1 mixture of 70% ethanol and 3% H_2O_2 for 5 min, and then washed three times with sterile distilled water. Seeds were germinated in sterilized Petri dishes containing sterile wattman filter paper, distilled water and then incubated at 22°C for 3 days. After germination, about 60 seedlings per autoclaved glass pot were inoculated with 1 ml of bacterial culture. One percent NaCl solution was used to mimic salt stress condition in the test vessels. Pots with the same bacterial isolate under 0% NaCl and pots without bacterial cultures served as controls. Physical (root length, shoot length, fresh weight) and biochemical (photosynthetic pigment, protein and proline content) parameters were measured at days 3, 5 and 7.

IAA content in bacterial medium, supplemented with 200 µg/ml of L-tryptophan and 0.5 or 1.0% NaCl, was determined with the Salkowski's reagent (50 ml of 35% $HClO_4$+1 ml of 0.5M $FeCl_3$) [12]. Chlorophyll a, b and total carotenoids were extracted with 90% acetone and determined according to Lichtenthaler [13]. The Bradford protein assay was used to measure the total protein concentration [14]. Proline was determined according to Bates and expressed as µg/g of fresh weight [15]. Initial qualitative estimation of isolates' phosphate-solubilizing activity was carried out on Pikovskaya agar with 0.01% methyl red. Solubilizing activity was visualized by the clear/red zone developed around the colony [16]. Three to five replicates were used in each test. Microsoft Office Excel and Grapher 10 were used for statistical analysis, standard deviation calculation and chart drawing.

3. Results

Growth of two isolated bacterial strains occurred at 4–37°C (optimal temperature 27°C). Both strains were able to grow on nitrogen free medium and did not grow in the presence of more than 5% NaCl. In the phosphate solubilizing activity assay after 10 days of incubation, isolates developed red or/and clear zones around colonies on Pikovskaya agar medium. Surface areas of red zones were 20 and 12 cm^2 (under 0% NaCl) and 14 and 5 cm^2 (under 1% NaCl) for A1 and A15, respectively.

In the liquid culture medium, the maximum accumulation of IAA occurred when the investigated isolates reached their stationary growth phase (Figure 1). Duration of IAA accumulation period in the culture medium of test variants equaled 6 days, only in case of strain *Pantoea vagans* A15 at 1% NaCl, robust inhibition of IAA synthesis appeared within 4 days. The diminishing of concentration of synthesized IAA in the medium occurred after the bacterial strains attained stationary growth phase within 4 days of cultivation. Also it can be assumed that bacterial cells utilized some amount of auxin from the solution to synthesize secondary metabolites. Increasing of the medium mineralization from 0.5% to 1% NaCl led to decrease in maximal IAA amount: A1 – from 64 to 49 µg/ml; A15 – from 43 to 35 µg/ml. Finally, maximal values of IAA synthesis were detected at days 4 and 5 for A15 and at day 6 for A1.

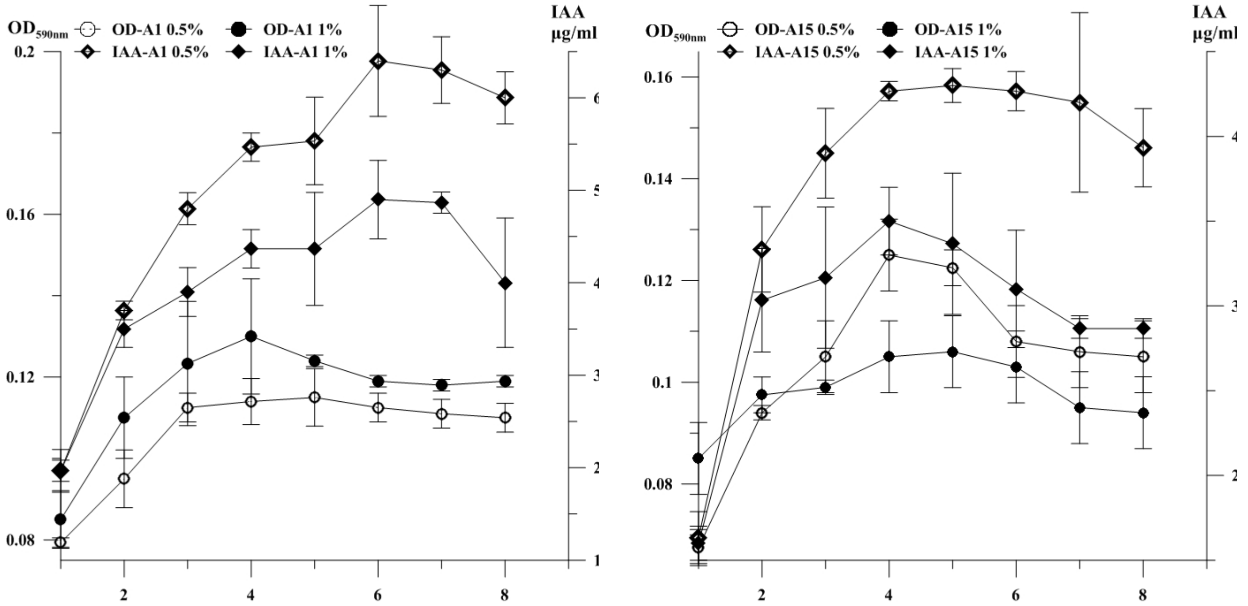

Figure 1 – Optical density and IAA synthesis rate for bacterial cell cultures in the liquid media supplemented with L-tryptophan under 0.5 and 1.0% NaCl during 8-day cultivation

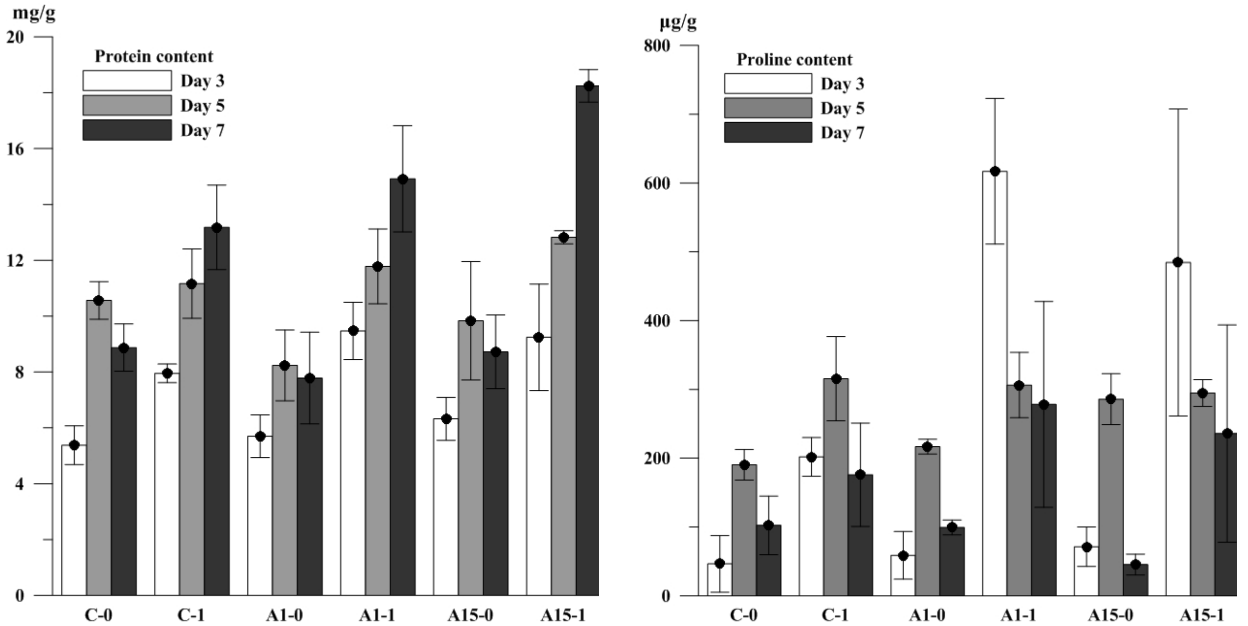

Figure 2 – Protein and proline content in fresh weight of the wheat seedlings under non-stress (“0”) and 1% NaCl (“1”) and with respective (A1 and A15) *Pantoea* strains for 3, 5, 7 days, “C”- control variants. Graphs show average values (n=3) and their standard deviations

In the experiment on the effect of isolated *Pantoea* strains on wheat seedlings under 0 and 1% NaCl there were no statistically significant differences with the control samples in morphometric parameters (length and weight of shoots and roots) and pigment content.

Evaluation of proline content in seedlings’ fresh weight (Figure 2) showed that in the salt-containing control the amino acid concentration was 1.5-3-fold higher than in the saltless control. Proline amount in seedlings inoculated with A1 strain without salt did not differ from the salt free control but in case of the A15 without salt, proline values were just slightly higher than in control. In case of both *Pantoea vagans* strains concentration of proline in the seedlings’ fresh weight on day 3 were 3 and 2.4-fold higher than the control ones with salt, reaching 616 and 485 µg/g for A1 and A15 with 1% NaCl respectively. Control value on day 3 under 1% NaCl was equal to 201 µg/g. On day 5, there was a sharp decline in proline concentration appeared on chart as equalization with the control values. During the next two days there was a further decrease in concentration of this amino acid (158% and 134% of the control values for A1 and A15 with 1% NaCl respectively).

In contrast to all saltless variants (experimental and control) samples with NaCl showed a gradual significant increase in protein content of the seedlings’ fresh weight (Figure 2). The maximum values at 7 day were 14.9 and 18.2 mg/g for samples inoculated with A1 and A15, respectively. Thus, their total protein amount equaled 113% and 138% of the control sample containing 1% NaCl.

4. Discussion

Two strains of salt tolerant bacteria isolated on nitrogen-free medium identified as *Pantoea vagans* A1 and *Pantoea vagans* A15. Isolates were able to synthesize large amount of IAA and release phosphorus from insoluble forms that could be explained by production of organic acids in the medium. Similarly, phosphate dissolution by IAA-excreting salt-tolerant (7% of NaCl) *Pantoea* strains was shown by Dastager et al., 2009 [17]. Chen reported that halotolerant (9% of NaCl) *Pantoea* strain with multiple PGP properties was able to promote the wheat growth by enhancing its resistance to drought stress [18]. Cherif-Silini isolated phosphate solubilizing IAA-synthesizing strain of *Pantoea agglomerans* from wheat rhizosphere using nitrogen-free medium [19]. The strain could tolerate to 300 mM NaCl and temperature up to 41°C.

Despite all growth promoting traits of A1 and A15, under salt stress we found no statistically significant differences in morphometric parameters (length, fresh weight of seedlings) or pigment content between inoculated and non-inoculated variants. Albeit there are a number of studies reported about more or less significant positive effect of crop inoculation with *Pantoea* isolates on shoot and root length and fresh weight as well as chlorophyll concentration in both the absence of salt stress and at 100 – 400 mM of salt [18, 19, 20]. A more long-lasting field experiment should be fulfilled to study the effects on morphometric parameters.

Species belonging to the genus of *Pantoea* are commonly isolated from various ecological niches and hosts involving plants, animals, humans, soil samples and other natural environments. Several species are known as plant pathogens [17], [21], [22]. It have been reported that some *Pantoea* strains were identified as rhizospheric, epiphytic and endophytic colonizers of many crops possessed multiple plant growth traits including IAA production and releasing phosphorus from insoluble forms being tolerant to moderate levels of environmental stresses like heat, high salt concentration and extreme pH ranges [17], [20], [23]. In addition, several *Pantoea* strains can act as biological control agents against plant pathogens [24]. Maximum accumulation of IAA at the stationary growth phase corresponds to the data of other researchers who studied the relation between auxin synthesis and bacterial growth phase where highest auxin levels were also detected during the stationary growth phase [9], [25]. Various researchers reported variable IAA production ability of *Pantoea* strains. Common values span from 20 – 60 and up to 100 µg/ml that are similar to those reported in this study [17], [25], [26]. In addition, Apine and Jadhav noticed that medium optimization gave maximum production of IAA for *Pantoea agglomerans* about 2 mg/ml [9].

It has been shown that wheat seedlings inoculated with two *Pantoea* isolates A1 and A15 exhibited considerable influence on proline content in fresh weight of wheat seedlings under salt stress in comparison to saltless control or uninoculated seedlings. Proline accumulation is a common response to abiotic stress in many plants, but the extent of proline accumulation varies in different plant species. There are many papers pointing out that the accumulation of proline in plants in general and particularly in wheat might reach great values and apparently depends on stress type, exposure time and in case of osmotic stress – on salt concentration [11], [27]. In our study, three-day salt stress had resulted in a two- or three-fold increase in proline level compared to the control with 1% NaCl. The similar findings were stated in several reports where accumulation of crucial amount of proline in tissues of various plants occurs during the first 72 h [10], [27]. Finally, gradual and significant increase in protein content matching with decline in proline concentration and possibly indicates the utilization of synthesized proline for the production of polypeptides. On the other hand, some representatives of *Pantoea* genus had positive effect on crop plants (promoting the growth, pigment content, phosphate solubilization, production of IAA, siderophores, etc.), but negatively affected proline accumulation. While accumulation of proline is a widespread plant response to environmental stresses, it is still controversial if its accumulation is a symptom of stress damages or an indication of stress tolerance and plant resistance [18], [20].

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

References

1. Johari-Pireivatlou M. Effect of soil water stress on yield and proline content of four wheat lines / M. Johari-Pireivatlou // Afr.J.Biotechnol. – 2010. – Vol.9. – P.36–40.

2. Kaur G. Modulation of proline metabolism under drought and salt stress conditions in wheat seedlings / G. Kaur, B. Asthir, N.S. Bains // Ind.J.Biochem.Biophys. – 2018. – Vol.55. – P.114–124.

3. Gopalakrishnan T. Modeling and mapping of soil salinity and its impact on paddy lands in jaffna peninsula, Sri Lanka / T. Gopalakrishnan, L. Kumar // Sustainability. – 2020. – Vol.12. doi:10.3390/su12208317.

4. Sankar B. Drought-induced biochemical modifications and proline metabolism in Abelmoschus esculentus (L.) / B. Sankar, C.A. Jaleel, P. Manivannan and others // Moench.Acta Bot.Croat. – 2007. – Vol.66. – P.43–56.

5. Singh M. Proline and salinity tolerance in plants / M. Singh, J. Kumar, V.P. Singh et al. // Biochem.Pharmacol. – 2014. – Vol.3. doi: 10.4172/2167-0501.1000e170.

6. Nia S.H. Yield and yield components of wheat as affected by salinity and inoculation with Azospirillum strains from saline or non-saline soil / S.H. Nia, M.J. Zarea, F. Rejali et al. // JSSAS. – 2012. – Vol.11. – P.113–121.

7. Asuming-Brempong S. Isolation of phosphate solubilizing bacteria from tropical soil / S. Asuming-Brempong, N.K. Aferi // Glob.Adv.Res.J.Agric.Sci. – 2014. – Vol.3. – P.8–15.

8. Saharan B.S. Plant growth promoting rhizobacteria: A critical review / B.S. Saharan, V. Nehra // LSMR. – 2011. – Vol.11: LSMR-21.

9. Apine O.A. Optimization of medium for indole-3-acetic acid production using Pantoea agglomerans strain PVM / O.A. Apine, J.P. Jadhav // J.Appl.Microbiol. – 2011. – Vol.110. – P.1235–1244.

10. Maghsoudi K. P5CS expression level and proline accumulation in the sensitive and tolerant wheat cultivars under control and drought stress conditions in the presence/absence of silicon and salicylic acid / K. Maghsoudi, Y. Emam, A. Niazi et al. // J.Plant Interact. – 2018. – Vol.13. – P.461–471.

11. Poustini K. Proline accumulation as a response to salt stress in 30 wheat (Triticum aestivum L.) cultivars differing in salt tolerance / K. Poustini, A. Siosemardeh, M. Ranjbar // Genet.Resour.Crop.Evol. – 2007. – Vol.54. – P.925–934.

12. Gordon S.A. Colorimetric estimation of indole-acetic acid / S.A. Gordon, R.P. Weber // Plant Physiol. – 1951. – Vol.26. – P.192–195.

13. Lichtenthaler H.K. Chlorophylls and carotenoids, the pigments of photosynthetic biomembranes. In: Douce R. and Packer L. (eds.) / H.K. Lichtenthaler // Methods Enzymol. – 1987. – Vol.148. – P.350–382.

14. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding / M.M. Bradford // Anal.Biochem. – 1976. – Vol.72. – P.248–254.

15. Bates L.S. Rapid determination of free proline for water stress studies / L.S. Bates, R.P. Waldern, I.D. Teare // Plant and Soil. – 1973. – Vol.39. – P.205–207.

16. Pikovskaya R.I. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species / R.I. Pikovskaya // Mikrobiologiia. – 1948. – Vol.17. – P.362–370.

17. Dastager S.G. Isolation and characterization of plant growth-promoting strain Pantoea NII-186 from western ghat forests oil, India / S.G. Dastager, C.K. Deepa, S.C. Puneet and others // Lett.Appl.Microbiol. – 2009. – Vol.49. – P.20–25.

18. Chen C. Pantoea alhagi, a novel endophytic bacterium with ability to improve growth and drought tolerance in wheat / C. Chen, K. Xin, H. Liu and others // Sci Rep. – 2017. – Vol.7. doi:10.1038/srep41564.

19. Chérif-Silini H. Isolation and characterization of plant growth promoting traits of a rhizobacteria: Pantoea agglomerans lma2 Pak / H. Chérif-Silini, A. Silini, M. Ghoul et al. // J.Biol.Sci. – 2012. – Vol.15. – P.267–276.

20. Chérif-Silini H. Durum wheat stress tolerance induced by endophyte Pantoea agglomerans with genes contributing to plant functions and secondary metabolite arsenal / H. Cherif-Silini, B. Thissera, A.C. Bouket et al. // Int.J.Mol.Sci. – 2019. – Vol.20. doi: 10.3390/ijms20163989.

21. Brady C. Phylogeny and identification of Pantoea species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA) / C. Brady, I. Cleenwerck, S. Venter et al. // Syst.Appl.Microbiol. – 2008. – Vol.31. – P.447–460.

22. Egorova M. First report of Pantoea ananatis causing grain discolouration and leaf blight of rice in Russia / M. Egorova, E. Mazurin, A.N. Ignatov // New Disease Reports. – 2015. – Vol.32. – P.21.

23. Kageyama B. Pantoea punctata sp. nov., Pantoea citrea sp. nov., and Pantoea terrea sp. nov. isolated from fruit and soil samples / B. Kageyama, M. Nakae, S. Yagi et al. // Int.J.Syst.Bacteriol. – 1992. – Vol.42. – P.203–210.

24. Azizi M.M.F. The emergence of Pantoea species as a future threat to global rice production / M.M.F. Azizi, S.I. Ismail, M.Y. Ina-Salwany et al. // J.Plant Prot.Res. – 2020. doi: 10.24425/jppr.2020.133958.

25. Tsavkelova E.A. Bacteria associated with orchid roots and microbial production of auxin. / E.A. Tsavkelova, T.A. Cherdyntseva, S.G. Botina et al. // Microbiol.Res. – 2007. – Vol.162. – P.69–76.

26. Acuña J.J. Indole acetic acid and phytase activity produced by rhizosphere bacilli as affected by pH and metals / J. J. Acuña, M.A. Jorquera, O.A. Martínez et al. // J.Soil Sci.Plant Nutr. – 2011. – Vol.11. – P.1–12.

27. Huang Z. Salt stress encourages proline accumulation by regulating proline biosynthesis and degradation in Jerusalem artichoke plantlets / Z. Huang, L. Zhao, D. Chen et al. // PLoS ONE. – 2013. – Vol.8. doi:10.1371/journal.pone.0062085.