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INFLUENCE COLLOIDAL SOLUTIONS OF NANOMOLYBDENUM ON THE EFFICIENCY OF SYMBIOTIC NITROGEN FIXATION IN LEGUMES (PEA, CHICKPEA)

SUMMARY

The strategic goal to the solving problem of dietary and fodder protein and restoring fertility of Ukrainian soils is optimal expansion of sowing areas traditional and non-traditional legumes. Creation of materials which are easily assimilated by living creatures and not harmful to the environment is one of the important issues of modern nanotechnologies. The aim of our study was the comparative evaluation of pre-sowing treatment with nanomolybdenum and microbiological preparation for impact on the efficiency of symbiotic nitrogen fixation in pea and chickpea plants. Field studies were conducted in the separated subdivision of the National University of Life and Environmental Sciences of Ukraine «Agronomic Experiment Station» on the typical black soils in the northern part of Forest Steppe of Ukraine. It was study of the influence of biological preparations on the nitrogen fixation capacity of pea (var. Tsarevych, Deviz) and chickpea plants (var. Rozanna, Triumf). The nitrogenase activity of the nodules in the root system of legumes was determined by acetylene-ethylene method. The efficiency of legume-rhizobia symbiosis depends on the number and virulence of symbiotic bacteria, which makes fixation of atmospheric nitrogen. Pre-sowing seeds treatment by strain of microorganisms enhances the quantitative and qualitative increase in the efficiency of the legume-rhizobia symbiosis. Using colloidal solution of molybdenum without seed inoculation also influences the number and diversity of rhizobia in the soil. Number, weight and symbiotic activity of nodules of pea and chickpea plants varied depending on the weather conditions. In the flowering stage the effects from pre-sowing treatment by bacterial inoculants and molybdenum solutions was most notable – the number of nodules was greater at 50-150 % compared with control, using inoculation this figure was higher at 8-9 %.

Keywords: pea, chickpea, nitrogenase activity, colloidal solutions of nanomolybdenum.

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INTRODUCTION

The problem of global agriculture in the past and the beginning this century is the problem of protein production. In deciding this important problem have place increasing plant protein production, the special place belongs to legumes, including peas (Lopatko et al., 2009). According to many researchers, one of the most objective criteria for the best use of chemicals in growing technologies is microbiological testing (Honchar 2013, Priestera et al., 2012). However, according to the researchers of the Institute of Agricultural Microbiology NAAS, considering the close interaction of certain types of microorganisms with cultivated plants and chances of forming close plant-bacterial associations in the soil, only rhysospheric (root microorganisms) can display the system response to certain factors, closest to response of a plant itself (Lin and Xing, 2007; Lopatko, 2012). This perspective is highlighted in the works of many authors of the last century, and in more modern materials of foreign and domestic scholars (Kaplunenko et al., 2008).

We know, that productivity of legumes, is determined by many factors, and oxide plays a primary role to. In order to obtain high yields of crops one should constantly take care of the replenishment of nitrogen in the soil by nitrogen-fixing bacteria (Lopatko et al., 2013). Based on the lack of studies about the impact of mineral fertilization on the formation and activity of symbiotic nodule bacteria, the practical value of this work was established (Frantiichuk et al., 2012, Lopatko et al., 2013b).

The strategic goal to the solving problem of dietary and fodder protein and restoring fertility of Ukrainian soils is optimal expansion of sowing areas traditional and non-traditional legumes. The biological nitrogen accumulate in the soils through fixation from the atmosphere by nodule bacteria upon interaction with plants, secures the increased yield of the major crops, retains the soils fertility and improves ecological condition (Pylypenko and Honchar, 2016).

Traditionally in Ukraine and worldwide the problem of fertilizer and animal feed enrichment with vital micronutrients solve by salts of heavy metals and chelating compounds which on the structure and properties are of small biological meet the needs of plants and animals, and only slightly absorbed by the latter. The result is the accumulation of heavy metals in the environment, ecological condition worst, decreased quality of received food (Taran et al., 2014a).

On applying nutrients Ukraine significantly lags behind Western countries. There is a steady increasing in production and a wider outspread of micronutrients over the last 30-40 years in almost all developed countries. However, there is a reverse process in our country: the production of micronutrients by domestic industry takes place in small quantities, without purpose. Therefore, for our country production of a wide range of micronutrients with balanced content of elements and their application in the production of environmentally friendly products with high content nutrients is an actual problem (Kaiser et al., 2005).

Breeders of our country intensively work to create new varieties of pea and chickpea. Annually is replenished public register varieties of these cultures, so it is important for them to pick up effective strains of rhizobia. As shown by our research and experiments of other researchers at the expense successful selection of macro- and microsymbiotic treatments is possible to increase yield legumes on 0.3-0.5 t / ha without disturbing the ecological balance of the environment (Kalenska et al., 2016). Creation of materials which are easily assimilated by living creatures and not harmful to the environment is one of the important issues of modern nanotechnologies. The colloidal solutions of metals as micronutrients enhance the plant resistance to unfavorable environmental conditions and ensure high yields of food crops (Panyuta et al., 2016).

Nanotechnologies in agriculture involve the use of fertilizing and plant protection preparations latest generation. Colloidal solutions of biogenic metals become widely used to enhance productivity and resistance to abiotic and biotic environmental factors.

The aim of our study was the comparative evaluation of presowing treatment with nanomolybdenum and microbiological preparation for impact on the efficiency of symbiotic nitrogen fixation in pea and chickpea plants.

MATERIAL AND METHODS

Field studies were conducted in the separated subdivision of the National University of Life and Environmental Sciences of Ukraine «Agronomic Experiment Station» on the typical black soils in the northern part of Forest Steppe of Ukraine. It was study the influence of biological preparations on the nitrogen fixation capacity of pea (var. Tsarevych, Deviz) and chickpea plants (var. Rozanna, Triumf). The nitrogenase activity of the nodules in the root system of legumes was determined via acetylene-ethylene method. Using colloidal solutions of nanomolybdenum as micronutrients enhances plant resistance to unfavorable environmental conditions and ensures high yields of food crops due to the active penetration of nanoelements into the plant cells (Lopatko et al., 2013).

Sowing area is 30 m2, accounting area – 25 m2. Repetition – quadruple, accommodation options consistent. Seeding rate amounted 1.2 million of seeds per 1 ha of pea and of the chickpea – 500 thousand seeds / ha. On the day of sowing, the bacterization with ryzohumin suspension was carried out. Suspension in amount of 900 g per1 t of seeds was diluted in 8-10 l of water, and then immediately treated. The rate of colloidal solution of nanoparticles of molybdenum was 1 liter of solution per ton of seed, working solution meets metal concentration of 0.8 mg / l.

The rate of ryzobofit and ST 282 strain in liquid form per hectare was 100 ml; 1 ml contained 6.7 billion nodule bacteria. On the day of sowing, the treatment chemicals were diluted in 1.7 l of water, and then seeds were treated with this solution. During the main cultivation, one had added granular superphosphate (P_2O_5 19.5 %) and potassium salt (K_2O 40.0%) at a rate of 60 kg / ha s. d., Ammonium nitrate (N 34.4 %) 30 kg / ha in the spring (Volkogon, 2006, Volkogon, 2007).

The obtained results were processed by computer program Statistics 6.0.

RESULTS AND DISCUSSION

Pre-sowing treatment of seeds nodule bacteria formation provide the required number of nodules on the roots of plants chickpea. During the growing season in the versions without the use of nodule bacteria inoculated on the roots of plants nodules were not formed, indicating a lack of native soil chickpeas rhizobia that can form nodules on the roots of culture (Shcherbakova et al., 2017).

In experiment indicators number and weight nodules varied depending on investigated factors. Symbiotic activity of chickpea plants largely depend on the climatic conditions of the investigated year. The formation of nodules on the roots of chickpea depends of options on pre-sowing treatment of seeds. The largest number of nodules 18.7-24.6 pcs. / plant and mass 823-976 mg / plant was marked by pre-sowing treatment of seeds with strain ST 282 and CSM.

The use of colloidal solution of molybdenum contributed to increasing nitrogenase activity by 27-28% in variety Rosanna and by 19-20% in variety Triumph. In 2013, the dynamics nitrogenase activity was higher than in previous years. Low temperatures and excessive rainfall that occurred in the flowering period of chickpeas, adversely affected the nodules formation nitrogenase activity.

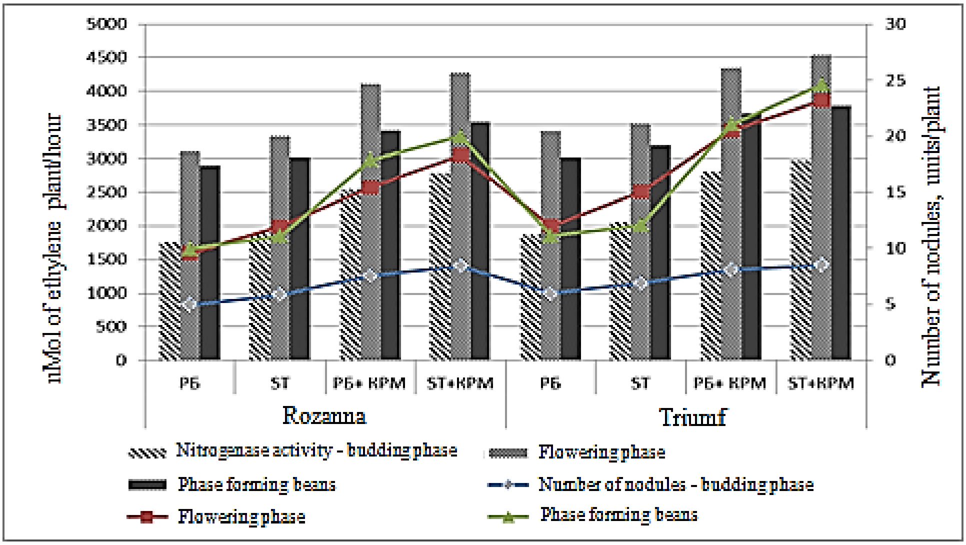
We obtained detailed data on the composition of low molecular weight organic components (organic acids, sugars and amino acids) of chickpea root exudates after inoculation with *M. ciceri* bacteria combined with treatment with CSM. According to our data, the introduction of rhizobia and CSM into the system resulted in changes in the composition of low molecular weight compounds in root exudates (Ryibalkina, 2005, Schwarz et al., 2009).

Qualitative composition and exudation intensity of the major carbon compounds differed in the variants inoculated with *M. ciceri* and treatment with CSM (Fig. 1). Organic acids were the 385 dominant fraction of root exudates in all variants. This fraction, in its turn, was dominated in all variants by succinic and malic acids; on the average, they could represent up to 90% of the total amount of organic acids depending on the variant. Similar data have been obtained in a study of pea, where the amount of malic acid was also high (from 45 to 71%).

However, instead of large amounts of lactic acid characteristic of pea roots (from 49 to 72% of the total amount of organic acids depending on the cultivar) the roots of chickpea produce much succinic acid (Sozer and Kokini, 2009, Taran et al., 2014b, Taran et al., 2016).

Seed inoculation with rysohumin had a positive influence on the formation of symbiotic apparatus of the leafless pea. The number and weight of nodules mg

/100 plants varied during the study (Tab. 1) depending on the use of technological measures, fertilization and varietal characteristics.



Note*: РБ - Inoculation by Ryzobofit; ST - Inoculation by strain ST 282; РБ+ CSM - Treatment CSM + Ryzobofit; ST+ CSM - Treatment CSM + strain ST 282

Figure 1. Number of nodules and nitrogenase activity of chickpea roots, depending of the preplant treatment of seeds options 2014-2016.

Table 1. Symbiotic activity of pea plants nodules depending on fertilizer, foliar application and seed inoculation (average for 2014-2016)

Variant of fertilizing	Stages of plant growth and development					
	BBCH 12-19		BBCH 55-59		BBCH 61-71	
	Deviz	**Tsarevych**	**Deviz**	**Tsarevych**	**Deviz**	**Tsarevych**
C	7.1	8.6	181	19.5	22.2	25.2
PH	17.4	18.6	36.1	38.6	50.6	53.1
ST	10.5	11.2	27.3	29.4	34.3	37.4
CSM	7.2	8.7	27.6	28.5	41.5	44.1
PH+ CSM	18.0	19.6	39.5	41.8	52.4	55.2
ST+ CSM	18.5	19.5	32.4	34.7	54.8	57.0
HIP_{05} for factor «fertilization»					*1.3*	
HIP_{05} for factor «inoculation of seeds»					*0.6*	

Note*: PH - Inoculation by Ryzohumin; ST - Inoculation by strain ST 238; РБ + CSM - Treatment CSM + Ryzohumin; ST+ CSM - Treatment CSM + strain ST 238

Conditions for the formation of symbiotic apparatus of Deviz pea variety were the most favorable while sowing inoculated seeds after presowing treatment of seeds by strain ST 238 and CSM. Thus, the number and weight of nodules were the highest and ranged from 52.4 to 54.8 pc / plant, and from 26.2 to 27.4 g /100 plants.

It had been established, that the varietal characteristics also have an impact on the formation and symbiotic activity of nodule bacteria. After a comparative analysis of the studied varieties we have noted, that Tsarevych variety had formed more nodules and greater weight, respectively.

CONCLUSIONS

The efficiency of legume-rhizobial symbiosis depends on the number and virulence of symbiotic bacteria which makes fixation of atmospheric nitrogen. Presowing seeds treatment by strain of microorganisms enhances the quantitative and qualitative increase in the efficiency of the legume-rhizobiales symbiosis. Using colloidal solution of molybdenum without seed inoculation also influences for number and diversity of rhizobia in the soil. Number, weight and symbiotic activity of nodules of pea and chickpea plants varied depending on the weather conditions. In the flowering stage the effects from presowing treatment by bacterial inoculants and molybdenum solutions was most notable – the number of nodules was greater by 50-150 % compared with control, using inoculation this figure was higher by 8-9 %.

The most favorable conditions for the formation of symbiotic apparatus had been created by the combination of inoculated seed sowing with application of CSM. While applying inoculated and CSM, the formation and performance of the device symbiotic apparatus of pea plants increase. The mentioned rate of mineral nutrition is effective on sowing by seeds, which are not inoculated.

REFERENCES

Frantiichuk V.V., Kovalenko M.S., Honchar L.M. and other (2012). The impact of non-ionic colloidal solution of nanoparticles of metals on the growth and development of winter wheat Collected Works “Institute of bioenergy crops and sugar beets”, № 14, 119–123.

Honchar L.M.(2013). The use of nanoscale biogenic metals at winter wheat growing technologies in the conditions of the Right-Bank Forest-steppe Ukraine. Engineering science from theory to practice, № 26, ANS «SibAK», 25–27 (in Russia).

Kaiser B.N, Gridley K.L, Brady J.N, Phillips T, Tyerman S.D (2005). The role of molybdenum in agricultural plant production. Ann Bot 96:745–54

Kalenska S., Nidzelskiy V., Pylypenko V., Taran N., Storozhenko V. (2016). Surface lipids and plant resistance of leguminous crops to stress factors in the conditions of Right-Bank Forest-Steppe of Ukraine. Electronic Journal «Scientific reports NULES Ukraine», №3(60). Access mode: http://journals.nubip.edu.ua/index.php/Dopovidi/issue/view/289.

Kaplunenko V.G., Kosinov N.V., Bovsunovskiy A.N., Chernyiy S.A. (2008). Nanotechnology in agriculture. Zerno, № 4 (25), 47–54.

Lin D., Xing B. (2007) Phytotoxicity: of Nanoparticles: inhibition of seed germination and root growth, Environ. Pollut, № 150, 243–250.

Lopatko K.H. (2012). Physical modeling the process of formation metal nanoparticles in spark processing. Scientific Bulletin NULES Ukraine 174:132–9

Lopatko K.H., Aftandiliants I. H., Kalenska S. M., Tonkha O. L. (2009) Patent number 38459 from 12.01.2009.

Lopatko K.H., Melnichuk M.D., Aftandilyants Y.G., Gonchar E.N., Boretskij V.F., Veklich A.N. et al (2013a) Obtaining of metallic nanoparticles by plasma-erosion electrical discharges in liquid mediums for biological application. Ann Warsaw Univ Life Sci 61:105–15, SGGW Agriculture.

Lopatko K.H, Melnychuk M.D. (2013b). Physics, synthesis and biological functionality of nanoscale objects. Publishing center NULES Ukraine, Kyiv (ukr)

Panyuta O., Belava V., Fomaidi S., Kalinichenko O., Volkogon M., Taran N. (2016). The effect of presowing seed treatment with metal nanoparticles on the formation of the defensive reaction of wheat seedlings infected with the eyespot causal agent. Nanoscale Res Lett 11:92

Priestera J., Gea Y., Mielkea R., Horst A., Moritz S., Espinosa K. et al (2012). Soybean susceptibility to manufactured nanomaterials with evidence for food quality and soil fertility interruption. Proc Natl Acad Sci U S A 109:2451–6

Pylypenko V., Honchar L. (2016) Symbiotic activity of nodule bacteria of pea plants at the territory of the Forest-Steppe of Ukraine. Scientific Journal NULES Ukraine, Series «Agronomy», № 235, 71-78.

Ryibalkina M. (2005). Nanotechnology for all. M.: Nanotechnology News Network, 444 s.

Schwarz G., Mendel R., Ribbe M. (2009) Molybdenum cofactors, enzymes and pathways. Nature 460:839–47

Shcherbakova E. N., Shcherbakov A. V., Andronov E.E. and other (2017). Combined preseed treatment with microbial inoculants and Mo nanoparticles changes composition of root exudates and rhizosphere microbiome structure of chickpea (Cicer arietinum L.) plants. Symbiosis. Published online: http://rdcu.be/oi6o

Sozer N., Kokini J.L. (2009). Nanotechnology and its applications in the food sector. Trends Biotechnol, 27, 82–89.

Taran N, Batsmanova L, Konotop Y, Okanenko A (2014a). Redistribution of elements of metals in plant tissues under treatment by non-ionic colloidal solution of biogenic metal nanoparticles. Nanoscale Res Lett 9:354–7

Taran N., Gonchar O., Lopatko K., Batsmanova L., Patyka M., Volkogon M. (2014b). The effect of colloidal solution of molybdenum nanoparticles on the microbial composition in rhizosphere of Cicer arietinum L. Nanoscale Res Lett 9:289–95

Taran N., Batsmanova L., Kosyk O. and other (2016). Colloidal Nanomolybdenum Influence upon the Antioxidative Reaction of Chickpea Plants (Cicer arietinum L.). Nanoscale Research Letters, T. 11(1), 476-480.

Volkogon V. (2006). Microbial preparations in crop production. Theory and practice. Agricultural science, Kyiv, ukr

Volkogon, V. (2007). Microbiological aspects of optimizing nitrogen fertilization of crops. Agricultural science, 144.

Volkogon, V. ed. (2006). Microbial preparations in agriculture. Theory and practice. Agricultural science, 312.