ENZYME ACTIVITY IN SOYBEAN ROOT-ADHERING SOIL IN DEPENDENCE ON NUTRITION AND WATER CONTENT CONDITION

Ecaterina EMNOVA[1], Simion TOMA[1], Oxana DARABAN[1], Iana DRUTA[1]

e-mail: kateemnova@yahoo.com

Abstract

Exo-enzymes secreted by plant roots in common with microbial exo-enzymes are involved in soil biochemical processes, namely the processes of mineralization of soil organic matter and providing by mobile species of nutrients. Rhizosphere soil enzyme activity is less studied than the bulk soil. Research aim was to assess the enzyme activity involved in nitrogen and phosphorus cycles in rhizosphere soil of soybean plants under different conditions of nutrition and soil water content. The greenhouse experiment was conducted in plastic pots (5 kg of dry soil - calcareous chernozem, humus content 1.85%, pH 7.93), in four replicates. The two soil water content were examined: optimal (70% WHC) and low (35% WHC), both in unfertilized and fertilized soil. Water stress period was 17 days. Enzyme activity of cultivated soil had higher values than the soil without plants, i.e. it is confirmed that rhizosphere soil is influenced by root exudation and rhizospheric microorganisms increased activity. In most cases in the rhizosphere of soybean variety Aura the enzymatic activities had a higher level in comparison with Clavera variety. In the soybean rhizosphere soil fertilized with mineral forms of N and P urease activity reached a higher level, but protease and phosphatase activities decreased compared with unfertilized soil. The mineral N and P amended before seeding regulate the processes of mineralization of soil organic matter and reduce soil enzymatic activity according to the feedback principle. Phosphatase activity determined at different pH values, showed the highest alkaline phosphatase activity in calcareous chernozem under soybean plants. Soil enzyme activity was negatively influenced by low soil moisture. One exception was the soil urease activity with significantly higher values ($p<0.05$) at low soil water content. The changes in soil enzyme activity coincided with the concentration of mobile forms of N and P determined in rhizosphere soil. After water stress the green mass of Aura variety was higher than those of variety Clavera, with 2.3 to 13.1%. But the difference was not supported statistically ($p>0.05$). The research results contribute to understanding the biochemical processes in rhizospheric soil to realize the potential of soybean productivity and to maintain the soil fertility.

Key words: calcareous chernozem, soybean rhizosphere, enzyme activity

Biochemical mineralization is the release of nitrogen, phosphorus and sulfur from soil organic compounds by the action of soil extracellular hydrolytic enzymes [Tate R., 2000]. The plant mass formation rate is limited by the rate of mineralization processes of plant debris, organic fertilizers, and/or soil organic matter (SOM) by the soil microbial community. Also, large quantities of plant nutrients, immobilized directly in microbial biomass, as a part of SOM, can serve as an additional source of mineral nutrition for crops. The release of mineral nutrients from plant and microbial biomass depends primarily on the number of soil microbial populations producing the complex hydrolytic enzymes necessary for transformation, decomposition and mineralization of SOM.

Plants of different varieties or genotypes can affect through the quantity and quality of root exudation (diffusates) the structure and functions of microbial community, which colonizes the rhizosphere (root-adhering soil), [Brimecombe M., 2001]. Besides that, the soil extracellular enzymes can originate from both the microorganisms, as well as root exudation of plants (secretions) [Martens D., 1992, R. Yang, 2007]. Being secreted, the extracellular enzymes are not under the control of microorganisms and roots that were responsible for their production [Schloter M., 2003]. Their activity and persistence are influenced by soil physical condition and chemical properties.

The climatic conditions throughout the Republic of Moldova can be characterized as unstable. Annual potential evaporation exceeds the rainfall, which creates soil water content deficit [Ursu A., 2011].

Soya is a crop with specific requirements to nutrient and water regime, and reacts poorly to the direct application of fertilizer, especial in areas of unstable wetting [Novak A., 1964, Toma S., 2008]. All soybean varieties cultivated in Republic of Moldova are vulnerable in drought condition

[1] Institute of Genetics and Plant Physiology, MAS, Chisinau, Republic of Moldova

[Vozian, 2010]. Compared with nutrition of legumes by fixing of atmospheric N the soybean root nutrition is poorly studied, including at different soil water content.

The aim of research was to assess the enzyme activity involved in nitrogen and phosphorus cycles in the rhizosphere soil (root-adhering) of soybean plants of two varieties (genotypes) in various conditions of soil water content and nutrition.

MATERIAL AND METHOD

The experiment was conducted under greenhouse condition in plastic pots (5 kg of absolutely dry soil - calcareous chernozem with humus content 1.85% and pH value 7.93), in four repetitions. Two soil water content levels were examined: optimal (70% WHC) and low (35% WHC) in the unfertilized soil, as well as fertilized one. Fertilizers were introduced 3 days before sowing. The load was N = 50 mg N kg^{-1} soil in the form of salt $Ca(NO_3)_2$, and P = 100 mg P_2O_5 kg^{-1} soil - salt KH_2PO_4. Two varieties of soybean seeds Clavera and Aura were processed before sowing with nitrogen-fixing bacteria *Rhizobium japonicum.* Water stress period constituted 17 days. The object of study was the enzymatic activity of rhizosphere soil.

Urease activity (Ure) was determined with urea as the substrate and reagent Nesler [Mineev V., 1989]. The activity of protease (Pro) was measured with Na-casein (substrate) and Folin-Ciocalteu reagent according to the method of Ladd and Batler (1972) [Alef K., Nannipieri, P.), 1995]. Phosphatase activity (Ph) acidic and alkaline were determined in the presence of buffer solutions (respectively, pH 5.0, 10.2) using the method proposed by Tabatabai and Bremner (1969) with p-nitrophenol phosphate disodium salt (pNP) as substrate [Khaziev F. 1990; Teylor J., 2002; Daraban O., 2012]. Actual Ph was measured under natural soil pH 7.5 (without buffer). Agro-chemical parameters were analyzed according to known methods [Mineev V., 1989].

The program Microsoft Excel for Windows XP (Microsoft Office) was used for data analysis. The arithmetic means were appreciated by standard deviation (σ). Reliability of differences between arithmetic averages was analyzed by Student t-test (bilateral test, type 3 with unequal variation, p <0.05).

RESULTS AND DISCUSSIONS

Soil urease activity (Ure) was positively influenced by the presence of used mineral fertilizers, with maxim value of 139 mg NH_4^+ g^{-1} dry soil h^{-1} in soil without plants, 175 mg NH_4^+ g^{-1} dry soil h^{-1} in soil under soybean variety Aura, and 179 mg NH_4^+ g^{-1} dry soil h^{-1} - under Clavera soil (*tab 1*). In unfertilized soil, soil urease activity in indicated treatments was respectively 124, 163, and 165 mg NH_4^+ g^{-1} dry soil h^{-1}. The presence of plants cultivated without and with fertilization both favored the mineralization of soil organic nitrogen with over 20% compared with soil without plants.

Table 1

Enzymatic activity of N and P cycles in soybean rhizosfere soil

Treatments	Nutritive regime	Soil water content, % WHC	Ure	Pro	acidic Ph, pH 5,0	actual Ph, pH 7,5	alkaline Ph, pH 10,2
			Mean values ± S.D. (n = 4)				
Soil without plants (control)	NP	70%	139±3	34.8±3.0	108±6	151±12	201±4
		35%	146±2***	33.0±7.6	96±4***	131± 6***	193±4***
	non-fertilized	70%	124±2	41.4±2.8	121±10	157± 9	205±13
		35%	149±2***	37.9±2.0	126±5	153±18	193±4***
Soybean variety Aura	NP	70%	175±3	33.8±6.3	114±6	159± 5	215±8
		35%	180±1***	33.5±4.4	113±12	151±12	198±5***
	non-fertilized	70%	163±3	34.8±6.2	136±5	189±13	228±5
		35%	187±4***	30.9±3.6	134±12	167±11***	189±14***
Soybean variety Clavera	NP	70%	179±2	34.4±4.9	122±8	182±14	212±4
		35%	184±2***	31.2±4.4	119±7	167±17***	210±7
	non-fertilized	70%	165±3	35.7±4.2	116±8	172± 6	225±7
		35%	173±3***	33.0±4.9	112±6	156±10***	196±5***
Initial soil			154±1	30.6±2.0	102±1	130± 2	175±2

Note. NP – respective, 50 mg N kg^{-1} dry soil and 100 mg P_2O_5 kg^{-1} dry soil. Ure – soil urease activity, µg NH_4^+ g^{-1} dry soil h^{-1}; Pro **–** soil protease activity, µg tyrosine g^{-1} dry soil h^{-1}; Ph – soil phosphatase activity, µg p-nitrophenol (pNP) g^{-1} dry soil h^{-1}. S.D. - standard deviation, σ. * Significant difference appreciation of parameters is shown for – 70% WHC versus 35% WHC,*(P<0.05); **(P<0.01); ***(P<0.001)

Table 2

Agrochemical parameters of soybean rhizosphere soil

Treatments	Nutritive regime	Soil water content % WHC	SOM	mobile NH_4^+	mobile NO_3^-	P_2O_5
			Mean values ± S.D. (n = 4)			
Soil without plants (control)	NP	70%	1.89±0.04	3.4±0.10	0.142±0.010	8.5±0.3
		35%	1.76±0.03***	2.5±0.07***	0.114±0.009***	8.4±0.8
	non-fertilized	70%	1.80±0.04	3.1±0.16	0.042±0.002	1.1±0.2
		35%	1.84±0.06	3.9±0.18***	0.033±0.011	0.9±0.1**
Soybean variety Aura	NP	70%	1.88±0.04	2.5±0.16	0.003±0.000	6.3±0.8
		35%	1.83±0.06	2.2±0.05**	0.005±0.002**	8.7±0.9***
	non-fertilized	70%	1.86±0.04	2.8±0.24	0.005±0.002	0.8±0.1
		35%	1.87±0.07	2.6±0.08*	0.004±0.001	0.7±0.1
Soybean variety Clavera	NP	70%	1.83±0.04	2.8±0.14	0.003±0.001	6.8±0.7
		35%	1.87±0.03	2.2±0.15***	0.006±0.002**	8.8±0.4***
	non-fertilized	70%	1.92±0.03	3.0±0.13	0.002±0.001	0.8±0.2
		35%	1.79±0.05***	2.7±0.21**	0.008±0.013	0.8±0.0
Initial soil			1.88±0.02	1.2±0.02	0.033±0.002	0.7±0.0

Note. NP – respective, 50 mg N kg^{-1} dry soil and 100 mg P_2O_5 kg^{-1} dry soil. SOM **–** Soil Organic Matter (humus), %; NH_4^+ – ammoniacal nitrogen, mg 100 g^{-1} dry soil; NO_3^- - nitrate nitrogen g. mg 100 g^{-1} dry soil; P_2O_5 – anorganic phosphorus (Machigin), mg 100 g^{-1} dry soil; S.D.- standard deviation, σ. *Significant difference appreciation of parameters is shown for 70% WHC versus 35% WHC, *(P<0.05); **(P<0.01); ***(P<0.001)

Plant variety had no apparent effect on urease activity. Difference, although statistically supported (p <0.001), was very small, indicating an increase of 1-2% in soil under the variety Clavera versus Aura. The Ure activity values were significant higher (p <0.001) at low soil water content (35% CTAs) than at optimal soil moisture, as it was also demonstrated in previous study of typical and calcareous chernozem [Emnova E., 2010; Toma S., 2011}. The difference becomes more pronounced in treatments in which fertilizers were introduced. The increase of 20.6% in soil without plants, 15.3% in soil cultivated with variety Aura and 4.5% - with variety Clavera was observed at soil water stress. The fact that the biggest difference is characteristic to soil without plants allows assume that it is not mainly due to root exudation but to a greater extent, to indigenous soil microorganisms.

Protease activity (Pro) of the studied calcareous chernozem soil (with low level of total soil organ**ic** matter 1.85%) was decreased in all treatments (*tab 1*) in comparison to typical chernozem (SOM 4.5%) [Emnova E., 2011]. At the same time, unlike the soil urease activity the protease activity of cultivated soil had lower values than in soil without plants, but the difference was not significant (p> 0.05). At soil water content 35% CTAs the protease activity tended to decrease, but again without the significant difference compared to optimal water supply. Cultivated soybean varieties influenced insignificantly the soil protease reactions of rhizosphere soil to environmental conditions.

Soil phosphatase activity (Ft) determined in alkaline medium had the highest values (*tab. 1*). Analyzing the three types of phosphatase which were analyzed in studied calcareous chernozem, the following relationship can be schematically represented: alkaline Ph (189 to 228 mg pNP g^{-1} dry soil h^{-1}) > actual Ph (130 to 189 mg pNP g^{-1} dry soil h^{-1}) > acidic Ph (96 to 136 mg PNP g^{-1} dry soil h^{-1}). The mineral forms of phosphorus introduced in soil inhibited the soil phosphatase activity, especially the acid and actual ones in soil without plants and cultivated with soybean variety Aura. These findings correspond to those obtained in previous research of typical chernozem soil cultivated in 6-years crop rotation [Emnova E., 2010; Daraban, 2012]. Otherwise, the Ph activity of NP fertilized calcareous chernozem soil, in which soybean variety Clavera plants was grown, increased respectively by 5% and 6.3% under optimum and low soil water content. Insufficient water content decreased the soil Ph activity, although not drastically, as it was also observed in previous research on calcareous chernozem without plants [Toma S., 2011]. Plant growing contributed to achieving the higher values, the exception made only the variety Clavera. On alkaline phosphatase activity, although it showed the highest values, the effect of fertilization (increase by 5.8%) was shown clearly ($p < 0.001$), only in treatments with optimum water content, and where the plants have been cultivated.

Changes in soil enzyme activity in general coincide with the concentration of mobile forms of N and P determined in rhizosphere soil (*tab. 2*). The higher concentrations of inorganic P and nitric N were found in fertilized rhizosphere soil of both soybean varieties, cultivated at 35% CTAs, but ammoniacal N concentrations, as a rule, were reduced compared with soil - at 70%CTAs. These concentrations can be the outcome of two processes - income of nutrient forms due to enzymatic digestion processes, and their consumption by plant roots. After water stress the green mass of Aura variety was higher than those of variety Clavera, with 2.3 to 13.1%. But the difference was not supported statistically ($p>0.05$).

CONCLUSIONS

The enzymatic activity of cultivated soil had higher values than the soil without plant, namely, the rhizosphere soil is influenced by the root exudation – directly, and by increased activity of rhizosphere microorganisms - indirectly. In most cases the enzymatic activities of soil under soybean variety Aura had a higher level in comparison with Clavera variety.

The urease activity reached a higher level in soil fertilized with mineral forms of N and P, but protease and phosphatase activities decreased compared with unfertilized soil. Mineral fertilizers containing N and P (respectively, 50 and 100 mg kg^{-1} dry soil) regulated the processes of proteins and of organic phosphorus forms mineralization in the soil by reducing the corresponding enzymatic activity according to the feedback principle.

The reduced soil water content negatively influenced the soil enzyme activity. The exception was the soil urease activity, which in all treatments had higher values as a response to low soil water content.

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