ENZYME ACTIVITY INVOLVED IN N AND P CYCLES, OF SOYBEAN PLANT RHIZOSPHERE SOIL, ROOTS AND LEAVES

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Abstract

The research aim was to evaluate the influence of exogenous nitrogen sources on key-enzyme activity involved in nitrogen (urease) and phosphorus (phosphatase) metabolism in the rhizosphere soil, roots and leaves of soybean plants at bud-flowering stage. The field experiment was designed on 4 plots (12 m^2, 60 plants/ m^2): two fertilization treatments x four replicates. The soil was fertilized in starting rates with mineral forms of N and P ($N_{20}P_{60}$): KH_2PO_4; NH_4NO_3 (ammonium nitrate) and $(NH_2)_2CO$ (urea). Urease and phosphatase activity (acid, alkaline and total) were determined in the studied material. Application of additional sources of mobile nitrogen (N) led to changes of enzymatic activities determined in the rhizosphere soil and soybean plant organs. Urease activity showed an increase in soil treated with urea, which probably served as a substrate for increased ammoniacal nitrogen amount and uptake, respectively. The increasing of the phosphatase activity in the rhizosphere soil and roots, in the treatment with ammonium nitrate as soil fertilizer, showed an intensification of the phosphorus mobilization. The obtained data indicated that there was an increased need in phosphorus assimilation for plants, in this treatment. Phosphatase activity (acid and total) in leaves was not influenced by the type of applicated fertilizer. Cultivation of soybean plants on soil fertilized with urea led to increased productivity elements compared to ammonium nitrate. Experimentally, it has been argued, the significant role of nitrogen sources in achieving mechanisms of functional integration and optimization of relations between roots and rhizosphere processes driven by microorganisms, thus, contributing to increase the productivity potential of soybean plants.

Key words: soybean plants, ammonium nitrate, urea, urease activity, phosphatase activity

In the soil, soluble forms of nitrogen are in a continuous processing. Nitrogen is absorbed by plants in the form of ammonium ions, nitrate and to a lesser extent in the amide form (Toma S.I., 2008). Thus, there are plant species that give precedence to ammonia nitrogen nutrition and others of nitric nitrogen. According to published data (Li S.X. et al., 2013) indicated ions can express different effects on nutrition, growth and productivity of various plant species. Different types of fertilizers can exert different impact on metabolism and on the quality of the harvest, respectively. Relationships established between plants (roots) and edaphic microbiota that synthesize and release various types of soil enzymes, have an important role in the process of plant nutrition. Soil enzymes have a major contribution in mobilizing soil nutrients, being responsible for soil organic matter decomposition (Emnova E.,Toma S., 2010; Tate R. III, 2001). As a result, the activity of hydrolytic enzymes is a representative biochemical indicator of the soil quality (Emnova E.,Toma S., 2010). Urease (urea-amidohidrolaza) [EC 3.5.1.5], plays a significant role in the nitrogen cycle, it catalyzes the hydrolytic cleavage of urea to NH_3 and CO_2, releasing thereby the inorganic N (which can be volatilized into the atmosphere) with simultaneous increase the pH of the soil (Khaziev F.H., 1990). Soil urease activity values characterize the rate of mineralization of substances containing nitrogen (Mihailovskaia N. et al., 2008). Phosphatase [EC 3.1.3.1-2] is a enzyme that catalyzes hydrolysis phosphoesteric linkages of diverse labile organic molecules. Phosphatase activity contributes to the degradation of organic phosphates and their conversion into forms easily assimilated by plants (Nannipieri P., et al., 2011; Kiss Şt., Ştefanic Gh., et al., 1991; Khaziev F.H., 1990). The mechanisms by which the soybean roots ensure the integrity of the function of suppling the plants with nitrogen, in drought conditions, using the biochemical activity of microorganisms in the rhizosphere are not fully studied and thus, require further investigation.

The *research aim* was to evaluate the influence of exogenous nitrogen sources on key-enzyme activity involved in nitrogen (urease) and phosphorus (phosphatase) metabolism of the

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rhizosphere soil, roots and leaves of soybean plants.

MATERIAL AND METHOD

The research was conducted on IGFPP ASM experimental basis. In the field experiment four plots were investigated (two variants of fertilization x 4 replicates). Each plot with an area of 12 m^2 (5 m x 2.4 m) consisted of 3 experimental lines and 2 protection lines, the distance between rows: 0.45 m, between plots in width and length, respectively, 0.6 m and 1 m. Soybean seeds were sown in the calculation of the 60 plants / m^2.

The soil was fertilized 14 days before sowing with mineral forms of N and P in starting rates, recommended for soybean (Boaghii I., 2010; Toma S., 2008; Corobco V., 1982). Phosphorus fertilizers were introduced into the soil, as the background in the form of KH_2PO_4 (60 kg P_2O_5 / ha). Nitrogen fertilizers have been amended in salt form NH_4NO_3 (ammonium nitrate) and $(NH_2)_2CO$ (urea) in a dose of 20 kg N / ha. Roots, leaves, and rhizosphere soil of soybean plants, variety Indra were used as study objects. Each agronomic sample was a mix of rhizosphere soil from 9 soybean plants (3 plants from each of 3 experimental line). Plants with roots were collected at the stage of bud-flowering. Seed material was provided by ICCC "Selectia", Balti, RM. Indra soybean variety was approved in Republic of Moldova since 2006 and is a mid-early variety with a vegetation period between 115-122 days. It is a productive variety with a yield potential of 4390-4500 kg / ha (Iacobuță M., et al., 2010).

The activity of urease from the soil (UreS) was determined by urea solution used as the substrate and Nesler reagent (Mineev V., 1989), the urease activity in roots (UreR) and leaves (UreL), according to the methods described by Pocinoc (1976) and Pleshcov (1968) (Emnova E. et al., 2012; Emnova E. et al., 2014).

To estimate the activity of phosphatase (Pht) it was used acidic or alkaline buffer solution (pH 5.0 and 10.2, respectively) by the method proposed by Tabatabai and Bremner (1969) with disodium p-nitrophenyl phosphate (pNP) as the substrate (Khaziev F. 1990; Taylor J., et al., 2002; Daraban O., 2012). Total Pht were determined with distilled water, at the natural pH of the soil (pH= 7.5). Soil chemical parameters were analyzed according to the recommended methods (Mineev V., 1989). Poductivity items were established at harvest.

Experimental data were statistically interpreted using Microsoft Office applications. The veracity of the difference between arithmetic means was analyzed by Student t-test (bilateral test, type 3, with unequal variations).

RESULTS AND DISCUSSION

General characteristics of the soil. Determinations of the soil samples taken before fertilizers introduction, allowed to assess the overall soil condition at the time of the initial investigation. It is known that calcareous chernozem differs from other types and subtypes of chernozem by higher pH value (Ursu A., 2011); in the soil from experimental field it was 7.26; soil water content was 20.07%. Organic matter content of carbonate chernozem was 2.87%, so the given soil is characterized as moderate humified. The content of mobile forms of nitrogen namely of ammonia nitrogen ($N\text{-}NH_4$) was 0.77 mg/100 g soil, and of nitrate nitrogen ($N\text{-}NO_3$) 0.41 mg/100 g soil, indicating the prevalence of ammoniacal nitrogen in the soil. The coefficient of variation of 14.6 to 18.8% average batch uniformity proved experimentally. The soil of the experimental field was characterized by a very low content of mobile forms of phosphorus (P_2O_5). The results of the enzyme analysis showed that the hydrolytic processes in the soil were triggered over (*tab. 1*)

Urease activity. Fertilization of the calcareous cernozem with urea has led to authentic increase of soil urease activity (Ure S) which recorded significantly higher values by 23.2% ($p \leq 0.001$) compared with ammonium nitrate *(tab. 2)*). The increase of enzyme activity can be explained by the need to increase nitrogen assimilation from the soil by plants.

Table 1

General characteristic of unfertilized calcareous chernozem

	H	SOS	pH	$N\text{-}NH_4$,	$N\text{-}NO_3$	P_2O_5	UreS	Pht S		
	%			mg in 100 g a.d.s.				acid	total	alkaline
mean	**20.07**	**2.87**	**7.26**	**0.77**	**0.41**	**0.90**	**86.1**	**118.4**	**123.1**	**378.1**
SD	0.74	0.04	0.02	0.11	0.08	0.09	6.2	4.7	3.9	14.4
CV, %	3.69	2.42	0.28	14.61	18.76	10.1	7.2	4.0	3.2	3.8

Note: H – soil water content; SOM – soil organic matter; $N\text{-}NH_4$ – ammonia nitrogen; $N\text{-}NO_3$ – nitrate nitrogen; a.d.s. – absolutely dry soil; UreS - soil urease activity, NH_4, mg in 1 g a.d.s./ 1 h at 37˚C; Pht S – soil phosphatase activity, pNP, µg in 1g a.d.s./1 h at 37°C; SD – standard deviation; CV - coefficient of variation,%.

Table 2

Enzyme activity of N and P cycles in the rhizosphere soil, roots and leaves of soybean plants, variety Indra

		Pht						Ure	
		acid		total		alkaline			
	treatment	Ammonium nitrate	Urea	Ammonium nitrate	Urea	Ammonium nitrate	Urea	Ammonium nitrate	Urea
Soil [1]	mean	**121.8**	**110.6**	**130.3**	**116.4**	**359.5**	**330.4**	**79**	**97**
	SD	4.8	2.8	8.4	5.5	22.3	17.0	2.6	2.6
	CV, %	4.0	2.6	6.5	4.8	6.2	5.1	3.2	2.7
Roots[2]	mean	**181.6**	**156.1**	**64.6**	**52.7**	**7.4**	**5.6**	**2767**	**3818**
	SD	16.2	14.1	5.5	4.8	1.2	0.7	157	447
	CV, %	8.9	9.0	8.5	9.1	16.4	12.9	5.7	11.7
Leaves [2]	mean	**430.1**	**416.1**	**218.8**	**230.7**	**18.3**	**16.4**	**1689**	**1805**
	SD	38.6	39.3	33.8	25.5	2.5	0.7	119	125
	CV, %	9.0	9.5	15.4	11.1	13.7	4.2	7.0	6.9

Note: Pht – phosphatase activity; Ure – urease activity; SD – standard deviation; CV – coefficient of variation; a.d.s. – absolutely dry soil; index [1] (referring to Pht activity) – pNP, **µg** 1 g a.d.s../1 h at 37°C; index [2] (referring to Pht activity) – pNP, **mg** 1 g a.d.s../1h at 37°C.

Table 3

Elements of productivity

treatment	Seed weight		Pod weight		Pod number	
	mean per 1 plant, g	SD	**mean** per 1 plant, g	SD	**mean** per 1 plant, g	SD
Ammonium nitrate	**10.8**	2.1	**18.2**	3.4	**49.4**	21.1
Urea	**13.9**	2.3	**22.7**	3.6	**57.7**	31.4

Urease activity in root tissues (**Ure R**) of plants, variety Indra went much more intense than that of rhizosphere soil and was favored by the application of urea fertilizers. Thus, the agrofond with urea fertilizer led to a genuine increase of UreR by 38%. A similar trend of increase observed in plant leaves Indra (**Ure L**), where, in the presence of urea it was noticed an increased nitrogen mineralization process by 6.8%.

After analyzing the results we can assume that the introduction of fertilizers in the soil as urea led to increased urease activity in both the soil and the roots and leaves, serving as a substrate for formation and increased nitrogen uptake.

***Phosphatase activity*.** The results indicate that fertilization with ammonium nitrate contributed to the increased Ft activity of rhizosphere soil (**Pht S**), as compared to the treatment with urea *(tab. 2)*. The created difference was 10.1% ($p \leq 0.001$), 11.9% ($p \leq 0.001$) and 8.8% ($p \leq 0.01$) for acid, total and alkaline Pht, respectively. In rhizosphere soil phosphatase activity determined in different pH environments, has established the following relationship: acid Pht < total Pht < alkaline Pht. The values of total Pht activity was closer to the acid Pht, alkaline Pht having the highest level.

Phosphatase activity determined in root tissue (**Pht R**) of soybean plants, variety Indra grown on agrofond with ammonium nitrate showed higher values than those grown in soil fertilized with urea. It was established a reliable increase ($p \leq 0.001$) of analyzed parameter in this treatment in comparision to fertilization with urea by 16.3%, 22.6% and 33.4% for acid, total and alkaline Pht, respectively. Between plant roots' phosphatase activities determined in media with different pH values was obtained following inequality: alkaline Pht < total Pht < acid Pht. Total Pht activity values were closer to the alkaline Pht ones.

The fertilization with various forms of nitrogen did not follow with increase of acid Fht and total activity in the leaves of soybean plants (**Pht L**). Only the alkaline Pht activity, with the lowest values, presented an increase at fertilization ammonium nitrate, by 11.8% ($p \leq 0.05$). Alike to the plant roots, between the phosphatase activity determined in environments with different pH values the following relationship was obtained: alkaline Pht < total Pht < acid Pht.

Comparing the values of Pht activity determined in the three subjects it was found that both acid and alkaline Pht activity increases towards soil → root → leaves.

Harvest items (*tab. 3*). In estimating the average number of pods per plant was noted that in the agrofond with urea it was higher by 16.8% (not statistically significant) than the one with ammonium nitrate. For variety Indra the kind of fertilizer significantly influenced the mass of pods per plant. Thus, it achieved a 24.7% increase ($p \leq 0.05$) for plants grown in soil fertilized with urea compared to ammonium nitrate, which confirmed the results of 2012: the beneffic effect of urea on plant productivity of variety Indra (Emnova E. et al., 2014).

CONCLUSIONS

Additional introduction of mobile nitrogen led to changes in enzymatic activities determined in rhizosphere soil and soybean plant organs, variety Indra. Urease activity in both the soil and vegetal parts (roots and leaves) showed an increase in case of urea treatment, which probably served as a substrate after increased ammoniacal nitrogen amount and uptake, respectively.

The increasing of the phosphatase activity in the rhizosphere soil and roots, in the treatment with ammonium nitrate as soil fertilizer, showed an intensification of the phosphorus mobilization. The obtained data indicated that there was an increased need in phosphorus assimilation for plants, in this treatment. Phosphatase activity (acid and total) in leaves was not influenced by the type of applicated fertilizer.

Cultivation of soybean plants on soil fertilized with urea led to increase productivity elements compared to ammonium nitrate. Experimentally, it has been argued, the significant role of nitrogen sources in achieving mechanisms of functional integration and optimization of relations between roots and rhizosphere microorganisms, thus, contributing to increase the productivity potential of soybean plants.

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