BIOLOGICAL ACTIVITY OF SOYBEAN RHIZOSPHERE SOIL IN DEPENDENCE ON WATER CONTENT AND NUTRITIONAL STATUS

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Abstract

Transformations of soil organic matter into easily assimilated nutrient forms by plants are mediated, to a greater extent, by soil microbiota. Soil biological activity characterizes both its fertility and the degree of environmental factors' influence. The aim of the current study was to explore the influence of suboptimal soil water content, mineral fertilizers, and plant variety on biological potential of soybean rhizospheric soil. Experiment was set up in greenhouse complex. Two soil water content levels were examined: optimal – 70% water holding capacity (WHC) and reduced – 35% WHC, established in the blossom–flowering stage. The treatments were: (1) NP soil fertilization and (2) deficient nutrient content. Soybean plants were represented by two varieties, also, the soil without plants were taken into account. Following biotic parameters were analyzed in the rhizosphere soil: C-MB (microbial biomass carbon), BSR (basal soil respiration) and the H-FDA (fluorescein diacetate hydrolysis). The results highlights that C-MB and the general hydrolytic activity (H-FDA) were reduced under temporary (17 days) soil water content deficiency. Estimated BSR as functional activity of microbiota, showed higher values at 35% WHC. This could indicate that additional energy expenses of bacterial community to maintain vital processes. Soil biological activity depended on the plant nutritional condition, showing a clear increase at NP fertilization, especially at soil water content deficiency. It was found that the examined parameters had greater levels in rhizosphere soil of Aura variety, compared to Clavera one. In soil without plants values were the lowest.

Key words: soybean, rhizosphere soil, microbial biomass, soil basal respiration, FDA hydrolysis

Legumes and especially soybean plants have a major role in promoting sustainable agriculture, both globally and on the national level (Rotaru V., 2010; Soy Stats, 2012). Although economic and ecological benefits of the soybean crop are well-known, areas occupied by this plant in Republic of Moldova are very small. In creating the given situation, along with economic and technological problems, the soil and climate ones occure most commonly (Bucur Gh. et al, 2007; Boaghii I., 2004).

In recent years, more frequently, adverse environmental conditions affect crop productivity. According to researches carried out all varieties of soybeans grown in Moldova are vulnerable to drought (Vozian V. et al., 2010).

Providing the optimal and balanced nutrition, contributes to achieve the potential production and adaptation of crop plants to unfavorable environmental conditions (Toma S., Roşca A., 1999). The biological potential of the soil can give a characteristic of fertility levels and the degree of influence of environmental factors. It was established that the flow of organic substrates coming from the roots, together with the physical, chemical and biological specific factors influence more significant the bacterial community structure and activity from the rhizosphere than in the bulk-soil (Brimecombe M. et al., 2001).

Microbial biomass (MB) as a living and active fraction of soil organic matter plays an important role in the soil ecosystem development and functioning. Microorganisms contributes to the maintenance of soil fertility and quality by its control over major key biochemical processes taking place in soil (Tate R. III, 2001; Emnova E., Toma S., 2010). Basal soil respiration (**BSR**) as an indicator of metabolic activity of microorganisms also serves as a parameter of the organic carbon content potentially mineralized up to CO_2 (Emnova E., 2012). It is considered that the hydrolysis of fluorescein diacetate (**H-FDA**), which includes the activity of proteases, lipases and esterases reflects the total microbial activity and can be active outside the cells, forming stable complexes with soil colloids (Alef K., Nannipieri P., 1995).

For this reason both microbial biomass carbon (**C-MB**) and metabolic activity of the microorganisms in the rhizosphere soil have been proposed as basic parameters of state and direction

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of total soil organic matter transformations (Hu C., Cao Z., 2007; Vallejo V. et al., 2010; Emnova E., 2012).

The aim of the current study was to assess the influence of suboptimal soil water content, mineral fertilizers, and plant variety on biological potential of soybean rhizospheric soil.

MATERIAL AND METHOD

The experiment was set up in greenhouse complex, in plastic pots, in four replicates, on the calcareous chernozem soil. Two soil water content levels were examined (70% and 35% water holding capacity – WHC), water stress lasted for 17 days and was established in the active stage of plant growth – blossom–flowering. The treatments were: 1) fertilized soil with the most important macronutrients $N_{50}P_{100}$ (mg/kg) and 2) deficient nutrient content. Soybean plants were represented by two varieties: Aura and Clavera, with different drought tolerance degree. Also, the samples of the soil without plants were taken into account. Experiment scheme was described in detail by Emnova E. et al. (2012).

Following biotic parameters were analyzed in the rhizosphere soil: C-MB, BSR and H-FDA.

C-BM was estimated according to the method of rehydration, elaborated by Blagodatsky et al., 1987. The C content extracted with K_2SO_4 was determined using bichromatic oxidation method.

BSR was determined by the method proposed by Isermeyer H. (1995), taking into consideration the changes introduced by Dilly O., Nannipieri P. (2001).

The rate of H-FDA was determined by the method described by Schnurer J. and Rosswall T. in 1982 (Alef K., Nannipieri P., 1995).

Experimental data were subjected to statistical processing to calculate the following parameters: arithmetic mean, standard deviation, reliability of differences between arithmetic averages analyzed by Student t-test (bilateral test, type 3 with unequal variations), the correlation coefficient. The Microsoft Excel program was used for data analysis.

RESULTS AND DISSCUSIONS

The biological activity of the soil. ***I.*** *The content of microbial biomass carbon (C-MB).* The low soil water content affects microbial growth in rhizosphere soil (*tab. 1*). C-MB in fertilized soil, at 35% WHC was lower than at the optimum soil water content, by 5.1% for Aura variety, 6.9% - Clavera variety and 5.2% for soil without plants. In the soil with low nutrient content decrease was by 3.2% (Aura variety) and 7.3% (Clavera). In the soil where there is no rhizospheric effect C-MB showed stability against short-term drought and has not changed significantly.

The introduction of mineral fertilizers contributed to the MB increase in both rhizosphere soil and in that without plants, with 3.8%...9.5%. Higher C-MB values were established in the rhizosphere of Aura variety than of Clavera one, for the fertilized soil by 4.0% (70% WHC) and 5.8% (35% WHC); for unfertilized soil, by 2.6% and 6.7%, respectively. In general, the MB accumulation depended on the presence of the plants. Thus, a tendency of MB increase was observed in the soil in which soybean plants were grown as compared to the soil without plants. This may be due to rhizosphere effect exercised on soil bacterial community.

II*. Basal soil respiration (BSR)*. The soil water conditions significantly influenced on basal soil respiration, indicating a higher level ($p < 0.001$) of 24.2% at low soil water content (35% WHC) in soil under soybean variety Aura, treatment with NP, compared with optimal water supply (70% WHC) (*tab. 1*). But in fertilized soil without plants the intensity of CO_2 elimination highlighted a significant decrease ($p < 0.05$) at 35% WHC, being lower by 20.4% than at 70% WHC.

The application of fertilizers in soil cultivated with variety Aura, at 35% WHC also led to a significant increase of respiration process by 31.1% compared to unfertilized soil. But, in soil without plants BSR showed a significant reduction of 16.6% at 70% WHC and 31.9% at 35% WHC compared with NP deficiency.

In unfertilized calcareous chernozem with optimal soil moisture the quantity of CO_2 elimination was higher, indicating an increase of 17.5% in soil under the variety Aura *versus* Clavera.

The repeated analysis of these soil samples incubated for 3 months, revealed the highest values of BSR in NP treatments, without plants at low soil water content, being with 39.5% significantly higher than at optimal soil water content. In unfertilized soil intensity of CO_2 emission was statistically lower with 24.5% at 70% WHC than at 35% WHC. Fertilization also had a significant influence on the BSR and showed a higher level in soil without plants, by increasing by 52.8%, at low water soil content and by 27.9% in the rhizosphere of Clavera plants compared to the NP deficiency. However in NP treatments without plant CO_2 elimination decreased significantly by 17.3% at 70% WHC.

Data analysis revealed no significant differences between variety of cultivated soybean plants impact on BSR regardless of the soil water level and nutritional conditions.

Table 1

Influence of water and nutrient regimes on rhizosphere soil biological activity and soybean plant biomass

Treatments	Nutritive regime	Soil water content, % WHC	C-MB	BSR^1	BSR^2	H-FDA	Green mass, g per plant
			Mean values ± SD				
Soil without plants (control)	NP	70	323 ± 10	7.8 ± 1.3	0.42 ± 0.02	21.2 ± 2.1	
		35	307 ± 7 ***	6.2 ± 1.6*	0.59 ± 0.03***	19.3 ± 0.5 *	
	non-fertilized	70	295 ± 20	9.3 ± 1.2	0.51 ± 0.06	16.9 ± 1.0	
		35	291 ± 15	9.1 ± 1.5	0.39 ± 0.03***	17.2 ± 1.3	
Soybean variety Aura	NP	70	345 ± 11	10.0 ± 0.8	0.39 ± 0.02	22.6 ± 1.9	14.1 ± 2.5
		35	327 ± 9 ***	12.4 ± 0.9***	0.44 ± 0.04**	21.7 ± 1.2	8.9 ± 3.5 ***
	non-fertilized	70	326 ± 4	10.6 ± 1.8	0.39 ± 0.04	20.7 ± 0.7	7.9 ± 2.0
		35	315 ± 3 ***	9.4 ± 0.8	0.37 ± 0.03	18.8 ± 0.5***	5.7 ± 1.8 **
Soybean variety Clavera	NP	70	331 ± 8	9.5 ± 1.4	0.36 ± 0.04	20.8 ± 2.4	13.5 ± 2.8
		35	308 ± 11 ***	10.0 ± 3.1	0.48 ± 0.03***	19.3 ± 0.9	7.8 ± 1.5 ***
	non-fertilized	70	317 ± 4	8.8 ± 1.1	0.37 ± 0.03	19.9 ± 0.6	7.0 ± 1.9
		35	294 ± 8 ***	8.9 ± 1.5	0.37 ± 0.03	16.3 ± 2.7 **	5.6 ± 1.1 *

Note. C-MB – microbial biomass carbon, µg C /1g dry soil; BSR – basal soil respiration, µg C-CO_2 /1g dry soil / 1 day, the index 1 – incubation lasting for 14 days; 2 – incubation lasting for 107 days; H-FDA – the rate of capacitatea de hidroliză a fluorescein diacetate hydrolysis, µg fluorescein /1g dry soil /1 hour; SD – 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)

Tablel 2

Correlational relationships between the biotic parameters (r - Pearson coefficient)

	BSR^1	BSR^2	H-FDA	Green mass
C-MB	0.35	-0.31	*0.95* **	*0.83* **
BSR^1		-0.40	0.30	0.19
BSR^2			-0.13	-0.06
H-FDA				*0.75* **

Note. │r│< 0.45 – very low relationship; 0.45 ≤│r│< 0.70 – average relationship (*); 0.70 ≤│r│≤1 – high relationship (**).

The results showed that the BSR has registered higher values at low soil water content. This fact could indicate that bacterial community incurred additional energy expenses maintain life processes.

III*. FDA hydrolysis (H-FDA)*. Low soil water content, established in the active phase of plant growth, caused a decrease of soil overall hydrolytic activity (*tab. 1*). In fertilized rhizosphere soil H-FDA decreased by 4.0% and 7.1% for Aura and Clavera variety, respectively. In unfertilized soil, consequences of suboptimal soil water supply were more drastic and of a higher degree of significance (p≤0.001). Therefore, the decrease of H-FDA values was by 8.8% and 17.9%, respectively. In the control, soil without plants, H-FDA was affected only in mineral fertilized soil.

Soil fertilization contributed to the accumulation of a larger pool of hydrolytic enzymes which led to record a higher rate of H-FDA (increase by 4.3%…25.5%). The established effect was more pronounced at 35% WHC (with the exception of the soil without plants).

The analized activity in the rhizosphere soil of Aura variety was higher (p<0.05) in both *optimal water supply* (by 8.2% and 3.7%, for soil with and without mineral fertilization) and *35% WHC* (by 11.3% and 13.3%, respectively). H-FDA activity did not show a clear regularity in comparison of soil without plants *versus* rhizosphere soil.

Green mass of soybean plants. Mineral fertilization in recommended doses, contributed to a significant increase in green mass of both varieties, by 39.4%…92.7%, p≤0.001 (*tab. 1*). Plant biomass of Aura variety was higher than those of the Clavera one by 2.3%…13.1%, however, the certified differences was not supported statistically (p>0.05).

At establishing of temporary low soil water conditions plant biomass has been considerable decreased by 20.5%...42.5%, p≤0.001. It was noted that even if the losses were more pronounced for plants from NP treatment, their weight was higher than those from 70% WHC, unfertilized soil. In conditions of severe soil water defficinecy plants reacted more quickly and more drastically than edaphic microorganisms. Adverse environmental conditions affected soil microbiota by reducing their activity, but if a short-term drought had sometimes catastrophic impact for plants, then for microorganisms, not really. Smolander A. et al. (2005) determined that drought conditions lasting less than 2 months were not enough to destroy soil microbial biomass. It has been found that the biological activity of the soil decreased, but it was totally restored at the soil rewetting.

Correlational analysis revealed a very high Pearson coefficient of relationship between C-BM, H-FDA and soybean plant green mass (*tab. 2*). The parameter that indicates the general soil biological activity estimated by BSR poorly but positive correlated with other parameters, while BSR determined after 3 months established negative correlation.

CONCLUSIONS

Soil biological activity depended on the plant nutritional regime, showing a clear increase in the NP treatment. Both microbial biomass size (C-MB) and total hydrolytic activity (H-FDA) have been reduced at low soil water content. By contrast, functional activity of microbiota estimated by basal soil respiration showed higher values at 35% WHC. Optimal soil fertilization contributes to increased microbiota tolerance in the rhizospheric soybean soil under low soil water supply. Examined parameters had higher values in rhizosphere of Aura variety compared to Clavera one and soil without plants.

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