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 dependent 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; Boaghi 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., Roșca 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 CO₂ (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\textsubscript{100}P\textsubscript{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\textsubscript{2}SO\textsubscript{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.,

The rate of H-FDA was determined by the
method described by Schnurer J. and Rosswall T.

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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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.
Influence of water and nutrient regimes on rhizosphere soil biological activity and soybean plant biomass

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nutritive regime</th>
<th>Soil water content, % WHC</th>
<th>C-MB</th>
<th>BSR¹</th>
<th>BSR²</th>
<th>H-FDA</th>
<th>Green mass, g per plant</th>
<th>Mean values ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil without plants (control)</td>
<td>NP</td>
<td>70</td>
<td>323 ± 10</td>
<td>7.8 ± 1.3</td>
<td>0.42 ± 0.02</td>
<td>21.2 ± 2.1</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>307 ± 7**</td>
<td>6.2 ± 1.6*</td>
<td>0.59 ± 0.03***</td>
<td>19.3 ± 0.5 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>non-fertilized</td>
<td>70</td>
<td>295 ± 20</td>
<td>9.3 ± 1.2</td>
<td>0.51 ± 0.06</td>
<td>16.9 ± 1.0</td>
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<tr>
<td></td>
<td></td>
<td>35</td>
<td>291 ± 15</td>
<td>9.1 ± 1.5</td>
<td>0.39 ± 0.03***</td>
<td>17.2 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean variety Aura</td>
<td>NP</td>
<td>70</td>
<td>345 ± 11</td>
<td>10.0 ± 0.8</td>
<td>0.39 ± 0.02</td>
<td>22.6 ± 1.9</td>
<td>14.1 ± 2.5</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>327 ± 9***</td>
<td>12.4 ± 0.9***</td>
<td>0.44 ± 0.04**</td>
<td>21.7 ± 1.2</td>
<td>8.9 ± 3.5***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>non-fertilized</td>
<td>70</td>
<td>326 ± 4</td>
<td>10.6 ± 1.8</td>
<td>0.39 ± 0.04</td>
<td>20.7 ± 0.7</td>
<td>7.9 ± 2.0</td>
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<tr>
<td></td>
<td></td>
<td>35</td>
<td>315 ± 3***</td>
<td>9.4 ± 0.8</td>
<td>0.37 ± 0.03</td>
<td>18.6 ± 0.5***</td>
<td>5.7 ± 1.8**</td>
<td></td>
</tr>
<tr>
<td>Soybean variety Clavera</td>
<td>NP</td>
<td>70</td>
<td>331 ± 8</td>
<td>9.5 ± 1.4</td>
<td>0.36 ± 0.04</td>
<td>20.8 ± 2.4</td>
<td>13.5 ± 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>308 ± 11***</td>
<td>10.0 ± 3.1</td>
<td>0.48 ± 0.03***</td>
<td>19.9 ± 0.9</td>
<td>7.8 ± 1.5***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>non-fertilized</td>
<td>70</td>
<td>317 ± 4</td>
<td>8.8 ± 1.1</td>
<td>0.37 ± 0.03</td>
<td>19.9 ± 0.6</td>
<td>7.0 ± 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>294 ± 8***</td>
<td>8.9 ± 1.5</td>
<td>0.37 ± 0.03</td>
<td>16.3 ± 2.7**</td>
<td>5.6 ± 1.1*</td>
<td></td>
</tr>
</tbody>
</table>

Note. C-MB – microbial biomass carbon, µg C/g dry soil; BSR – basal soil respiration, µg CO₂/g dry soil / 1 day; the index 1 – incubation lasting for 14 days, 2 – incubation lasting for 70; H-FDA – the rate of captabitarea dehidrlază a fluoroscell dicideate hydrolysis, µg fluoroscell /g dry soil /1 hour; WHC – 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)

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

<table>
<thead>
<tr>
<th></th>
<th>BSR¹</th>
<th>BSR²</th>
<th>H-FDA</th>
<th>Green mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-MB</td>
<td>0.35</td>
<td>-0.31</td>
<td>0.95 **</td>
<td>0.83 **</td>
</tr>
<tr>
<td>BSR¹</td>
<td>-0.40</td>
<td>0.30</td>
<td>-0.13</td>
<td>0.19</td>
</tr>
<tr>
<td>BSR²</td>
<td>-0.13</td>
<td>0.30</td>
<td>-0.06</td>
<td></td>
</tr>
<tr>
<td>H-FDA</td>
<td></td>
<td></td>
<td>0.75 **</td>
<td></td>
</tr>
</tbody>
</table>

Table 1

Table 2

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 analyzed 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 deficiency 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 Clamera one and soil without plants.

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