Symbiotic activity of leguminous crops depending on the variety and growing conditions

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Abstract. The article considers the symbiotic activity of chickpeas and soybean crops depending on the action of various herbicides and fertilizers. In the field conditions of the forest-steppe zone of North Ossetia-Alania, the effect of mineral fertilizers on the symbiotic activity of promising varieties of chickpeas was studied and it was established that mineral fertilizers had a significant effect on the formation of the symbiotic chickpeas apparatus. Mineral fertilizers contributed to the increase in the average mass of nodules and their number. According to the mass of active nodules formed by Vilana soybean plants, increasing the dose of complex fertilizer Nagro to 0.5 l/ha is justified, since the mass of nodules on one plant in this variant increased in development phases. All options with the introduction of bio fertilizer Nagro for soybean crops were characterized by a significant increase in the mass of nodules in comparison with the control variant. Control variants formed more inactive nodules on the lateral roots, which were also characterized by smaller sizes.

1. Introduction
As it is known modern agriculture is impossible without the widespread use of chemicals. In the preservation of the crop, they play an important role, largely determining the effectiveness of protection from pests, weeds and diseases from an economic point of view. However there are also negative sides to this process. Some herbicides have a negative effect on nitrogen-fixing activity, which does not always correlate with a decrease in yield, since such treatment improves the conditions of illumination and mineral nutrition of cultivated plants as a result of weed death. However, the share of “biological” nitrogen in the crop decreases, and, consequently, the value of legumes as nitrogen accumulators decrease [1–4].

The role of fertilizers in the process of symbiotic nitrogen fixation is ambiguous. Large doses of nitrogen fertilizers inhibit the processes of nitrogen fixation, while phosphorus is an important component of the nitrogen fixation process [5].

2. Methods and materials
The experiments were carried out in the forest-steppe zone of North Ossetia-Alania, at an altitude of 600 m above sea level, on the basis of North Caucasian Research Institute of Mining and Piedmont Agriculture of VSC RAS.

The soils of the experimental plot are represented by medium-power heavy loamy leached chernozems, underlain by pebbles, with a humus content of 6.3 %. The reaction is slightly acidic...
(pH 5.48). They are distinguished by a high content of gross and available reserves of nitrogen and phosphorus. According to the content of mobile potassium, the soils are moderately provided.

The average annual temperature is 9.3 °C, the average long-term sum of positive temperatures for a year is 3240 (3637) °C. The number of days with temperatures above 0° is 279. The average daily air temperature of more than 15 °C is most favorable for cultivating crops. The duration of the period with such a temperature is 117 days, and the sum of the temperatures is 2963 °C. In general, the area is hot, abundantly moist.

In a field experiment, various doses of herbicides and mineral fertilizers on the symbiotic activity of chickpeas crops, as well as the effect of complex bio-energy fertilizer on soybean crops, were studied.

In the process of research work, the following accountings, analyzes and observations were carried out:

- The determination of planting density was carried out at five standard sites located diagonally on the plot.
- The consideration of the biomass and the number of nodules, active (ASP) and total (TSP) symbiotic potentials, the concentration of leghemoglobin in nodules was determined by the phases of plant development according to G.S. Posypanov. The value of the specific activity of symbiosis was used to calculate the amount of symbiotically fixed nitrogen [6].
- The harvest was recorded by the method of test sites from six points of the plot with its subsequent conversion to 100 % purity and conditioned humidity.

3. Results

As our studies showed, the first nodules on the roots of chickpeas and soybeans appeared in 22–24 days after germination – in the branching phase. Within four to six weeks, their mass and quantity increased until the flowering phase and the formation of beans. The next three-four weeks nodules slightly changed downward. After, as a result of plant aging, the quality parameters of nitrogen fixation were reduced. During the initial stages of development, nodules were scattered throughout the root system and were small, nitrogen fixation was weak. Then their number increased sharply, and they were located on the main root, as well as around it [7].

During flowering and the formation of beans, nodules were located mainly on the main root and were characterized by a pink color at the break, which indicated the presence of leghemoglobin, and therefore, the activity of nodules. Nitrogen was absorbed from the air more actively, when the mass of nodules having this substance was greater. Nitrogen was fixed more actively, when the sowing of plants with red or pink nodules was greater [8].

Leghemoglobin lost its pink color with increasing soil moisture or the appearance of soil crust, the content of the nodule turned grayish. At the same time, oxygen access to the roots decreased, this caused its immediate transition to inactive cholelobin. A decrease in its content in nodules was observed during the period of seed formation, which was the reason for the weakening of nitrogen-fixing ability. As we approach the ripening of plants, this process intensified.

As it is known, legumes, in particular chickpeas and soybeans, require potassium and phosphorus for active symbiosis. With an average content of these elements in the soil, the introduction of additional phosphorus-potassium fertilizers increases productivity and improves symbiotic conditions [9].

Our studies showed that the increase in the average weight of one chickpeas nodule and their total amount was promoted by mineral fertilizers. The variety of Privo 1 against an unfertilized background in 2016 during the period of maximum development gave 35.6 pcs with an average weight of 35.4 mg. During the growing season: 25.3 pcs and 32.2 mg respectively. During the period of maximum development of the symbiotic system according to the background P90K45, the number of nodules was 43.4 pcs (increased by 7.6 pcs.), the mass increased by 4.6 mg. 34.3 pcs – the average number of nodules during the growing season, weight – 32.8 mg. The control values exceeded 9 pieces and 5.7 mg. In 2017–2018, we also observed approximately the same dynamics.
It is necessary to note that the nodules on the control were located throughout the root system of chickpeas. After the application of phosphorus-potassium fertilizers, the situation changed – they were localized on the main root or next to it, which indicates that nitrogen fixation intensified.

Therefore, the increase in doses of phosphate fertilizers had a beneficial effect on the formation of nodules; the nitrogen fixation process was intensified, since phosphorus was needed as an energy material for the symbiotic system.

The mass of one nodule and the number of nodules for 2016–2018 on average, depending on the level of herbicides and the mineral nutrition of chickpeas and soybeans, are shown in Table 1.

### Table 1. Number (pcs) and weight of one nodule (mg) on chickpeas and soybean plants, depending on the fertilizers and herbicides (2016–2018)

| Variant | The 1st (branching) | The 3rd (flowering) | The 4th (bean formation) |
|---------|---------------------|---------------------|-------------------------|
|         | mass of 1 nodule, mg| mass of 1 nodule, mg| mass of 1 nodule, mg    |
|         | raw nodules on 1 plant, pcs. | raw nodules on 1 plant, pcs. | raw nodules on 1 plant, pcs. |
| No fertilizer (chickpeas – Privo 1) | 8.7 | 12.3 | 32.8 | 33.4 | 34.1 | 33.9 |
| Harnes 3.0; Pivot 0.7 | 10.8 | 14.9 | 49.8 | 40.7 | 52.9 | 41.9 |
| Dualgold 1.5; Pivot 0.7 | 10.6 | 14.6 | 48.5 | 39.8 | 50.3 | 40.5 |
| No fertilizers (chickpeas – Volgogradsky 10) | 6.5 | 7.3 | 18.8 | 20.1 | 21.6 | 22.6 |
| Harnes 3.0; Pivot 0.7 | 8.9 | 11.0 | 39.5 | 29.9 | 40.9 | 34.9 |
| Dualgold 1.5; Pivot 0.7 | 8.3 | 9.8 | 34.4 | 29.1 | 28.9 | 31.7 |
| P90K85 (chickpeas – Privo 1) | 17.9 | 19.5 | 38.1 | 40.2 | 42.3 | 38.9 |
| Harnes 3.0; Pivot 0.7 | 38.0 | 43.4 | 50.1 | 57.5 | 50.4 | 58.0 |
| Dualgold 1.5; Pivot 0.7 | 28.9 | 30.2 | 32.9 | 34.1 | 33.9 | 36.2 |
| P90K85 (chickpeas – Volgogradsky 10) | 13.8 | 15.6 | 35.2 | 37.6 | 38.7 | 36.2 |
| Soy beans – Slavia | 7.1 | 27.5 | 12.5 | 31.4 | 17.6 | 44.2 |
| Nacro 0.3 | 7.1 | 30.4 | 13.8 | 34.5 | 19.3 | 48.8 |
| Nacro 0.5 | 7.5 | 31.5 | 15.7 | 34.9 | 20.8 | 53.1 |
| Soy beans – Chara | 6.4 | 35.0 | 10.8 | 44.2 | 16.4 | 49.5 |
| Nacro 0.3 | 6.5 | 35.7 | 12.6 | 47.5 | 17.8 | 53.7 |
| Nacro 0.5 | 6.9 | 36.2 | 13.6 | 48.0 | 19.1 | 54.7 |
| Soy beans – Vilana | 8.1 | 29.9 | 13.2 | 38.2 | 20.3 | 42.3 |
| Nacro 0.3 | 8.5 | 30.9 | 14.8 | 41.9 | 21.4 | 55.4 |
| Nacro 0.5 | 8.8 | 32.6 | 16.9 | 44.1 | 22.8 | 54.9 |

It was found that the amount and fresh weight of chickpeas nodules increased with increasing rates of mineral fertilizers. Regarding control, these indicators also increased some combinations of herbicides.

According to the mass of active nodules formed by Vilana soybean plants, increasing the dose of complex bioorganic fertilizer Nacro to 0.5 l/ha was justified.

The mass of nodules on one plant in this variant increased in development phases from 195.2 mg in the branching phase to 1252.8 mg in the phase of the onset of fruit formation.

All the variants with the introduction of bio fertilizer Nacro were characterized by a significant increase in the mass of nodules in comparison with the control variant. Control variants formed more
inactive nodules on the lateral roots, which were also characterized by smaller sizes. An insignificant mass of nodules occurs in the initial phases of plant development and growth. In particular, in the control version of 2016, in the phase of chickpeas branching according to Privo 1 variety, it amounted to 4 kg/ha. The same indicator was in 2017 3 kg/ha – 2018 (Table 2) 11, 9 and 8 kg/ha, respectively, according to the P90K45 variant. Before the bean formation phase, the mass of nodules increased (reached a maximum), after which a gradual decrease began. In 2018 in the control in the flowering phase, it amounted to 26 kg/ha.

It increased with the use of phosphorus-potassium fertilizers for 26 kg/ha. In the Volgogradsky 10 variety, the dynamics of the accumulation of mass by nodules was similar. However, it was characterized by lower indicators in quantitative terms. (Table 2).

In order to characterize the nitrogen-fixing ability of leguminous crops, not only the number and mass of nodules formed is of great importance, but also the duration of their active functioning. These indicators are combined active symbiotic potential (ASP). The active symbiotic potential during the growing season is the sum of ASP indicators for interphase periods. The overall symbiotic potential is calculated in the same way. It takes into account the mass of all nodules. This indicator has more theoretical significance. It is determined in those situations when it is necessary to show the influence on the activity of symbiosis of individual environmental factors, since they affect more the mass of nodules containing hemhemoglobin, and not the total mass of all nodules.

Table 2. Dynamics of the mass of chickpeas nodules depending on the mineral background and variety (kg/ha)

| Indicator             | 2016       | 2017       | 2018       | Average for 3 years |
|-----------------------|------------|------------|------------|---------------------|
|                       | Control    | P90K45     | Control    | P90K45             | Control    | P90K45   |
| Branching Privo 1     | 4          | 11         | 4          | 9                   | 3          | 8        | 3.7       | 9         |
| Budding               | 11         | 36         | 9          | 30                  | 8          | 27       | 9.3       | 31        |
| Flowering             | 31         | 70         | 27         | 53                  | 26         | 52       | 28.0      | 58        |
| Bean Formation Phase  | 64         | 149        | 61         | 138                 | 60         | 129      | 61.7      | 139       |
| Start of seed filling | 60         | 138        | 54         | 120                 | 50         | 111      | 54.7      | 123       |
| Seed Pouring          | 42         | 61         | 38         | 56                  | 30         | 51       | 36.7      | 56        |
| Ripening              | 21         | 32         | 14         | 30                  | 9          | 21       | 14.7      | 28        |
| Volgogradsky 10       |            |            |            |                     |            |          |           |           |
| Branching             | 3          | 8          | 2          | 7                   | 2          | 5        | 2.3       | 7         |
| Budding               | 9          | 29         | 8          | 28                  | 8          | 26       | 8.3       | 28        |
| Flowering             | 27         | 61         | 22         | 59                  | 20         | 46       | 23.0      | 55        |
| Bean Formation Phase  | 50         | 120        | 46         | 111                 | 40         | 99       | 45.3      | 110       |
| Start of seed filling | 46         | 104        | 40         | 96                  | 40         | 90       | 42.0      | 97        |
| Seed Pouring          | 31         | 40         | 29         | 38                  | 26         | 33       | 28.7      | 37        |
| Ripening              | 18         | 19         | 9          | 16                  | 6          | 20       | 11.0      | 18        |

As it was revealed, the highest ASP was formed in the fruiting phase of Vilana variety.

The lowest indices of active symbiotic potential were characterized by all three studied varieties in the early phases of vegetation. By the flowering phase, this indicator increases, and it reaches its maximum by the beginning of the bean formation phase (7431.1 kg × day/ha). The most effective option was the introduction of Nagro at a rate of 0.5 l/ha.

The positive effect of the introduction of integrated biofertilizer Nagro was traced on all studied options.

In chickpea plants of Privo 1, the duration of the general symbiosis varied between 98–107 days depending on the variant and year, active symbiosis was 74–84 days. Accordingly, for the variety Volgogradsky 10, these data was 97–104 and 74–81 days.

As it was found, the beginning of the formation of nodules to a decisive extent is determined by the parameters of the main environmental factors: temperature, moisture supply and soil pH. Nodules die in adverse humidity, sometimes completely. After restoration of optimum humidity they reappear.
As it is known, the mass of nodules depends on the growing conditions of the plants and the phase of their development. It can remain unchanged for no more than 7–10 days. The fixation of atmospheric nitrogen, as shown by numerous studies, occurs only in nodules containing hemhemoglobin. Therefore, it is most important to consider their mass with hemoglobin. The total mass is relevant only for the characterization of the symbiotic apparatus and its activity.

For Privo 1 grade, it was found that ASP ranged between 1968.4–2664 units and 4389–5964 units for years in the control variant for phosphorus-potassium fertilizers. For Volgogradsky 10, the corresponding indicators were: 1548.8–1972.5 and 3648–4414.5 units. In the control variant, nitrogen consumption for Privo 1 variety was 67.8 kg/ha in 2016; it was 32.53 kg/ha more according to the P\textsubscript{90}K\textsubscript{45} variant.

Within the range of 1968.4–5964.0 kg * days/ha, Privo 1 varied the active symbiotic potential, 8.45–10.42 g/kg *day – an indicator characterizing the specific activity of symbiosis.

The specific activity of symbiosis and the value of ASP determined the amount of chickpeas nitrogen fixed in crops by air, depending on the mineral nutrition. The amount of nitrogen fixation was calculated with these existing indicators (Table 3).

Table 3. Amount of fixed air nitrogen by chickpeas crops depending on the variety and growing conditions of the forest-steppe zone of North Ossetia-Alania

| Indicator | 2016 Control | 2016 P\textsubscript{90}K\textsubscript{45} | 2017 Control | 2017 P\textsubscript{90}K\textsubscript{45} | 2018 Control | 2018 P\textsubscript{90}K\textsubscript{45} | Average for 3 years |
|-----------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------------|
| Privo 1   |             |                 |             |                 |             |                 |                   |
| N sowing, kg/ha | 64.8 | 97.33 | 59.6 | 86.08 | 61.92 | 82.37 | 62.1 | 88.6 |
| N fixed., kg/ha | 26.1 | 58.4 | 22.8 | 49.3 | 16.6 | 37.1 | 21.8 | 48.3 |
| ASP, kg day/ha | 2664.0 | 5964.0 | 2190.4 | 4734.8 | 1968.4 | 4389.0 | 2274.3 | 5029.3 |
| SAS, g/kg day | – | 9.8 | – | 10.42 | – | 8.45 | – | 9.6 |
| Volgogradsky 10 |             |                 |             |                 |             |                 |                   |
| N sowing, kg/ha | 59.2 | 87.42 | 56.99 | 76.44 | 77.35 | 89.78 | 64.5 | 84.5 |
| N fixed., kg/ha | 22.8 | 51.0 | 14.2 | 33.6 | 9.12 | 21.6 | 15.4 | 35.4 |
| ASP, kg day/ha | 1972.5 | 4414.5 | 1650.2 | 3911.6 | 1542.8 | 3645.0 | 1721.8 | 3990.4 |
| SAS, g/kg day | – | 11.56 | – | 8.60 | – | 5.91 | – | 8.7 |

According to chickpeas of Privo 1, the amount of fixed nitrogen in the air, in the control group varied between 16.6–26.1 kg/ha. According to the P\textsubscript{90}K\textsubscript{45} variant, the indicators were 37.1–58.4 kg/ha. For all varieties, the amount of nitrogen fixation in the control was 2–2.5 times less than in the P\textsubscript{90}K\textsubscript{45} variant (Table 3).

The main indicator for the assessment of the studied herbicides and fertilizers is the productivity of legumes. As Figure 1 shows, the most productive was the Vilana variety – 1.09–1.49 t/ha, which was 0.08–0.31 t/ha more than the varieties Slavia and Chara.

The introduction of integrated bio fertilizer Nagro in the norms of 0.3 l/ha and 0.5 l/ha increased the productivity of all studied varieties (increase – 0.08–0.40 t/ha), due to the content of a wide range of microelements in it, as well as the ability to increase the utilization rate of the main macronutrients from the soil – nitrogen, phosphorus and potassium.

Privo 1 was the most productive of the varieties of chickpeas under study (Fig. 2).

The application of phosphorus-potassium fertilizers stimulated the productivity of chickpeas in all the studied options – the increase was 0.23–0.26 t/ha. The most effective combination was Harnes 3.0; Pivot 0.7, which made it possible to obtain yields on the Privo 1 variety over 2.5 t/ha.
Figure 1. Productivity of soybean crops using bio fertilizer Nagro, t/ha

Figure 2. Productivity of chickpeas crops of Privo 1 depending on fertilizers and herbicides, t/ha

4. Conclusion
The increase in the average mass of nodules and their number was promoted by the norms of applied fertilizer. When P90K45 was added to the chickpeas crops, the symbiotic apparatus was most developed. In the context of the control their number increased by 20–27 % during the period of maximum development of the symbiotic system.

Before the phase of the formation of beans and reaching a maximum, the mass of nodules increased during it, after which a gradual decrease began. In the variant where phosphorus fertilizers were applied, in 2016 it increased by 85 kg/ha relative to control, by 77 kg/ha in 2017, by 69 kg/ha in 2018 for Privo 1 chickpeas variety. For Volgogradsky 10 variety the situation was approximately the same.

For Privo 1, the amount of fixed air nitrogen in the control varied between 16.6–26.1 kg/ha, 37.1–58.4 kg/ha – according to the P90K45 variant. For all the varieties, the amount of nitrogen fixation in the control was 2–2.5 times less than the P90K45 variety.

The introduction of integrated bio fertilizer Nagro in the norms of 0.3 l/ha and 0.5 l/ha increased the productivity of all studied varieties (increase – 0.08–0.40 t/ha). The application of phosphorus-potassium fertilizers stimulated the productivity of chickpeas in all the studied options — the increase of 0.23–0.26 t/ha. The most effective combination was Harnes 3.0; Pivot 0.7 with yield increase over 0.87 t/ha.

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