Study of symbiotic nitrogen fixation of chickpea (*Cicer arietinum* L.) in the Oryol region

M V Donskaya and M M Donskoy

1Federal Scientific Center of Legumes and Groat Crops, 302502, 10, building 1, Molodezhnaya St., Streletsky village, Oryol district, Oryol region, Russia

E-mail: office@vniizbk.orel.ru

Abstract. Modern agriculture tends to reduce chemogenic loads on the environment and becomes more environmentally oriented. In this regard, increasing the yield of agricultural crops through the use of useful soil microflora is an urgent direction in research. In the field conditions of the Oryol region, the features of the formation and operation of symbiotic systems of 13 chickpea varieties under mono- and double inoculation with different strains of nitrogen-fixing bacteria *Mesorhizobium ciceri* and a soil-root mixture containing a mix of arbuscular mycorrhizal fungi *Glomus spp* were studied. The use of microbiological drugs had a positive effect on the growth and development of most chickpea varieties, which contributed to a more intensive accumulation of green mass of plants, led to an increase in the duration of the growing season by 1...7 days compared to the control. At the same time, the plants exceeded the control by 0.5...23.4% in height. There was an increase in the weight of dry plants by 0.7...70.1%, the number of seeds per plant by 0.3...69.1%, the weight of seeds per plant by 0.6...71.4%, and the weight of 1000 seeds by 0.4...36.9%.

It was found that the most powerful and active symbiotic apparatus in chickpeas is formed during the flowering period – the beginning of bean formation. The maximum number of nodules was formed in the Avatar variety in the variant with mono inoculation of *Mesorhizobium ciceri* strain 065 and in the Krasnokutsky 123 variety in the variant with *Mesorhizobium ciceri* strains 527 and 522. The highest values of nitrogenase activity (109.23...155.79 mg N₂/plant h) were recorded in the varieties Avatar, Krasnokutsky 123 and Kostyuzhansky 27 in the variants with double inoculation.

1. Introduction

Legumes have the ability to form symbioses with beneficial soil microorganisms: nodule bacteria, arbuscular mycorrhizal fungi, and growth-stimulating rhizosphere microorganisms. Legume-rhizobial symbiosis and arbuscular mycorrhiza are of great practical importance, as a result of which plants can develop in conditions of nitrogen deficiency, assimilation of hardly soluble phosphates occurs, and plant growth conditions improve [1].

The genetic features of both types of symbiosis on the example of pea culture have been studied quite widely [2], despite this, there is not enough data for their direct use in agricultural production. When cultivating crops in non-traditional regions, it is necessary to develop methods for creating symbiotic systems in the field, study the features of inoculation, and establish patterns of influence of soil and climatic conditions on the work of the symbiotic apparatus.

Chickpeas (*Cicer arietinum* L.) are the oldest food and fodder crop cultivated in the world on an area of 17 million hectares. In symbiosis with nodule bacteria of the *Mesorhizobium ciceri* species,
chickpea plants are able to absorb up to 120-150 kg/ha of air nitrogen during the growing season [3]. The presence of specific virulent rhizobia strains in the soil is the main condition for active symbiosis. Chickpeas have a high degree of mycorrhization [4]. Pre-sowing seed inoculation with biologic drugs can significantly increase the symbiotic activity and crop yield [5].

Chickpeas were not widely cultivated in the Oryol region. Its implementation is hindered, on the one hand, by a small number of varieties adapted to the conditions of the region, on the other hand, by the lack of adaptive cultivation technology, including the creation of effective symbiotic systems.

The aim of the research was to screen the source material of chickpeas (Cicer arietinum L.) for inclusion in breeding programs to increase the effectiveness of symbiosis and determine the effectiveness of microbiological drugs for cultivation in the Oryol region.

2. Materials and methods

The work was performed in 2014...2016. The material for research was 13 varieties of chickpeas (Cicer arietinum L.) genotypes were evaluated for the ability to nodulation and nitrogen fixation in a field experiment.

The experimental scheme included control and variants with mono - and double inoculation of seeds with rhizotrophin (strains 527, 522, 065 based on nodule bacteria Mesorhizobium ciceri) and implementation of a soil-root mixture containing arbuscular mycorrhizal fungi (Glomus intraradices, strain 8, and Glomus fasciculatum, strain 7) into the soil before sowing. The area of experimental plots is 5 m². Microbiological specimens were obtained from the Russian Research Institute of Agricultural Microbiology (Saint-Petersburg-Pushkin). Treatment with drugs was carried out according to the recommended technologies. Field and laboratory studies were performed using generally accepted methods [6-8].

Mathematical data processing was performed using methods of biological statistics using Microsoft Office Excel 2010.

3. Results

Soil and climatic conditions play an important role in the formation of an active symbiosis between legumes and beneficial soil microorganisms. In general, the conditions of the growing seasons of 2014...2016 were favorable for normal growth and development of chickpeas, the temperature indicators did not deviate much from the long-term average values.

3.1. Development dynamics

It was found that the use of microbiological drugs led to a significant accumulation of green mass, while the duration of the growing season in these variants increased by 1...7 days compared to the control. The largest increase in biomass (+49.2% to the control) was observed in the Avatar variety in the variant with inoculation with strain 065 (figure).
3.2. Structural analysis

It was found that inoculation of chickpea seeds before sowing with various strains of *Mesorhizobium ciceri* increased the plant height by 0.5...23.4%, compared with the control, in the varieties Privo 1, Zolotoy Yubiley, Krasnokutsky 36, Krasnokutsky 123, Avatar, Kostyuzhansky 27, Yubeley, Smachny, Ornament, Pegas, Ustoychivy 02 (table 1). The use of AM fungi increased the height of plants by 2.1...9.5% in comparison with the control in the varieties Zolotoy Yubiley, Krasnokutsky 36, Avatar, Smachny, Ornament, Ustoychivy 02, Colorit; double inoculation led to an increase in plant height by 0.7...19.2% in all varieties.

Mono- and double inoculation of chickpea seeds with different strains of *Mesorhizobium ciceri* and implementation of a substrate containing AM fungi into the soil before sowing affected positively the main elements of the crop structure in most of the studied varieties. The increase in dry weight of plants in all variants, amounted to 0.7...70.1% compared to control, maximum increase recorded at Avatar variety in the variant with inoculation strain 065; increase in the number of seeds per plant was in the range 0.3...69.1% (maximum recorded in the variety Pegas in variant with dual inoculation strain 065 and AM fungi); the increase in seed productivity was at the level of 0.6...71.4%, the greatest positive effect was observed in the Krasnokutsky 36 variety in the variant with inoculation with strain 522. The use of different strains of rhizotorphin and AM fungi increased the seed size of different chickpea varieties by 0.4...36.9% compared to the control. The maximum positive effect was observed in the Krasnokutsky 36 variety in the variant with rhizotorphin strain 065.

**Table 1.** Influence of microbiological preparations on the elements of the structure of the yield of chickpea varieties*, 2014...2016.

| Varieties         | Zolotoy yubiley | Krasnokutsky 36 | Krasnokutsky 123 | Avatar | Kostyuzhansky 27 | Yubeley niy | Pamyat’ | Pegas |
|-------------------|-----------------|------------------|------------------|--------|------------------|-------------|---------|-------|
| Control           | 71.6            | 54.8             | 75.4             | 62.4   | 69.6             | 77.9        | 71.0    | 51.4  |
| Strain 527        | 73.9            | 55.3             | 75.8             | 69.1   | 68.4             | 72.0        | 69.2    | 53.2  |
| Strain 522        | 73.5            | 67.6             | 77.9             | 70.8   | 71.4             | 82.4        | 65.4    | 58.9  |

**Figure 1.** Reaction of chickpea plants (Avatar) on application of microbial preparations: 1 - Control; 2 - *Mesorhizobium ciceri* (strain 065); 3 - Mix of arbuscular mycorrhizal fungi *Glomus* spp.; 4 - Double inoculation.
Varieties showed the most positive effect from the influence of microbiological preparations.  

|          | 2014  | 2015  | 2016  |
|----------|-------|-------|-------|
| Control  | 2014  | 2015  | 2016  |
| Control  | 47.6  | 36.7  | 50.9  |
| Strain 527 | 38.6  | 56.7  | 49.4  |
| Strain 522 | 52.5  | 59.5  | 54.2  |
| Strain 065 | 56.8  | 61.5  | 57.7  |
| St. 527+AM | 66.4  | 53.0  | 45.6  |
| St. 522+AM | 57.9  | 59.2  | 51.9  |
| St. 065+AM | 60.2  | 28.4  | 44.3  |
| AM       | 48.7  | 36.0  | 46.0  | 45.2  | 26.8  | 67.2  | 38.3  | 25.3  |

|          | 2014  | 2015  | 2016  |
|----------|-------|-------|-------|
| Control  | 2014  | 2015  | 2016  |
| Control  | 79.5  | 64.2  | 68.6  | 66.7  | 78.9  | 86.8  | 47.5  | 36.9  |
| Strain 527 | 53.2  | 90.9  | 62.0  | 69.3  | 88.3  | 87.1  | 79.1  | 45.8  |
| Strain 522 | 101.3 | 107.8 | 78.9  | 68.2  | 93.1  | 89.8  | 56.3  | 57.6  |
| Strain 065 | 92.4  | 93.9  | 79.1  | 69.1  | 59.2  | 66.0  | 68.2  | 53.7  |
| St. 527+AM | 106.4 | 73.1  | 54.4  | 77.5  | 70.2  | 93.2  | 64.5  | 46.3  |
| St. 522+AM | 100.6 | 85.1  | 69.4  | 73.2  | 89.2  | 76.8  | 73.2  | 51.3  |
| St. 065+AM | 102.5 | 42.0  | 65.3  | 76.4  | 62.6  | 79.2  | 55.4  | 62.4  |
| AM       | 85.5  | 46.5  | 60.7  | 70.3  | 59.8  | 80.4  | 50.3  | 44.2  |

|          | 2014  | 2015  | 2016  |
|----------|-------|-------|-------|
| Control  | 2014  | 2015  | 2016  |
| Control  | 17.4  | 14.7  | 19.2  | 16.4  | 17.9  | 23.1  | 13.6  | 10.8  |
| Strain 527 | 12.4  | 20.8  | 19.0  | 17.3  | 19.6  | 22.7  | 21.2  | 12.6  |
| Strain 522 | 23.3  | 25.2  | 21.6  | 19.5  | 21.6  | 24.7  | 18.3  | 14.0  |
| Strain 065 | 21.9  | 25.0  | 24.4  | 21.3  | 13.5  | 17.4  | 16.4  | 14.7  |
| St. 527+AM | 26.1  | 18.9  | 16.5  | 16.3  | 17.9  | 24.3  | 19.6  | 10.3  |
| St. 522+AM | 22.5  | 19.0  | 20.2  | 18.9  | 23.0  | 20.1  | 14.4  | 14.4  |
| St. 065+AM | 24.8  | 10.4  | 16.8  | 20.3  | 16.0  | 19.3  | 13.9  | 16.3  |
| AM       | 22.2  | 11.8  | 16.6  | 14.8  | 15.9  | 16.4  | 16.1  | 13.8  |

Note: Varieties showed the most positive effect from the influence of microbiological preparations.
3.3. Nodule forming ability and activity of nitrogenase

It was found that the maximum number of nodules was formed in the Avatar variety in the variant with rhizotorphin strain 065 – 134 and in the Krasnokutsky 123 variety in the variant with rhizotorphin strain 522 – 127 (table 2). The varieties Avatar, Krasnokutsky 123 and Kostyuzhansky 27 in variants with double inoculation – 109.23...155.79 micrograms of N2/plant h differed with maximum values of nitrogenase activity.

| Varieties          | Zolotoy yubile | Krasno kutsky 36 | Krasnoku tsky 123 | Avatar | Kostyuzhans ky 27 | Yubile y | Pamyt' | Pegas |
|--------------------|----------------|------------------|-------------------|--------|-------------------|----------|--------|-------|
| Variants           | Control        | Strain 527       | Strain 522        | Strain 065 | St. 527+AM | St. 522+AM | St. 065+AM | AM    |
| Number of nodules  | 0 2            | 11 31            | 14 25             | 18 35  | 39 35            | 98 24    | 25 35  | 2 0   |
| Nitrogenase activity, мкг N2/раст./час | - - | 26.96 21.16 28.56 35.95 | 9.82 35.95 91.38 16.48 | 11.98 8.99 21.16 35.95 | 5.27 17.25 35.95 116.48 | 9.82 41.94 62.92 31.46 | 21.16 12.25 41.94 88.99 | 21.16 12.25 41.94 88.99 |

HCP05(varieties): 2014 = 6.5; 2015 = 7.0; 2016 = 6.9
HCP05(preparations): 2014 = 8.4; 2015 = 8.2; 2016 = 9.2

4. Discussion

As a result of the research, it was shown that mono - and double inoculation with different strains of Mesorhizobium ciceri and AM fungi had a positive effect on the dynamics of chickpea development, the plants generally looked healthier than in control plots, which confirms the positive effect of soil symbiotic microorganisms on plant resistance to biotic stresses [9].

More intense leaf color in variants using microbial drugs indicates increased photosynthesis activity, and an increase in the duration of the growing season indicates a longer accumulation of biomass, which is consistent with similar studies conducted on peas [10].

The maximum effect of double inoculation was observed in most varieties in 2014 and 2016, which were characterized by an increased temperature regime. This can be explained by the fact that mycorrhizal fungi during settlement increased the absorption surface of the roots and the area of nutrition of chickpea plants, as arbuscular mycorrhiza, spreading in the intercellular space inside the

5
root, forms a mycelium outside, through which additional moisture and nutrients dissolved in it (mainly hard-to-reach phosphorus and nitrogen) enter the plant through the roots [11, 12].

In the conditions of the Oryol region, the most powerful and active symbiotic apparatus in chickpeas is formed during the flowering period – the beginning of bean formation. Treatment with biologics contributed to the formation of nodules in all varieties, but it was noted that in the control variant, most varieties of nodules on the roots of plants were formed in a single amount, or did not form at all). This is due to the fact that chickpeas have been introduced to the conditions of the Oryol region relatively recently, so there are still no nitrogen-fixing bacteria specific to them in the soil. At the same time, the maximum number of nodules and high nitrogenase activity do not always indicate high productivity of varieties, which is consistent with studies conducted on peas, and shows that it is incorrect to use only these features to assess the effectiveness of the symbiotic system without taking into account additional criteria [11].

5. Summary

As a result of the research, it was found that the use of microbiological drugs had a positive effect on the growth and development of plants in most chickpea varieties, significantly increased the productivity of varieties (up to 71.4%) and increased the duration of the growing season.

It was found that the efficiency of the symbiotic system is significantly affected by both weather and climate conditions and genotype.

The most powerful and active symbiotic apparatus in chickpeas is formed during the flowering period – the beginning of bean formation. The maximum number of nodules (134) was formed in the Avatar variety in the variant with nitrogen-fixing bacteria Mesorhizobium ciceri strain 065, the highest value of nitrogenase activity (155.79 mg N2/plant h) was observed in the same variety in the variant with double inoculation with strain 527 and AM fungi.

Genotypes characterized by high responsiveness to the use of microbiological drugs are included in breeding programs to increase the effectiveness of symbiosis of variety-microbial systems.

6. References

[1] Vance C P 2001 Symbiotic nitrogen fixation and phosphorous acquisition. Plant nutrition in the world of declining renewable resources (Plant Physiology) 127 390-397

[2] Borisov A Yu, Shtark O Yu, Zhukov V A, Nemankin T A, Naumkina T S [et al.] 2011 Interaction of legumes with useful soil microorganisms: from plant genes to varieties (Agricultural biology) 3 41-47

[3] Stolyarov O V, Fedotov V A, Demchenko N I 2004 Chickpeas (Cicer arietinum L.) Voronezh, Publishing house of Voronezh State University (VSU)) 256

[4] Solaiman A R M, Molla M N, Hossain M D 2006 Response of chickpea to dual inoculation with Rhizobium and arbuscular mycorrhiza, nitrogen and phosphorus (Korean J. Crop Sci.) 51(6) 527-533

[5] Erman M, Demir S, Oacak E, Tufenkci S, Oguz F, Akkopru A 2011 Effects of Rhizobium, arbuscular mycorrhiza and whey applications on some properties in chickpea (Cicer arietinum L.) under irrigated and rainfed conditions. 1. – Yield, yield components, nodulation and AMF colonization (Field crops research) 122(1) 14-24

[6] Vishnyakova M A, Buravtseva T V, Bulyntsev S V [et al.] 2010 Methodological guidelines collection of world genetic resources of grain legumes of RICP: replenishment, conservation and study (St. Petersburg, LLC “Kopi-R Group”) 141

[7] Orlov V P, Orlova I F, Shcherbina E A, Guryev G P, Vasilchikov A G 1984 Method for evaluating the activity of symbiotic nitrogen fixation of legume breeding material by the acetylene method (Orel, RRI of LGC) 16

[8] Posypanov G S 1991 Methods for studying biological fixation of air nitrogen (Moscow, “Agropromizdat”) 300
[9] Tikhonovich I A, Provorov N A 1998 Genetics of symbiotic nitrogen fixation with the basics of breeding (Saint Petersburg, “Nauka”) 208

[10] Stark O Yu, Danilova T N, Naumkina T S, Vasilchikov A G, Chebotar V K [et al.] 2006 Analysis of the source material of seed peas (Pisum sativum L.) for breeding varieties with high symbiotic potential and selection of parameters for its evaluation (Ecological genetics) 4(2) 22-28

[11] Smith S E, Read D J 2008 Mycorrhizal symbiosis 3rd Edition London, UK, «Academic Press» 800 p.

[12] Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N and Zhang L 2019 Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance (Front. Plant Sci.) 10:1068. doi: 10.3389/fpls.2019.01068

[13] Polukhin A A, Panarina V I 2020 Main problems of selection and seed production of agricultural crops and ways to solve them (Zernobobovye i krupyanye kul’tury) 3(35) 5-11

[14] Grudkina M, Polukhin A, Grudkina T 2019 Factors increasing the effectiveness of state support in agriculture (IOP Conference Series: Earth and Environmental Science) 274 012113

[15] Hamitowa S M, Glinushkin A P, Avdeev Y M, Naliukhin A N, Kostin A E, Kozlov A V, Uromova I P, Rudakov V O, Tesalovskiy A A, Protopopova E V, Pigorev I Y, Polukhin A A, Sycheva I I 2017 Condition Assessment Of Tree Plantations and Phytosanitary Properties of Soils in Cedar Groves (International Journal of Pharmaceutical Research & Allied Sciences) 6(4) 1-7

[16] Medvedev A V 2020 Improving the innovative and entrepreneurial potential of irrigated agriculture in the South of Russia (Russian Economic Bulletin) 3 (3) 149-153

[17] Bayanova O V 2020 Mechanism for ensuring the growth of feed crop production (Russian Economic Bulletin) 3 (1) 124-127