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Efficiency of bacterization of seeds of chick pea of grade Skarb with new strain of Mesorhizobium ciceri

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The purpose. To study effect of bacterization of seeds of chick pea of grade Skarb with new strain of Mesorhizobium ciceri upon efficiency of fabaceous-rhizobial symbiosis. **Methods.** Microbiological, gas-chromatographic, field experiment, statistical. Objects of researches — chick pea of grade Skarb, strains of nodule bacteria Mesorhizobium ciceri. Field researches in 2018 were carried out on experimental fields of Selection-genetic institute — National center of seeds growing and strain investigation (SGI-NCSGSI, Odessa oblast) and Institute of agricultural microbiology and agroindustrial production of NAAS (IAMAIP, Chernihiv oblast). Sowing of chick pea was realized manually on plots with area 5 m2 (SGI-NCSGSI) and 8 m2 (IAMAIP). Frequency of experiments — 4, disposition of alternatives — randomized. Mathematical processing was made by means of program Microsoft Office Excel 2007. **Results.** New strain M. ciceri 2 is secreted from nodules of plants of chick pea of grade Pamiat, taken on fields with active local population of rhizobia, which was generated as a result of long-term growing of chick pea in crop rotation. Preseeding bacterization of seeds of chick pea with strain M. ciceri 2 at growing this crop both on fields without local population of nodule bacteria of chick pea, and on the background of active population of M. ciceri positively influenced formation of fabaceous-rhizobial symbiosis. So, average height of plants increased on 10 – 17%, amount of legumes for a plant — on 23 – 55%, amount of seeds for a plant — on 27 – 46%, mass of seeds foe a plant — on 27 – 35%. **Conclusions.** New strain M. ciceri 2, secreted from nodules of chick pea of grade Pamiat, forms efficient symbiosis with plants of chick pea of grade Skarb both at absence and presence in soil of active rhizobia population of chick pea.

Key words: *fabaceous-rhizobial symbiosis, nodule bacteria, plants, seed inoculation.*

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A promising agricultural crop for growing in arid conditions of steppe zone of Ukraine is chickpea [1, 2]. Tendency to change integral indicators of weather conditions contributed to a significant expansion of chickpea crops in forest-steppe zone and in Polissya [3, 4]. In 2019, there are 15 varieties of chickpea registered in the State Register of Plant Varieties Suitable for Distribution in Ukraine, which is twice as much as last year. Light seed varieties Odysei, Budzhak, Triumf and Pam'iat', were bred at the Plant Breeding and Genetics Institute — National Center of Seed and Cultivar Investigation are intended for cultivation in the steppe zone of Ukraine, and varietie Skarb - for forest-steppe [5].

The successful introduction of chickpeas in different soil and climatic zones of Ukraine and especially in regions where previously where this culture has not been cultivated before, it is necessary to conduct pre-sowing bacterization seed active strains of rhizobia chickpea to facilitate the implementation of symbiotic potential of plants, increase resistance to adverse soil and climatic conditions and productivity growth [6, 7].

The purpose of research: investigate the effect bacterization of chickpea seeds varieties Skarb a new strain *Mesorhizobium ciceri* on the formation of effective legume- rhizobia symbiosis.

Materials and methods. Field research was conducted in 2018 in the fields of Plant breeding and genetics institute — national center of seed and cultivar investigation are intended (Odessa region) and the Institute of agricultural microbiology and agricultural manufacture NAAS of Ukraine (Chernihiv region).

Cultivation chickpea was carried out by conventional method for steppe zone [8]. In the fields of PBGI-NCSCI the soil cover is represented by mid-humus, important loamy black soil on loess deposits. Predecessor - winter wheat.

Experimental field IAMAM NAAS is characterized by the following factors agrochemical: soil - shallow black soil leached on loess loam. Predecessor - is spring oats. Planting chickpeas is carried out in a wide-row way (spacing - 45 cm).

In both experiments, bacterial suspension of industrial (*Mesorhizobium ciceri* H-12) and new strains of *Mesorhizobium ciceri* were used for bacterization of chickpea seeds, which were grown on a liquid bean medium on a rocking chair at 220 rpm for 3 days. Bacterial load is 10^6 cells/seed [9].

The results of research. The experiments were conducted in 2016–2018. During of 2016-2017 we have selected 58 bacterial isolates from chickpea nodules of varieties Skarb, Admiral, Odysei, Budzhak, Triumf and Pam'iat' selected in the fields PBGI-NCSCI (Odessa region), where an active rhizobia population was formed in the soil as a result of many years of cultivation (over 10 years) of this crop [10]. Another 11 isolates were isolated from nodules chickpea varieties Triumf and Pam'iat' that selected at new areas of cultivation of this crop in the fields of the Institute of agriculture in the Carpathian region NAAS (Lviv region). The resulting isolates for cultural-morphological characteristics answered characteristics of the genus *Mesorhizobium* [11].

Under the conditions of vegetation experiments on sterile vermiculite we analyzed the ability of new isolates to form active symbiosis with chickpea plants and selected the two most effective strains isolated from chickpea nodules of the varieties Skarb (*M. ciceri* 1) and Pam'iat' (*M. ciceri* 2) which differ in seed size and bush form. *M. ciceri* 1 and *M. ciceri 2* are characterized by a moderate growth rate (colonies appear on bean agar within 4-5 days), do not grow on meat-peptone agar, when grown on litmus milk, they produce acid. According to the analysis of the results of studies of the physiological and biochemical properties of the new strains, they were identified as *Mesorhizobium ciceri* [12].

The symbiotic properties of rhizobia were studied in field experiments in the absence of chickpea rhizobia in soil (IAMAM NAAS) and in fields with an active population of *Mesorizobium ciceri* (PBGI-NCSCI).

Thus, bacterization of chickpea seeds of Skarb with new strains of *M. ciceri* in the absence of chickpea bacteria in soil (IAMAM NAAS) contributed to the increase of the number of nodules on chickpea roots by 14% (*M. ciceri* 1) and 77% (*M. ciceri* 2) in compared to the positive control, and their mass was 2 - 3 times larger, respectively (Table 1).

1. Symbiotic properties of new strains of rhizobia chickpeas in terms of field experiments (2018)

Variant	Number of nodules, units / plant		Weight nodules, mg / plant	
	Chernihiv region	Odessa region	Chernihiv region	Odessa region
Control (Without bacterization)	-	14,0 ± 0,6	-	108,9 ± 2,2
Bacterization: *M. ciceri* H-12	13,2 ± 1,1	14,6 ± 0,4	215 ± 5	121,1 ± 4,8
M. ciceri 1	15,1 ± 2,1	14,8 ± 0,4	435 ± 32,5	123,3 ± 8,8
***M. ciceri* 2**	**23,3** ± 2,1	**15,3** ± 0,7	**636** ± 30	**133,3** ± 5,8

Against the background of an active local population of chickpea rhizobia, the number and weight of nodules with *M. ciceri* 1 bacterization was at the level with a positive control, while *M. ciceri* 2

bacterization contributed to an increase in the number of nodules by 10 and 5% compared with the absolute and positive controls and the mass of nodules - by 22 and 10%, respectively.

The data obtained indicate a high symbiotic activity and competitiveness of a new bacterial strain of *M. ciceri* 2 isolated from the chickpea potatoes Pam'iat'.

The next stage of our work was to study the effect of bacterization of chickpea seeds of Skarb variety of new strains *M. ciceri* on the structural parameters of yield and plant productivity.

In the field of field experiment, in the absence of active rhizobia population of chickpea in the soil, the bacterization of strain *M. ciceri* 2 of chickpea seeds of Skarb variety was the most effective. Thus, the observed increase in plant height by 17% and 10% compared to the Variant without bacterization and with bacterization *M. ciceri* H-12. Increasing number of beans per plant by 55% and 28%, the number of seeds per plant - 43% and 27% mass and seeds of plants - 35% and 27%, respectively (Table 2).

2. Influence of pre-sowing bacterization of seeds of *M. ciceri* strains on the structural indicators of chickpea of Skarb variety cultivar yield in the absence of active rhizobia population of this culture in soil (IAMAM NAAS, 2018)

Variant	Height, cm	Number beans units. / plant	Number of seeds / plant	Weight seeds g / plant
Control (Without bacterization)	52,53 ± 0,73	25,13 ± 0,85	13,40 ± 0,74	4,04 ± 0,24
Bacterization: *M. ciceri* H-12	56,03 ± 0,71	30,37 ± 1,40	15,10 ± 0,73	4,29 ± 0,22
M. ciceri 1	57,57 ± 0,67	31,67 ± 1,13	17,60 ± 0,99	4,80 ± 0,20
***M. ciceri* 2**	**61,60 ± 0,98**	**38,97 ± 2,99**	**19,17 ± 1,98**	**5,44 ± 0,60**

The cultivation of chickpeas on soils with an active population of *M. ciceri* and bacterization by the studied strains bacteria did not significantly increase the height of the inoculated plants relative to control. However, the treatment of seeds with a suspension of *M. ciceri* 2 contributed to the increase in the number of beans on the plant by 23% compared with the option without bacterization, the number of grains from the plant - by 46%, the mass of seeds from the plant - by 39% (Table 3).

3. Influence of pre-sowing bacterization of seeds of M. ciceri strains on the structural indicators of chickpea yield of Skarb variety against the background of an active population of rhizobia of this culture (PBGI-NCSCI, 2018)

Variant	Height, cm	Number beans pcs. / Plant	Number of seeds, pcs. / Plant	Weight seeds g / plant
Control (Without bacterization)	43,2 ± 1,72	21,6 ± 2,83	13 ± 1,87	4,76 ± 1,06
Bacterization: *M. ciceri* H-12	43,1 ± 0,92	24,6 ± 1,66	19,6 ± 1,32	5,64 ± 0,66
M. ciceri 1	37,1 ± 1,49	25,1 ± 5,88	17,1 ± 4,98	4,37 ± 1,38
***M. ciceri* 2**	**43,1 ± 0,94**	**26,8 ± 2,77**	**28,7 ± 2,78**	**6,63 ± 0,87**

Thus, the results of field experiments show that the new strain of *M. ciceri* 2, isolated from nodules chickpeas of Pam'iat' variety, is characterized by the ability to form effective symbiosis with plants of the new large-seeded Skarb variety in conditions different soil and climatic of Ukraine.

Conclusions

Pre-sowing bacterization of chickpea seeds with a new strain of *Mesorhizobium ciceri* 2 for the cultivation of this culture in fields where there was no local population of chickpea bacteria and on the background of an active population of *M. ciceri* positively influenced the formation of effective legume-rhizobial symbiosis. In the soils of the Odessa region against the background of the local rhizobia population, on the roots of inoculated chickpeas plants number of nodules grew by 5%, their weight by 10% relative to positive control. The formation of active symbiosis of chickpea plants with *M. ciceri* 2 contributed to an increase in the number of beans on the plant by 23% compared with the option without bacterization, the number of grains from the plant — by 46%, the mass of seeds from the plant - by 39%. Use for inoculation of the seeds of the test strain and chickpea cultivation on soils, where the background of *M. ciceri* has not yet formed, increased the number of nodules by 77%, and their mass by 3 times compared with the positive control. There was also an increase in plant height (by 10-17%) and an increase in structural indicators of chickpea crop: the number of beans per plant — by 23-55%, the number of seeds per plant - by 27-46% and the weight of seeds - by 27-35%.

In view of the above, a new strain of *M. ciceri* 2 is a promising bioagent of a microbial preparation for bacterium chickpea in order to form an effective legume-rhizobial symbiosis and increase the productivity of this culture.

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