Mesorhizobium ciceri ND-64 – HIGHLY EFFICIENT SYMBIONT OF CHICKPEA PLANTS, CULTIVATED IN POLISSIA AND STEPPE ZONE OF UKRAINE

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Abstract. In the cultivation of legumes, microbial preparations based on beneficial soil microorganisms are becoming increasingly important. Due to the mechanisms of biological fixation of molecular nitrogen and its conversion into a form accessible to plants, nodule bacteria are able to meet significantly the needs of crops in this element. The objective of our work was to carry out analytical selection of chickpea nodule bacteria and to study the effect of bacterization with new rhizobia strains on the structural parameters of chickpea yield in different soil and climatic zones of Ukraine. Inoculation of chickpea seeds of Skarb variety with a new strain of *M. ciceri* ND-64, which isolated from chickpea nodules of Pamiat variety, contributed to the increased number, weight of nodules and nitrogenase activity compared to the control (without inoculation), positive control (inoculation by *M. ciceri* H-12) and *M. ciceri* ND-101, isolated from chickpea nodules of Skarb variety. It was established that the bacterization of *M. ciceri* ND-64 contributed to the increase of productivity and yield of chickpea plants when grown in Polissia and Steppe zone of Ukraine. Considering the obtained data, the new strain *M. ciceri* ND-64 is a promising bioagent of a microbial preparation for bacterization of chickpea in order to form an effective legume-rhizobial symbiosis and increase the productivity of this culture.

Key words: *Mesorhizobium ciceri*; chickpea; symbiosis; nitrogen-fixing activity; yield.

INTRODUCTION

In the cultivation of legumes, microbial preparations based on beneficial soil microorganisms are becoming increasingly important [11, 24]. Due to the mechanisms of biological fixation of molecular nitrogen and its conversion into a form accessible to plants, nodule bacteria are able to meet significantly the needs of crops in this element [6, 13]. This helps to increase plant productivity and soil fertility [18].

One of the most common legumes in the world is chickpea [8, 19, 22]. Chickpea seeds are superior to other legumes in terms of availability and amount of amino acids, especially methionine and tryptophan [3, 7, 12], and are an important source of vegetable protein.

The areas of chickpea crops in Ukraine are mostly concentrated in the southern regions of the country, however changes in weather conditions contribute to their expansion in the central and even northern regions [2].

According to the State Statistics Service of Ukraine, the manufacture of chickpea has increased 2 times – from 19.2 thousand tonnes in 2017 to 41.2 thousand tonnes in 2019.

Cultivating chickpeas in different soil and climatic zones, especially in new areas, involves the inclusion of such an agricultural measure as pre-sowing bacterization of seeds by active and highly efficient strains of *Mesorhizobium ciceri* in the technology of cultivation [10, 16]. This will contribute to the implementation of the symbiotic potential of plants, increase their resistance to adverse soil and climatic conditions and increase yields [4, 21].

The objective of our work was to carry out analytical selection of chickpea nodule bacteria and to study the effect of bacterization with new rhizobia strains on the structural parameters of chickpea yield in different soil and climatic zones of Ukraine.

MATERIALS AND METHODS

Study objects

The object of the study were *M. ciceri* H-12 (patent UA 17664 U) [25], *M. ciceri* ND-101, *M. ciceri* ND-64 (patent UA 141783 U) [15], chickpea plants of Kabul-type varieties: large-seeded variety Skarb (registered in the State Registry of Plant Varieties in 2017) and medium-seeded variety Pam'iat' (registered in 2008).

Field experiments

The field experiments were conducted in the fields of the Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation of the National Academy of Agricultural Sciences of Ukraine (PBGI-NCSCI) in the Steppe zone of Ukraine (Odesa region). Soil cover – southern medium humus hard loamy chernozems on loess deposits. Chickpeas was cultivated in the experimental fields of PBGI-NCSCI for over 20 years, which contributed to the formation of active populations of nodule bacteria – microsymbionts of this legume [2, 14].

The field experiments were also conducted at the Institute of Agricultural Microbiology and Agro-industrial Manufacture of the National Agrarian Academy of Sciences of Ukraine (IAMAM NAAS) in the Polissia zone (Chernihiv region). Soil cover – leached low loamy chernozem on loess - like loams. A population of chickpea rhizobia began to form in the soil of the experimental field due to the cultivation of chickpeas in adjacent areas during 2 years of experiment.

Studies under conditions of field experiments were performed in 4 repetitions. The area of the accounting plot is 5 m^2 (PBGI-NCSCI) and 8 m^2 (IAMAM NAAS), the placement of variants is randomized [5]. Chickpea was sown manually in the wide rows (width between rows – 45 cm).

Field experiments in 2018-2019 were conducted as follows (Skarb variety):

1. Control (without seed inoculation);
2. Inoculation by *M. ciceri* H-12 (positive control);
3. Inoculation by *M. ciceri* ND-101 (strain isolated from the nodules of Skarb chickpea variety);
4. Inoculation by *M. ciceri* ND-64 (strain isolated from the nodules of Pam'iat' chickpea variety).

For bacterization of chickpea seeds, bacterial suspension of the strain *M. ciceri* grown on the rocking device at 220 rpm during 3 days on a liquid legume medium of the following composition, g/L: $(NH_4)_2SO_4$ – 1.0; K_2HPO_4 – 0.5; KH_2PO_4 – 0.5; $MgSO_4×7H_2O$ – 0.2; $CaCO_3$ – 1.0; sucrose – 10.0; pea decoction – 10 mL, water – 900 mL; pH = 6.8-7.0 was applied. Sterilization of the medium – 1.0 atm., 40 min.

Determination of nitrogen-fixing activity of microorganisms was performed by the acetylene method [9] on a gas chromatograph HP 4890A.

Statistical analysis

The processing of experimental data was carried out using a table editor Microsoft Excel. Data were subjected to factorial analyses of variance (ANOVA). Differences were considered significant when the value of the significance level was $p \leq 0.05$.

RESULTS

Active selection work on the isolation of new highly efficient strains of chickpea rhizobia was carried out at IAMAM NAAS in 2016-2017. A total of 69 isolates from chickpea nodules of 6 varieties were obtained, which according to cultural and morphological characteristics corresponded to the characteristics of bacteria of the genus *Mesorhizobium* [17].

In the conditions of vegetation experiments on sterile vermiculite, it was shown that the most effective symbionts of chickpea was *M. ciceri* ND-101, isolated from chickpea nodules of Skarb variety (deposited in the Collection of Beneficial Soil Microorganisms at IAMAM NAAS under № 273) and *M. ciceri* ND-64, which was isolated from chickpea nodules of Pamiat variety (deposited in the Collection of Beneficial Soil Microorganisms at IAMAM NAAS under № 274).

The symbiotic properties of rhizobia were studied in the field experiments in the where the population of chickpea nodule bacteria began to form in the soil in the Polissia zone (Chernihiv region) and against the background of the active local population of chickpea rhizobia in the Steppe zone (Odesa region). In the Polissia zone, bacterization of chickpea seeds with new strains of *M. ciceri* contributed to an increase in the number of nodules on chickpea roots by 8.8% (*M. ciceri* ND-101) and by 68.8% (*M. ciceri* ND-64) compared to the positive control (Table 1). The weight of nodules was 1.3-2 times higher, respectively and nitrogenase activity by 15.5-70.5% compared with the reference strain *M. ciceri* H-12. The results indicate a high symbiotic activity of *M. ciceri* ND-64.

In the Steppe zone, against the background of the active local population of rhizobia, the number and weight of nodules after bacterization by *M. ciceri* ND-101 was at the level of positive control, while bacterization by *M. ciceri* ND-64 increased the number of nodules by 69 and 29% compared to absolute and positive controls and nodule weight – was 1.9-2.1 times respectively. Nitrogenase activity in the variant with inoculation by *M. ciceri* ND-101 increased by 42% relative to control and by 21% relative to positive control. The highest nitrogenase activity was reported after inoculations by *M. ciceri* ND-64 (86% and 58%, respectively) (Table 1). This indicates the high competitiveness of *M. ciceri* ND-64.

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

The efficiency of chickpea seed inoculation with the reference strain *M. ciceri* H-12 and new strains *M. ciceri* ND-101, *M. ciceri* ND-64, was studied in a field experiment in two different regions of Ukraine for two years. Tables 2 and 3 present the results.

In Polissia zone, bacterization of chickpea seeds by *M. ciceri* ND-64 proved to be the most efficient. Thus, an increase in plant height by 11 and 8% was observed compared to the variants without bacterization and with bacterization by *M. ciceri* H-12. The number of beans per plant increased by 48 and 23%, the number of seeds per plant – by 39 and 17% and the weight of seeds – by 39 and 15%, weight of 1000 seeds – by 19 and 12%, respectively (Table 2).

Cultivating chickpeas bacterized with the studied strains on soils with an active population of nodule bacteria (Odesa region) did a increase in the height of inoculated plants compared to the control by 17%. Treatment of seeds with a suspension of *M. ciceri* ND-64 also increased the number of beans per plant by 44% compared to the variant without bacterization, the number of grains from the plant – by 53%, the weight of seeds from the plant – by 16%, weight of 1000 seeds – by 9% (Table 2).

Table 1. Symbiotic properties of the strains of chickpea nodule bacteria under conditions of field experiments (2018-2019)

Variant	Number of nodules, pcs/plant		Weight of nodules, mg/plant		Nitrogenase activity, nmol/plant*h	
	Polissia zone	Steppe zone	Polissia zone	Steppe zone	Polissia zone	Steppe zone
Control (without inoculation)	4.2 ± 1.3	15.0 ± 0.5	102.2 ± 2.4	210.9 ± 1.2	638.1 ± 38.4	1020.0 ± 26.0
Inoculation by *M. ciceri* H-12	17.0 ± 2.0	15.6 ± 0.6	355.3 ± 4.9	215.1 ± 2.9	1449.6 ± 66.3	1200.0 ± 25.0
Inoculation by *M. ciceri* ND-101	18.5 ± 2.2	22.8 ± 0.4	476.5 ± 22.2	323.0 ± 4.8	1675.5 ± 22.9	1450.0 ± 25.0
Inoculation by *M. ciceri* ND-64	**28.7 ± 2.2**	**25.3 ± 0.5**	**749.8 ± 17.0**	**450.5 ± 5.7**	**2472.5 ± 90.1**	**1900.0 ± 19.0**

Table 2. Effect of chickpea seed inoculation by the new strains of *M. ciceri* on the structural parameters of chickpea yield (2018-2019)

Variants of experiment	Height, cm	Number of beans, pcs/plant	Number of seeds, pcs/plant	Weight of seeds, g/plant	Weight of 1000 seeds, g
Polissia zone					
Control (without inoculation)	49.8 ± 0.7	22.3 ± 0.8	16.0 ± 0.7	5.1 ± 0.2	404.0 ± 14.0
Inoculation by *M. ciceri* H-12	51.0 ± 0.7	26.8 ± 1.2	19.0 ± 0.9	6.2 ± 0.3	429.0 ± 12.0
Inoculation by *M. ciceri* ND-101	52.2 ± 0.5	26.6 ± 1.0	18.8 ± 0.9	6.0 ± 0.2	450.0 ± 10.0
Inoculation by *M. ciceri* ND-64	**55.1 ± 0.6**	**33.1 ± 2.0.**	**22.2 ± 1.5**	**7.1 ± 0.5**	**480.0 ± 20.0**
Steppe zone					
Control (without inoculation)	45.2 ± 0.7	20.5 ± 0.8	19.0 ± 0.9	6.4 ± 0.1	450.0 ± 16.0
Inoculation by *M. ciceri* H-12	47.1± 0.9	25.8 ± 0.7	24.6 ± 0.3	6.6 ± 0.1	465.0 ± 20.0
Inoculation by *M. ciceri* ND-101	49.1± 0.5	27.1 ± 0.6	26.1 ± 0.9	6.9 ± 0.2	477.0 ± 18.0
Inoculation by *M. ciceri* ND-64	**53.1 ± 0.8**	**29.5 ± 0.8**	**29.1 ± 0.8**	**7.4 ± 0.3**	**490.0 ± 21.0**

Table 3. Effect of seed inoculation by the strains of *M. ciceri* on chickpea yield (2018-2019)

Variants of experiment	Yield, t/ha	Increment	
		t/ha	%
Polissia zone			
Control (without inoculation)	1.23	-	-
Inoculation by *M. ciceri* H-12	1.41*	0.18	14.6
Inoculation by *M. ciceri* ND-101	1.45*	0.22	17.8
Inoculation by *M. ciceri* ND-64	**1.69***	**0.46**	**37.4**
LCD_{05}	0.06		
Steppe zone			
Control (without inoculation)	1.02	-	-
Inoculation by *M. ciceri* H-12	1.17*	0.15	14.7
Inoculation by *M. ciceri* ND-101	1.23*	0.21	20.5
Inoculation by *M. ciceri* ND-64	**1.36***	**0.34**	**33.3**
LCD_{05}	0.03		

* = significant differences at the level of 0.05

During two years of studies, it was shown that the yield of Skarb variety chickpea plants, cultivated in the Steppe zone (with an active population of chickpea rhizobia in the soil) and in the Polissia zone of Ukraine (on soils where the rhizobia population of this culture has just begun to form), was the highest after inoculation by *M. ciceri* ND-64 (Table 3).

DISCUSSION

Chickpea plants are able to enter into a symbiotic relationship with specific nodule bacteria *M. ciceri*, resulting in the formation of nodules of nondeterministic type [1, 20, 23]. According to Didovych, there are no aboriginal chickpea nodule bacteria in the soils of most soil and climatic zones of Ukraine [4]. Therefore, formation and functioning of effective legume-rhizobial symbiosis in order to obtain high yields of this culture requires pre-sowing bacterization of seeds by active and highly efficient strains of *M. ciceri*.

M. ciceri fix atmospheric nitrogen and convert it into an accessible form for plants, enrich the soil with nitrogen, promoting more intensive plant growth [6]. This process plays an important role in the nitrogen balance of the biosphere and contributes to the improvement of the ecological environment. With the use of inoculants, it is possible to reduce the use of mineral nitrogen while reducing energy costs for crop production [6, 11, 24].

Our data are consistent with the data of researchers which show that inoculation of chickpea seeds with effective strains of nodule bacteria promotes an increase in the number, mass of nodules and nitrogen fixing activity in comparison with the control [4, 10].

This work shows that the nitrogen fixing activity ranged from 1,900.0 to 2,472.5 nmol C_2H_4/plant/hour under the conditions of pre-sowing bacterization by *M. ciceri* ND-64 cells, both when grown in different soil and climatic zones, in the absence or presence of active population of rhizobia of this culture in the soil.

It is known that *M. ciceri* – based microbial inoculants actively influence the growth and development of plants, providing an increase in the yield [13, 18]. We have first investigated the efficiency of inoculation of chickpea seeds by the reference strain *M. ciceri* H-12 in the Polissia zone of Ukraine and shown that it is 14.6%. The obtained results are consistent with the results of Didovych et al. [4, 25]; the researchers have found that the effect of inoculation by *M. ciceri* H-12 on the yield of chickpeas when cultivating in the Steppe zone is 8.8% to 24.7%.

When inoculating chickpea seeds with *M. ciceri* ND-64, the increase in yield ranged from 33.3% to 37.4% when cultivating against the background of the active population of chickpea rhizobia in the Steppe zone and Polissia zone, where chickpeas were not previously cultivated.

Considering the obtained data, the new strain of *M. ciceri* ND-64 is a promising bioagent of a microbial preparation for chickpea bacterization in order to form an efficient legume-rhizobial symbiosis and increase the productivity of this culture.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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