Australian Journal of Crop Science AJCS

AJCS 15(01):129-136 (2021) ISSN:1835-2707
doi: 10.21475/ajcs.21.15.01.2952

Dynamics of formation and functioning of legume-rhizobial symbiosis *Mesorhizobium ciceri-Cicer arietinum* (variety Pam'iat')

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Abstract

The study of the dynamics of formation and functioning of symbiosis between nodule bacteria and chickpea plants was carried out in the northern region of Ukraine in the experimental plots, where the local population of chickpea mesorhizobia began to form. A new strain of Me*sorhizobium ciceri* ND-64 and a reference *Mesorhizobium ciceri* H-12 were used to inoculate *Cicer arietinum* seeds. The field study determined the number, weight, nitrogenase activity of nodules on the chickpea roots, dry green mass and mass of roots of chickpea plants of Pam'iat' variety. Samplings were carried out nine times in different phases of plant vegetation, starting from the branching phase and ending with the phase of complete maturity of seeds. The study showed that round and oval nodules appeared on the roots of chickpea plants during the branching stage. Gradually, they increased in size and in the phase of budding formed splices. The largest number of nodules (43.1 units/plant) was formed in the bean formation phase. The highest nitrogenase activity of the nodules was registered in the flowering phase. It was found that the bacterization of *M. ciceri* ND-64 promotes earlier formation and long-term functioning of the symbiotic apparatus in comparison with the reference strain and the control variant. The highest symbiotic rates under inoculation with *M. ciceri* ND-64 were registered at the beginning of the bean formation phase. In the control variant and with inoculation of *M. ciceri* H-12, these parameters were the highest later (at the end of the bean formation phase) and rapidly decreased in the subsequent phases, while the nodules formed by *M. ciceri* ND-64 continued to functioning actively. The positive effect of inoculation of chickpea seeds with a suspension of *M. ciceri* ND-64 on the increase in the number of beans and seeds per plant, weight of 1000 seeds and yield of chickpea plants was shown. Considering the obtained data, the new strain *M. ciceri* ND-64 is a promising bioagent of a microbial preparation for bacterization of chickpea.

Keywords: Legume-rhizobial symbiosis; nodules; nitrogenase activity; chickpea; *Mesorhizobium ciceri;* yield.
Abbreviations: NA_nitrogenase activity.

Introduction

One of the most widespread legumes is chickpea, which ranks third in the world in terms of sown area. The main producers and consumers of this crop are countries with arid climates (Gangola et al., 2014; Rubio et al., 2014; Fierro et al., 2019; Olika et al., 2019), which is associated with high drought resistance of plants and biological value of products. Chickpea seeds are superior to other legumes in terms of availability and amount of amino acids, especially methionine and tryptophan. Therefore, chickpea is an important source of vegetable protein, which determines the special importance of this crop in the food industry (Frias et al., 2010; Jadhav et al., 2015; Cobos et al., 2016).
The areas of chickpea crops in Ukraine are predominantly concentrated in the southern regions of the country. However, change of integrated parameters of weather conditions contributes to their expansion in the central and northern regions (Bushulyan et al., 2015).
Chickpea plants are able to enter into a symbiotic relationship with specific nodule bacteria *M. ciceri* (Nour et al., 1994; Jarvis et al., 1997).
There are no indigenous chickpea nodule bacteria in the soils of the majority of soil-climatic zones of Ukraine, therefore formation and functioning of effective legume-rhizobial symbiosis in order to obtain high yields of this crop require for pre-sowing bacterization of seeds with active and highly effective strains of *M. ciceri* (Didovich et al., 2006).
The specific virulent strain of rhizobia and its activity are the main conditions for the formation of active symbiosis (Elias et al., 2019). However, the formation and functioning of an effective symbiotic system are also influenced by environmental factors (Gibson A., 1971; Streeter J.G., 2003). Temperature factor is of particular importance for the development of legume-rhizobial symbiosis (Kishinevsky et al., 1992; Michiels et al., 1994). Therefore, the objective of our study was to establish formation and functioning of the symbiosis of *M. ciceri – C. arietinum* over time when grown in the northern region of the country and to study the activity and efficacy of a new strain of *M. ciceri* ND-64 (Pat. UA 141783 U) compared to the reference strain *M. ciceri* H-12 (Pat. UA 17664 U).

Results

Study of the formation of the symbiosis of M. ciceri – C. arietinum

A study of the formation of the symbiosis of M. *cicero –C. arietinum* over time showed that the nodules on the roots of chickpeas begin to form in the branching phase. Thus, at day 14 after emergence, the number of nodules was insignificant (0.2 – 2.1 units/plant) on the roots of non-inoculated plants and in the variant with inoculation with the production strain of *M. ciceri* H-12 (Fig. 1). Inoculation of seeds with *M. ciceri* ND-64 contributed to the earlier formation of the symbiotic apparatus in the branching phase, the number of nodules was 10.6 units/plant, which is 5 times higher than this parameter in the positive control.

The flowering phase was characterized with a sharp increase in the number of nodules: 32.3 units/plant following inoculation of *M. ciceri* ND-64, 14.2 units/plant – in the positive control and 6.9 units /plant in the control variant.

The highest number of nodules in the variant with inoculation using *M. ciceri* ND-64 was registered at the beginning of the bean formation phase – 43.1 units/plant, which is 3.2 times higher than the parameter in the positive control (inoculation with *M. ciceri* H-12).

Under bacterization with *M. ciceri* H-12 and in the control variant, the number of nodules was the highest later (at the end of the bean formation phase) and it was 27 and 17 units/plant, respectively. In subsequent phases, the number of nodules in the control and positive control decreased rapidly, while the number of nodules formed by *M. ciceri* ND-64 remained large (35.4-20.2 units/plant).

In the middle of the ripening phase, the number of nodules on the plants of all variants increased slightly due to the heavy rainfall.

At the beginning of the vegetation of chickpea plants, nodules were characterized by an oval or round shape. Nodules larger in size were divided into 2-3 partially separated nodules. There were located mainly along the main root (Fig. 2).

Starting from the budding phase, nodules grew due to the intensive development of the meristem. Thus, the formation of adhesions from 5-10 oval nodules collected in the form of a ball or fan was registered.

The flowering phase was characterized by nodule enlargement. Their adhesions also were fan- or ball-shaped, but the nodules acquired a more elongated shape. It should be noted that signs of aging of the nodules already began to appear in the flowering phase (Fig. 3). The highest values of nodule weight during seed inoculation with *M. ciceri* ND-64 were registered in the flowering phase – beginning of bean formation – 1.06 g/plant. During the subsequent stages of plant development, these parameters slowly decreased, and in the phase of bean filling and seed ripening were still quite high (the number of nodules was 27.80 units/plant, their weight – 0.77 g/plant). In the control variant, as well as during inoculation of seeds with *M. ciceri* H-12, the highest symbiotic parameters were registered later (in the bean formation phase): the number of nodules was 16.6 and 27.9 units/plant, weight 0.42 and 0.65 g/plant, respectively. There was also a dramatic decrease in the weight and number of nodules (up to 11.13 units/plant and 0.28 g/plant) after bean formation phase in variants after inoculation with *M. ciceri* H-12 (Fig. 4).

NA during the entire growing season was also highest in the variant with inoculation using *M. ciceri* ND-64 and increased rapidly from the budding phase to the bean formation phase, and at the beginning of the bean formation phase it was 3597 nmol ethylene/plant • hour (Fig. 5). In the subsequent phases of ontogenesis of chickpea plants, this parameter decreased moderately, however at the end of the chickpea vegetation the NA in the variant with bacterization using *M. ciceri* ND-64 was at the level of 558 nmol ethylene/plant • hour. Under inoculation with *M. ciceri* H-12 and in the control variant, the highest parameters of NA (1611 and 981 nmol ethylene /plant • hour) were registered at the end of the bean formation phase. From the bean filling phase, this parameter decreased in all variants of the experiment. It should be noted that at the end of the ripening phase (before harvest), the NA of the nodules of the inoculated plants was still registered, while nodules ceased to functioning in the control variant. Throughout the period of plant development, dry green mass increased quite evenly, and only in the ripening phase a dramatic increase for inoculated plants was registered (Fig. 6). These parameters in all variants of the experiment in the early phases did not scarcely differ, and in the flowering phase were still the highest under inoculation with *M. ciceri* ND-64.

The weight of roots increased significantly from the beginning of the budding phase, however the rate of its growth slowed down from the phase of bean formation (Fig. 7). It should be noted that the root weight of inoculated plants continued to increase, even in the phase of bean ripening (before harvest), while this parameter in the control variant has been already declined.

Study of the effect of inoculation of chickpea seeds with M. ciceri on the structural parameters of yield of Pam'iat' variety

We have studied the effect of inoculation of chickpea seeds with *M. ciceri* on the structural parameters of yield of Pam'iat' variety (UD0500736, UKR) under the conditions of field experiment in 2019. For example, when inoculating seeds of this variety with *M. ciceri* ND-64, the number of beans and seeds from the plant significantly increased by 39.8 % and 42.3 % compared to the control variant (without inoculation) and by 13.3 % and 12 % compared to the positive control (inoculation with *M. ciceri* H-12) (Table 1). The weight of seeds from the plant and the weight of 1000 seeds was significantly increased by 44 % and 13 % relative to control and by 18 % and 10 % relative to positive control.

During 3 years we have studied the effect of inoculation of chickpea seeds with a suspension of *M. ciceri* ND-64. The average significant increment in yield of chickpea plants was 0.31 t/ha or 26.5%, which is 12.8% higher than this figure in the positive control (Fig. 8).

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 (Nour et al., 1994; Jarvis et al., 1997; Rivas et al., 2009; Andrews and Andrews, 2017). According to various researchers in the field, the nodules appear about 20-30 days after the appearance of plants, which are usually in the upper soil

Table 1. Effect of inoculation of chickpea seed with *M. ciceri* strains on the structural parameters of yield of Pam'iat' variety (*2017-2019*)

Variant	Number of beans per plant, pcs	Number of seeds per plant, pcs	Weight of seed per plant, g	Weight of 1000 seeds, g
Control	17.70 ± 0.75[a]	16.80 ± 0.72[b]	4.76 ± 0.24[c]	273.70 ± 4.22[d]
Inoculation with *M. ciceri* H-12	21.85 ± 0.76[a]	21.33 ± 0.75[b]	5.82 ± 0.22[c]	280.90 ± 8.02[e]
Inoculation with *M. ciceri* ND-64	24.75 ± 0.72[a]	23.90 ± 0.67[b]	6.88 ± 0.20[c]	309.40 ± 5.40[e]

Different letters in each row of the same variant represent a significant difference at $p \leq 0.05$

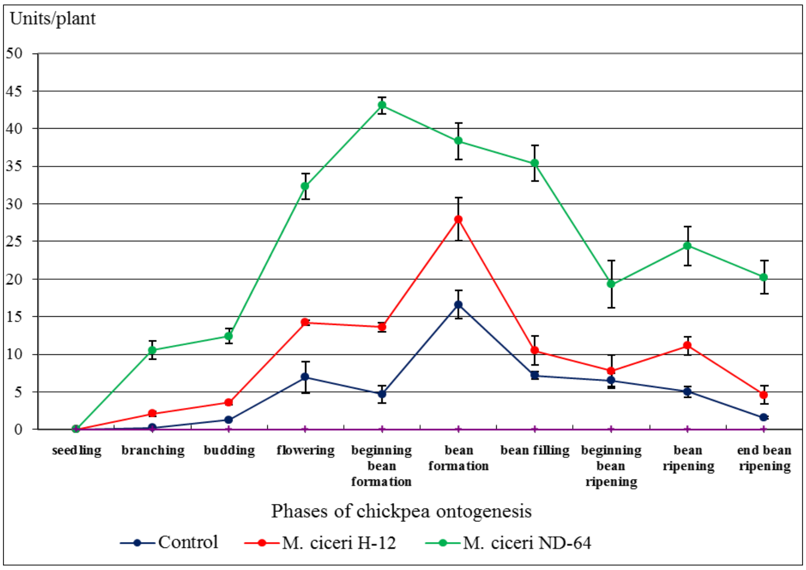

Fig 1. Number of nodules on the chickpea roots of Pam'iat' variety under inoculation with *M. ciceri.* Data shows mean values of three replicates ± SD.

Table 2. Initial soil physico-chemical composition (before experiment)

Characteristic	Arable layer	Mobile forms of phosphorus	Exchangeable potassium	pH of water
Feature value	3.6%	210-240 mg P_2O_5 per 1 kg of soil	160-170 mg K_2O per 1 kg of soil	6.5

1 **2** **3**

Fig 2. Nodules on chickpea roots (*branching phase*):1 - control (without inoculation), 2 - *M. ciceri* H-12, 3 - *M. ciceri* ND-64.

Table 3. Meteorological conditions during the study *(Chernihiv, Ukraine 2017-2019).*

Month	Air temperature, ºC				Amount of rainfalls, mm			
	Long-term average	2017	2018	2019	Long-term average	2017	2018	2019
April	8.4	9.1	11.2	9.5	41.0	16.2	1.5	26.8
May	14.7	13.8	17.8	16.4	55.0	24.5	22.2	74.5
June	17.9	18.2	19.3	22.7	71.0	43.1	100.6	15.9
July	19.9	18.9	20.4	18.6	71.0	66.0	147.1	63.1
August	18.8	22.1	20.4	18.6	59.0	52.8	13.9	43.4
September	13.2	15.0	16.3	15.0	57.0	39.7	32.0	39.7

1 2 3

Fig 3. Nodules on chickpea roots (*flowering phase*):1 - control (without inoculation), 2 - *M. ciceri* H-12, 3 - *M. ciceri* ND-64.

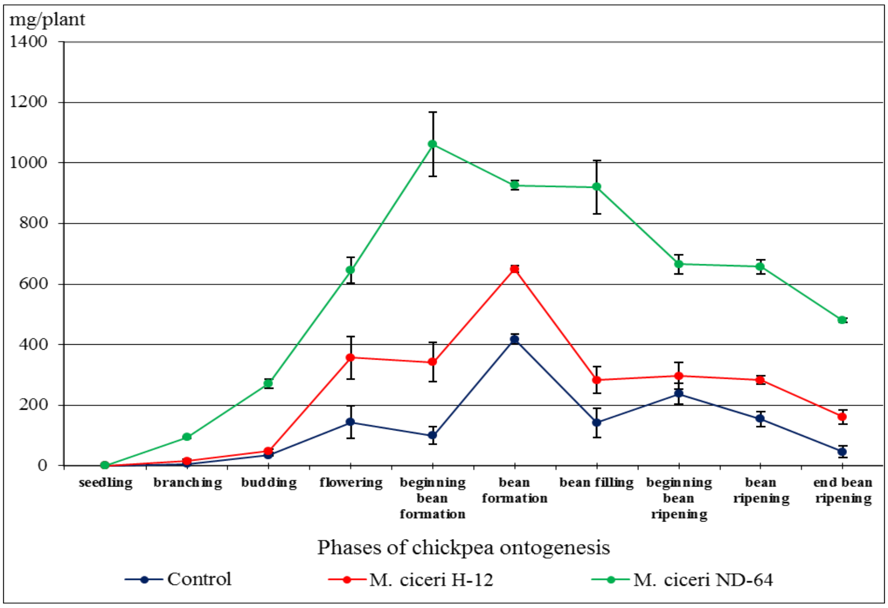

Fig. 4. Weight of nodules on the chickpea roots of Pam'iat' variety under inoculation with *M. ciceri.* Data shows mean values of three replicates ± SD.

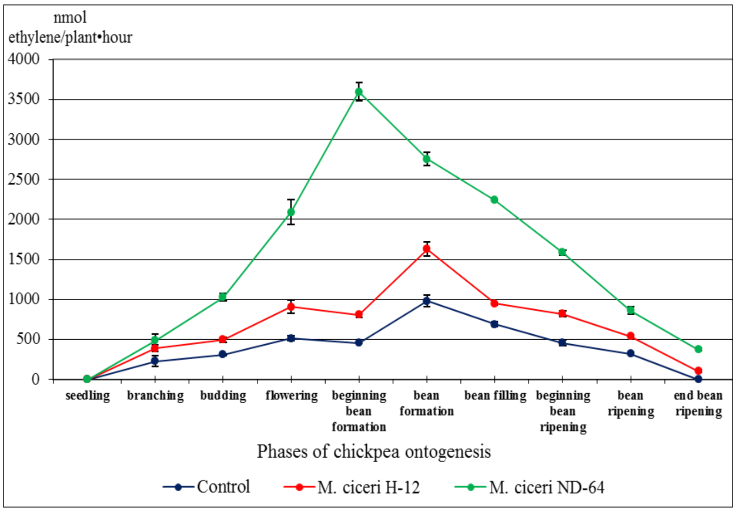

Fig 5. Nitrogenase activity of nodules on the chickpea roots of Pam'iat' variety under inoculation with *M. ciceri.* Data shows mean values of three replicates ± SD.

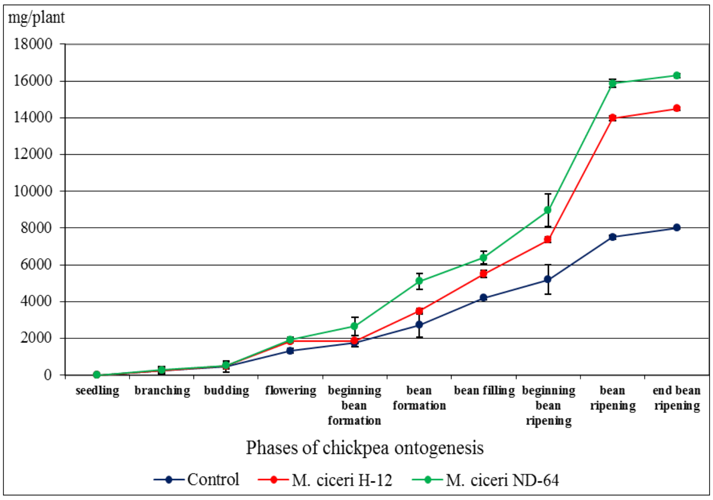

Fig 6. Dry green mass of chickpea plants of Pam'iat' variety during vegetation period. Data shows mean values of three replicates ± SD.

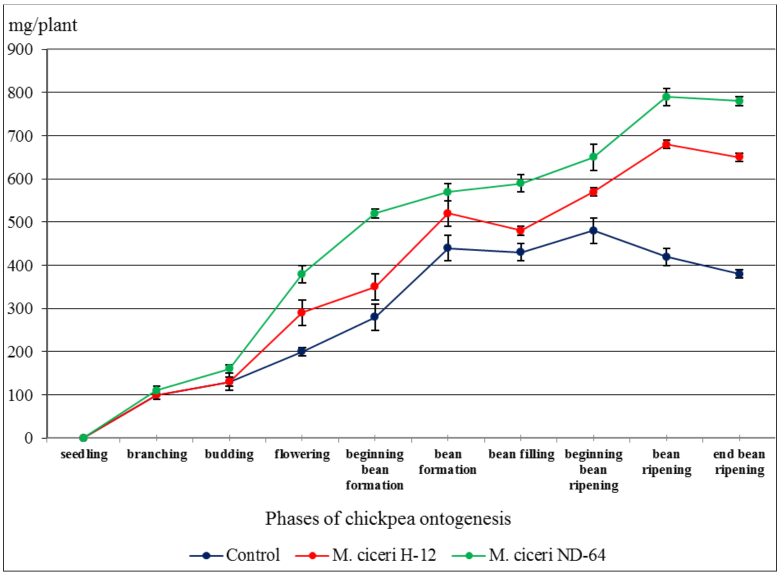

Fig 7. Dry mass of roots of chickpea plants of Pam'iat' variety during vegetation period. Data shows mean values of three replicates ± SD.

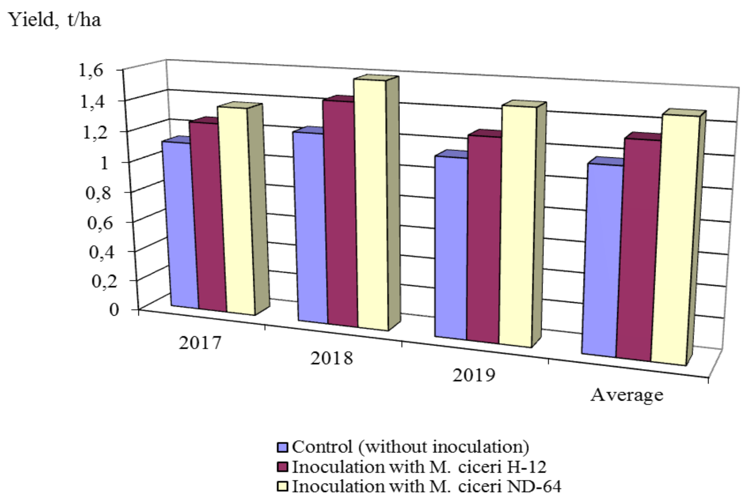

Fig 8. Effect of inoculation of seeds with *M. ciceri* on the yield of chickpea of Pam'iat' variety.

layer at a depth of 15 cm (Rupela and Dart, 1979; Kryvoruchko, 2017). We have shown that by inoculation with the active strain of *M. ciceri* ND-64 on the roots of chickpea nodules are formed as early as 14 days after germination.

The number of nodules and the mass of nodules on the plant, which are formed by inoculation of chickpea seeds depends on the genotype of chickpeas, rhizobia strain and soil and climatic conditions and can be in the following range: 1-9 nodules weighing 0.142-0.215 g/plant (Zaman et al., 2011), 20.3–36.8 nodules weighing 0.72–1.05 g/plant (Aouani et al., 2001), 47-65 nodules weighing 0.32–0.37 g/plant (Chaudhary and Sindhu, 2015), 42-69 nodules weighing 0.43-0.89 g/plant (Khaitov et al., 2016), 121-196 nodules weighing 1.53-1.76 g/plant **(**Nasr Esfahani et al., 2014). We have shown that in the northern region of Ukraine during inoculation of chickpea seeds with the active strain *M. ciceri* ND-64 on the roots formed nodules in the amount of 10.6-43.1 units/plant weighing 0.09-1.06 g/plant in different phase of ontogenesis of chickpea plants.

It is known that there is a correlation between NA (plant • hour) and the weight and number of nodules (Rupela and Dart, 1979, Rupela, 1990). At the beginning of the bean formation phase NA was 3597 nmol ethylene/plant • hour, the number and weight of nodules was higher than in other phases of chickpea plant ontogenesis.

The duration of functioning of chickpea nodules depends on the activity of strains and soil and climatic conditions. A field study in India (Rupela and Dart, 1979) showed that nitrogen-fixing nodulesactivity was lost 89 days after planting (south-central India), whereas in northern India nodules activity persisted for up to 145 days after planting (3 weeks before collecting seeds). We recorded the NA of the nodules of inoculated plants of *M. ciceri* ND-64 (374.27 nmol/plant • hour), and the reference strain of *M. ciceri* H-12 (102.07 nmol/plant • hour), while in the control version the nodules were absent at the end of the ripening phase (1 week before harvest). That is, the symbiotic properties of the strain are of great importance for the long-term existence of symbiosis.

It is known that microbial inoculants based on *M. ciceri* bacteria actively affect the growth and development of chickpea plants. Thus, according to studies by Khaitov et al., 2016, inoculation of chickpea seeds with rhizobia strains increases the dry weight of plants by 27-38%, dry weight of roots by 42-64%. We showed that when inoculating chickpea seeds with strain *M. ciceri* ND-64, the dry weight of plants in different phases of ontogenesis increased by 7-87%, dry weight of roots by 10-105% relative to control in other phases of chickpea plant ontogenesis.

Inoculation of chickpea seeds with effective strains increases the number of beans and seeds from the plant, the mass of seeds from the plant, which increases plant yields (according to Gan et al., 2005; Khattak et al., 2006; Zaman et al., 2011; Nasr Esfahani et al., 2014; Gul et al., 2014; Khaitov et al., 2016). We showed that by inoculation of chickpea seeds with strain *M. ciceri* ND-64 increased, the number of pods and seeds from the plant by 39.8% and 42.3%, the weight of seeds from the plant by 44%, the increase in yield was up to 37.4 % for growing in the area of Polissya, where chickpeas have not been grown before. Based on the data obtained, the new strain of *M. ciceri* ND-64 is a promising bioagent of a microbial preparation for bacterization of chickpeas in order to form an effective legume-rhizobial symbiosis and increase the productivity of this culture.

Materials and methods

Plant materials

The study was carried out with chickpea (*Cicer arietinum* L.) plants of Pam'iat' variety (UD0500736, UKR), selected at the Plant Breeding and Genetic Institute – National Center of Seed and Cultivar Investigation of the National Academy of Agricultural Sciences of Ukraine. It belongs to the type Kabuli, is light-seeded, with an average seed size (weight of 1000 seeds is about 315 g).

Bacterial strains

Mesorhizobium ciceri ND-64 was isolated in 2016 from chickpea nodules of Pam'iat' variety (UD0500736, UKR), which was grown in the southern region of Ukraine (Region of Odesa) with a large population of chickpea rhizobia in the soil, which was formed as a result of long-term cultivation of this culture. The strain *M. ciceri* ND-64 is deposited in the Depository of the Danylo Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the registration number *M. ciceri* B-7835 (patent UA 141783 U).

Reference strain – *Mesorhizobium ciceri* H-12 (patent UA 17664 U).

Seed inoculation and sowing

For bacterization of chickpea seeds, bacterial suspension of the reference *M. ciceri* H-12 and new *M. ciceri* ND-64 strain 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; $MgSO_4 \times 7H_2O$ – 0.2; $CaCO_3$ – 1.0; sucrose (sugar) – 10.0; pea decoction – 100; pH = 6.8–7.0 was applied. Sterilization of the medium – 1.0 atm., 40 min.

Chickpea seeds were inoculated for 2 hours, and then sowing was performed by hand.

Bacterial load – 10^6 cells/seed.

Conduction of study and experimental design

Field experiment was conducted at the Institute of Agricultural Microbiology and Agroindustrial Manufacture of National Academy of Agricultural Sciences of Ukraine in Chernihiv (Northern region of Ukraine). The experimental field is characterized by the following agrochemical parameters: soil – leached shallow slightly-loamy chernozem on loess clay loam (Table 2). 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 3 years of experiment.

The investigation of the dynamics of formation and functioning of legume-rhizobial symbiosis of *M. ciceri* with chickpea plants of Pam'iat' variety was carried out in the field experiment in 4 repetitions. The area of the accounting plot is 10 m^2, placement of variants is randomized. Predecessor – spring oats. Chickpea was sown manually in a wide row method (width between rows is 45 cm) (Gaur et al., 2010).

Samplings were carried out nine times in different phases of plant vegetation with an interval of 6-12 days, in phases: the branching, budding, flowering, beginning bean formation, bean filling, beginning andend bean ripening. 10 plants with each of the 4 replicates of each variant were selected for analysis. Harvest was gathered manually from 10 m^2 each of the 4 replicatesin sheaves, dried before threshing.

Nitrogenase activity of nodules was determined by acetylene method (Hardy et al., 1968) using gas chromatograph HP 4890A.

Weather conditions

According to the Chernihiv Regional Center for Hydrometeorology, the air temperature in northern Ukraine exceeded the long-term average rate by 1.2-4.7°C during April-June 2019 (Table 3). It should be noted that chickpea is a cold- and drought-resistant plant that requires significant soil moisture at the stage of seed germination. The amount of rainfall in May exceeded the average rate, and in June it was much lower than the average long-term rate. Thus, the weather conditions for spring-summer 2019 were favourable for the normal growth and development of chickpea plants.

Statistical analysis

The treatments were manipulated in completely randomized design (CRD) with 3 replicates. Statistical analysis was performed using one-way analysis of variance (ANOVA). The data were presented as means ±SD. Significance at a P value ≤ 0.05 differences among means were determined by LSD test.

Conclusion

The study of the dynamics of formation of symbiosis of nodule bacteria with chickpea plants in the conditions of the northern region of Ukraine showed that inoculation of seeds with active strain *M. ciceri* ND-64 contributes to earlier formation and long-term functioning of symbiotic apparatus compared to inoculation with reference strain *M. ciceri* H-12 and control variant.

It was shown that round and oval nodules appear on the roots of chickpea plants during the branching stage. Gradually, they enlarged and formed a fusion in the budding phase. The largest number of nodules was formed in the bean formation phase. The highest NA of the nodules was reported in the budding phase. The highest symbiotic parameters during inoculation with *M. ciceri* ND-64 were observed at the beginning of the bean formation phase: the number of nodules – 43.1 units/plant, the weight of nodules – 1.06 g/plant, NA – 3597 nmol C_2H_2/plant • hour. When inoculated with *M. ciceri* H-12 and in the control variant, these parameters were the highest later (at the end of the bean formation phase) and rapidly decreased in the subsequent phases, while the nodules formed by *M. ciceri* ND-64 continued to functioning actively.

Inoculation of chickpea seeds with a suspension of *M. ciceri* ND-64 contributed to an increase in the number of beans and seeds per plant by 39.8% and 42.3%, the weight of 1000 seeds – by 13%.

The yield of chickpeas of Pam'iat' variety increased as a result of inoculation of seeds with *M. ciceri* ND-64 by 26.5% when grown on soils where the formation of the local mesorhizobia population begins (northern region of Ukraine).

Acknowledgments

This research received no specific grant from any funding, agency, commercial or not-for-profit sectors. No conflicts ofinterest have been declared.

References

Andrews M, Andrews ME (2017) Specificity in legume-rhizobia symbioses. Int J Mol Sci. 18(4), 705.

Aouani ME, Mhamdi R, Jebara M, Amarger N (2001) Characterization of rhizobia nodulating chickpea in Tunisia. Agronomie. 21:577-581.

Bushulyan OV, Sichkar VI, Bushulyan MA, Pasichnyk SM (2015) Results and prospects of the chickpea breeding in Ukraine. Legumes Cereals. 4(16):49-54.

Chaudhary D, Sindhu S (2015) Inducing salinity tolerance in chickpea (*Cicer arietinum* L.) by inoculation of 1-aminocyclopropane-1-carboxylic acid deaminase containing *Mesorhizobium* strains. Afr J Microbiol Res. 9:117-124.

Cobos MJ, Izquierdo I, Sanz MA, Tomás A, Gil J, Flores F, Rubio J (2016) Genotype and environment effects on sensory, nutritional, and physical traits in chickpea (*Cicer arietinum* L.). Span J Agric Res. 14(4), e0709: 10 p.

Didovich SV, Tolkachov MZ, Kameneva IO (2006) Efficient strains of *Mesorhizobium ciceri* for preparation of rhizobofit for chickpea (Cicer arietinum). Agricul Microbiol: Interdepart Them Scientif Collect. 4:147-158.

Elias NV, Herridge DF (2014) Naturalised populations of mesorhizobia in chickpea (*Cicer arietinum* L.) cropping soils: effects on nodule occupancy and productivity of commercial chickpea. Plant Soil. 387(1-2):233-249.

Fierro M, Palmieri D, De Curtis F, Vitullo D, Rubio J, Gil J, Lima G, Millan T (2019) Genetic andagronomic characterization of chickpea landraces for resistance to *Fusarium oxysporum* f. sp. *ciceris*. Phytopathol mediterr. 58(2):239-248.

Frias J, Vidal-Valverde C, Sotomayor S, Diaz-Pollan C, Urbano G (2009) Influence of processing on available carbohydrate content and antinutritional factors of chickpeas. Eur Food Res Technol. 210:340-345.

Gan Y, Selles F, Hanson KG, Zentner RP, McConkey BG, McDonald CL (2005) Effect of formulation and placement of *Mesorhizobium* inoculants for chickpea in the semiarid Canadian prairies. Can J Pl Sci. 85:555-560.

Gangola MP, Båga M, Gaur PM, Chibbar RN (2014) Chickpea – nutritional quality and role in alleviation of global malnourishment. Legume Perspectives. 3:33-35.

Gaur PM et al (2010) Chickpea Seed Production Manual. Patancheru 502 324, Andhra Pradesh, (ed) India: International Crops Research Institute for the Semi-Arid Tropics. 28 pp.

Gibson A (1971) Factors in the physical and biological environment affecting nodulation and nitrogen fixation by legumes. Plant Soil. 35:139–152.

Gul R, Khan H, Khan N, Khan FU (2014) Characterization of chickpea germplasm for nodulation and effect of rhizobium inoculation on nodules number and seed yield. J Anim Plant Sci. 24(5):1421-1429.

Hardy RWF, Holsten RD, Jackson EK, Burns RC (1968) The acetylene-ethylene assay for nitrogen fixation: laboratory and field evaluation. Plant Physiol. 43(8):1185-1207.

Jadhav AA, Rayate SJ, Mhase LB, Thudi M, Chitikineni A, Harer PN, Jadhav AS, Varshney RK, Kulwal PL (2015) Marker-trait association study for protein content in chickpea (*Cicer arietinum* L.). J Genet. 94(2):279–286.

Jarvis BDW, Van Berkum P, Chen WX, Nour SM., Fernandez MP, Cleyet-Marel J-C, Gillis M (1997) Transfer of *Rhizobium loti, Rhizobium huakuii, Rhizobium ciceri, Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int J Syst Bacteriol. 47:895-898.

Kishinevsky BD, Sen D, Weaver RW (1992) Effect of high root temperature on Bradyrhizobium-peanut symbiosis. Plant Soil. 143:275–282.

Khaitov B, Kurbonov A, Abdiev A, Adilov M (2016) Effect of chickpea in association with Rhizobium to crop productivity and soil fertility. Eurasian J Soil Sci. 5(2):105-112.

Khattak SG, Khan DF, Shah SH, Madani MS, Khan T (2006) Role of rhizobial inoculation in the production of chickpea crop. Soil Environ. 25:143-145.

Kryvoruchko I (2017) Zn-use efficiency for optimization of symbiotic nitrogen fixation in chickpea (*Cicer arietinum* L.). Turk J Bot. 41(5):423-441.

Michiels J, Verreth C, Vanderleyden J (1994) Effects of temperature stress on bean-nodulating Rhizobium strains. Appl Environ Microbiol. 60:1206–1212.

Nasr Esfahani M, Sulieman S, Schulze J, Yamaguchi-Shinozaki K, Shinozaki K, Tran L-S (2014) Approaches for enhancement of N2 fixation efficiency of chickpea (*Cicer arietinum* L.) under limiting nitrogen conditions. Plant Biotechnol J. 12:387-397.

Nour SM, Fernandez MP, Normand Ph, Cleyet-Marel J-C (1994) *Rhizobium ciceri* sp. nov., consisting of strains that nodulate chickpeas (*Cicer arietinum* L.). Int J Syst Bacteriol. 44(3):511-522.

Olika E, Abera S, Fikre A (2019) Physicochemical properties and effect of processing methods on mineral composition and antinutritional factors of improved chickpea (*Cicer arietinum L.*) varieties grown in Ethiopia. Int J Food Sci. 2019 (961457):1-7.

Patent Ukraine 141783. Tolkachov MZ, Didovich SV, Kameneva IO (2006) Strain of nodule bacteria *Mesorhizobium ciceri* H-12, active symbiotic nitrogen fixer, which is used for the preparation of a bacterial preparation that increases the yield of chickpeas. Bull. №10, 4 p.

Patent Ukraine 141783. Lohosha OV, Vorobei YuO, Usmanova TO (2020) Strain of nodule bacteria *Mesorhizobium ciceri* ND-64 (IMB-7835) for bacterial preparations for chickpea. Bull. №8, 4 p.

Rupela OP, Dart PJ (1979) Research on symbiotic nitrogen fixation by chickpea at ICRISAT. In: Green JM, Nene YL, Smithson JB (ed) Proceedings of the International Workshop on Chickpea Improvement, 28 February–2 March 1979, Hyderabad, India. Patancheru, India: ICRISAT, pp. 161-167.

Rupela OP (1990) A visual rating system for nodulation of chickpea. Int Chick News. 22:22-25.

Rubio J, Millán T, Gil J (2014) An overview of chickpea breeding programs in Spain. Legum Perspectiv. 3:69-70.

Streeter JG (2003) Effects of drought on nitrogen fixation in soybean root nodules. Plant Cell Environ. 26(8):1199–1204.

Zaman S, Abdul Mazid M, Kabir G (2011) Effect of *Rhizobium* inoculant on nodulation, yield and yield traits of chickpea (*Cicer arietinum* L.) in four different soils of Greater Rajshahi. J Life Earth Sci. 6:45-50.