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Soybean growing under inoculation by *Bradyrhizobium japonicum* strains in the Forest-steppe and Steppe zones of Ukraine

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Abstract

The strategic task of soybean (*Glycine max* (L.) Merr.) breeding today is to create highly adaptive cultivars that have a high level of genetic protection from biotic and abiotic environmental factors and are able to maximise the yield potential in combination with a high grain quality. The use of inoculants is an environmentally safe measure that can significantly increase the soybean yield and reduce the level of plant damage by pests. The aim of the experiment was to evaluate the biological inoculants based on *Bradyrhizobium japonicum* strains under different cultivation conditions and dry-resistant soybean cultivars 'Almaz' and 'Antracite'. *B. japonicum* strains 634b for the 'Almaz' were the most effective in the Forest-steppe, as the yield increased by 0.11 t ha^{-1} compared to that of the control treatment. At the same time, the yield increased by 0.05 t ha^{-1} due to the application of *B. japonicum* strains M8. Under these cultivation conditions, for the 'Antracite', the *B. japonicum* strains M8 provided the greatest growth of yield (increased by 0.23 t ha^{-1} compared to that of the control) and by 0.14 t ha^{-1} when the *B. japonicum* strains 634b were used. On the average, over the experimental years, soybean cultivars in the Forest-steppe had a much higher yield (2.70–2.88 t ha^{-1}) compared to that of the Steppe (1.28–1.88 t ha^{-1}). Treatment with the *B. japonicum* strains 634b significantly increased the yield for the 'Almaz' and 'Antracite' in the Steppe. In the Forest-steppe, the treatment with *B. japonicum* strains 634b was more effective for the 'Almaz' and the one with *B. japonicum* strains M8 for the 'Antracite'. Quantitative symbiotic indices of soybeans were higher in the treatment with *B. japonicum* strains 634b for the 'Almaz' in the Forest-steppe and Steppe. In the Steppe, the application of *B. japonicum* strains M8 was more effective for the 'Antracite'.

Keywords: *Glycine max*, inoculation, *Bradyrhizobium japonicum*, quantitative indexes of plant, yield, conditions of cultivation.

Introduction

Soybean (*Glycine max* (L.) Merr.) is the most important legume crop worldwide (Salvucci et al., 2012). Soybean plays an important role in the balance of food resources improving agricultural productivity, soil nitrogen balance, and crop structure (Pandey et al., 2017; Möller, 2018; Sun et al., 2018; Didovich et al., 2020; Biliavska et al., 2021). The world soybean production in 2017 was 352.6 million tons. In the years 2016–2017, the average soybean grain yield in the entire European Union (3.0 t ha^{-1}) was 10% higher than the yield in Germany (2.7 t ha^{-1}), 20% lower than in the USA (3.4 t ha^{-1}), and about 13% lower than in Brazil (3.1 t ha^{-1}) (FAOSTAT, http://www.fao.org/faostat/en/#data/QC). Ukraine has the greatest opportunities in Europe for soybean cultivation and can provide its own needs (Biliavska, 2008).

The production of soybeans can play a substantial role in the food chain as a healthy food. In different agricultural systems, crops are produced without the application of chemical materials, and the systems severely depend on ecologically and environmentally controlled processes (Zaefarian, Rezvani, 2016). Accordingly, a basic rule is the production of healthy food and the preservation of agroecosystems and the environment. The managing strategies for controlling nutrients, pests, and weeds are eco-friendly and environmentally sound. In modern agriculture, it is usually provided either by chemical or biological fertilisation (Salvucci et al., 2012). Soybean can be an alternative for decreasing of environment contamination and use at the sustainable agricultural management system, because nitrogen is

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one of the most important nutrients that often limits plant growth and thus yield. Hence, soybean production under different systems is highly dependent on the ecological knowledge of field managers (Faezeh, Rezvani, 2016).

An actual issue is the study of the environmental stress of soybean plants. Soybean yield largely depends on meteorological factors and the level of plant reaction to environmental conditions. For some regions, environmental stress factors such as salinity, a low pH, and a low root-zone temperature are actual and can cause a poor nodulation in the presence of otherwise compatible symbionts. Elements of plant and noodle symbiosis such as signal production and excretion, rhizobial attachment, root hair curling, infection thread formation, and nodule initiation are particularly sensitive to these stresses (Duzan et al., 2004).

In Ukraine and other regions, the strategic task of soybean breeding nowadays is to create highly adaptive cultivars that have a high level of genetic protection from biotic and abiotic environmental factors (such as a high temperature and a low amount of precipitation) and can maximise the yield potential in combination with a high grain quality (Belyavskaya, Belyavsky, 2016). Inoculation of soybean (*Glycine max* (L.) Merr.) with *Bradyrhizobium japonicum* strains is a common practice of significant agronomic benefit in areas, where the crop is planted for the first time (Batista et al., 2007; Cerezini et al., 2016; Zimmer et al., 2016; Toleikiene et al., 2021) or in soils, where the number of *B. japonicum* strains is too low to allow an efficient nodulation of the plant.

The experiment conducted on the soils of the Forest-steppe and Steppe zones of Ukraine showed the absence of local nodule bacteria of soybean. However, local introduced populations of soybean rhizobia (microorganisms), which are able to form nitrogen-fixing root nodules during the next cultivation, are found in places, where soybean was grown before. However, due to global warming, the activity of nodule bacteria is decreasing and consequently the nitrogen fixation of plants is also decreasing (Libault, 2014).

The use of microbiological products is an inexpensive environmentally safe measure that can significantly increase the soybean yield and reduce the level of plant damage by pests (Schloter et al., 2000). The application of inoculants allows one to eliminate pesticide stress due to a high fungicidal and bacterial activity and stimulates the development of root system and leaf apparatus (Young, Hong, 1992). All this helps to optimise the ecological state of agrocenoses (Brockwell et al., 1985; Patyka et al., 2014). However, the issue of the complex application of biological products of different functionality has not been sufficiently investigated. Therefore, research on the effect of soybean inoculation is extremely relevant and is aimed at improving the elements of soybean cultivation technologies. In addition to the economic benefits, organic farming has a clear social aspect since its main purpose is to improve the soil environment and provide the population with safe food.

Therefore, the aim of the study was to evaluate the application of *B. japonicum* strains under different cultivation conditions (Forest-steppe and Steppe zones of Ukraine) and dry-resistant soybean cultivars 'Almaz' and 'Antracite'.

Material and methods

Site description and conditions of the experiment. The experiment was conducted in the two sites of different zones of Ukraine in 2013–2018.

Site#1: Forest-steppe zone of Ukraine (49.61354 N, 34.54942 W). The size of the experimental area was 50.0 m^2. The main soil characteristics at the site (0–30 cm depth) were the following: soil type – grey podzolic middle loam; soil organic matter 2.87%; NH_3-N 81 mg kg^{-1} dry soil; P_2O_5 139 mg kg^{-1} dry soil; K_2O 118 mg kg^{-1} dry soil; acidity (pH) 6.8. During the experimental period, the weather conditions were favourable. In some periods, the air temperature was higher than the annual average, and during the years of experiment it ranged from 9.4°C to 22.9°C (Figure 1). The amount of precipitation was close to the annual average and during this period varied greatly from 12.5 to 134.3 mm per month (Figure 2). The hydrothermal coefficient (HTC) calculated according to Selyaninov varied from 0.7 to 1.2. According to the HTC, the weather conditions of the experimental site#1 were close to the optimal ones (HTC = 1.2–1.3) for the growth and development of soybeans in 2013 and 2016. In 2014 and in 2017–2018, the weather conditions were favourable (HTC = 1.1), in 2015 the weather conditions of experimental site were dry (HTC = 1.0) (Figure 3).

Site#2: Steppe zone of Ukraine (44.93330 N, 34.08283 W). The size of the experimental area was 50.0 m^2. The main soil characteristics at the 0–30 cm depth were the following: soil type – *Calcic Chernozem*, low humus; soil organic matter 2.08%; NH_3-N 150 mg kg^{-1} dry soil; P_2O_5 90 mg kg^{-1} dry soil; K_2O 389 mg kg^{-1} dry soil; acidity (pH) 6.8. During the experimental period, the weather conditions were favourable in 2013–2017. In some periods, the air temperature was higher than the annual average, and during the years of experiment it ranged from 8.8°C to 23.8°C (Figure 1). The amount of precipitation was close to the annual average and during this period varied greatly from 2.0 to 126.0 mm per month (Figure 2). According to the HTC, the weather conditions of experimental site#2 were close to the optimal ones with a high moisture content (HTC = 1.1–1.5) for soybean vegetation in 2014–2017. In 2013 and 2018, the weather conditions were dry (HTC = 0.5–0.7) (Figure 3).

Field measurements and analyses. The experiment was based on four replications with a plot area of 50 m^2 and were carried out by common methods (Dospekhov, 1985). The experiment included three factors: Factor A – soybean (*Glycine max* (L.) Merr.) cultivation conditions: Forest-steppe zone and Steppe zone; Factor B – soybean cultivars 'Almaz' and 'Antracite'; Factor C – biological products based on *Bradyrhizobium japonicum* (Bj) strains: Bj0 – without inoculation and with water (control treatment), M8 – *B. japonicum* strains M8, and 634b – *B. japonicum* strains 634b.

Strains from the collection of beneficial soil microorganisms of the Institute of Agricultural Microbiology and Agroindustrial Production of NAAS in Chernihiv, Ukraine, were used. For the pre-sowing treatment of soybean seeds, the aqueous bacterial suspension of strains with an inoculation load of 10^9 cells ml^{-1} in an amount of 1.5% of the working solution by seeds weight were used. The numbers and the weight of nodules were determined according to the Posypanov (1991) method in the flowering stage of soybeans. Counting was carried out from ten plants in four replications. In the experiment, such biometric indicators as the number of nitrogen-fixing nodules from 10 plants and their biomass were taken into account. The grain yield of soybeans was determined by weighing in accordance with the field agronomic practice (Lukomets, 2010).

Agricultural operations for the soybean cultivation under different environmental conditions are described in Table 1.

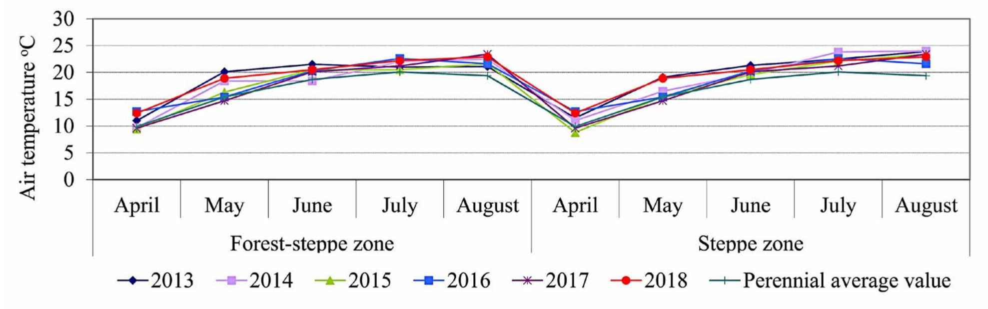


Figure 1. Average air temperature during the soybean cultivation (2013–2018)

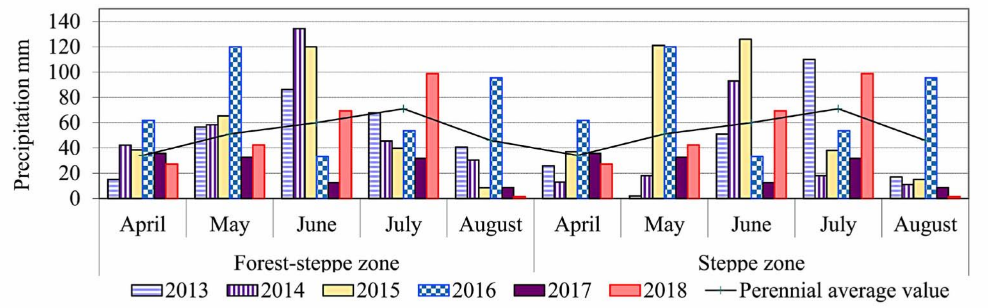
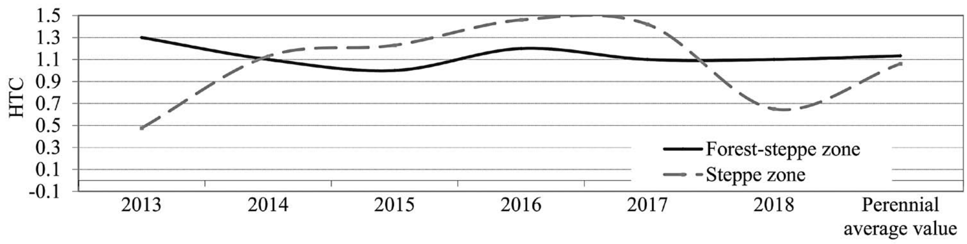


Figure 2. Amount of precipitation during the soybean cultivation (2013–2018)



HTC: <0.4 – very dry; 0.4–0.5 – dry; 0.6–0.7 – middle dry; 0.8–0.9 – weak humidity; 1.0–1.5 – quite humidity; >1.5 – excessive humidity

Figure 3. Hydrothermal coefficient (HTC) during the soybean cultivation (2013–2018)

Table 1. Agricultural operations for the soybean cultivation

Agricultural operation	Forest-steppe zone of Ukraine	Steppe zone of Ukraine
Disking	2nd ten-day period of September	2nd ten-day period of September
Ploughing	3rd ten-day period of September	3rd ten-day period of September
Disking with harrowing	1st ten-day period of April	1st ten-day period of April
Tillage with harrowing	3rd ten-day period of April	2nd ten-day period of April
Pre-sowing seed treatment	1st ten-day period of May	3rd ten-day period of April
Sowing	1st ten-day period of May	3rd ten-day period of April
Sowing depth	3–5 cm	3–5 cm
Width of row-spacing	45 cm	45 cm
Care for crops	During vegetation	
Soybean harvesting	1st ten-day period of September	2nd ten-day period of August

Note. No fertilisers were used during the soybean cultivation.

During the experiment, the *B. japonicum* strains M8 and 634b were used as inoculants of the soybean cultivars ‘Almaz’ and ‘Antracite’, which were bred at Poltava State Agrarian University.

Cultivar ‘Almaz’. The plant height 60–70 cm; the height of the lower bean attachment 12–14 cm. The weight of 1 000 seeds 190–220 g. The cultivar is of early maturity, cold-resistant, and drought-resistant. The vegetation period 100–105 days. The seeding rate is 70–75 kg ha^{-1}. The content of crude protein in seeds 37–39% and fat 24–26%. The cultivar is highly resistant to lodging and cracking of beans. The cultivar of grain type of use is resistant to main diseases and pests. It is recommended for growing in the Forest-steppe and Steppe zones of Ukraine.

Cultivar 'Antracite'. The plant height 80–100 cm; the height of the lower pod attachment 12–14 cm. The weight of 1 000 seeds 180–200 g. The cultivar is of early maturity. The vegetation period 95–105 days. The seeding rate 70–80 kg ha^{-1}. The content of protein in seeds 37–39% and fat 24–26%. The potential grain yield in the Steppe and Forest-steppe zones of Ukraine 3.0–4.0 t ha^{-1}. The cultivar is resistant to lodging and cracking of beans, bacterial and viral diseases, and weakly damaged by pests. It is an optimal preceding crop for winter wheat. Agricultural operations of 'Antracite' are generally accepted in the Forest-steppe and Steppe zones of Ukraine.

Statistical analysis of the experimental results was carried out for four replicates by methods of dispersion (MANOVA) and correlation analysis using the software Statistica, version 6.0 (Informer Technologies Inc.). The significances of differences between the means were tested at the $p < 0.05$ probability level.

Results and discussion

Quantitative symbiotic indices of the soybean cultivars with the application of B. japonicum strains under different cultivation conditions. Quantitative symbiotic indices of soybean (the number and weight of nodules per plant) varied widely and depended on both the cultivation conditions and application of *B japonicum* strains (Tables 2–3).

On the average, over the experimental years, both cultivars grown in the Forest-steppe had the largest number of nodules on the roots. This indicator increased compared with that of the control treatment with an inoculant based on the *B. japonicum* strains M8 and 634b. In the Steppe, the number of nodules varied from 25.4 to 41.3 units per plant for the 'Almaz' and from 29.6 to 39.5 units per plant for the 'Antracite'. This number of nodules was significantly higher when *B. japonicum* strains were used: from 35.1 to 53.9 units per plant for the 'Almaz' and from 47.8 to 61.4 units per plant for the 'Antracite'. In the Steppe, for the soybean cultivar 'Almaz', the number of nodules on the roots exceeded that

Table 2. Dependence of the number (units per plant) of the nodules of soybean on the cultivation conditions, cultivar, and application of *Bradyrhizobium japonicum* strains

Cultivation conditions (Factor A)	Cultivar (Factor B)	Inoculant (Factor C)	Year						Average
			2013	2014	2015	2016	2017	2018	
Steppe zone	Almaz	Bj0	26.9	25.4	28.8	29.8	27.3	25.7	27.3
		M8	33.0	29.7	33.8	34.3	33.1	33.1	32.8
		634b	39.0	39.1	40.8	41.3	38.8	39.2	39.7
	Antracite	Bj0	30.5	30.2	31.1	31.6	30.4	30.1	30.7
		M8	37.3	37.1	39.2	39.5	35.9	35.6	37.4
		634b	30.6	30.2	31.0	31.3	29.8	29.6	30.4
Forest-steppe zone	Almaz	Bj0	36.5	36.9	35.7	38.9	35.1	38.3	36.9
		M8	42.6	42.6	43.0	45.7	42.1	45.2	43.5
		634b	47.5	49.1	52.4	53.9	48.0	48.7	49.9
	Antracite	Bj0	47.8	48.2	50.6	51.5	48.3	48.4	49.1
		M8	53.1	53.2	54,4.	55.3	53.4	53.3	53.8
		634b	59.7	59.8	60.7	61.4	59.8	59.9	60.2
	LSD_{05} (Factor A)		3.6	3.6	3.8	3.5	3.7	3.3	–
	LSD_{05} (Factor B)		5.4	5.8	5.7	5.9	5.6	5.8	–
	LSD_{05} (Factor C)		3.9	3.0	3.7	3.9	3.0	3.2	–
	LSD_{05} (Factors A × B)		4.1	4.5	4.7	4.3	4.2	4.1	–
	LSD_{05} (Factors A × C)		5.5	5.6	5.8	5.1	5.7	5.0	–
	LSD_{05} (Factors B × C)		10.0	10.7	10.4	10.1	10.2	10.8	–

of the control treatment by 5.5 units per plant (M8) and by 6.9 units per plant (634b). Under the same conditions, the inoculation with *B. japonicum* strains M8 (growth by 6.7 units per plant compared with that of the control) was more effective for the 'Antracite'; the use of the *B. japonicum* strains 634b did not significantly increased the number of nodules. The weight of nodules on soybeans depended on both conditions of the cultivation of the year and the applied inoculants and differed among the studied cultivars (Table 3).

In the Steppe, the weight of nodules of 'Almaz' varied from 0.303 to 0.512 g per plant and that of 'Antracite' from 0.307 to 0.521 g per plant. On the average, the use of *B. japonicum* strains 634b were more effective (0.175 g

Table 3. Dependence of the fresh biomass (g per plant) of the nodules of soybean on the cultivation conditions, cultivar, and application of *Bradyrhizobium japonicum* strains

Cultivation conditions (Factor A)	Cultivar (Factor B)	Inoculant (Factor C)	Year						Average
			2013	2014	2015	2016	2017	2018	
Steppe zone	Almaz	Bj0	0.309	0.303	0.311	0.315	0.307	0.306	0.309
		M8	0.331	0.326	0.337	0.340	0.323	0.325	0.330
		634b	0.505	0.503	0.509	0.512	0.501	0.502	0.505
	Antracite	Bj0	0.321	0.318	0.325	0.326	0.317	0.315	0.320
		M8	0.514	0.508	0.517	0.521	0.505	0.504	0.512
		634b	0.317	0.313	0.320	0.322	0.311	0.307	0.315
Forest-steppe zone	Almaz	Bj0	0.568	0.554	0.570	0.574	0.557	0.567	0.565
		M8	0,779	0.785	0.798	0.803	0.782	0.791	0.790
		634b	0.975	0.977	0.987	0.992	0.980	0.983	0.982
	Antracite	Bj0	0.575	0.577	0.588	0.591	0,573	0.578	0.580
		M8	0.897	0.895	0.906	0.912	0.892	0.898	0.900
		634b	1.061	1.059	1.080	1.084	1.068	1.066	1.070
	LSD_{05} (Factor A)		0.087	0.088	0.089	0.088	0.089	0.088	–
	LSD_{05} (Factor B)		0.153	0.154	0.156	0.157	0.155	0.156	–
	LSD_{05} (Factor C)		0.171	1.181	0.174	0.175	0.173	0.175	–
	LSD_{05} (Factors A × B)		0.136	0.137	0.137	0.138	0.134	0.132	–
	LSD_{05} (Factors A × C)		0.075	0.074	0.072	0.074	0.071	0.072	–
	LSD_{05} (Factors B × C)		0.278	0.280	0.284	0.285	0.271	0.274	–

per plant more compared to that of the control) compared with the *B. japonicum* strains M8 (0.021 g per plant more compared to that of the control). In the Forest-steppe, the weight of nodules of 'Almaz' varied from 0.554 to 0.992 g per plant and that of 'Antracite' from 0.573 to 1.084 g per plant. On the average, the *B. japonicum* strains M8 were more effective over the years (nodule weight increased by 0.192 g per plant compared to that of the control), while the effectiveness of *B. japonicum* strains 634b was low.

The climatic conditions of soybean cultivation are dry in some parts (southern regions) of the country. Significant changes in the climate system have been observed in Ukraine in recent 10 years. The average temperature over the last 10 years has increased by 0.77°C, which is almost three times higher than the rate of change of the average annual global air temperature (0.21°C per 10 years). According to the NOAA (US National Oceanic and Atmospheric Administration), Ukraine is one of the countries with the highest temperature. Due to rising air temperature, there was a redistribution of precipitation between seasons and months. A significant increase in their amount by 19.2% and 28.5%, respectively, was observed in autumn, September and October. The decrease of precipitation occurred in winter and summer by 4.6% and 7.8%, respectively. Therefore, there is a need to investigate different soybean cultivars, which are adapted to the environmental conditions.

The research results of Whaley and Eskandari (2019) showing the soybean growing conditions including the environment determine the breeding direction, and this in turn depends on the geographic region and the proposed cultivation of specific varieties. Genetic background and environmental conditions influencing soybean seed protein content and its relationship with yield can facilitate the development of superior high-protein cultivars. This study describes the effect of genotype, environment, and GE interactions on both traits that was significant in both populations. A significant ($P < 0.05$) phenotypic variation for seed protein content and yield in both populations within and across environments was shown by Sobko et al. (2020). They obtained a significantly positive correlation with the seed yield solar radiation ($r = 0.32$) and precipitation ($r = 0.33$). The cultivars of experimental years from MG 00 were less significantly correlated with the tested environmental factors than the cultivars from the maturity group (MG 000). These results were confirmed by MANOVA, which showed a significant impact on the soybean crop yield of the cultivation conditions (Factor A) and the cultivar (Factor B).

Based on the obtained results by Jarecki (2002), it was found that the inoculation significantly increased the number and dry weight of nodules on soybean roots compared to that of the control. The bacterial inoculants significantly increased the number of pods per plant. As a result, a significant increase in the seed yield (0.58 t ha^{-1}) was obtained compared to that of the control. Protein content and fat yield were higher after the seed inoculation by 318 and 101 kg ha^{-1}, respectively, compared to those of the control.

Effect of the cultivation conditions and application of B. japonicum strains on the soybean yield. It was found that in the control treatment (without seed inoculation), both soybean cultivars had from 1.5 to 2.0 times less of nitrogen-fixing nodules, which affected the growth and development of soybeans and the level of grain yield of the investigated cultivars. The results show that the cultivation conditions and the application of *B. japonicum* strains have a great effect on the soybean yield (Table 4, Figures 4–6).

The statistical analysis of experimental data showed the highest soybean yield in the Forest-steppe compared to that in the Steppe during the application of inoculants (in 2013–2016 research years). Statistically reliable differences of yields between the studied cultivars were mostly manifested in 2015–2018 research years in the Steppe conditions. Significant increasing of the soybean yield was evaluated for the cultivar 'Almaz'. In the Forest-steppe conditions, the effectivity of inoculant application was found for the 'Antracite' in 2016, but a significant increasing of the soybean yield was observed for the 'Almaz'.

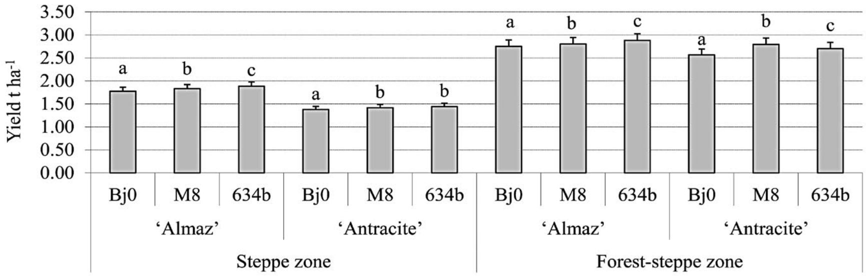
In the Steppe conditions, the efficiency of *B. japonicum* strains 634b was significant for the cultivar 'Almaz' in 2016. In the Forest-steppe conditions, the efficiency of *B. japonicum* strains 634b was significant for the 'Almaz' in 2016 and 2018, and the efficiency of *B. japonicum* strains M8 only in 2018: yield increasing by 0.23 t ha^{-1} and 0.29 t ha^{-1} at $LSD_{05} = 0.19$ (Factor C). For the 'Antracite', a significant efficiency was shown by the *B. japonicum* strains 634b in 2013, 2014, and 2018, and the *B. japonicum* strains M8 showed a reliable efficiency only in 2016 at $LSD_{05} = 0.18$ (Factor C). Over the experimental years, in the Steppe, with seed inoculation, the yield increase of the 'Almaz' was observed. The application of *B. japonicum* strains compared to the control treatment increased the yield by 0.06 t ha^{-1} (M8)

Table 4. Dependence of the soybean yield (t ha^{-1}) on the cultivation conditions, cultivar, and application of *Bradyrhizobium japonicum* (Bj) strains

Cultivation conditions (Factor A)	Cultivar (Factor B)	Inoculant (Factor C)	Year						Average
			2013	2014	2015	2016	2017	2018	
Steppe zone	Almaz	Bj0	1.70	1.60	1.80	2.13	1.72	1.71	1.77
		M8	1.73	1.64	1.82	2.28	1.74	1.76	1.83
		634b	1.78	1.64	1.91	2.34	1.80	1.83	1.88
	Antracite	Bj0	1.40	1.29	1.40	1.90	1.09	1.18	1.38
		M8	1.46	1.31	1.44	1.95	1.15	1.19	1.42
		634b	1.51	1.31	1.39	2.04	1.19	1.21	1.44
Forest-steppe zone	Almaz	Bj0	2.69	2.62	2.70	3.10	2.50	2.90	2.75
		M8	2.69	2.58	2.70	3.21	2.50	3.13	2.80
		634b	2.80	2.68	2.74	3.36	2.51	3.19	2.88
	Antracite	Bj0	2.40	2.50	2.61	2.89	2.49	2.50	2.56
		M8	2.54	2.63	2.64	3.25	2.50	2.50	2.70
		634b	2.94	3.02	2.62	2.98	2.50	2.70	2.79
	LSD_{05} (Factor A)		0.18	0.17	0.09	0.10	0.13	0.16	–
	LSD_{05} (Factor B)		0.36	0.39	0.31	0.30	0.33	0.41	–
	LSD_{05} (Factor C)		0.25	0.29	0.20	0.18	0.12	0.19	–
	LSD_{05} (Factors A × B)		0.24	0.24	0.05	0.10	0.06	0.09	–
	LSD_{05} (Factors A × C)		0.31	0.31	0.17	0.16	0.24	0.29	–
	LSD_{05} (Factors B × C)		0.65	0.72	0.57	0.55	0.59	0.74	–

and by 0.11 t ha^{-1} (634b). Under the same conditions, for the 'Antracite', application of *B. japonicum* strains M8 increased the yield by 0.04 t ha^{-1}, and the application of strains 634b by 0.06 t ha^{-1}. For the 'Almaz', the use of *B. japonicum* strains 634b was the most effective in the Forest-steppe, as the yield increased by 0.11 t ha^{-1} compared to that of the control. The yield increased by 0.05 t ha^{-1} due to the application of *B. japonicum* strains M8. Under these conditions, for the 'Antracite', the application of *B. japonicum* strains M8 provided the greatest growth of yield (increased by 0.23 t ha^{-1} compared to that of the control) and 0.14 t ha^{-1} when the strains 634b were used.

On the average, over the experimental years, the soybean cultivars had a much higher yield in the Forest-steppe (2.70–2.88 t ha^{-1}) compared to that of the Steppe (1.28–1.88 t ha^{-1}). 'Almaz' provided a significantly higher yield in the Forest-steppe (2.88 t ha^{-1}) compared to that in the Steppe (1.88 t ha^{-1}); the 'Antracite' was more productive in the Forest-steppe (2.79 t ha^{-1}) compared with the Steppe (1.42–1.44 t ha^{-1}). In the Steppe, the application of *B. japonicum* strains 634b significantly increased the yield of 'Almaz' and 'Antracite'. In the Forest-steppe, the application of *B. japonicum* strains 634b was more effective for the 'Almaz' and the strains M8 for the 'Antracite' (Figure 4).



Note. Different letters indicate significant differences between treatments; error bars represent standard deviation of the mean; Bj0 – without inoculation (control), M8 – *B. japonicum* M8, 634b – *B. japonicum* 634b.

Figure 4. Dependence of the soybean yield on the conditions of Steppe and Forest-steppe zones, cultivar, and application of *Bradyrhizobium japonicum* strains (average for 2013–2018)

Thus, over six years of the experiment, the inoculation of seeds of 'Antracite' with the *B. japonicum* strains was the most effective in the Forest-steppe. Thereby, the maximum growth of the yield of 'Antracite' compared with that of the control treatment was noted. The application of *B. japonicum* strains M8 in the Forest-steppe and the strains 634b in the Steppe on the 'Almaz' increased the yield. So, the results of our experiment showed a substantial impact of the cultivation conditions, varietal characteristics, and the application of inoculants in the pre-sowing seed treatment on the soybean yield. This is confirmed by the results of multivariate analysis of variance (MANOVA) (Table 5) and graphs (Figures 5–7).

Table 5. Dependence of the soybean yield on the cultivation conditions, cultivar, and application of *Bradyrhizobium japonicum* strains according to MANOVA

	SS	df	MS	*F*	*p*
Intercept	1372.662	1	1372.662	16702.03	0.000000
Factor A	91.632	1	91.632	1114.94	0.000000
Factor B	5.387	1	5.387	65.55	<0.0001
Factor C	0.651	2	0.326	3.96	0.020089
Factors A × B	1.512	1	1.512	18.40	0.000025
Factors A × C	0.100	2	0.050	0.61	0.545861
Factors B × C	0.142	2	0.071	0.86	0.422806
Factors A× B × C	0.125	2	0.062	0.76	0.469483
Error	22.683	276	0.082		

SS – sum of squares, df – degree of freedom, MS – middle square, *F* – Fisher's criterion

The MANOVA showed a substantial impact of the cultivation conditions (Factor A) of the investigated cultivars (Factor B) and inoculants (Factor C) on the soybean crop yield ($p < 0.05$ and $F >$ Ft). The experimental data show that the treatments with inoculant application on the background under different cultivation conditions and cultivars greatly increased the soybean yield.

According to the graph analysis, it was determined that in two different cultivation conditions the variation range of soybean yield was considerable: it varies from 1.60 to 2.04 t ha^{-1} in the Steppe and from 2.50 to 3.25 t ha^{-1} in the Forest-steppe (Figure 5). It was also found that the average yield of the soybeans grown in the Steppe will be much lower than in the Forest-steppe.

The difference in the yield by the soybean cultivars is quite remarkable: this indicator varies from 1.60 to 3.36 t ha^{-1} for the 'Almaz' and from 1.09 to 3.25 t ha^{-1} for the 'Antracite'. So, the advantage of the 'Antracite' compared to the 'Almaz' has been approved.

The application of symbiotic microorganisms increased the yield from 0.04 to 0.23 t ha^{-1} (M8) and from 0.06 to 1.13 t ha^{-1} (634b) compared with that of the control treatment. The average value of soybean yield under the inoculation by *B. japonicum* strains had a significant difference compared to that of the control (Bj0). The average value of soybean yield was higher under the inoculation by *B. japonicum* strains 634b. Thus, the effectiveness of *B. japonicum* strains 634b compared to that of the strains M8 was approved. Therefore, the results of our experiment prove that the maximum realisation of the potential of the adapted cultivar is possible considering the plant-microbial interactions and the use of complementary pairs "cultivars – strains of microorganisms".

Many researches (Zimmer et al., 2016; Leggett et al., 2017; Adjetey, 2019) showed that the use of seed inoculation significantly increased the soybean yield. Under favourable conditions, this crop in symbiosis with nodule bacteria is able to form the yield up to 28–35 kg ha^{-1} and absorb from the atmosphere up to 70–280 kg ha^{-1} N during the growing season and is a good pre-crop for most field crops. On the contrary, Abou-Shanab et al. (2017) reported that inoculation did not always increase soybean seed yield. Kaschuk et al. (2016) found that nodule formation on soybean roots was limited by a high

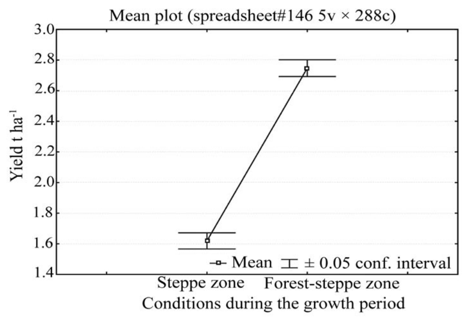


Figure 5. Dependence of the soybean yield on the conditions of Steppe and Forest-steppe zones (Factor A)

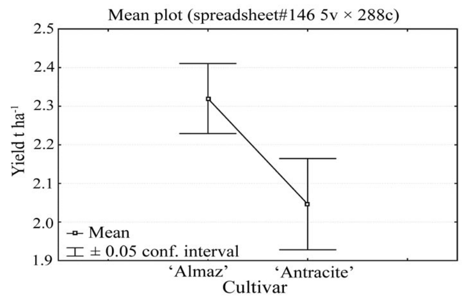
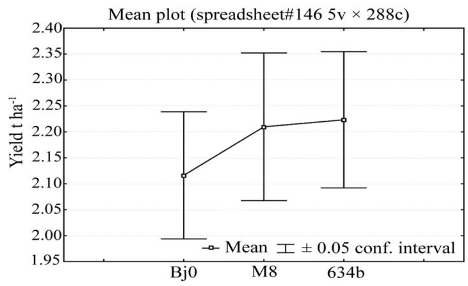


Figure 6. Dependence of the soybean yield on the cultivar (Factor B)



Bj0 – without inoculation (control), M8 – *B. japonicum* M8, 634b – *B. japonicum* 634b

Figure 7. Dependence of the soybean yield on the inoculation with *Bradyrhizobium japonicum* strains (Factor C)

total nitrogen content in the soil. Duzan et al. (2004) added that nodulation could be inhibited by abiotic stress such as a low soil pH and soil temperature or a high soil salinity. Narożna et al. (2015) presented interesting results on the survival of *Rhizobium* strains in the soil in the successive years following the soybean cultivation. In such situation, increasing the number of *B. japonicum* strains increases the number and/or weight of nodules and may improve the grain yield (Hume, Blair, 1992).

The pre-sowing treatment of seeds with biological products based on the strains of *B. japonicum* not only increased plant productivity but also promoted the introduction into soil microbiocenoses of highly effective strains of nodule bacteria (Brockwell et al., 1985). Therefore, experiments in this field should be considered as particularly important.

Conclusions

1. The research results established a substantial impact of cultivation conditions (factor A) on the soybean crop yield (p < 0.05 and F > Ft). The soybean cultivars 'Almaz' and 'Antracite' provide a significantly higher yield in the Forest-steppe.

2. The effectiveness of *Bradyrhizobium japonicum* strains as an inoculant for the pre-sowing seed treatment, which affects the formation of the weight and number of nodules on soybeans and increases the yield, was proved.

3. Quantitative symbiotic indices (the number and weight of the nodules of soybeans) were higher in the treatment with the *B. japonicum* strains 634b for the 'Almaz' in the Forest-steppe and Steppe. Inoculation with *B. japonicum* strains M8 was more effective for the 'Antracite' in the Steppe.

Effective strains of *B. japonicum* M8 and 634b will be studied as part of multifunctional complexes on different soils with pH 5–5.5. Research with a wider range of strains and the selection of effective strains for studies under different environmental conditions of the Forest-steppe zone of Ukraine will also be continued.

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Sojų auginimas taikant inokuliaciją *Bradyrhizobium japonicum* bakterijomis Ukrainos miškastepių ir stepių zonose

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Santrauka

Strateginis gauruotosios sojos (*Glycine max* (L.) Merr.) selekcijos uždavinys – sukurti adaptyvias veisles, pasižyminčias stipria genetine apsauga nuo biotinių ir abiotinių aplinkos veiksnių ir gebančias maksimaliai padidinti derliaus potencialą ir pagerinti grūdų kokybę. Inokuliantų naudojimas yra aplinkai saugi priemonė, galinti reikšmingai padidinti sojų pupelių derlių ir sumažinti kenkėjų augalams daromą žalą. Eksperimento tikslas – įvertinti biologinius *Bradyrhizobium japonicum* bakterijų pagrindu sukurtus inokuliantus įvairiomis sąlygomis auginant sausrai atsparių veislių 'Almaz' ir 'Antracite' sojas. *B. japonicum* 634b bakterijos buvo veiksmingiausias veislės 'Almaz' sojoms miškastepėse – derlius padidėjo 0,11 t ha^{-1}, palyginti su kontroliniu variantu. Derlius taip pat padidėjo 0,05 t ha^{-1} dėl *B. japonicum* M8 bakterijų panaudojimo. Tokiomis auginimo sąlygomis veislės 'Antracite' sojų inokuliacija *B. japonicum* M8 bakterijomis labiausiai padidino derlių iki 0,23 t ha^{-1}, palyginti su kontroliniu variantu, o panaudojus *B. japonicum* 634b bakterijas, derlius padidėjo 0,14 t ha^{-1}. Eksperimento laikotarpiu sojų pupelių vidutinis derlius miškastepėse buvo daug didesnis (2,70–2,88 t ha^{-1}) už derlių stepėse (1,28–1,88 t ha^{-1}). *B. japonicum* 634b bakterijų panaudojimas žymiai padidino veislių 'Almaz' ir 'Antracite' sojų pupelių derlių stepėse. Miškastepėse *B. japonicum* 634b bakterijos buvo veiksmingesnės veislės 'Almaz', o *B. japonicum* M8 bakterijos – veislės 'Antracite' sojoms. Inokuliuojant su *B. japonicum* 634b bakterijomis, miškastepėse ir stepėse veislės 'Almaz' sojų gauti didesni kiekybiniai simbioziniai rodikliai. Stepėse *B. japonicum* M8 bakterijų panaudojimas buvo efektyvesnis veislės 'Antracite' sojoms.

Reikšminiai žodžiai: *Glycine max*, inokuliacija, *Bradyrhizobium japonicum*, augalo kiekybiniai rodikliai, derlius, auginimo sąlygos.